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Original research article

Original research article: Histopathological study of endometrial carcinomas with special reference to immunohistochemical profile of Mismatch repair proteins

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Abstract

Background: Endometrial carcinoma is the 4th most commonly diagnosed gynecologic malignancy in the world and 11th most common in India [1]. Traditionally, endometrial carcinomas have been divided into two types. Type I-low grade, estrogen related, often clinically indolent, endometrioid carcinomas. Type II-non-endometrioid, clinically aggressive carcinomas that are unrelated to estrogen stimulation and include serous and clear cell carcinomas. This classification is not useful for tumor stratification because of significant overlap in various features with respect to high grade tumors of either types. Recent advances on genomic studies have classified endometrial carcinoma into 4 subtypes:

1. With Pole (DNA polymerase epsilon) mutations.
2. Microsatellite instability (MSI).
3. Showing low copy number alterations.
4. Tumors with high copy number alterations and TP53 mutations.

Integration of molecular characteristics with morphologic features helps to stratify patients to predict prognosis. MSI can be identified by IHC which is efficient, relatively simple and cost effective. The present study is proposed to evaluate the morphological features of endometrial cancers and to analyze their profile based on loss of immunostaining for MMR proteins.

Methods: A prospective study for a period of 2 years and 3 months from August 2020 to November 2022 was conducted in the Department of Pathology from Government General Hospital, Kakinada. A total of 50 endometrial samples were collected.

Results: On histopathological examination 80% of cases were diagnosed as Type 1 endometrial carcinoma and 20% were type 2 endometrial carcinomas. On IHC for MMR protein expression, 22% cases showed loss of one or more MMR proteins. All of these deficient MMR cases were endometrioid type. Loss of MLH1 and PMS2 was the most common abnormality detected in deficient MMR tumors.

Conclusion: Detecting MMR protein loss in endometrial carcinomas by IHC is an efficient, relatively simple and economical method. It needs to be routinely performed in all cases of endometrial carcinomas.

Keywords: Endometrial carcinoma, mismatch repair proteins, microsatellite instability

Introduction

The incidence and mortality rate of endometrial carcinoma has been registering an increasing trend in the world. Recent advances have been made in defining molecular genetic alterations that lead to endometrial tumorigenesis.

Among these well characterized is defect in DNA mismatch repair system which occurs predominantly in endometrioid carcinomas. Loss of MMR proteins leads to accumulation of single base pair mismatches, as well as small insertions and deletions in tandem repeats called as microsatellites, which manifest as Micro Satellite Instability [2]. MSI can be detected by IHC of MMR proteins MLH1, MSH2, MSH6 and PMS 2.

The purpose of this study was to perform a comprehensive investigation of association of histopathological variables with MSI in patients with endometrial carcinoma.

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Material and Methods

Source of data: Endometrial specimens received in the Department of Pathology from Government General Hospital, Kakinada.

Study design: Prospective study for a period of 2 years and 3 months from August 2020 to November 2022.

Sample size: 50.

Inclusion criteria: Endometrial scrapings, Total abdominal hysterectomy specimens.

Exclusion criteria: Inflammatory/ infective lesions of endometrium and Inadequate samples.

Method of data collection: Endometrial scrapings, hysterectomy and radical surgery specimens received with biopsy requisition form were fixed in 10% buffered formalin. Fixed specimens were then subjected to grossing, tissue processing and section cutting. Microscopic evaluation of Hematoxylin and Eosin stained sections of paraffin blocks was done. Sections from selected paraffin blocks were subjected to immunohistochemical staining for MMR proteins and analyzed.

Results

The present study was conducted in the department of pathology, Government general hospital, Kakinada. In this study a total of 50 cases were taken and histopathological examination was done. Among 50 cases, 40 cases were >50yrs and 10 cases were 50 and <50yrs. The majority of them were between the age group 51 to 60 years (58%). The percent of cases between age group 61-70 was 18 %, 71-80 was 4%, 51-60 was 18% and 35 to 40% was 2% (chart 1). In the present study 37 patients were presented with a chief complaint of post-menopausal bleeding, while 8 patients were presented with leukorrhea, 3 patients were presented with pain abdomen and 2 patients were presented with pyometra.

In the present study out of 50 cases, 40 cases were endometrioid carcinomas, 9 cases were serous carcinomas and 1 case was clear cell carcinoma. 80% of the cases were endometrioid carcinomas (fig 1), while 18% were serous carcinomas (fig 2) and 2% were clear cell carcinomas. Among endometrioid carcinomas 67% (27cases) were FIGO grade 1 carcinomas, 18% (7 cases) were FIGO grade 2 carcinomas and 15% (6 cases) were FIGO grade 3 carcinomas.

Among all these cases selected blocks were sent for IHC of MMR proteins MLH1, MSH 2, MSH 4 and PMS 2. Complete loss of nuclear staining of tumor cells was considered as MMR deficient.

Out of total 50 cases, 11 cases showed deficiency of one or more MMR proteins. Of these, all the 11 cases were Endometrioid carcinomas. The percent of MMR deficient tumors were 22% and MMR proficient tumors were 78% (chart 2,3). Among these 11cases of MMR deficient tumors, 7 cases showed loss of MLH1, PMS2 and 3 showed loss of MSH2, MSH6 and 1 case showed loss of all 4 MMR proteins (fig 3, 4, 5, 6).

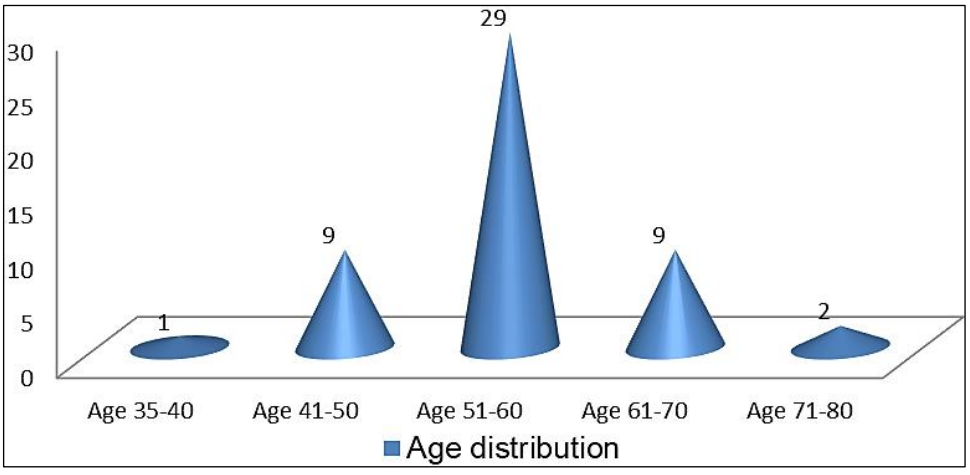

Chart 1: Age distribution (n=50)

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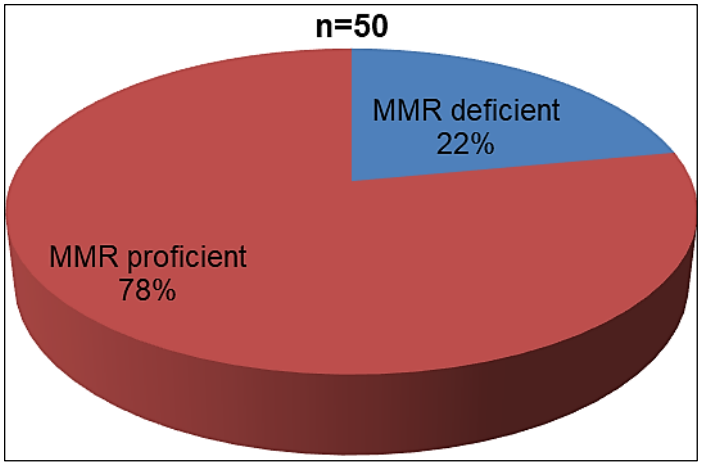

Chart 2: Distribution of cases based on MMR status (n=50)

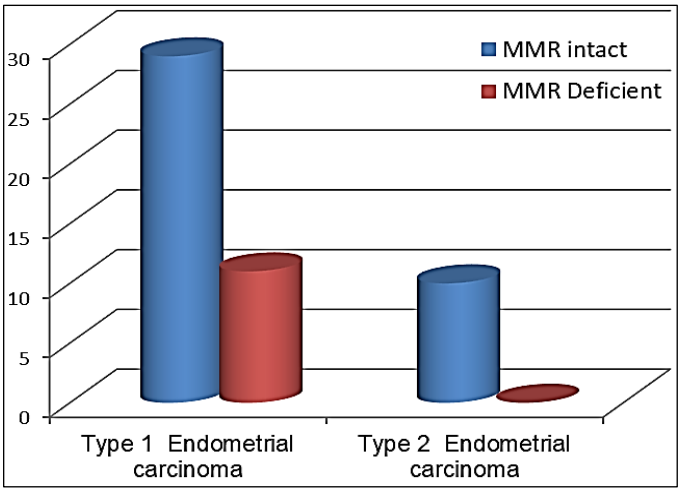

Chart 3: MMR protein status in endometrial carcinomas

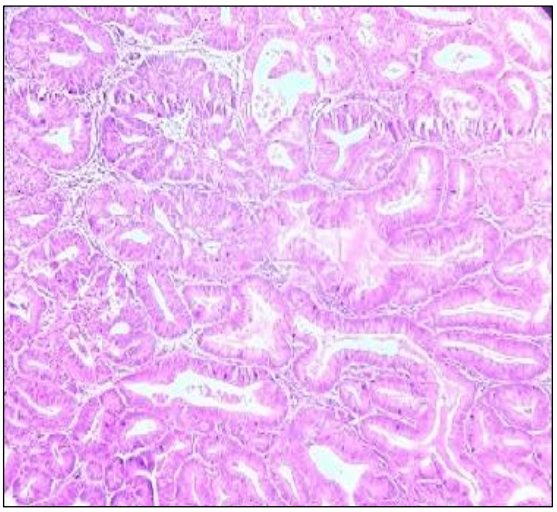

Fig 1: HPE-H&E-400x-Endometrioid carcinoma

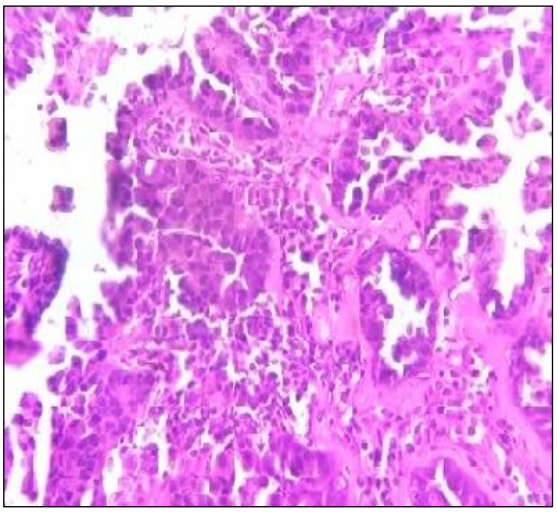

Fig 2: HPE-H&E-400x-serous carcinoma

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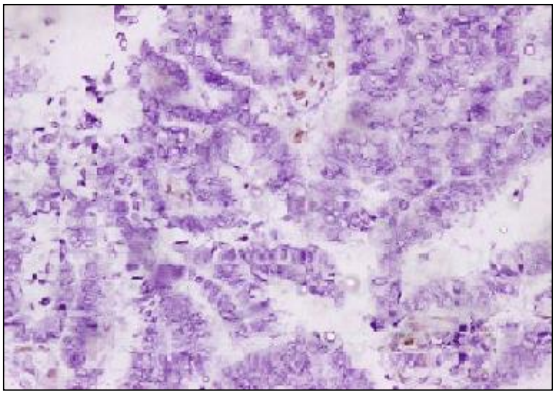

Fig 3: IHC-400x- loss of MLH1 expression

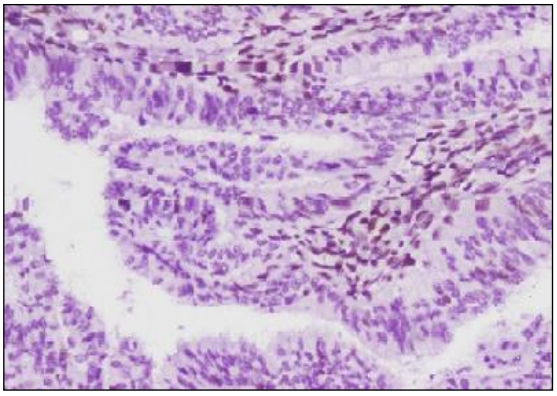

Fig 4: IHC-400x-loss of MSH2 expression

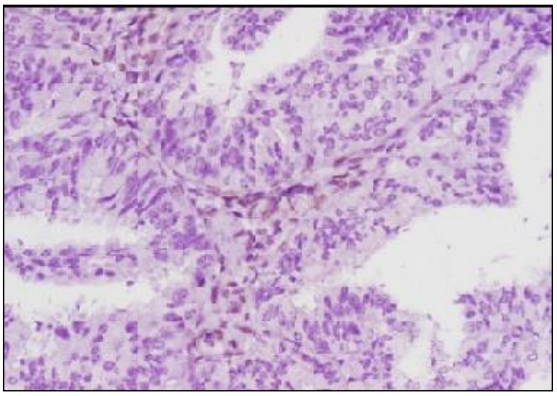

Fig 5: IHC-400x- loss of MSH6 expression

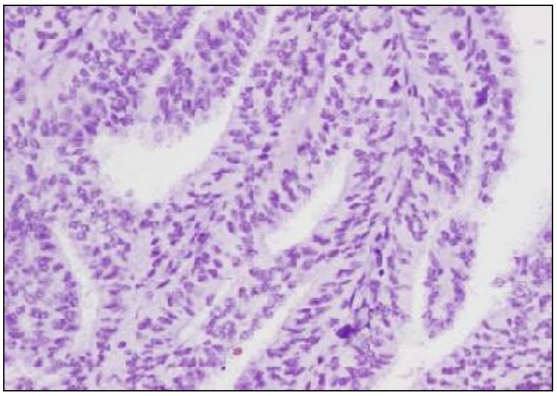

Fig 6: IHC-400x-loss of PMS2 expression

Discussion

Endometrial carcinoma is the most common malignancy of female genital tract in developed countries. In 2020 there was an estimated number of 417000 incident cases and 97,000 deaths from the disease worldwide [3]. Its incidence is rising alongside the growing obesity epidemic [4].

Most of the endometrial carcinomas are sporadic, an estimated 5% occur in the context of hereditary predisposition, most commonly Lynch syndrome [5].

Identification of microsatellite instability phenotype is important in endometrial carcinomas. DNA MMR deficiency is associated with increased risk of developing several types of cancer and is the most common cause of hereditary endometrial cancer. Although there has been extensive investigation of MMR deficiency in colorectal cancer, MMR deficiency in endometrial cancer is relatively under investigated.

In the present study, mean age of the patients is 56.9 years with extremes ranging from 36 to 75. In a study done by Doghri *et al*. [6] the mean age of patients was 58.6 years with extremes ranging from 34 to 80. The mean age of patients was 59.6years in a study done by Sharma *et al*. The mean age of patients was 63 years in a study done by Black *et al*.

Most of the women were diagnosed following routine investigations for post-menopausal bleeding, the cardinal symptom of the disease. In the present study the most common presenting symptom was post-menopausal bleeding followed by leukorrhea, pain abdomen and pyometra.

Histological subtype, FIGO grade, disease stage, presence of lympho-vascular space invasion and deep myometrial invasion are established prognostic biomarkers in endometrial cancer [7]. The histological subtypes of endometrial cancer include endometrioid carcinomas, which have a favourable prognosis, and non-endometrioid carcinomas (serous, clear cell, carcinosarcomas), which are biologically aggressive and associated with poor outcomes.

In the present study 80% of endometrial carcinomas were endometrioid type, 18% of the tumors were serous cell carcinomas and 2% of the tumors were clear cell carcinomas. The present study is in concordance with the study done by Black *et al*. with 80%-Endometrioid, 15%-serous, 4%-clear cell carcinomas, 1%-undifferentiated and Kaur *et al*. [8] with 81.7%-type 1 endometrial carcinomas and 18.3%-type 2 endometrial carcinomas (Table 1).

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Table 1: Comparision of histological types of endometrial carcinoma

Study	Type of carcinomas
Sharma *et al*.	85.1%-Type 1 endometrial carcinomas 9.8%-Type 2 endometrial carcinomas 4.9%-malignant mixed mullerian tumours
Black *et al*.,	80%-Endometrioid, 15%-serous 4%-clear cell carcinomas 1%-undifferentiated
Doghri *et al*.,	81.81%-Endometriod 4.54%-serous, 2.3%-clear cell 6.81-carcino sarcoma, 4.54%-mixed
Kaur *et al*.,	81.7%-Type 1 endometrial carcinomas 18.3%- type 2 endometrial carcinomas
Present study	80%-endometrioid type 18%-serous carcinomas 2%-clear cell carcinomas

In the present study endometrioid carcinomas are divided as follows: FIGO grade 1- 67%, grade 2-18% and grade 3-15%. The present study is correlated with the study of Sharma *et al*. The proportion of people affected by DNA mismatch repair in present study is 22%. All the cases of dMMR (22%) are endometrioid carcinomas which was correlated with the study of Joehlin-price *et al*., [9], with deficient MMR in 22.4% cases. The percentage of deficient MMR tumors is 21.6% in a study done by Sharma *et al*., 20% in a study by Black *et al*., 17% in a study by Kanopiene *et al*. [10], 22.7% in a study by Doghri *et al*., 21.5% in a study done by Kaur *et al*. In the present study 22% of carcinomas show dMMR % and these 22% are endometrioid type similar to the study done by Sharma *et al*. In the present study cases which are having dMMR expression are of endometrioid type similar to the study done by Sharma *et al*. (Table 2).

Table 2: Comparision of dMMR % in various studies and present study

Study	Deficient MMR%
Joehlin-Price *et al*.	22.4%
Sharma *et al*.	21.6%
Black *et al*.	20%
Kaur *et al*.	21.5%
Doghri *et al*.	22.7%
Kanopiene *et al*.	17%
Present study	22%

In the present study combined loss of MLH1/PMS 2 is seen in 7 cases (63.6%), combined loss of MSH2, MSH6 is seen in 3 cases (27.3%) and combined loss of MLH1, MSH2, MSH6, PMS2 is seen in 1 case (9.1%). The present study revealed several significant clinicopathological relationships: positive association of MMR deficient tumours with advanced pathological stage and deficient MMR expression being shown mostly in endometrioid carcinomas.

dMMR tumors have high frequency of inactivating frameshift mutations in genes with coding region microsatellite repeats which is a major mechanism through which dMMR contributes to tumorigenesis. Genetic instability is believed to be the hallmark of most human cancers, MSI is one of the well characterized forms of genetic instability, other being chromosomal instability.

Pembrolizumab an IgG4 isotype antibody has the potential to bind and block PD-1 receptors [11]. It is used as targeted therapy for endometrial carcinomas that are characterized by microsatellite instability or MMR deficiency. The treatment is proved to be particularly effective due to their augmented somatic hypermutation.

The incorporation of endometrial cancer molecular testing into routine clinical care has several additional advantages. It will allow for the early identification of women with an inherited defect affecting one of the four mismatch repair genes (Lynch syndrome) for whom cancer surveillance and aspirin chemoprevention may help to prevent future cancers, and cascade testing may identify other affected family members [12].

Summary

In the present study histopathological and immunohistochemical features of 50 endometrial carcinoma cases were analyzed. Most common affected age group was age 50 -60 years. Most common type of carcinoma was endometrioid type of carcinoma accounting to 80%. Most of the endometrioid carcinomas were of FIGO grade 1. Deficient MMR tumors constitute upto 22% of which all were endometrioid type. In the present study significant correlation of dMMR tumors with higher grade was seen. Loss of MLH1 and PMS2 ware more common in deficient MMR tumors in endometrial carcinoma.

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Conclusion

Analyzing immunohistochemistry status for evaluating the MSI in patients with Type I endometrioid adenocarcinomas is an alternative and efficient tool in predicting the prognosis for patients. MSI+ endometrial cancers have a high frequency of inactivating frameshift mutations in genes with coding region microsatellite repeats, and that this may represent the major mechanism through which MSI contributes to tumorigenesis.

Targeted therapy with Immune check point inhibitors like pembrolizumab is beneficial in advanced cancers with dMMR status. Genetic testing and counselling should be considered for women with endometrial carcinoma who are younger than 50years and those with a clinically significant family history of endometrial or colo-rectal carcinoma.

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List of Abbreviations Used

IHC - Immunohistochemistry.
HPE - Histopathological examination.
H&E - Hematoxylin and eosin.
MMR - Mismatch repair proteins.
dMMR - deficient Mismatch repair proteins.
MSI - Micro satellite instability.
Pole - DNA polymerase epsilon.