Original Article

Immunohistochemistry for Mismatch Repair (MMR) Proteins in Endometrial Cancer

Alamgir SN[1], Ferdous J[2], Faika MJ[3], Farhana K[4], Rahman KT[5], Rashid FB[6], Asaduzzaman M[7]

ABSTRACT

A cross-sectional study was conducted in the Department of Gynaecological Oncology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, between January and December of 2023, to evaluate the immunohistochemistry findings for mismatch repair (MMR) proteins in endometrial cancer. A total of 54 endometrial carcinoma patients diagnosed by fractional curettage or diagnostic curettage admitted into the BSMMU Hospital were enrolled in this study. Patient's history (including age, BMI, family history, parity, menstrual pattern, comorbidities, clinical complaints, investigations and operation notes) was recorded in data collection sheet. After surgery, blocks were made from pathological specimens for histopathological examination. After regular histopathological examination, assessment of the MMR status of each patient's tumour was determined by immunohistochemistry by using monoclonal mouse antibodies against MMR proteins (MLH1, MSH2, MSH6, and PMS2) following the Dako EnVision method according to manufacturer protocol (in the Department of Pathology of the same institution). Immunohistochemistry findings revealed that two-thirds (63%) of the patients had intact expression of all MMR protein (MMRp), while 37% had a loss of expression of any of the proteins, which is known as mismatch repair deficiency (dMMR) state. Among them, the most common MMR defect identified was combined loss of MLH1/PMS2 in 9(16.67%) cases, followed by isolated PMS2 loss or isolated MSH2 loss in 6(11.11%) cases, combined MSH2/MSH6 loss in 4(7.4%) cases, while loss of all four proteins was observed in only 1(1.85%) case (Table-III). Deeper myometrial invasion (e"50%) was more evident in MMRp tumours compared to dMMR tumours ($p<0.01$). Cervical extension was also found more frequent in MMRp tumours ($p<0.05$). In contrast, dMMR tumours were found more frequently having lower tumour grade and lower FIGO stage compared to MMRp tumours ($p<0.05$). However, no differences were observed in tumour type, size, adnexal involvement, lymphovascular space invasion (LVSI), and lymph node involvement ($p>0.05$)

Keywords: *Endometrial cancer, histopathology, immunohistochemistry, mismatch repair protein.*

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1. Dr. Sadia Nusrat Alamgir, Assistant Professor, Department of Gynaecological Oncology, National Institute of Cancer Research and Hospital (NICRH), Dhaka-1212. Email: sadianusrat77@yahoo.com (Corresponding author).
2. Prof. Jannatul Ferdous, Chairman, Department of Gynaecological Oncology, Bangladesh Medical University (BMU), Dhaka-1000.
3. Dr. Mst. Jakanta Faika, Assistant Professor, Department of Gynaecological Oncology, National Institute of Cancer Research and Hospital (NICRH), Dhaka-1212.
4. Dr. Kaniz Farhana, Assistant Professor, Department of Gynaecological Oncology, National Institute of Cancer Research and Hospital (NICRH), Dhaka-1212.
5. Dr. Khandker Tafriha Rahman, Assistant Professor, Department of Gynaecological Oncology, National Institute of Cancer Research and Hospital (NICRH), Dhaka-1212.
6. Dr. Farhana Binty Rashid, Assistant Professor, Department of Obstetrics & Gynaecology, Dhaka Medical College & Hospital, Dhaka-1000.
7. Dr. Mohammad Asaduzzaman, Assistant Registrar, Department of Medical Oncology, National Institute of Cancer Research and Hospital (NICRH), Dhaka-1212.

INTRODUCTION

Endometrial cancer has emerged as a significant gynaecological malignancy in low- and middle-income countries (LMICs) like Bangladesh, frequently ranked as the second most common female genital tract malignancy after cervical cancer.[1,2] Recent advancement in genetic studies revealed that about 20–40% of endometrial cancer cases exhibit a mismatch repair deficiency (dMMR) phenotype. dMMR is caused by genetic or epigenetic alterations of any of the mismatch repair genes (*MLH1, MSH2, MSH6, PMS2*).[3,4] The MMR system is crucial for maintaining genomic stability by correcting DNA replication errors, which are more common in genome regions with short repetitive DNA sequences. The most relevant MMR proteins in humans are *MLH1, MSH2, MSH6, and PMS2*, which are coded by the corresponding genes. These genes are *MLH1* (mutL homolog 1), *MSH2* (mutS homolog 2), *MSH6* (mutS homolog 6), and *PMS2* (PMS1 homolog 2, mismatch repair system component), located in chromosomes 3, 2, 2, and 7, respectively.[5]

Detection of deficient mismatch repair function is used diagnostically, predictively, and prognostically in endometial cancer.[4] Immunohistochemistry (IHC) assay of MMR proteins has emerged as a widely employed method for detecting the dMMR phenotype in endometrial cancer. It serves as a molecular classification,[6,7] a companion diagnostic for immunotherapy,[6,8-10] a secondary screening for Lynch syndrome.[6,7,9] Regarding molecular classification, various guidelines, and the recently published FIGO 2023 staging encourage its implementation for all endometrial cancers, explicitly highlighting the requirement for dMMR assessment through IHC.[7] In immunotherapy applicability, MMR IHC is preferred over PCR-based microsatellite instability (MSI) testing for endometrial cancer.[8] As we mentioned earlier, universal screening for Lynch syndrome through immunohistochemistry of MMR protiens and mutations in all endometrial cancer cases is also suggested by certain guidelines.[6,7,9] Under the circumstances, the significance of the MMR-IHC assay in patients with endometrial cancer has become increasingly evident even in low-resource settings like Bangladesh. Hence, we propsed the present study to evaluate the immunohistochemistry findings for mismatch repair (MMR) proteins in endometrial cancer (tumours removed by surgery) in a tertiary level specialized hospital in Dhaka, Bangladesh.

METHODS

This cross-sectional study was conducted in the Department of Gynaecological Oncology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, between January and December of 2023. Sample selection was done following purposive sampling technique. A total of 54 endometrial carcinoma patients diagnosed by fractional curettage or diagnostic curettage admitted into the BSMMU Hospital were enrolled in this study. At the time of their entry into the study a complete personal and family history was taken and recorded. Their age, BMI, parity, menstrual pattern, comorbidities, clinical complaints were noted in the data collection sheet. Preoperative investigations included some imaging modalities like transvaginal sonography (TVS) and magnetic resonance imaging (MRI) scans. All of the enrolled patients were treated by hysterectomy with bilateral salpingo-oophorectomy and bilateral pelvic and para aortic lymphadenectomy and/omentectomy as per staging. Paroperative findings and the findings of the cut section of the uterus (myometrial invasion, cervical extension, tumour size, ascites, omental metastasis) were noted.

After surgery was done, pathological specimens were routinely fixed with formalin and embedded in paraffin; then blocks were prepared for histopathological examination. After that, for immunohistochemistry evaluation, from paraffin embedded blocks 4 micrometer thick sections were cut, deparaffinized with xylene and rehydrated through a graded series of alcohol. Assessment of the MMR status of each patient's tumour was determined by immunohistochemistry (*MLH1, MSH2, MSH6,* and *PMS2*) by using monoclonal mouse antibodies against MMR proteins following the Dako EnVision method according to manufacturer protocol. Antibodies, source, clone and localisation for mismatch repair (MMR) proteins are shown in Table-I.

Table-I: *Antibodies used, source, clone and localization for mismatch repair (MMR) proteins (Dako EnVision by Agilent Technologies, Inc., Santa Clara, CA, USA)*

Antibodies	Source	Clone	Localisation
MLH1	Mouse monoclonal	ES05	Nuclear
PMS2	Rabbit monoclonal	EP51	Nuclear
MSH2	Mouse monoclonal	FE11	Nuclear
MSH6	Rabbit monoclonal	EP49	Nuclear

Any nuclear staining even patchy was taken as "no loss of expression" for MMR protein and was reported as MMR proficient. On the other hand, only absolute absence of nuclear staining was considered as "loss of expression", according to the College of American Pathologists (CAP) guideline.[8] Molecular expressions are shown in Fig. 1–4. Both regular histopathological examination and immunohistochemistry were done in the Department of Pathology of the same institution. The analysis of immunoreactivity was done by the Consultant Pathologist. All were recorded in the data sheet.

After collection, data was checked for omission, inadequacy and inconsistency. Omission was corrected by re-taking history or re-examining the patient. Irrelevant and inconsistent data was discarded. Categorical variables was presented as frequency and percentage. Associations between molecular expression and clinicopathological factors was assessed using Pearson's chi-square test. Statistical significance was set at a two tailed p-value of <0.05. Data analysis was done using STATA software version 14 (StataCorp LLC, College Station, Texas, USA).

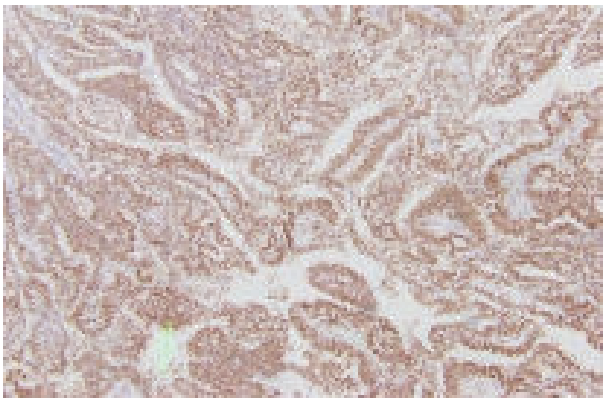

Fig. 1: *Photomicrograph showing intact expression of MLH1 in tumour cells (×100 magnification).*

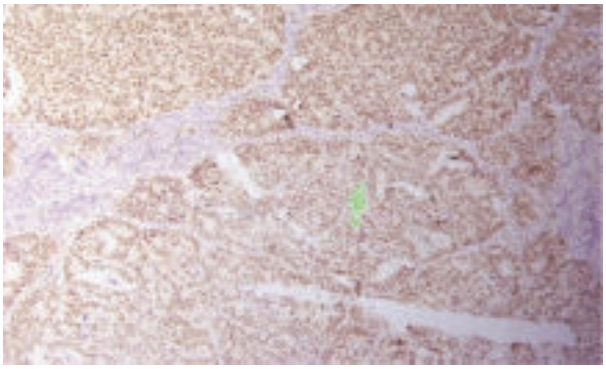

Fig. 2: *Photomicrograph showing intact expression of PMS2 in tumour cells (×100 magnification).*

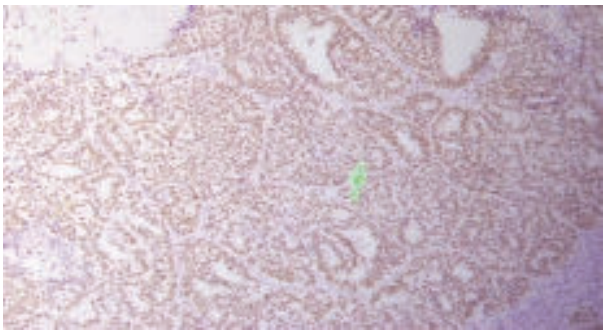

Fig. 3: *Photomicrograph showing intact expression of MSH2 in tumour cells (×100 magnification).*

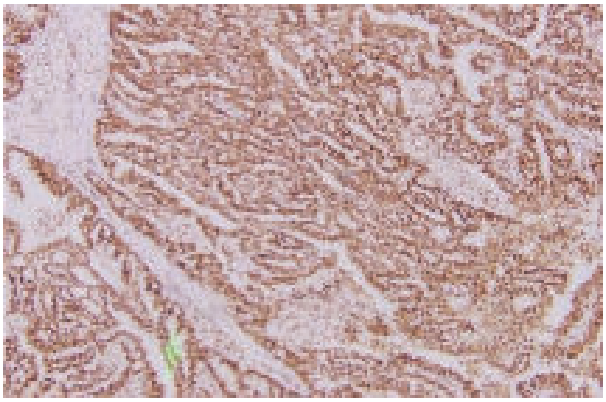

Fig. 4: *Photomicrograph showing intact expression of MSH6 in tumour cells (×100 magnification).*

RESULTS

Out of 54 patients, more than two-thirds (68.5%) were in the ≥60 years age group. Estimated BMI <25 was predominant (57.4%). The majority of the women were postmenopausal (79.6%), while most of them (61.1%) had both hypertension and diabetes mellitus as comorbidities. A positive family history of cancer was found in 20.4% cases (Table-II). Immunohistochemistry findings revealed that two-thirds (63%) of the patients had intact expression of all MMR

protein (MMRp), while 37% had a loss of expression of any of the proteins, which is known as mismatch repair deficiency (dMMR) state. Among them, the most common MMR defect identified was combined loss of *MLH1/PMS2* in 9(16.67%) cases, followed by isolated *PMS2* loss or isolated *MSH2* loss in 6(11.11%) cases, combined *MSH2/MSH6* loss in 4(7.4%) cases, while loss of all four proteins was observed in only 1(1.85%) case (Table-III). The majority of dMMR tumours (85%) and MMRp tumours (70.6%) were of endometroid variety; however, the difference was not significant ($p>0.05$). MMRp tumours were mostly larger in size, the difference was not significant though ($p>0.05$). Deeper myometrial invasion (≥50%) was more evident in MMRp tumours compared to dMMR tumours ($p<0.01$). Cervical extension was also found more frequent in MMRp tumours ($p<0.05$). In contrast, dMMR tumours were found more frequently having lower tumour grade and lower FIGO stage compared to MMRp tumours ($p<0.05$). However, no differences were observed in adnexal involvement, lymphovascular space invasion (LVSI), and lymph node involvement ($p>0.05$) (Table-IV).

Table-II: *Demographic characteristics of the patients (N=54)*

Variables	Frequency	Percentage
Age group (in years)		
<60	17	31.5
≥60	37	68.5
BMI (kg/m^2)		
≥25	23	42.6
<25	31	57.4
Menopausal history		
Premenopausal	11	20.4
Postmenopusal	43	79.6
Comorbidities		
HTN and DM	33	61.1
HTN	6	11.1
DM	6	11.1
Others	9	16.7
Family history of cancer		
Yes	11	20.4
No	43	79.6

Table-III: *Distribution of the MMR protein expression (N=54)*

Variables	Frequency	Percentage
Intact expression of all proteins/ MMR proficient	34	62.96
Loss of expression of one or more protein/ MMR deficient	20	37.04
i) Combined loss of *MLH1* & *PMS2*	9	16.67
ii) Isolated *PMS2* or *MSH2* loss	6	11.11
iii) Combined loss of *MSH2* & *MSH6*	4	7.41
iv) Loss of all proteins	1	1.85

Table-IV: *Comparison of clinicopathological factors between groups (N=54)*

Variables	dMMR (n=20) Frequency (Percentage)	MMRp (n=34) Frequency (Percentage)	p-value
Histological type			
Endometroid	17 (85.0)	24 (70.6)	0.232^{NS}
Non-endometroid	3 (15.0)	10 (29.4)	
Size of tumour (cm)			
<2	7 (35.0)	4 (11.8)	0.121^{NS}
2–4	6 (30.0)	13 (38.2)	
>4	7 (35.0)	17 (50.0)	
Myometrial invasion			
<50%	15 (75.0)	8 (23.5)	0.004^{S}
≥50%	5 (25.0)	26 (76.5)	
Cervical extension			
Yes	2 (10.0)	13 (38.2)	0.025^{S}
No	18 (90.0)	21 (61.8)	
Adnexal involvement			
Yes	2 (10.0)	9 (26.5)	0.147^{NS}
No	18 (90.0)	25 (73.5)	
Lymphovascular space invasion (LVSI)			
Yes	13 (65.0)	6 (17.6)	0.387^{NS}
No	7 (35.0)	28 (82.3)	
Lymph node involvement			
Yes	2 (10.0)	10 (29.4)	0.098^{NS}
No	18 (90.0)	24 (70.6)	
Grade			
Grade I	9 (45.0)	11 (32.3)	0.047^{S}
Grade II	9 (45.0)	9 (26.5)	
Grade III	2 (10.0)	14 (41.2)	
FIGO stage			
Stage I	13 (65.0)	10 (29.4)	0.033^{S}
Stage II	6 (30.0)	12 (35.3)	
Stage III	1 (5.0)	10 (29.4)	
Stage IV	-	2 (5.9)	

Chi-square test was applied to reach p-value; S=significant, NS=not significant.

DISCUSSION

We tried to adopt the molecular classification of endometrial carcinoma applied by the cancer genome atlas (TCGA), which has already been proved as more reproducible; valuable prognostic and predictive information can be obtained through this classification. For evaluation of the molecular status for patients with endometrial cancer genetic testing is required. In the present study, for detection of status of MMR protein, imunohistochemistry assay was used; its effectiveness was shown in several previous studies.[11-13] The four MMR proteins assessed are *MLH1, MSH2, MSH6, and PMS2*. These proteins form two heterodimers, which are *MLH1-PMS2* and *MSH2-MSH6*. When *MLH1* or *MSH2* are lost, there is a consequent loss of *PMS2* or *MSH6*, respectively. On the other hand, a loss of *MSH6* or *PMS2* expression can occur as an isolated event.[14] That is why IHC assessment of only *MSH6* and *PMS2* has been suggested to have the same accuracy as the full MMR panel in identifying dMMR cases. Moreover, IHC using antibodies against the four MMR proteins (*MSH2, MSH6, MLH1, and PMS2)* represents a relatively widely available, affordable, and easy to perform technique, compared to MSI testing.[13]

We observed a relatively high prevalence of abnormal expression of MMR proteins in our study population (37%). This finding was in congruence with the findings of several previous studies (25-37%).[11,15-17] However, other studies showed much lower incidence of dMMR tumours (17-20%).[18,19]

Concerning the relationship of MMR proteins and other clinicopathological features, we observed that the dMMR endometrial tumours presented with less aggressive endometrioid histology at lower tumour stages and grades compared to MMR-proficient tumours. Hashmi et al. reported that dMMR was related to high FIGO stage; any relation with tumour grade was not established.[20] However, Tangjitgamol et al. found that early stage, more endometrioid histology, and lower grade tumour were associated with MMR deficiency.[21]

The lymphovascular space invasion (LVSI) is a marker of metastatic potential in cancer patients. In the present study, LVSI was observed in 65% of endometrial cancers with dMMR suggesting that metastasis was more likely in dMMR cases.[22] However, dMMR endometrial cancers have more favourable prognosis compared to their MMR-proficient counterparts. Microsatellite instability (MSI) phenotype or dMMR endometrial tumours have a protective immunephenotype and positively correlate with high immune infiltration. In theory, this protective immune phenotype can counteract the poor clinicopathological parameters that co-exist in dMMR tumours.[22]

Last but not the least, our study has several limitations. The study population was selected from one selected hospital in Dhaka city; hence, the results of the study may not reflect the exact picture of the country. A small sample size was another limitation, which was due to time and budget constraint. Further studies are recommended with large sample size and involving multicentre from different regions of the country.

CONCLUSION

In this study, two-thirds of the study patients (EC) had intact expression of all MMR proteins and the rest had a loss of expression of any of the proteins, i.e., mismatch repair deficiency (dMMR). MMR deficiency is common in EC, between 20–40%, and impacts the screening for Lynch syndrome (LS), therapeutic decision-making, risk stratification, and inclusion in clinical trials. With the increasing emphasis on molecular classification in EC, accurate and reproducible testing is paramount to improving patient outcomes.

Conflict of interest: None declared.

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Ethical approval: This study was approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh (BSMMU/2022/9561-IRB Reg. No. 3956).

Authors' contribution: Conceptualization and design of the study: SN Alamgir, J Ferdous; patient selection, data collection and compilation: SN Alamgir, J Ferdous, MJ Faika, K Farhana, KT Rahman, FB Rashid; data analysis: SN Alamgir, M Asaduzzaman; supervision of the study: J Ferdous; manuscript preparation, editing and final submission: SN Alamgir, J Ferdous, MJ Faika, K Farhana, KT Rahman, FB Rashid, M Asaduzzaman.

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