Assessment of Microsatellite Instability in Endometrioid Carcinoma by Immunohistochemistry At Armed Forces Institute of Pathology, Rawalpindi

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ABSTRACT

Objective: To assess microsatellite instability in endometrioid carcinoma by immunohistochemistry expression of MLH1, PMS2, MSH2 and MSH6.

Study design: Case series.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, from Jun to Dec 2018.

Methodology: Thirty-two cases of endometrioid carcinoma were included in the study. Patient age and menopausal status were recorded. The grade of the tumour was ascertained by microscopic examination. Immunohistochemistry for MLH1, PMS2, MSH2 and MSH6 assessed microsatellite instability.

Results: In our study, eight females (25%) had premenopausal status while 24 (75%) had postmenopausal status. Out of 32 cases, 25 (78.1%) females had grade-I tumours, 4 (12.5%) had grade-II tumours, and 3 (9.45%) had grade-III endometrioid carcinoma. MMR status was proficient in 8 cases (25%) while deficient in 24 cases (75%). Among the 24 cases of MMR deficient endometrioid carcinoma, loss of expression of MLH1 was most frequent n=15 (62.5%), followed by PMS2 n=14 (58.3%) MSH2 n=7 (29.2%). MSH6 was retained in all cases. The most common pattern was a combined loss of MLH1/PMS2 in 12 cases (50%).

Conclusion: As depicted by the high percentage of MMR deficient tumours, microsatellite instability is observed in many endometrioid carcinomas. Patients suffering from endometrioid carcinoma should be screened for Lynch syndrome by testing for MMR status.

Keywords: Endometrioid carcinoma, Immunohistochemistry, Microsatellite instability, MLH1, MSH2, MSH6, PMS2.

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INTRODUCTION

The most frequent gynaecological cancer is endometrial cancer. After breast, lung, and colorectal cancer, it is the fourth most frequent cancer among women in the United States.[1] It was predicted that in the year 2020, an estimated 65620 cases of endometrial carcinoma will be diagnosed, and out of the approximately 12590 women will die of this cancer.[2] In Pakistan, the incidence of endometrial carcinoma is 3.6%.[3] Patients are on average 63 years old at the time of diagnosis, with 90% of instances occurring in women over 50 years old. Endometrial cancer is diagnosed in about 20% of women before they reach menopause .[4]

Unopposed oestrogen medication, early menarche, late menopause, Tamoxifen therapy, nulliparity, infertility or failure to ovulate, and polycystic ovarian syndrome are risk factors for type-I endometrial cancer.[5,6]

One of the underlying causes of endometrial cancer is a mutation of DNA mismatch repair (MMR) genes. Germline mutations in the mismatch repair genes lead to Lynch syndrome (LS), also known as hereditary nonpolyposis colorectal carcinoma syndrome.[7] LS is associated with increased oncogenesis for tumours in several organs or sites like large or small intestines, ovaries, vagina, endometrium, gastrointestinal tract, pancreas, renal pelvis, ureter, and brain.[8] It is responsible for 5% of all endometrial cancer cases and 4% of all colorectal cancer risks during a lifetime. Germline flaws in MMR are described in about 1.8-2.1 percent of random patients of endometrial carcinoma and in about 9% of positive patients of endometrial carcinoma aged <50 years.[9,10]

In case of detection of MSI on IHC/genetic testing, the ACOG/ SGO recommends risk reduction and surveillance of the patients by strategies like 1-2 year colonoscopy starting at age 20-25 years, 1-2 yearly endometrial sampling starting at age 30-35 years and

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prophylactic hysterectomy with bilateral salpingo-oophorectomy in early to mid-40s. This study evaluated the frequency of microsatellite instability in endometrioid carcinoma by immunohistochemical expression of four MMR proteins, MLH1, PMS2, MSH2 and MSH6. The purpose was to highlight the syndromic relationships of various cancers, as these have better survival than non-syndromic.

METHODOLOGY

This case series was carried out at the Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, from June 2018 to December 2018. After approval from the Institutional Review Board of Armed Forces Institute of Pathology, Rawalpindi (FC-HSP17-23/READ-IRB/18/904). Thirty-two cases of endometrioid carcinoma were selected by non-probability, consecutive sampling.

Inclusion criteria: Female patients aged 33 to 77 years diagnosed with endometrioid carcinoma on endometrial biopsy were included in the study.

Exclusion criteria: Cases with inappropriately fixed tissue, non-endometrioid histology, and patients who already received chemo, radiotherapy, or metastatic disease were excluded from the study.

Thirty-two cases of endometrioid carcinoma diagnosed on endometrial biopsy were included. Patients' data like name, age and menopausal status were recorded. Hem atoxylin and Eosin (H&E) slides were prepared from tissue sections of formalin-fixed paraffin-embedded (FFPE). Histologic tumour type and grade were recorded. Immunohistochemistry was performed using MLH1, PMS2, MSH2 and MSH6 antibodies (DAKO) on the diagnosed cases as per the manufacturer's protocol. Normal colonic tissue was used as the positive control. As per the College of American Pathologists, detection of nuclear staining in any number of tumour cells was taken as retained expression, while absent nuclear staining in tumour cells was taken as loss of expression of MMR proteins.[11] Expression of IHC of the four MMR proteins was grouped into two categories: no loss of expression of MMR proteins (MMR proficient) and loss of any MMR protein (MMR deficient). The isolated or combined loss of MMR proteins was also noted.

Statistical Package for Social Sciences (SPSS) version 22.0 was used for the data analysis. Mean and standard deviation was calculated for age. Frequency and percentage were calculated for a qualitative variables like menopausal status, tumour grade, MMR status and results of expression of MMR proteins. The menopausal status and tumour grade were compared with the MMR status, and the *p*-value of ≤0.05 was considered statistically significant using a chi-square test.

RESULTS

In our study, the age of the patients ranged between 30 and 77 years. Most of the patients belonged to the fifth decade of life. Most patients had postmenopausal status 24 (75%), while eight patients (25%) had premenopausal status.

Out of 32 cases, 25 (78.1%) had grade-I, 4 (12.5%) had grade-II, and 3 (9.4%) had grade-III endometrioid carcinoma. MMR status was proficient in 8 cases (25%) while deficient in 24 cases (75%) (Figure-1).

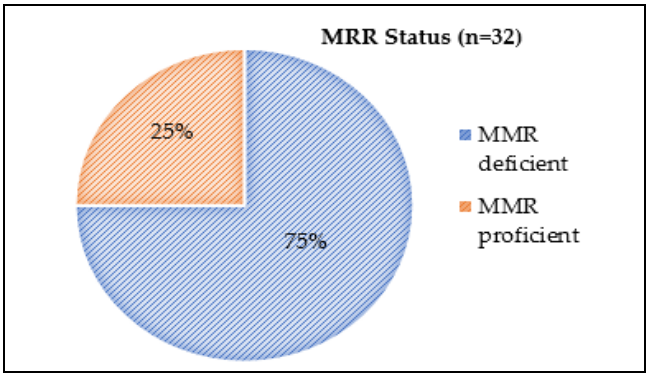

Figure-1.Mismatch repair status in endometrioid carcinoma by immunohistochemistry (n=32).

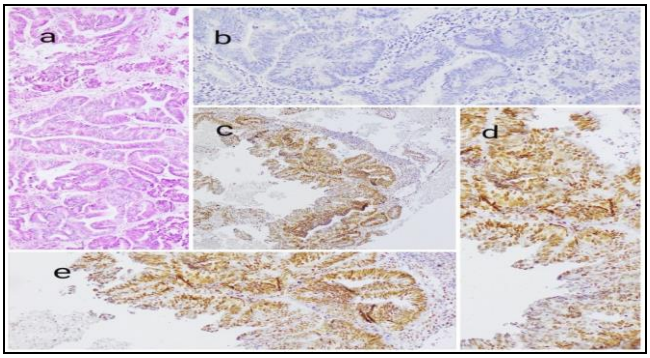

Figure-2. Immunohistochemical expression of MMR proteins in endometrioid carcinoma. (a) Endometrioid carcinoma, grade I (10x), (b) Loss of nuclear expression of MLH1 (10x), (c) Retained nuclear expression of PMS2 (10x), (d) Retained nuclear expression of MSH2 (10x), (e) Retained nuclear expression of MSH6 (10x).

Among the 24 cases of MMR deficient endometrioid carcinoma, loss of expression of MLH1 was most frequent n=15 (62.5%), followed by PMS2 n=14 (58.3%) MSH2 n=7 (29.2%). MSH6 was retained in all the cases. The most common pattern was the combined loss of

MLH1/PMS2 in 12 cases (50%), followed by isolated MSH2 loss, isolated MLH1 loss and isolated PMS2 loss (Table-I).

Table-I: Patterns of loss of expression of mismatch repair proteins (n=24).

Patterns	Number of Cases
MLH1, PMS2	12 (50.0%)
Isolated MSH2	7 (29.2%)
Isolated MLH1	3 (12.5%)
Isolated PMS2	2 (8.3%)

When MMR status was stratified with menopausal status and tumour grade, the association of MMR status with tumour grade was found to be statistically significant (<0.05) (Table-II).

Table-II: Comparison of mismatch repair status with tumour grade (n=32).

Characteristics	MMR Deficient	MMR Proficient	*p*-value
Premenopausal	8 (25%)	0 (0%)	0.059
Postmenopausal	16 (0.5%)	8 (25%)	
Tumour Grade			
Grade I	22 (68%)	3 (9.3%)	0.006
Grade II	1 (3.1%)	3 (9.3%)	
Grade III	1 (3.1%)	2 (6.2%)	

DISCUSSION

This study observed a significant number of cases of endometrioid carcinoma exhibiting loss of expression of either of the Mismatch Repair proteins, indicating microsatellite instability.

Microsatellite Instability (MSI), caused by defects in mismatch repair (MMR) genes, is one of the pathways in the development of endometrial carcinoma.[11] Microsatellites are short repetitive sequences of DNA predominantly found in the non-coding regions of genes. Due to MSI, errors are made in DNA repair during replication. This leads to the development of an increased number of repeat elements.[12] High levels of MSI (MSI-H) occur by one of the two pathways. It is either due to germline or sporadic mutations in at least one of the DNA mismatch repair proteins (MLH1, PMS2, MSH2, and MSH6) or *MLH1* gene hypermethylation. It is noteworthy that the risk of development of endometrial carcinoma is also dependent on the type and number of MMR genes that are mutated. For example, the risk of developing endometrial carcinoma is 40-60% when there is the mutation of MLH1 and MSH2 genes, while it is 71% due to mutation in the MSH2 gene.

MSI is more frequently found in endometrioid type endometrial carcinoma than non-endometrioid carcinomas. Non-endometrioid endometrial cancer commonly occurs due to *p*53 gene mutations. Various studies have been carried out to determine the association of MSI status with clinical and pathologic parameters and its impact on the prognosis of endometrial cancer. In a case study by NRG oncology/gynaecology, MSI-related endometrial cancer was found was to be affecting a younger age group and mostly presenting at an advanced grade and stage. It was also shown to have a higher incidence of lymphovascular invasion and myometrial invasion.[13] However, despite linkage to poor prognostic factors, the MSI-related tumours had a favourable outcome.

Current guidelines recommend LS screening for all patients with newly diagnosed colorectal cancer.[14] Similarly, the strong relationship between endometrial cancer and LS requires screening of the endometrial carcinoma samples to determine the defects in DNA MMR pathway.[15] Therefore, in 2014, it was recommended by the American College of Obstetricians and Gynecologists (ACOG) and the Society of Gynecologic Oncology (SGO) recommended that all endometrial cancers should be screened for mismatch repair (MMR) deficiency.[16] It was suggested that all women diagnosed with endometrial cancer undergo clinical screening for endometrial cancer by reviewing personal and family history and molecular testing for Lynch Syndrome. In addition to this, it was recommended that all asymptomatic women with a first-degree relative be diagnosed before the age of 60 years with colorectal or endometrial cancer should also be tested to rule out the future possibility of developing Lynch syndrome-related cancers.

The identification of MSI and screening of LS are made by several methods to indirectly or directly recognize the mutations in MMR genes. The most decisive and accurate technique is germline mutation analysis of the four MMR genes MutL homolog 1 (MLH1), PMS1 homolog 2 (PMS2), MutS homolog 2 (MSH2), MutS homolog 6 (MSH6), and epithelial cell adhesion molecule (EPCAM).[17,18,19] However, immunohistochemical staining obliges as a more cost-effective method for screening.[20,21] It has a sensitivity of 94% and specificity of 91%. Therefore immunohistochemistry can be used as a surrogate test for the detection of MSI.22A suitable method of screening patients for a possibility of Lynch Syndrome is to perform IHC for MMR proteins in the first instance. Those patients who show MMR deficiency on immuno-histochemistry can be selected for genetic testing to confirm Lynch Synd-

rome. For example, if there is a loss of MLH1 on IHC, MLH1 hypermethylation analysis can be performed to confirm the sporadic cause of MLH1 loss. In this way, immunohisto-chemistry can be used to avoid the cost and time implied by unnecessary genetic testing of patients with MMR proficient endometrial cancer.

In Lynch syndrome, endometrial carcinoma can be associated with other malignancies, including the gastrointestinal tract, pancreas, ovaries, vagina, ureter, renal pelvis and brain. With the detection of mismatch repair protein deficiency through immunohistochemistry and MSI testing, diagnosis of Lynch syndrome has become possible. The detection of individuals with the potential risk of Lynch syndrome will be helpful in the prevention of cancer development, early diagnosis of cancers by thorough screening, prevention of cancer -related mortality and patient management through personalized therapies.

The American society of clinical oncology recently released new evidence-based guidelines emphasizing the importance of using immunohistochemistry (IHC) procedures in patients with primary or metastatic colon cancer when looking for mismatch repair gene mutations.[14]

In certain countries, a genetic test combined with IHC analysis to detect MSI is already being used to identify probable LS patients in endometrial cancer patients under the age of 50 years.

Endometrial cancers are a major cause of morbidity and mortality in South Asia.[15] Literature review shows that limited studies have been perfor-med to evaluate the status of MSI in endometrial cancer in Pakistan. Therefore, this study was performed to determine the frequency of MMR status in our population.

In our study, the mean age of the patients was 54.53 ± 11.18 years, and the majority of them were postmenopausal (75%). This was in concordance with the study conducted at Liaquat National Hospital Karachi, where the mean age was 54.53 ± 20.18 years and had postmenopausal status.[22] The mean patient age observed in an Indian study was 59.6 years.[23] While the mean age was ten years younger (44.4 years, range 32-50 years) in a study carried out by Pecorino *et al*.[13]

In our study, abnormal expression of MMR proteins was observed in 75% of cases (n=24) which was considerably higher than the study conducted at Liaquat National Hospital Karachi, where it was noted in 44.4% of cases (n=56).[22] 21.6% cases (n=22) showed loss of expression of MMR proteins in an Indian study, while 22.4% showed the loss in the study carried out by Joehlin-Price *et al*.[24] The study by Pecorino et al, showed that 46% of samples showed loss of MMR proteins by IHC analysis.[13] Gordhandas *et al*, reviewed 29 articles regarding MMR testing in endometrial carcinoma. They found out that among 6,325 patients who underwent MMR assessment via IHC, 1,612 patients (25.4%) showed abnormal staining for MMR proteins.[25]

In our study, among the 24 cases of MMR deficient endometrioid carcinoma, loss of expression of MLH1 was most frequent n=15 (62.5%), and the most common combined pattern of loss of MMR proteins was the loss of MLH1/PMS2 in 12 cases (50%). This was in concordance with the data reported previously in Karachi by Hashmi et al, and in India by Sharma *et al*, 27% and 50%, respectively.[22,23] However, in contrast to both studies, we also observed isolated loss of PMS2 and MSH2 in our cases which need further investigation by molecular testing. MLH1 and PMS2 were the most frequent markers with an abnormal expression on IHC as detected by Pecorino *et al.* in 31 and 30% of cases respectively.[13] MLH1 was also the most frequently lost protein (n=1,162 cases) as depicted in the study carried out by Gordhandas *et al*, followed by MSH2 (n=450).[25]

Comparing the clinical and pathological characteristics with MMR status, the mean age for MMR deficient patients was 52.33 years, while that of MMR proficient tumours was 61.25 years. This is in concordance with the mean age reported by Gord-handas *et al*, In our study, MMR deficient cases showed grade I histology in 22 cases (91.6%) while grade II (4.2%) and III (4.2%) was observed in 1 case each. 25 MMR proficient cases showed grade I histology in 3 cases (37.5%), grade II in 3 cases (37.5%) and grade III in 2 cases (25%). In the study conducted by Gordhandas *et al*, among MMR deficient tumours, 31 cases (39%) were grade I, 25 cases (32%) were grade II, and 23 (29%) cases were grade III. For MMR proficient tumours, 58% (n=471) were grade I, 22% (n=175) were grade II and 20% (n=165) were grade III.[25] However, it is noteworthy that histologic types other than endometrioid carcinoma were also included in their study.

This study will help to identify the association of cancer with various syndromes that will early identify the disease and helps in the prognosis and treatment.

STUDY LIMITATIONS

Affordability issues, following up and post-treatment changes were the main limitations observed in our study.

CONCLUSION

Our study concluded that a significant number of cases of endometrioid carcinoma show loss of expression of either of the Mismatch Repair proteins, indicating microsatellite instability.

RECOMMENDATION

The MMR deficient cases can be subjected to germline testing to confirm Lynch syndrome and genetic counselling of the affected patients.

Conflict of Interest: None**.**

Authors' Contribution

AA: Writing, FA: Topic selection, AA: Editing, HUD: Final correction, RA: Path correction, AA: Editing.

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