Annals of Diagnostic Pathology 47 (2020) 151558

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Clinicopathological features of 50 mismatch repair (MMR)-deficient endometrial carcinomas, tested by immunohistochemistry: A single institutional feasibility study, India

Bharat Rekhi[a,*], Santosh Menon[a], Kedar K. Deodhar[a], Jaya Ghosh[b], Supriya Chopra[d], Amita Maheshwari[c]

[a] Department of Surgical Pathology, Tata Memorial Hospital, HBNI University, Mumbai, India
[b] Department of Medical Oncology, Tata Memorial Hospital, HBNI University, Mumbai, India
[c] Department of Radiation Oncology, Tata Memorial Hospital, HBNI University, Mumbai, India
[d] Department of Surgical Oncology (Gynecology), Tata Memorial Hospital, HBNI University, Mumbai, India

ARTICLE INFO

Keywords:
Endometrioid carcinoma
Mismatch repair proteins
MMR deficient endometrial carcinoma
Microsatellite instability
Uterine malignancy

ABSTRACT

There are few comprehensive studies from Asia on clinicopathologic features of mismatch repair (MMR)-deficient endometrial carcinomas, including rarely from our country.

One hundred and four cases of endometrial carcinomas were tested for four MMR proteins by immunohistochemistry.

Among 50 MMR-deficient (MMRd) tumors(48%), age-range was 27–68 years(median = 53) and tumor size (n = 34) varied from 1.2–10 cm(average = 4.6). Lower uterine segment(LUS) was involved in 21/31 cases (67.7%). Histopathologically, all cases were endometrioid adenocarcinomas(EMACs), of FIGO grade 2(low-grade)(18 cases) and 3(high-grade)(32 cases), displaying de-differentiated, undifferentiated and lymphoepithelioma(LE)-like patterns, in 24 cases(48%). Tumor infiltration ≥ half of myometrium was seen in 30/44 cases (68.1%); lymphovascular emboli in 19/43 cases(44.1%); and lymph node metastasis in 7/22(31.8%) cases. Uncommonly, clear cell component(n = 2) and focal neuroendocrine differentiation (n = 2) were observed. Immunohistochemically, tumor cells showed paired loss of MLH1 and PMS2 in 33(66%) and MSH2 and MSH6 in 14(28%) cases, along with loss of MSH2 and PMS2, in two and a single case, respectively. Nine patients(18%) were treated for another cancer and 9/33(27.2%) disclosed familial history of cancer. MSH2 was the most frequently lost MMR protein in those cases. Additionally, tumor cells displayed ER positivity in 41/50 cases (82%), PR in 38/41cases(92.6%) and wild-type p53 staining in 24/28 cases(85.7%). Tumor with LE-pattern showed PDLI immunoexpression.

Certain clinicopathologic features suggestive for MMRd associated ECs, such as relatively large-sized tumors, involving LUS; especially high-grade, infiltrative EMACs, with undifferentiated/de-differentiated, and LE-like patterns; showing deep muscle invasion, frequent PR immunoexpression and invariably, wild-type p53 immunostaining can be useful in screening cases of Lynch syndrome. This constitutes the first report on these tumors from our country.

1. Introduction

Endometrial carcinoma (EC) constitutes the most common invasive neoplasm of the female genital tract in the developed nations, and is the fourth most frequently diagnosed cancer among North American females [1]. It is increasing world-wide.

According to the cancer genome atlas (TCGA), there are 4 molecular subtypes of ECs. One of these is microsatellite instability (MSI) hypermutated subtype, arising from defects in the DNA mismatch repair (MMR) system, which can further be inherited(Lynch syndrome), acquired(somatic) or epigenetic, in up to 30% cases of EC [2]. ECs can be tested for MMR-deficiency(MMRd), either by DNA extraction, or by demonstration of the loss of immunohistochemical expression of MMR proteins, namely MLH1, PMS2 and MSH2 and MSH6, mostly identified

* Corresponding author at: Department of Surgical Pathology, Tata Memorial Hospital, Room Number: AB-818, 8th Floor, Annex Building, Dr E.B. Road, Parel, Mumbai 400012, India.
E-mail address: rekhi.bharat@gmail.com (B. Rekhi).

https://doi.org/10.1016/j.anndiagpath.2020.151558

in the form of paired absence; uncommonly as absence of single protein and rarely in the form of absent immunoexpression of all 4 proteins [3-5]. It has been reported that MSI and IHC analysis for MMRd are highly concordant in ECs [3].

More recently, certain clinicopathological features have been found to be associated with MMR-deficient ECs, but in limited number of studies [5,6]. MMRd has been reported to have prognostic and predictive effect in ECs [7,8]. While there are documentations of MMRd associated ECs in the western population, there are few similar studies from Asia, including an occasional report on these tumors from our country [3,9-13].

Herein, we present clinicopathologic features of 50 MMRd associated ECs from our country, constituting the first such report from our subcontinent.

2. Material and methods

The Institutional Electronic Search Engine was used to retrieve cases using key words “MMR” and “endometrial carcinoma” for cases registered from January 2016, until date. Clinical details were obtained from Electronic Medical Records (EMR) and case files from the medical records department.

The study included cases referred to us in the form of paraffin blocks with or without slides, specimens, as well “in-house” operated specimens. Fifteen referral cases were submitted for only histopathologic opinion. All cases were critically reviewed by the first author (B.R.).

Various clinicopathologic parameters were recorded and analyzed. Tumor grading was performed as per the recommendations of the International Society of Gynecological pathologists, including the FIGO grading system [14].

Based on clinical requisitions and clinicopathological features, 104 cases of EC (mostly ‘sign-outs’ of the first author) were tested for four MMR proteins. Immunohistochemistry was performed on Ventana Biosystems, Benchmark, XT (Roche, Basel, Switzerland) on formalin fixed paraffin embedded whole tissue sections. Apart from MMR proteins, most tumors were also tested for estrogen(ER), along with progesterone receptor (PR) and p53. Details of various antibody markers have been listed in Table 1. Loss of immunoexpression of any one of the four MMR proteins was interpreted as MMRd. Immunoexpression of one or more of the MMR proteins were considered as negative or lost, when all the tumor cells showed its negative/absent staining, while interspersed stromal and inflammatory cells revealed positive staining (internal control). Presence of any staining in the tumor cells was interpreted as normal or retained staining. Cases where both the tumor cells and internal control were negative were interpreted as equivocal or uninterpretable. [3-5,10]. Five cases were uninterpretable, therefore excluded from the study. Further, various clinicopathological features were analyzed in all MMRd cases.

Table 1
List of various immunohistochemical antibody markers tested in the present study.

SR. No.	Antibody marker	Clone	Dilution	Manufacturer
1	MLH1	G168-728	1:25	Cell Marque, USA
2	PMS2	EPR3947	Ready to use (RTU)	Cell Marque
3	MSH2	G219-1129	1:50	Cell Marque
4	MSH6	44	1:50	Cell Marque
5	ER	SP1	1:50	Biocare, CA, USA
6	PR	G175-405	1:10	Ventana, Arizona, USA
7	P53	DO7	1:400	Dako, Produkionsveg, Glostrup, Denmark
8	Napsin A	TMU_Ad02	1:100	Biocare
9	PDL1	SP263	RTU	Roche, USA

3. Results

There were 50 cases of MMR-deficient endometrial carcinomas. Age-range was 14–65 years, median = 53 years, average = 51.9 years. Eighteen patients were less than 50 years of age, while 31 patients were more than 50 years of age, along with a single patient of 50 years age. In 9/33 patients (27.2%), there was familial history of cancer, including colon cancer (n = 4) (father and brothers) and throat cancer (mothers of 2 and grandmother of 1 patient). A single patient disclosed history of stomach cancer in her brother and endometrial cancer in her sister and another patient revealed history of leukaemia in her sister.

Nine patients (18%) revealed history of treatment for another cancer. Two patients were previously treated for breast carcinoma (triple negative, 11 years ago and 21 years ago). Two patients (cases 29 and 46) had caecal adenocarcinoma, 15 and 5 years back, respectively. A single patient, each, was previously treated for a gastrointestinal stromal tumor(GIST) of the stomach(one year back), respectively. A single patient had breast carcinoma, 6 years back, followed by synchronous colon and endometrioid adenocarcinoma (case 41). Two patients (cases 25 and 32) had simultaneous ovarian involvement.

Tumor size (known in 34 cases) varied from 1.2–10 cm (average = 4.6). Lower uterine segment (LUS) involvement was seen in 21/31 cases (67.7%).

Details regarding tumor staging were available in 27 cases (55.1%). There were 13 cases, who presented with stage I, including IA(n = 6), IB(n = 6), IBB2(n = 1); 10 cases, who presented with stage III(n = 2), including IIIA(n = 1), IIIB(n = 1), IIIC(n = 1), IIIC1(n = 3), IIIC2(2); 3 cases, who presented with stage II and a single case, who presented with stage IVB disease.

Histopathologically, all cases were endometrioid adenocarcinomas (EMACs), with 18 cases of FIGO grade 2(low-grade) and 33 cases of FIGO grade 3(high-grade), displaying de-differentiated, undifferentiated and lymphoepithelioma (LE)-like pattern with tumor infiltrating lymphocytes, in 24 cases (48%). Uncommonly, clear cell (n = 2) component; focal neuroendocrine differentiation (n = 2) and spindle cell component (n = 1) were also observed. Two cases (2, 32) were initially considered as low grade. However, based on moderate nuclear atypia in both cases and papillary along with significant amount of clear cell component in the latter, grade 2 was assigned.

Tumor infiltration, more than or equal to half of myometrium was seen in 30/44 cases (68.1%); lymphovascular invasion in 19/42 cases (45.2%); and lymph node (pelvic and paraortic) metastasis in 7/22(31.8%) cases. Other metastatic sties were lung (n = 2), iliac fossa (n = 1), abdominal wall (n = 1), thigh(soft tissues) (n = 1), bone (n = 1), omentum (n = 1) and peritoneal fluid(n = 1). Cervical involvement was noted in 10/38 (26.3%) cases.

Immunohistochemically, tumor cells showed paired loss of MLH1 and PMS2 in 33(66%) and MSH2 and MSH6 in 14(28%) cases, along with loss of MSH2 and PMS2, in 2 and a single case, respectively. Among 9 cases with a family history of cancer, the most frequently lost protein was MSH2 (6 cases), followed by MSH6 (5 cases), MLH1 (3 cases) and PMS2 (3 cases). Similarly, among patients harbouring more than one cancer, the most frequently lost protein was MSH2 (5 cases), followed by MLH1 and PMS2, in 4 cases, each and MSH6 in 6 cases. Additionally, tumor cells displayed estrogen receptor (ER) positivity in 41/50 cases (82%), progesterone receptor (PR) positivity in 38/41 cases (92.6%) and wild type p53 staining in 24/28 cases (85.7%). Five cases (3 of EMAC grade 3 and 2 of grade 2) tested for PDL1 immunostaining, showed focal cytoplasmic membranous staining for PDL1 in the tumor cells and the interspersed immune cells (lymphocytes and macrophages) (Table 2) [Figs. 1–8].

Therapeutically (n = 49), 43 patients (86%) underwent total abdominal hysterectomy, followed by bilateral salpingo-oophorectomy (TAHBSO); 11 patients underwent total hysterectomy; three patients underwent endometrial curettage and a single patient underwent hysterectomy with bilateral salpingo-oophrectomy with

Table 2
Clinicopathological features, including immunohistochemical results of 50 MMRd endometrial carcinomas.

Age	T-size (cm)	Tumor grade	Tumor stage	Lower uterine segment	Thickness of myometrium	LVI	ER	PR	P53	MLH1	PMS2	MSH2	MSH6
34	NK	III	NK	P	H	P	N	NP		N	N	P	P
50	NK	II	NK	NK	NK	A	P	P		P	P	N	N
58	2.2	III	IB	A	MH	A	N	NP		N	N	P	P
57	NK	III	IA	NK	LH	A	N	N		P	P	N	N
44	6	III	IVB	A	MH	P	N	NP		N	N	P	P
43	NK	III	NK	NK	NK	NK	N	NP		N	N	P	P
44	NK	II	IIIC2	P	MH	P	P	NP		P	P	N	N
58	NK	III	NK	NK	LH	P	N	NP		N	N	P	P
59	9	III	NK	P	MH	P	FP	NP		N	N	P	P
56	NK	III	IB2	NK	MH	NK	FP	FP		N	N	P	P
47	2.5	II	IA	A	LH	A	P	P		N	N	P	P
55	10	III	IB	P	MH	A	P	P		N	N	P	P
53	5.4	III	NK	A	LH	A	P	FP		N	N	P	P
59	4.2	III	IA	A	H	P	P	P		N	N	P	P
65	NK	III	NK	NK	MH	A	FP	FP		N	N	P	P
60	2	II	IA	A	LH	A	N	N		N	N	P	P
48	NK	II	NK	P	MH	P	FP	FP		N	N	P	P
27	8	II	NK	P	MH	NK	P	P		N	N	P	P
53	7.1	III	IIIC1	NK	MH	A	P	P		N	N	P	P
57	3.2	III	IIB	P	MH	P	P	P		N	N	P	P
51	5.5	III	NK	P	MH	P	FP	FP		P	P	N	N
55	6	III	II	P	H	P	P	P		N	N	P	P
48	NK	III	III	NK	NK	NK	p	p		N	N	P	P
61	6.5	III	IB	P	MH	A	P	P		P	P	N	N
38	5	III	IIIA	P	LH	A	P	P		N	N	P	P
52	5	III	II	P	MH	P	P	P		P	P	N	N
64	4.5	III	IA	A	LH	A	P	P		P	P	N	N
46	3.5	II	NK	NK	H	A	P	P		N	N	P	P
51	5.3	II	IIIC1	P	MH	P	P	P		P	P	N	P
54	7	III	NK	NK	MH	P	N	N		N	N	P	P
53	NK	II	NK	NK	NK	NK	P	P		N	N	P	P
36	5.5	II	NK	P	NK	P	P	P		N	N	P	P
62	4	III	NK	NK	LH	A	P	P		N	N	P	P
41	4.2	II	II	P	H	P	P	P		P	P	N	N
68	NK	III	NK	NK	H	NK	P	P		N	N	P	P
60	6.5	III	IIIC1	NK	LH	P	P	P		N	N	P	P
40	NK	II	NK	NK	H	A	P	P		P	N	P	P
49	3	III	NK	NK	MH	P	P	P		P	P	N	N
55	NK	II	IA	NK	LH	A	P	P		P	P	N	N
64	5	III	III	P	MH	P	P	P		N	N	P	P
53	NK	III	NK	NK	MH	A	N	NP		N	N	P	P
60	3.8	III	1B	P	MH	A	P	P		P	P	N	N
54	3.5	III	IB	A	MH	A	P	P		N	N	P	P
64	2.7	III	II	P	MH	P	P	P		N	N	P	P
49	2.7	III	IB	NK	NK	NK	P	P		P	P	N	N
45	2	II	IIIC2	P	LH	A	P	NP		P	P	N	N
31	1.2	II	NK	A	LH	A	P	P		N	N	P	P
49	5.7	II	NK	A	H	A	P	P		P	P	N	P
65	6	II	NK	P	LH	A	P	P		N	N	P	P
52	NK	II	NK	P	LH	A	P	P		P	P	N	N

LVI: Lymphovascular invasion, NK: Not known, P: Positive, A: Absent, H: Half, MH: More than Half, LF: Less than Half, ER: estrogen receptor, PR: Progesterone receptor, N: negative, NP: Not performed/tested.

colpoperineorrhaphy. Thirteen patients underwent adjuvant radiotherapy (RT) and chemotherapy (CT); six patients underwent adjuvant RT and three patients underwent adjuvant CT, including a single case with palliative intent.

4. Discussion

There are fewer comprehensive studies on a sizable number of MMR deficient (MMRd) ECs from Asia, including none from our country as an indexed publication, except two case reports [9-13]. In one of the earlier studies on MMRd associated ECs, in a multi-ethnic Asian cohort, Woo et al. [9] reported 19.4% (15/77 cases) cases of EC, associated with MMR defects, including an overrepresentation of patients of Indian ethnic origin, compared with Chinese and Malays. As per the TCGA, up to 30% cases of EC are associated with MMRd [2].

Various authors have observed a prevalence of MMRd in cases of EC varying from 19.4% to 55.1%., the latter in a study from Thailand [9,10,12,13,15,16]. In a recent study, Pasanen et al. [6] reported MMRd in 35.8% cases of unselected ECs. The present study constituting the first of its kind from the Indian subcontinent showed MMRd in up to 48% in cases of EC. Some of the possible reasons could be ours being a tertiary cancer referral centre with a relatively higher percentage of high-grade cancers and certain cases included on the basis of morphological features, as well some other cases referred for MMR testing. MMRd manifests as a result of high levels of microsatellite instability (MSI-H) and/or by loss of MMR protein expression, the latter detected by immunohistochemistry [6,17,18]. At the population level, it has been observed that 13%–25% cases of MMRd EC occur as a result of germline pathogenic variants in *MLH1, MSH2, MSH6*, or *PMS2* genes (Lynch syndrome), whereas 62%–73% cases have been reported to arise from somatic hypermethylation of the promoter region of *MLH1* gene [19]. In another recent study, the authors observed MLH1 promoter methylation in 76% cases of MMRd associated ECs [6].

Among four paired MMR proteins tested in the present study, MLH1

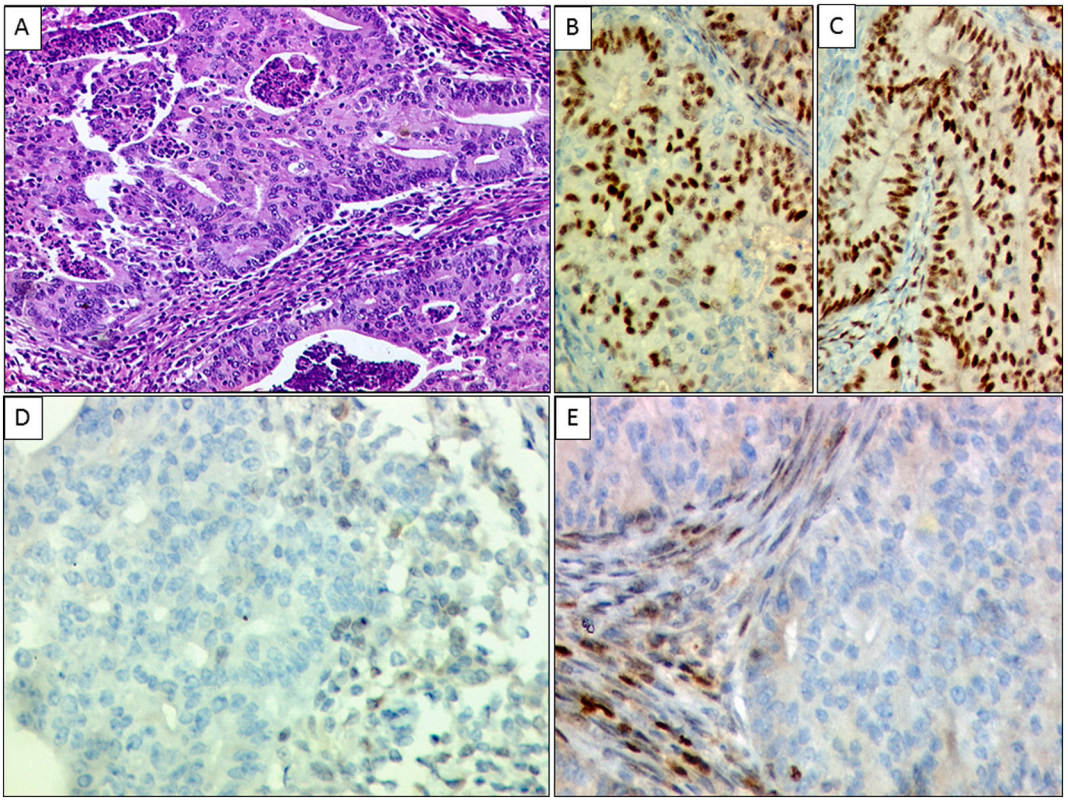

Fig. 1. Case 2. A. Endometrioid adenocarcinoma (EMAC). FIGO grade 2, in a patient with history of colon cancer in her father. H and E, ×200. B. Tumor cells showing immunopositivity for ER. Diaminobenzidine, ×400. C. PR positivity. DAB, ×400. D. Tumor cells showing loss of MSH2 immunostaining. DAB, ×400. E. Tumor cells displaying loss of MSH6 immunostaining. DAB, ×400.

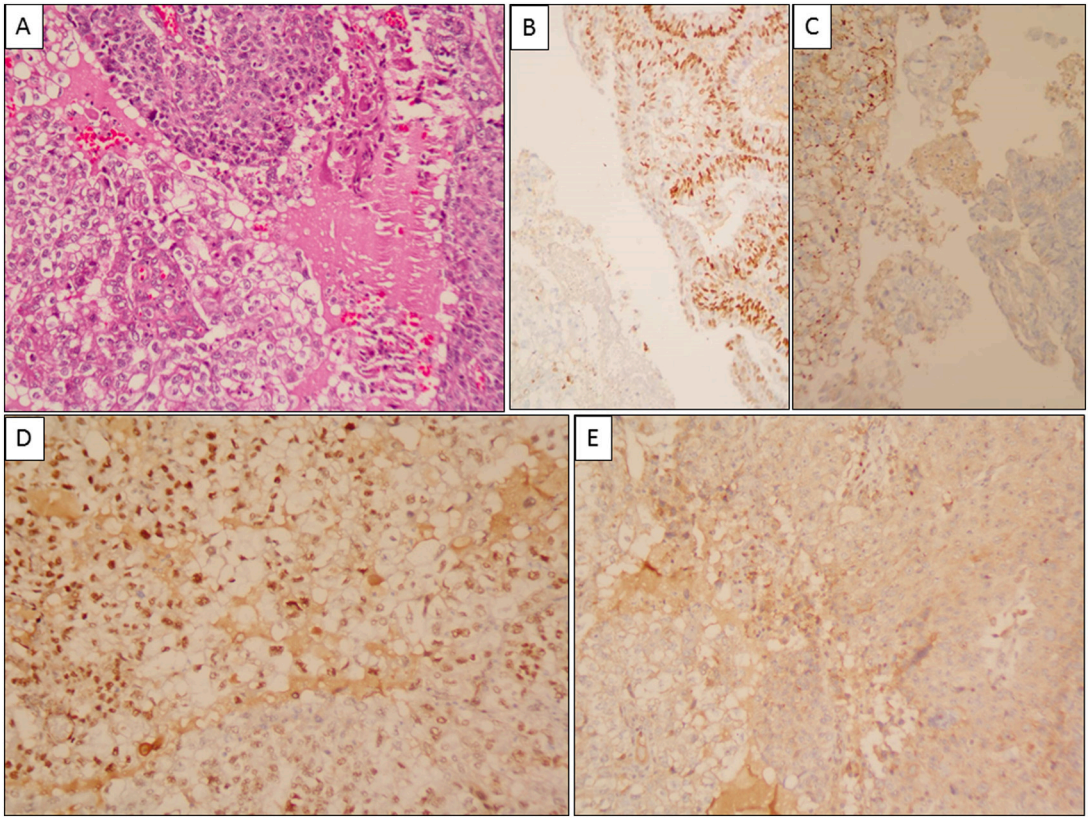

Fig. 2. Case 4. A. EMAC grade III with clear cell component. B. ER positivity in area of EMA. DAB, ×400. C. Area of clear cell component showing distinct positivity for NapsinA. DAB, ×400. C. D. Tumor cells showing retained expression of MLH1. DAB, ×400. Tumor cell showing loss of MSH6. DAB, ×400.

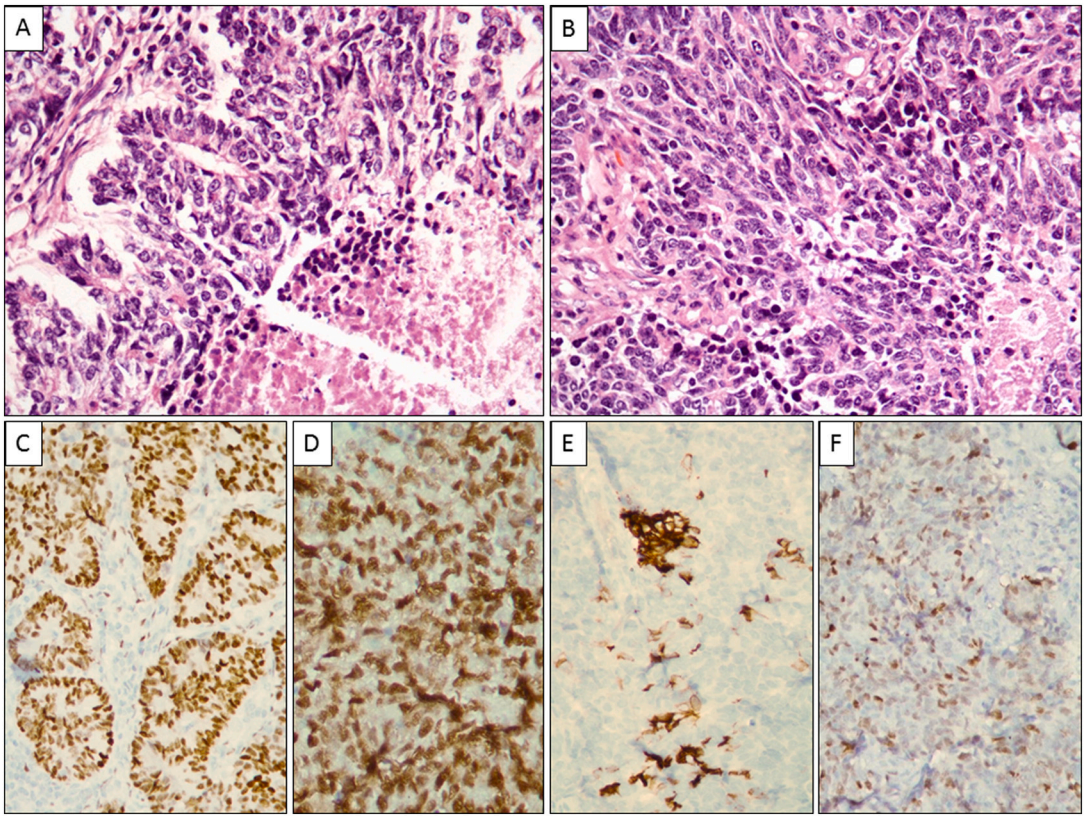

Fig. 3. Case 12. A–B. Endometrioid adenocarcinoma (De-differentiated) FIGO grade 3. A. Relatively differentiated areas with tumor necrosis and apoptosis. H and E, ×400 B. relatively undifferentiated tumor areas. H and E, ×400. C. Diffuse, intense immunopositivity for ER. DAB, ×400. D. Diffuse, intense PR positivity. DAB, ×400. E. Tumor cells showing focal positivity for synaptophysin. DAB, ×400. F. Focal p53 immunopositivity (Wild type). DAB, ×400.

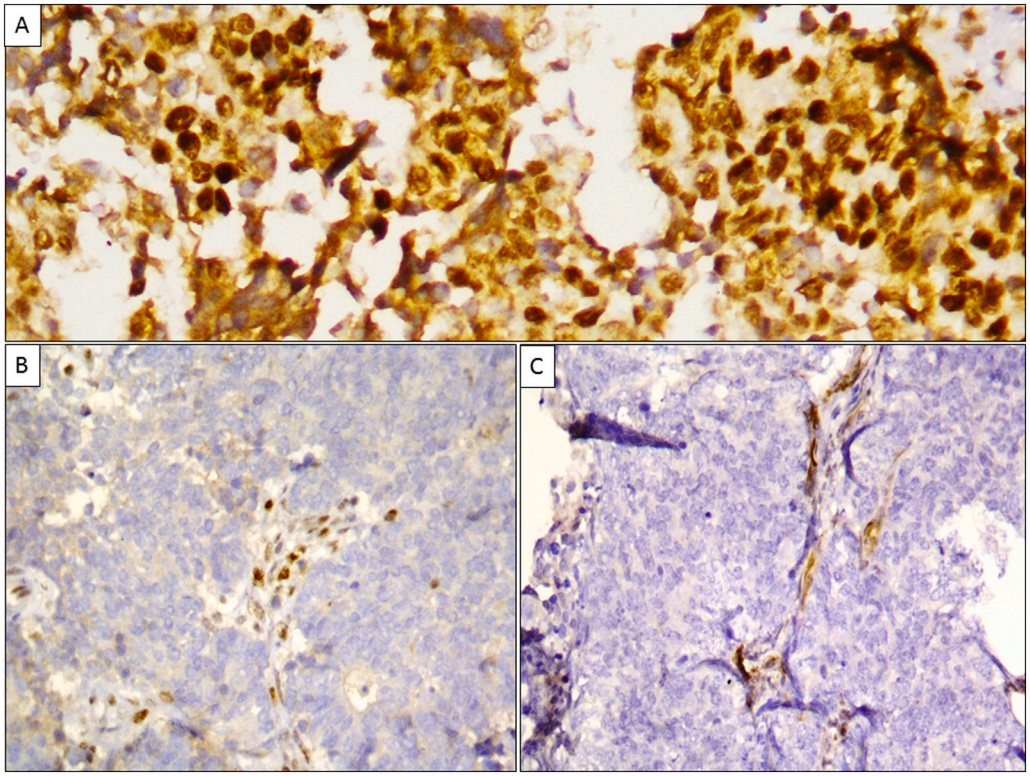

Fig. 4. Case 12. A. Tumor cells showing retained immunoexpression of MSH2. DAB, ×400. B. Tumor cells showing loss of PMS2. DAB, ×400. C. Loss of MLH1. DAB, ×400.

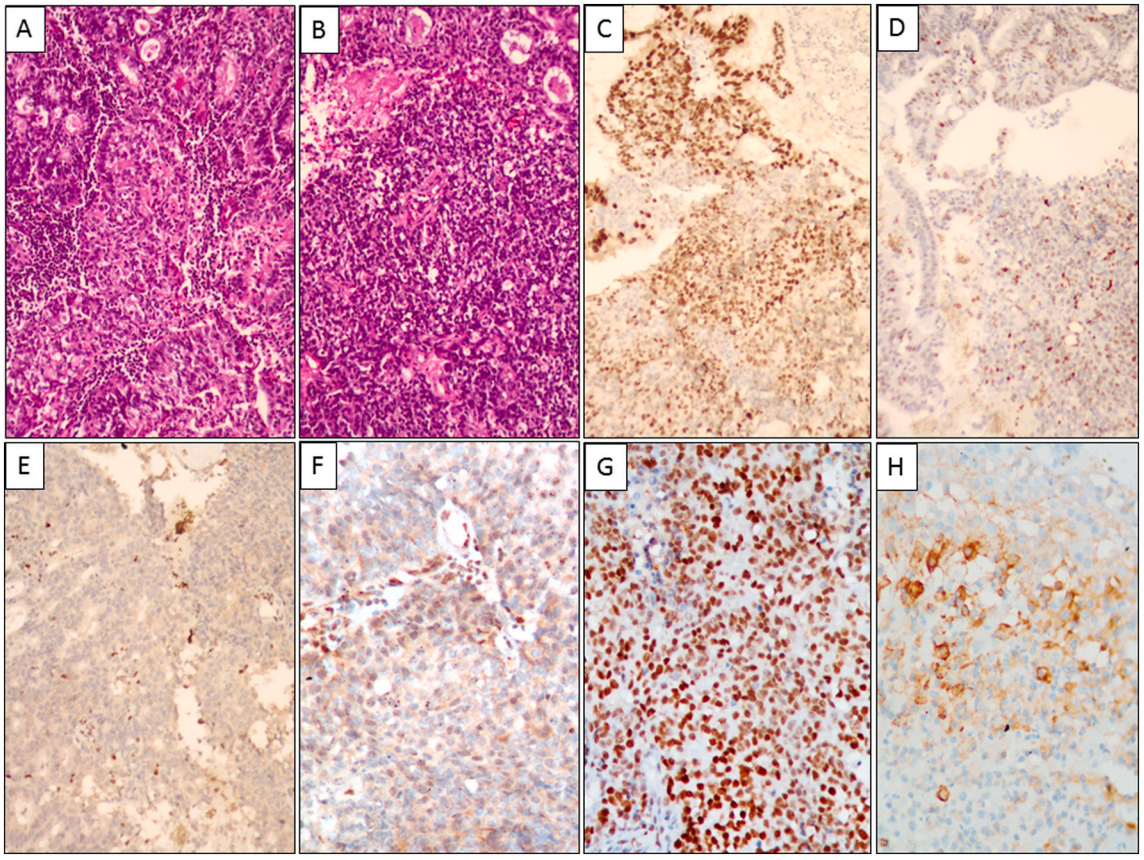

Fig. 5. Case 14. A–B. Endometrioid adenocarcinoma (dedifferentiated) FIGO grade 3with tumor infiltrating lymphocytes (TILs) H and E, ×200. C. ER positivity within tumor cells. H and E, ×400. D. Focal p53 positivity (Wild -type). DAB, ×400. E. Tumor cells showing loss of PMS2. DAB, ×400 F. Tumor cells showing loss of MLH1. DAB, ×400. G. Tumor cells displaying retained immunoexpression of MSH2 immunostaining. DAB, ×400. H. Tumor cells displaying membranous PDL1 immunostaining. DAB, ×400.

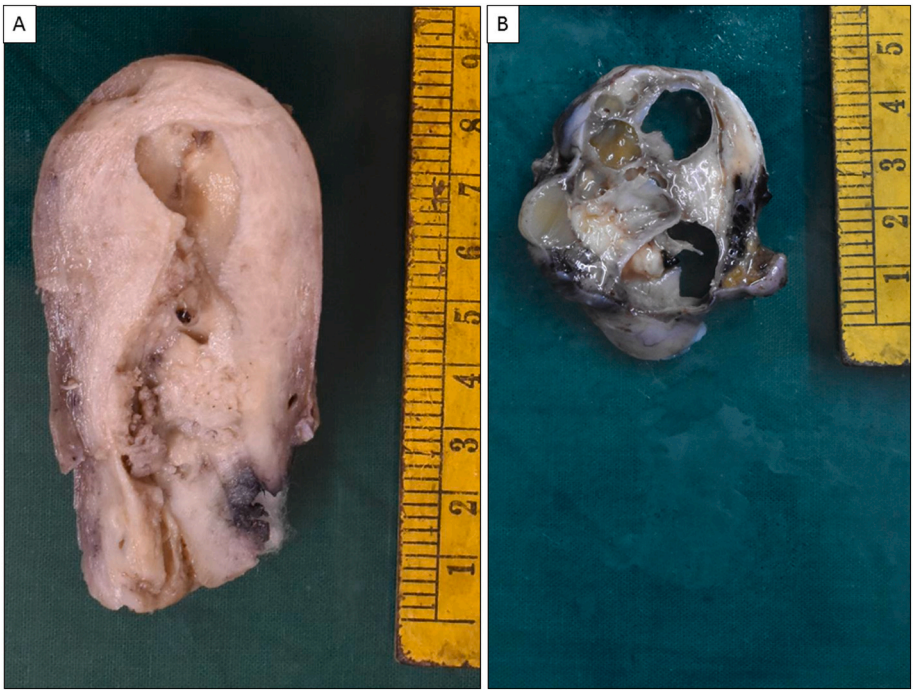

Fig. 6. Case 32. A. Gross specimen (fixed), showing a grey-white friable tumor filling the endometrial cavity, up until the lower uterine segment, sparing the endocervical canal. B. Section from the ovary, showing grey-white solid-cystic areas with tumor deposits.

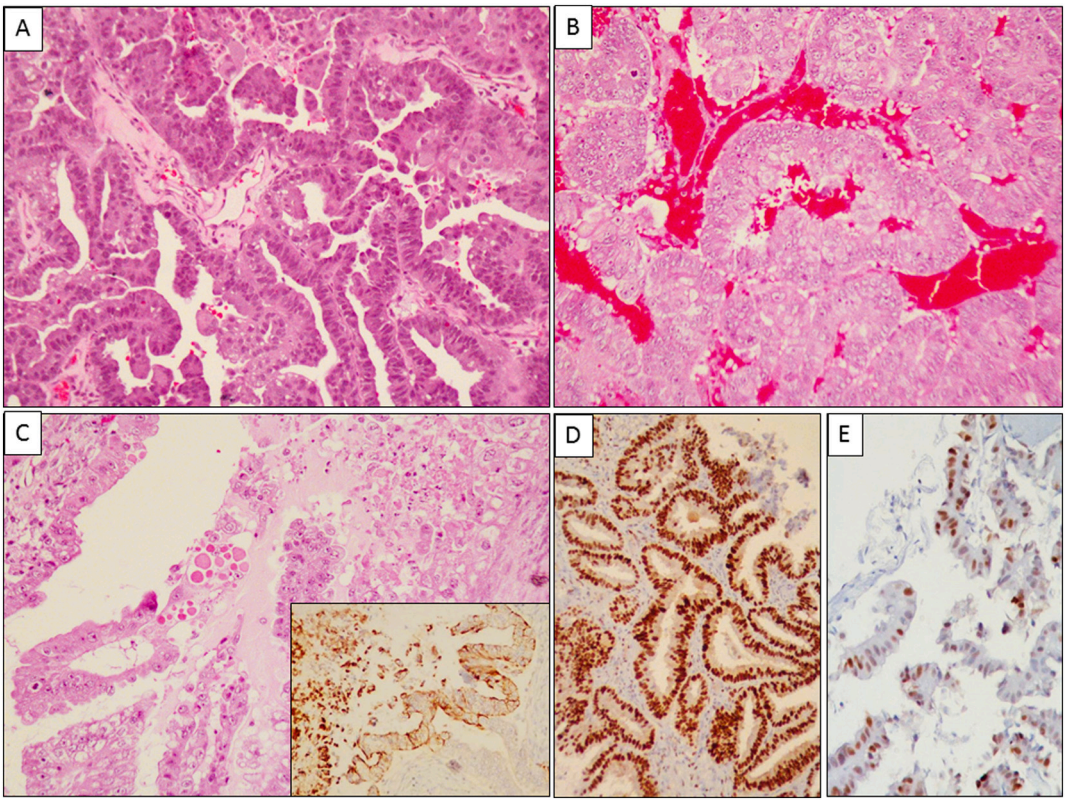

Fig. 7. Case 32. A. EMAC grade II with tumor cells arranged in a prominent papillary pattern. H and E, ×200. B. Tumor cells displaying moderate nuclear atypia. H and E, ×400. C. Distinct areas of clear cell component. H and E, ×400. Inset: Napsin A positivity within clear cell component. DAB, ×400. D. Areas of EMAC displaying ER positivity. DAB, ×400. E. Focal p53 immunopositivity (Wild type). DAB, ×400.

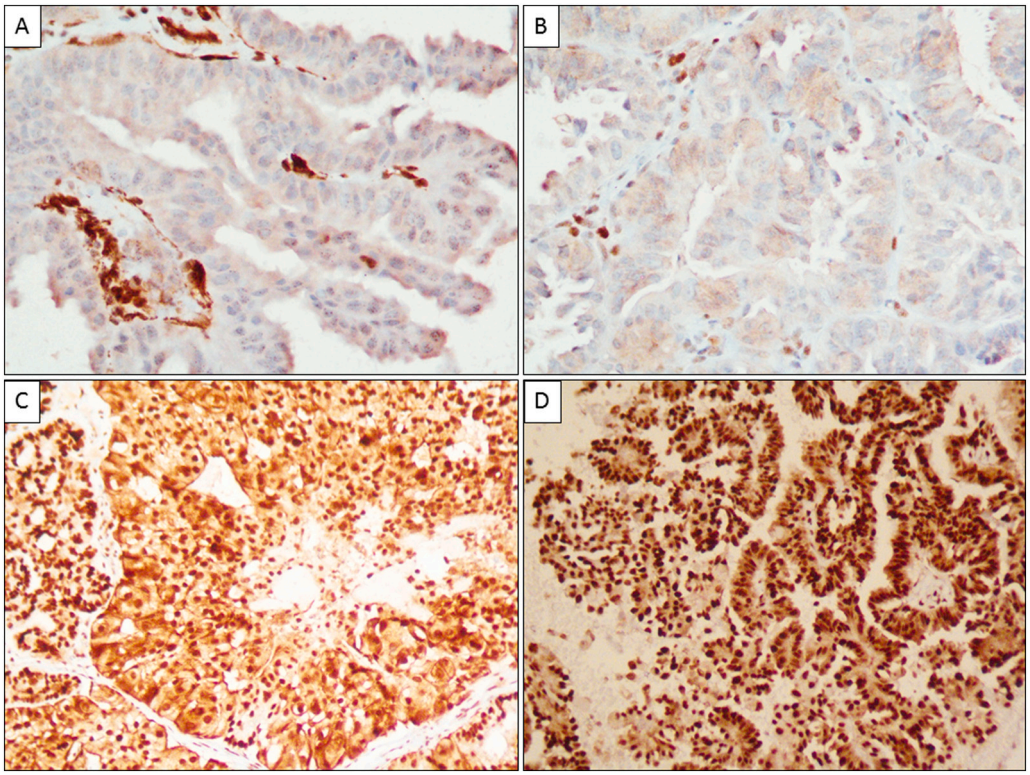

Fig. 8. A. Tumor cells showing loss of MLH1. DAB, ×400. B. Loss of PMS2. DAB, ×400. C. Tumor cells showing retaining immunoexpression of MSH2. DAB, ×400. Tumor cells showing retaining immunoexpression of MSH6. DAB, ×400.

and PMS2 together were lost in 66% cases, while MSH2 and MSH6 were lost in 28% cases. Remaining three tumors showed loss of MSH2 (n = 2) and PMS2 (n = 1), respectively. Similar to our results, Woo et al. [9] observed a much more frequent, combined loss of MLH1 and PMS2 in 86.6% cases and that of MSH2 and MSH6 in 13.3% cases. In another study, the authors observed most frequent loss of combined MLH1/PMS2, followed by MSH2/MSH6, MSH6 and isolated loss of PMS2 [20]. Contrastingly, Tangjitgamol et al. [10] observed the most frequent defect with MSH6 in 38.7% cases, followed by PMS2 (34.3%), MLH1 (33.2%), and MSH2 (16.4%). In a larger cohort, Aguilar et al. [15] observed loss of MLH1/PMS2 in 70%cases, loss of MSH2/MSH6 in 8.7% cases, loss of MSH6 in 10.5% cases, loss of PMS2 loss in 7.8% cases and other patterns in 2.6% cases of MMRd associated EC.

Average and median age of the patients with MMRd in the present study was 51.9 years and 53 years, respectively. This was slightly lesser to an average age of 58 years, in cases of EC, as per an earlier record from our Institution [21]. The mean age range observed in the previous studies was 57.3 years to 66 years, wherein some authors did not observe significant age difference between cases of EC, with or without MMRd [9,10,15]. In some studies, the authors suggested that chances of MMRd, especially Lynch syndrome, more in patients of EC, lesser than 40 years of age [6]. Pasanen et al. [6] reported association between MMRd methylated subgroup of tumors with older patients. As per the recent NCCN guidelines, MMRd testing and screening for Lynch syndrome has been recommended for all patients of EC, irrespective of their age [22].

Various other clinicopathological features were also analyzed in this study. Twenty-seven percent patients disclosed a family history of cancer, including colon cancer as the most frequent type. Average tumor size of 4.6 cm was slightly more than previously reported size in overall cases of EC from our Institution and also reported by others [6,19]. A significant percentage (67.7%) of cases had involvement of the lower uterine segment, as previously observed [6,23,24]. Stage-wise, most presents presented with stage I, followed by stage II disease. In their study, Tangjitgamol et al. [10] reported early stage disease in 59.2% cases of MMRd associated ECs. Histopathologically, most cases were of FIGO grade 3/high-grade(64% cases) and grade 2/low-grade(36%) endometrioid-type, which is similar to the histopathological type, but in contrast with the grade, observed in overall ECs [9,21]. Additionally, 48% tumors revealed histomorphologic heterogeneity, in the form of undifferentiated/dedifferentiated and lymphoepithelioma-like tumor areas with tumor infiltrating lymphocytes. Uncommonly, clear cell component was observed in two tumors; focal neuroendocrine differentiation in another two and spindly cells in another single tumor. Earlier, Soslow [23] and Carcangiu, et al. [25], in two different studies reported an increased frequency of non-endometrioid types, including clear cell carcinoma and high grade EMACs in cases of Lynch syndrome-related ECs. In another study, abnormal staining for MMR proteins was observed in 7/12 cases (58%) of undifferentiated endometrial carcinoma [26]. In an earlier report of 2 cases of MMRd associated ECs in relatively younger patients, tumor grade was high with presence of undifferentiated components and metastatic deposits in the adrenal gland and lymph nodes, respectively [11]. Various other histopathologic components reported as a part of in undifferentiated components, as also noted in some cases of the present study are neuroendocrine and sarcomatous differentiation [26]. Therefore, high grade and undifferentiated/dedifferentiated EMACs with morphologic heterogeneity, especially occurring in relatively younger women could possibly be predictive of MMRd associated ECs. Although there was morphologic heterogeneity, including an occasion case containing serous component, all cases were of endometrioid type, in congruence with most studies [9,10,16].

Tumor infiltration, more than or equal to half of myometrium was seen in 68.1% cases; lymphovascular invasion in 45.2% cases and lymph node (pelvic and paraortic) metastasis in 35% cases, in the present study. Cervical involvement was noted in 26.1% cases. Like-wise earlier investigators reported lymphovascular space invasion and deep muscle invasion, more frequently in cases of MMRd and Lynch syndrome than in patients harbouring MMR proficient (MMRp) ECs. (p = 0.01) [6,15]. In an earlier study, lymph nodes showed metastatic deposits in 41.9% cases of MMRd associated ECs.10 In a study on ECs occurring in women less than 40 years of age, Shih et al. [27] reported significant association between MMRd associated ECs with deep myometrial invasion.

Upon testing tumors with additional immunohistochemical markers, 82% tumors were positive for ER, 92.6% for PR and 85.7% tumors displayed wild type staining for p53. Likewise Pasanen et al. [6] observed association between wild type expression of p53 with MMRd associated ECs. However, they reported association between ER negativity and MMRd ECs. Among ER positive tumors in the present study, diffuse ER staining was frequently seen in low-grade tumors, while focal positivity was seen in high-grade tumors. Eight out of 9 cases of ER negative tumors were of high grade, as previously reported [28]. Two cases with clear cell component, displayed positive staining for Napsin A in the areas of clear cell differentiation [29]. In addition, five cases, especially the ones with “lymphoepithelioma(LE)-like” pattern, including tumor-infiltrating lymphocytes, tested for PDL1 immunostaining, showed variable expression of PDL1, in tumor cells as well as immune cells, as similarly reported in a recent study comprising a larger number of tumors.6 Previously, Kim et al. [20] reported that patients with MMRd or probably Lynch syndrome showed an increase in immune markers compared those with MMR proficiency or sporadic cancer, respectively. This seems to open avenues for consideration of immunotherapy in cases of recurrences and or advanced-stage disease [30].

Nine patients (18%) revealed an earlier history of treatment for another cancer, including breast carcinoma in two cases; caecal adenocarcinoma in two cases and synchronous ovarian involvement in two cases. One of the patients was treated for breast cancer and subsequently developed synchronous endometrial and colon cancer. In their study, Tangjitgamol et al. [10] observed 16.9% cases with history of another cancer. While some authors suggested the presence of synchronous endometrioid carcinomas of the ovary and endometrium with MSI, others did not conclude this result [31,32]. Interestingly, besides the study cases, we came across a 39 year-old patient with synchronous EMAC of the ovary and colonic carcinoma, displaying combined loss of MSH2 and MSH6. Upon extensive sectioning of the TAHBSO specimen, there was no tumor in the endometrium. Apparently, MMRd associated ovarian carcinomas have been reported, which are mostly non-serous type [33].

Therapeutically, most cases (86%) in this study were treated with TAHBSO, including adjuvant CT and RT offered in 13 cases. While some cases of EMAC, especially of early stage in younger women are treated with hormonal therapy, especially in those intending for fertility conservation; there is some evidence that cases with MMRd might not be suitable for the same, in view of more chances of myometrial invasion and associated higher grade/aggressive components in such cases as also noted in the present study as well as in some earlier reports [11,24,25]. In terms of clinical outcomes, while some studies showed a relatively worse survival in MMRd associated ECs, others revealed improved survival in these cases. Few studies showed no influence of MMRd in clinical outcomes of EC [27,33-35]. In a study, Steinbakk, et al. [36] demonstrated that high survivin, low p21 and microsatellite instability high, together led to identification of a small subgroup of FIGO stage 1 EMACs with a poor prognosis.

While a reasonable number of cases tested for MMRd constitute strength of our study, there are certain recognizable limitations. Clinical details, such as family history, tumor stage, T-size, involvement of LUS were not available for all cases and certain parameters such as lymphovascular invasion were not available in all cases, considering at times, referral cases are accompanied with limited details. Likewise, regarding the pathological parameters, the limitations were submission of fewer blocks, despite total hysterectomies, performed at the referring hospitals. In addition, there were some patients, who underwent curettage and were lost to follow-up.

To conclude, this constitutes the first comprehensive study on MMRd associated ECs from our subcontinent. Although, MMR testing has been

recommended in all cases of EC, this remains a limitation in this part of this world, in view of financial constraints, logistics, especially test validation, and quality related issues. Nonetheless, certain clinicopathologic features that can be used as trigger for MMRd testing include relatively large-sized tumors, involving LUS; frequently in ≤53 year-old patients, in our population, harbouring high-grade, deeply infiltrative EMACs, comprising heterogeneous tumor patterns, such as de-differentiated, undifferentiated and "LE-like" patterns. These tumors are frequently PR positive and display wild-type p53 immunostaining. MMRd testing is useful for screening cases of Lynch syndrome. Loss of MSH2 was most frequently associated MMRd in patients harbouring more than one cancer and also those with a family history of malignancy. PDL1 immunostaining in cases of MMRd EMACs reinforces the possibility of consideration of immunotherapy in select cases.

CRediT authorship contribution statement

Bharat Rekhi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing - original draft, Writing - review & editing. **Santosh Menon:** Data curation, Investigation, Methodology. **Kedar K. Deodhar:** Data curation, Investigation, Methodology. **Jaya Ghosh:** Data curation, Writing - review & editing. **Supriya Chopra:** Data curation, Writing - review & editing. **Amita Maheshwari:** Data curation, Writing - review & editing.

Funding source

None.

Declaration of competing interest

None.

Acknowledgement

The technical support of Immunohistochemistry Laboratory at Tata Memorial Mumbai. Dr. Ashwini Duggad, Resident, Department of Pathology, TMH for helping in retrieval of some cases. This study (in part) was presented by B.R. as a poster (physical) during the 31st European Congress of Pathology Meeting, held on 8th September 2019 in Nice, France. This study (in part) was presented by B.R. as a poster (physical) during the 31st European Congress of Pathology meeting, held on 8th September 2019 in Nice, France.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29.
[2] Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013;497:67–73.
[3] Stelloo E, Jansen AML, Osse EM, Nout RA, Creutzberg CL, Ruano D, et al. Practical guidance for mismatch repair-deficiency testing in endometrial cancer. Ann Oncol 2017;28:96–102.
[4] Hagen CE, Lefferts J, Hornick JL, Srivastava A. "Null pattern" of immunoreactivity in a lynch syndrome-associated colon cancer due to germline MSH2 mutation and somatic MLH1 hypermethylation. Am J Surg Pathol 2011;35:1902–5.
[5] Garg K, Soslow RA. Endometrial carcinoma in women aged 40 years and younger. Arch Pathol Lab Med 2014;138:335–42.
[6] Pasanen A, Loukovaara M, Bützow R. Clinicopathological significance of deficient DNA mismatch repair and MLH1 promoter methylation in endometrioid endometrial carcinoma. Mod Pathol Feb 14. doi: https://doi.org/10.1038/s41379-020-0501-8. [Epub ahead of print].
[7] Nelson GS, Pink A, Lee S, Han G, Morris D, Ogilvie T, et al. MMR deficiency is common in high-grade endometrioid carcinomas and is associated with an unfavorable outcome. Gynecol Oncol 2013;131:309–14.
[8] Kim SR, Pina A, Albert A, McAlpine J, Wolber R, Blake Gilks C, et al. Does MMR status in endometrial cancer influence response to adjuvant therapy? Gynecol Oncol 2018;151:76–81.
[9] Woo YL, Cheah PL, Shahruddin SI, Omar SZ, Arends M. The immunohistochemistry signature of mismatch repair (MMR) proteins in a multiethnic Asian cohort with endometrial carcinoma. Int J Gynecol Pathol 2014;33:554–9.
[10] Tangjitgamol S, Kittisiam T, Tanvanich S. Prevalence and prognostic role of mismatch repair gene defect in endometrial cancer patients. Tumour Biol 2017;39:1010428317725834.
[11] Rekhi B. Mismatch repair protein deficient endometrioid adenocarcinomas, metastasizing to adrenal gland and lymph nodes: unusual cases with diagnostic implications. Indian J Pathol Microbiol 2015;58:491–5.
[12] Long Q, Peng Y, Tang Z, Wu C. Role of endometrial cancer abnormal MMR protein in screening Lynch-syndrome families. Int J Clin Exp Pathol 2014;7:7297–303.
[13] Saeki H, Hlaing MT, Horimoto Y, Kajino K, Ohtsuji N, Fujino K, et al. Usefulness of immunohistochemistry for mismatch repair protein and microsatellite instability examination in adenocarcinoma and background endometrium of sporadic endometrial cancer cases. J Obstet Gynaecol Res 2019;45:2037–42.
[14] Soslow RA, Tornos C, Park KJ, Malpica A, Matias-Guiu X, Oliva E, et al. Endometrial carcinoma diagnosis: use of FIGO grading and genomic subcategories in clinical practice: recommendations of the International Society of Gynecological Pathologists. Int J Gynecol Pathol 2019;38:S64–74. Suppl 1(Iss 1 Suppl 1.
[15] Aguilar CMAO, Vroobel A, Lalondrelle S, Taylor A, Nobbenhuis M, Attygalle A, et al. Prevalence and clinical implications of mismatch repair (MMR) deficiency in unselected endometrial cancer (EC) patients. Ann Oncol 2018;29(Suppl. 8):viii342. [Abstract].
[16] Nagle CM, O'Mara TA, Tan Y, Buchanan DD, Obermair A, Blomfield P, et al. Australian Endometrial Cancer Study Group. Endometrial cancer risk and survival by tumor MMR status. J Gynecol Oncol 2018;29:e39.
[17] Boland CR, Koi M, Chang DK, Carethers JM. The biochemical basis of microsatellite instability and abnormal immunohistochemistry and clinical behavior in Lynch syndrome: from bench to bedside. Fam Cancer 2008;7:41–52.
[18] Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. J Clin Oncol 2006;24:4783–91.
[19] Buchanan DD, Rosty C, Clendenning M, Spurdle AB, Win AK. Clinical problems of colorectal cancer and endometrial cancer cases with unknown cause of tumor mismatch repair deficiency (suspected Lynch syndrome). Appl Clin Genet 2014;7:183–93.
[20] Kim J, Kong JK, Yang W, Cho H, Chay DB, Lee BH, et al. DNA mismatch repair protein immunohistochemistry and MLH1 Promotor methylation testing for practical molecular classification andthe prediction of prognosis in endometrial Cancer. Cancers (Basel) 2018;10(9). [pii: E279].
[21] Deodhar KK, Rekhi B, Menon S, Ganesh B. An audit of histopathology reports of carcinoma endometrium: experience from a tertiary referral center. J Postgrad Med 2015;61:84–7.
[22] National comprehensive cancer network (NCCN) guidelines version 1.2020. Uterine neoplasms. P. 1–108. https://www.nccn.org/professionals/physician_gls/default.aspx#site.
[23] Soslow RA. Endometrial carcinomas with ambiguous features. Semin Diagn Pathol 2010;27:261–73.
[24] Garg K, Leitao Jr. MM, Kauff ND, Hansen J, Kosarin K, Shia J, et al. Selection of endometrial carcinomas for DNA mismatch repair protein immunohistochemistry using patient age and tumor morphology enhances detection of mismatch repair abnormalities. Am J Surg Pathol 2009;33:925–33.
[25] Carcangiu ML, Radice P, Casalini P, Bertario L, Merola M, Sala P. Lynch syndrome–related endometrial carcinomas show a high frequency of nonendometrioid types and of high FIGO grade endometrioid types. Int J Surg Pathol 2010;18:21–6.
[26] Tafe LJ, Garg K, Chew I, Tornos C, Soslow RA. Endometrial and ovarian carcinomas with undifferentiated components: clinically aggressive and frequently underrecognized neoplasms. Mod Pathol 2010;23:781–9.
[27] Shih KK, Garg K, Levine DA, Kauff ND, Abu-Rustum NR, Soslow RA, et al. Clinicopathologic significance of DNA mismatch repair protein defects and endometrial cancer in women 40 years of age and younger. Gynecol Oncol 2011;123:88–94.
[28] Shen F, Gao Y, Ding J, Chen Q. Is the positivity of estrogen receptor or progesterone receptor different between type 1 and type 2 endometrial cancer? Oncotarget 2017;8:506–11.
[29] Fadare O, Desouki MM, Gwin K, Hanley KZ, Jarboe EA, Liang SX, et al. Frequent expression of napsin a in clear cell carcinoma of the endometrium: potential diagnostic utility. Am J Surg Pathol 2014;38:189–96.
[30] Ono R, Nakayama K, Nakamura K, Yamashita H, Ishibashi T, Ishikawa M, et al. Dedifferentiated endometrial carcinoma could be a target for immune checkpoint inhibitors (anti PD-1/PD-L1 antibodies). Int J Mol Sci 2019;20(15). [pii: E3744].
[31] Aysal A, Karnezis A, Medhi I, Grenert JP, Zaloudek CJ, Rabban JT. Ovarian endometrioid adenocarcinoma: incidence and clinical significance of the morphologic and immunohistochemical markers of mismatch repair protein defects and tumor microsatellite instability. Am J Surg Pathol 2012;36:163–72.
[32] Shannon C, Kirk J, Barnetson R, Evans J, Schnitzler M, Quinn M, et al. Incidence of microsatellite instability in synchronous tumors of the ovary and endometrium. Cancer Res 2003;9:1387–92.
[33] Xiao X, Melton DW, Gourley C. Mismatch repair deficiency in ovarian cancer – molecular characteristics and clinical implications. Gynecol Oncol 2014;132:506–12.
[34] Resnick KE, Frankel WL, Morrison CD, Fowler JM, Copeland LJ, Stephens J, et al. Mismatch repair status and outcomes after adjuvant therapy in patients with surgically staged endometrial cancer. Gynecol Oncol 2010;117:234–8.
[35] Black D, Soslow RA, Levine DA, Tornos C, Chen SC, Hummer AJ, et al. Clinicopathologic significance of defective DNA mismatch repair in endometrial carcinoma. J Clin Oncol 2006;24:1745–53.
[36] Steinbakk A, Malpica A, Slewa A, Skaland I, Gudlaugsson E, Janssen EA, et al. Biomarkers and microsatellite instability analysis of curettings can predict the behavior of FIGO stage I endometrial endometrioid adenocarcinoma. Mod Pathol 2011;24:1262–71.