SJIF Impact Factor: 6.711

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 9.
Issue: 10
Page N. 26-34
Year: 2025

Original Article ww.wjahr.com

EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF MISMATCH REPAIR PROTEINS IN ENDOMETRIAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN A SAMPLE OF IRAQI PATIENTS (A CLINICOPATHOLOGICAL STUDY)

***Alaa Rafid Faraj (M.B.Ch.B), Ikram Abdul Latif Hasan (M.B.Ch.B, F.I.B.M.S) (Pathology)**

Article Received date: 22 July 2025 **Article Revised date:** 12 August 2025 **Article Accepted date:** 02 September 2025

***Corresponding Author: Alaa Rafid Faraj (M.B.Ch.B)**

DOI: https://doi.org/10.5281/zenodo.17222644

ABTRACT

Background: Carcinoma of the endometrium is the most common gynecologic malignancy, and its increasing prevalence is attributed to various risk factors, including obesity, hypertension, and diabetes. Endometrial cancer is more frequently categorized by molecular subgroups based on mutation burden and copy number alterations rather than histological findings. Recent advancements in genomic analysis have uncovered that approximately 20–30% of endometrial cancer cases exhibit a mismatch repair deficiency phenotype. It serves as a molecular classification a companion diagnostic for immunotherapy, and a secondary screening for Lynch syndrome. **Aim of study:** To evaluate the expression of mismatch repair proteins on endometrial adenocarcinoma in a sample of Iraqi female patients and to correlate its expression with different clinic-pathological findings. **Patient and methods:** A retrospective study was carried out at Al-Yarmouk Teaching Hospital and private lab in Baghdad (from January 2024 to January 2025). It included fifty formalin- fixed, paraffin embedded tissue blocks of endometrial carcinoma female patients were included in this study. Five sections of 5µm thickness were acquired from each block. One of them was stained with hematoxylin and eosin stain (H and E) for histopathological revision, the other sections were stained immunohistochemically for MMR proteins expression (PMS2, MLH1, MSH2, MSH6), which was done in private lab in Baghdad in August 2024. **Results:** In this study, the most common histological type was endometrioid (84%). We noticed that 24% of patients showed negative PMS2 expression; 14% showed negative MLH1 expression; 20% showed negative MSH2 expression; and 20% showed negative MSH6 expression. Mismatch repair protein expression showed that 32% of patients had deficient mismatch repair protein expression. Deficient mismatch repair protein expression was significantly associated with younger age patients, positive family history; endometroid histology type; FIGO stage IA, FIGO grade I; and TNM stage T1A. Positive expressions of any protein were significantly associated with positive expressions of other proteins. **Conclusion:** Endometrial carcinomas occur mainly in postmenopausal women. The percentage of immunohistochemical expression of microsatellite instability in endometrial carcinoma in Iraqi females are like the results of other studies worldwide. Endometroid type of endometrial carcinoma is the most common type, and it is most associated with microsatellite instability. Mismatch repair proteins evaluation by immunohistochemistry is simple and affordable method for assessing microsatellite instability that is useful in the prognosis and treatment of endometrial carcinoma.

KEYWORDS: Endometrial, carcinoma, immunohistochemical, mismatch repair proteins, Iraq.

INTRODUCTION

Endometrial cancer is the second most prevalent gynecologic cancer in both resource-abundant and resource-limited countries. In the past decade, numerous studies have explored prognostic factors such as pathological type, histologic grade, lymph vascular involvement, and tumor staging but with insufficient reproducibility. Investigation has, therefore, turned to gene carcinogenesis such as molecular alterations to provide a new prognostic classification.[1]

Recent advancements in genomic analysis have uncovered that approximately 20–30% of endometrial cancer cases exhibit a mismatch repair deficiency (dMMR) phenotype, which is caused by genetic or epigenetic alterations of any of the mismatch repair genes (MLH1, MSH2, MSH6, or PMS2).[2]

Tumors without defects in MMR genes are called mismatch repair-proficient (MMRp). The accumulation of insertions or deletions of nucleotides into coding repeat sequences results in an increase in lymphocyte infiltration, and the phenotype is, therefore, a possible candidate for immunotherapy. Thus, identification of MMRd tumors has become critical for patients with EC for therapeutic management, clinical decision making, and prognosis.[3]

In addition to MMR testing and to better identify risk groups currently included in the latest European Society of Gynecological Oncology/European Society for Radiotherapy, immunohistochemistry (IHC) markers such as p53 or POL-E have been proposed in a diagnostic algorithm. Many oncologic centers use IHC for such testing as it is cheap and of high sensitivity, specificity, and reproducibility.[4]

This study aims to evaluate the expression of mismatch repair proteins on endometrial adenocarcinoma in a sample of Iraqi female patients and to correlate its expression with different clinic-pathological findings.

PATIENTS AND METHODS

Study design, setting and data collection time

This is a retrospective study that was carried out at Al-Yarmouk Teaching Hospital and private lab in Baghdad (from January 2024 to January 2025).

Study patients and Sample size

This study involved fifty cases diagnosed with endometrial carcinoma, collected from archived materials from Al-Yarmouk Teaching Hospital and private lab.

Inclusion criteria

Any female patient diagnosed with endometrial carcinoma with:

- Variable grades and stages.
- Accessible clinical data.
- Available formalin fixed paraffin embedded (FFPE) tissue blocks.

Exclusion criteria

- ✓ D & C specimens.
- ✓ Incomplete clinical data

Sampling of cases

The clinicopathological parameters including name, age, family history, clinical presentation, histopathological findings like tumor grade, stage, type, lympho-vascular invasion were obtained and recorded from the archived materials.

- ➢ Fifty formalin- fixed, paraffin embedded tissue blocks of endometrial carcinoma female patients were included in this study. The formalin-fixed paraffin embedded tissue samples were collected and stained with the routine H&E stain and re-examined by senior histopathologist for confirming histopathological diagnosis.
- ➢ Five sections of 5µm thickness were acquired from each block. One of them was stained with hematoxylin and eosin stain (H and E) for histopathological revision, the other sections were stained immunohistochemically for MMR proteins expression (PMS2, MLH1, MSH2, MSH6), which was done in private lab in Baghdad in August 2024.

Evaluation of Immunohistochemical staining and quality control

To interpret the efficacy of immunohistochemical staining, CAP protocol for immunohistochemistry interpretation is used, it states that any nuclear staining, is taken as ―no loss of expression‖ even if it was focal or patchy and only complete absence of nuclear staining was regarded as ―loss of expression, ‖ presuming internal controls are positive.

2.7. Statistical Analysis

The data analyzed using Statistical Package for Social Sciences (SPSS) version 26. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. The Chi square test was used to assess the association between categorical variables, while fisher exact test was used instead when the expected frequency was less than 5. A level of P – value less than 0.05 was considered significant.

2.8. Ethical approval

Official approval was granted from the Scientific Council of Iraqi Board of Pathology / Histopathology Specialization and from the Scientific Committee of Al-Yarmouk Teaching Hospital.

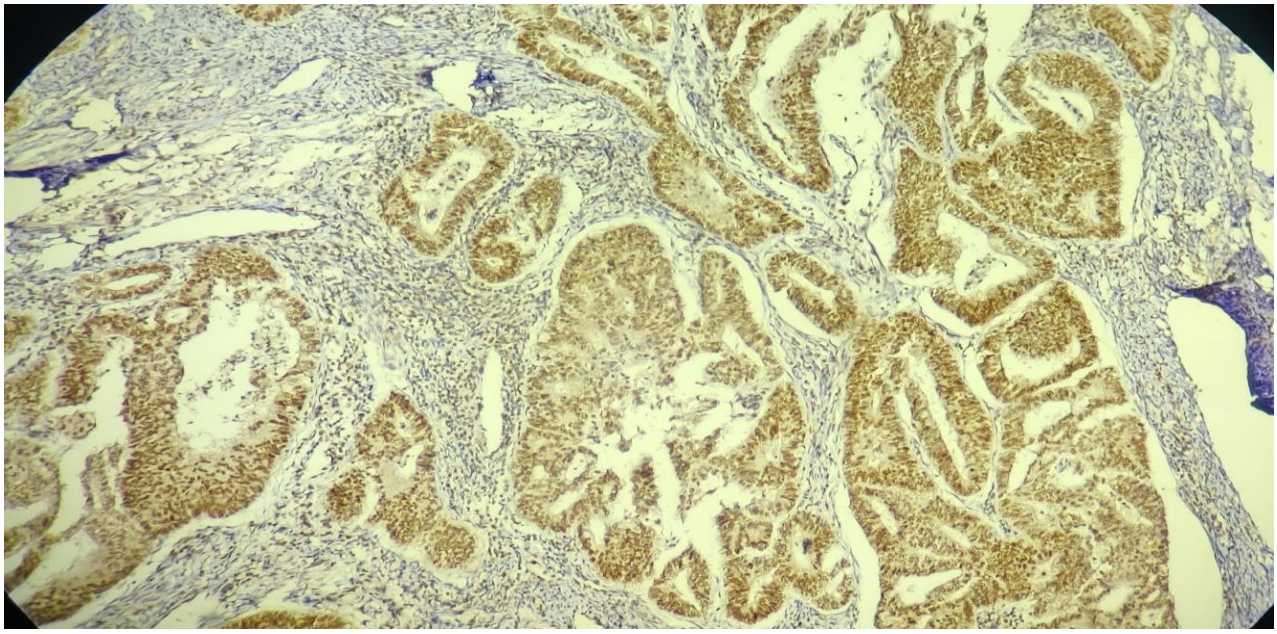

Figure 1: Showing medium power (10x), positive staining with MSH2 in endometrial carcinoma.

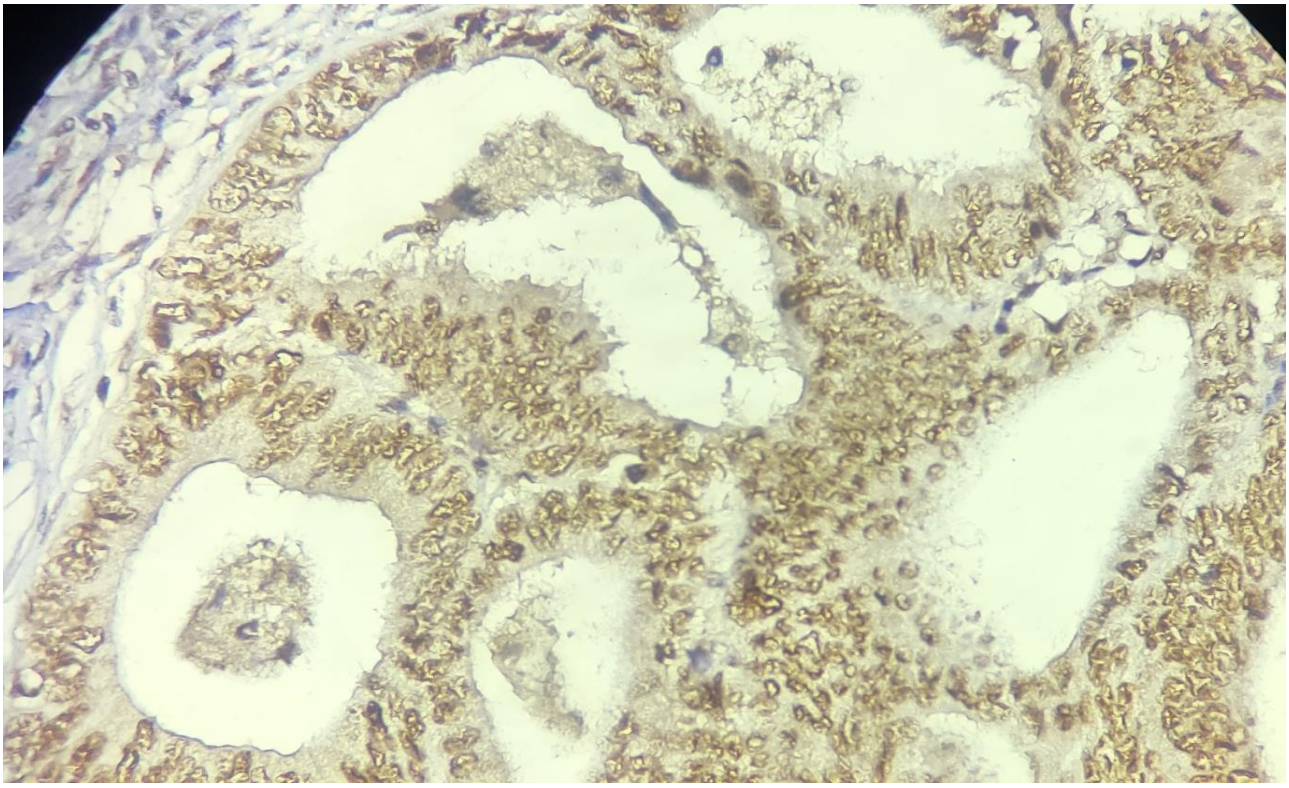

Figure 2: showing high power (40x), MLH1+ve staining of endometrial carcinoma.

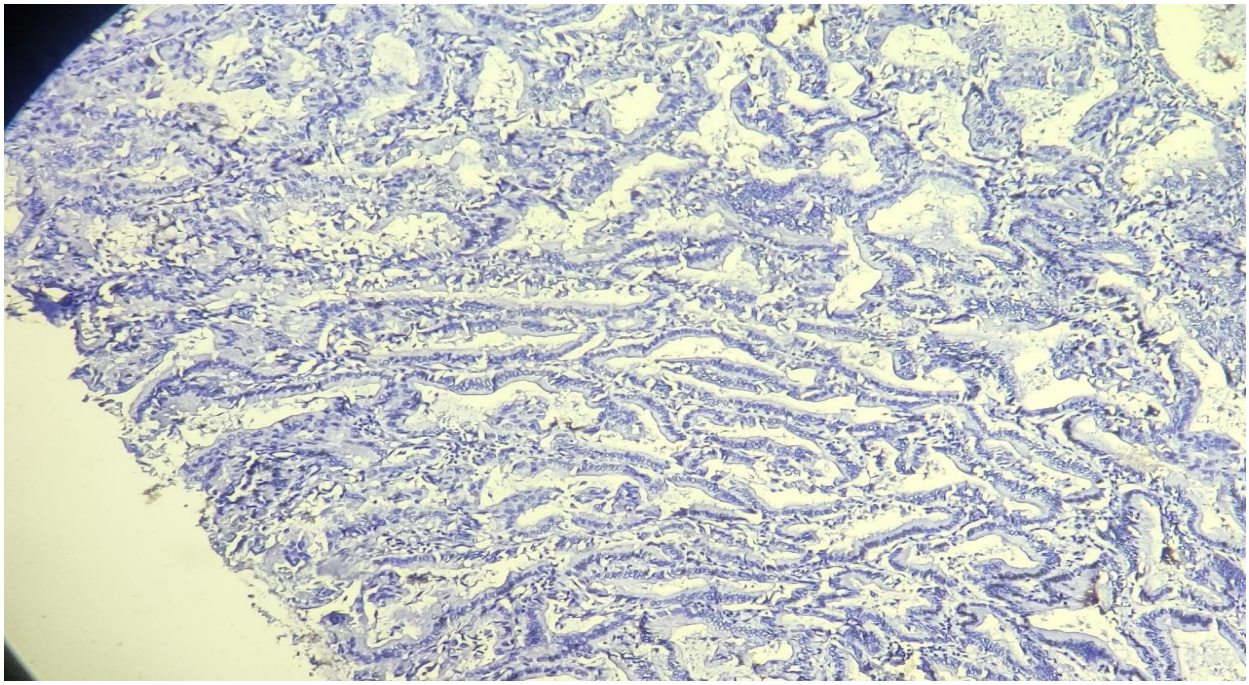

Figure 3: showing medium power (10x), -ve staining of MLH1 in endometrial ca.

RESULTS

The total number of study patients was 50 blocks. All of them were females diagnosed with endometrial carcinoma underwent laboratory workup to determine the expression of mismatch repair proteins on them.

General characteristics

The distribution of study patients by general characteristics is shown in figure and table (1). The study patients' age ranged from 47 to 69 years with a mean of 62.5 years and a standard deviation (SD) of ± 6.55 years. The highest proportion of study patients was aged ≥ 60 years (56%).

We noticed that 78% of study patients were currently married; 22% had positive family history; and 86% presented with postmenopausal bleeding.

Figure 4: Distribution of study patients by age.

Table 1: Distribution of study patients by general characteristics.

Variable	No. (n= 50)	Percentage (%)
Marital status		
Currently married	39	78.0
Unmarried	11	22.0
Family hx		
Positive	11	22.0
Negative	39	78.0
Presentation		
Postmenopausal bleeding	43	86.0
Menorrhagia	7	14.0

Tumor characteristics

Histological type

As shown in table (2), the most common histological type was endometrioid (84%) and 16% were serous.

Table 2: Distribution of study patients by histological type.

Histological type	No. (n= 50)	Percentage (%)
Endometrioid	42	84.0
Serous	8	16.0

Invasion

Table 3 shows the distribution of study patients by invasion. Myometrial invasion was > 50% in 28% of patients, while 14% of them had lymphovascular invasion.

Table 3: Distribution of study patients by invasion.

Invasion	No. (n= 50)	Percentage (%)
Myometrial invasion		
> 50%	14	28.0
< 50%	36	72.0
Lymphovascular invasion		
Positive	7	14.0
Negative	43	86.0

Grade and stage

As shown in table 4, 56% of patients were graded I by FIGO grade; 72% were staged 1A by FIGO stage; and 72% were staged T1A by TNM stage.

Table 4: Distribution of study patients by grade and stage of tumor.

Grade and stage	No. (n= 50)	Percentage (%)
FIGO grade		
I	28	56.0
II	19	38.0
III	3	6.0
FIGO stage		
1A	36	72.0
1B	8	16.0
II	3	6.0
III	3	6.0
TNM stage		
T1A	36	72.0
T1B	8	16.0
T2	3	6.0
T3A	3	6.0

Mismatch repair protein expression

Figure 2 shows the distribution of study patients by negative expressions of mismatch repair proteins. We noticed that 24% of patients showed negative PMS2 expression; 14% showed negative MLH1 expression; 20% showed negative MSH2 expression; and 20% showed negative MSH6 expression.

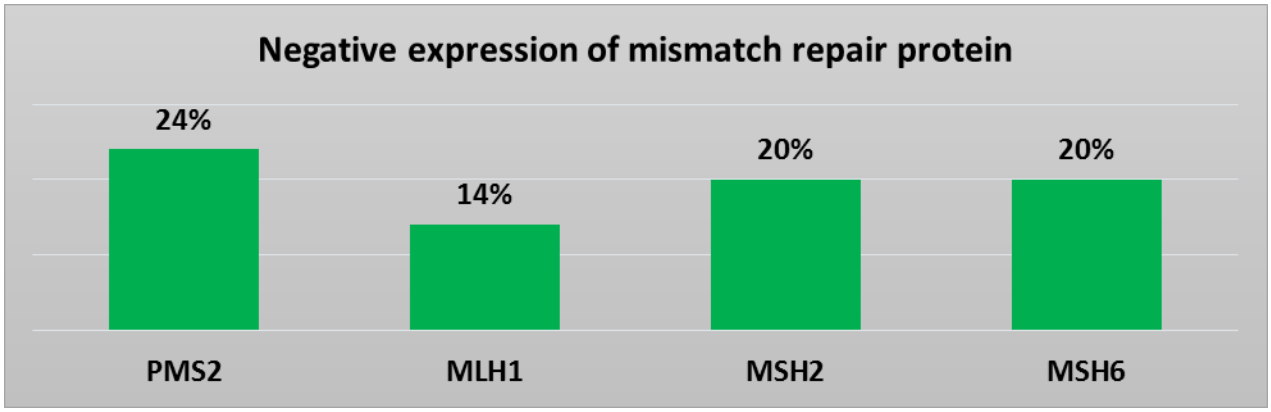

Figure 5: Distribution of study patients by negative expression of mismatch repair proteins.

Mismatch repair protein expression showed that 32% of patients had deficient mismatch repair protein expression and 68% of patients had retained mismatch repair protein expression as shown in figure (6).

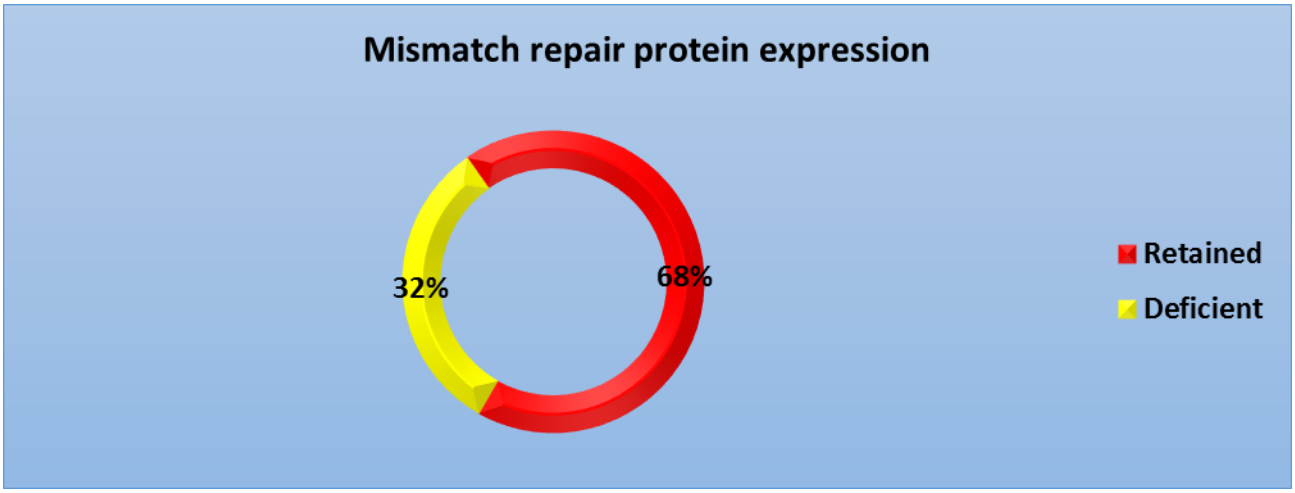

Figure 6: Mismatch repair protein expression.

Table 5 shows that positive deficient mismatch repair protein expression was significantly associated with younger age patients (89.7%, P= 0.007), positive family history (93.8%, P= 0.043); endometroid histology type (84.1%, P= 0.001); FIGO stage IA (90.3%, P= 0.004), FIGO grade I (90.3%, P= 0.001); and TNM stage T1A (94.7%, P= 0.002).

Table 5: Association between Mismatch repair protein expression and clinicopathological characteristics of patients.

Variable	Mismatch repair protein expression		Total (%) n= 50	P- Value
	MMRr (%) n= 34	MMRd (%) n= 16		
Age (Year)				
< 60	9 (40.9)	13 (59.1)	22 (44.0)	0.001
≥ 60	25 (89.3)	3 (10.7)	28 (56.0)	
Family history				
Positive	2 (18.2)	9 (81.8)	11 (22.0)	0.001
Negative	32 (82.1)	7 (17.9)	39 (78.0)	
Histology type				
Endometroid	26 (61.9)	16 (38.1)	42 (84.0)	0.034
Serous	8 (100.0)	0 (0)	8 (16.0)	

Presentation				
Postmenopausal bleeding	31 (72.1)	12 (27.9)	43 (86.0)	0.124
Menorrhagia	3 (42.9)	4 (57.1)	7 (14.0)	
Myometrial invasion				
> 50%	7 (50.0)	7 (50.0)	14 (28.0)	0.088
< 50%	27 (75.0)	9 (25.0)	36 (72.0)	
Lymphovascular invasion				
Positive	3 (42.9)	4 (57.1)	7 (14.0)	0.124
Negative	31 (72.1)	12 (27.9)	43 (86.0)	
FIGO grade				
I	13 (46.4)	15 (53.6)	28 (56.0)	0.001
II	18 (94.7)	1 (5.3)	19 (38.0)	
III	3 (100.0)	0 (0)	3 (6.0)	
FIGO stage				
1A	21 (58.3)	15 (41.7)	36 (72.0)	0.044
1B	7 (87.5)	1 (12.5)	8 (16.0)	
II and III	6 (100.0)	0 (0)	6 (12.0)	

Table 6 shows that positive expressions of any protein were significantly associated (P < 0.05) with positive expressions of other proteins.

Table 6: Association between Mismatch repair proteins expressions.

		MLH1		MSH2		MSH6	
		+ve	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**
PMS2	**+ve**	36	2	34	4	35	3
	-ve	7	5	6	6	5	7
MSH2	**+ve**	39	1			38	2
	-ve	4	6			2	8
MLH1	**+ve**					38	5
	-ve					2	5

DISCUSSION

Tumor characteristics

In the current study, the most common histological type was endometrioid among 84% of participants, while the remaining 16% had serous type.

In Yoshida et al study, 50 cases of endometrial carcinoma were enrolled. Of them 41 cases were endometrioid carcinoma (82%), 4 cases (8%) of mixed carcinoma (serous carcinoma and endometrioid carcinoma), 3 cases of serous carcinoma (6%), 1 case of dedifferentiated carcinoma (2%), and 1 case of carcinosarcoma (2%).[5] A close results obtained in Loukovaara et al study, in which endometrial carcinoma was the most prevalent histological type among 88.3% of patients diagnosed with endometrial carcinoma, while the serous type observed among 3.6%.[6] In Jain et al study, high grade serous carcinoma represented 37.5% of the enrolled cases, Clear cell carcinoma found 25%, mucinous carcinoma in 25% also and Poorly differentiated carcinoma with neuroendocrine differentiation in 12.5%.[7]

Regarding the invasion of tumors, the present work reported that myometrial invasion was > 50% in 28% of patients, while 14% had lymphovascular invasion. Different results observed in Jain et al study, in which myometrial invasion >50% was observed in majority of cases involved, with lymphovascular invasion presented in 44.4% of the participated cases.[7]

Concerning the grade and stage of disease in this study, 56% of patients were graded I; 72% were staged 1A by FIGO; and 72% were staged T1A by TNM stage.

A close results published in Loukovaara et al study, in which stage-IA endometrial carcinoma was the commonest stage among 54.2% of participants, while stage-II was prevalent among 6.8% of cases according to FIGO grade.[6] In a different manner, Yoshida and colleagues made a study on 50 cases of endometrial carcinoma, in which 53.6% of cases were graded as grade-I, 30% as grade-II, 8% as a grade-II.[5]

The vast majority of endometrial carcinoma cases enrolled in Kato et al study was in stage I and II (90.4%) versus 9.6% of cases presented with stage III of disease. On the other hand, 86.8% of patients were graded I and II by FIGO grade with the remaining distributed between grade III/IV (13.2%).[9] Hashemi and colleagues observed that FIGO stage-I was the most frequent stage at presentation (61.9%) and cervical or adnexal involvement was noted in a minority of cases (31.7% and

9.5% respectively). Similarly, 7.1% of cases were found to be at high grade/ grade III.[8]

The variation in the characteristics of endometrial cancer across studies is influenced by several factors, including histological subtypes (low or high grade), diagnostic tools, diagnostic accuracy, differences in surgical staging (total hysterectomy versus biopsy), variability in tumor aggressiveness across populations, geographic or ethnic differences, obesity and metabolic disorders and different sample size or study design.

Mismatch repair protein expression

This study reported that 12 patients (24%) showed negative PMS2 expression; 7 patients (14%) showed negative MLH1 expression; 10 patients (20%) showed negative MSH2 expression; and 10 patients (20%) showed negative MSH6 expression.

The results of MMR immunohistochemical staining in Yoshida et al study showed that negative MLH1 expression was observed in 52% of cases, negative MSH2 expression in 24% of cases, negative MSH6 expression in 46% of cases, and negative PMS2 expression in 56% of cases.[5] The most common MMR defect identified among 82 cases of endometrial carcinoma participated in Jain et al study was combined MLH1/PMS2 in 21% of cases. This was followed by isolated negative MSH6 (4%), combined MSH2/MSH6 loss in 4%, isolated negative PMS2 (2%) and isolated negative MSH2 in only 2% of cases.[7]

Among 191 cases evaluated in Kato et al study, frequencies of MMR-related protein loss were observed in 28% cases by MLH1, 15% of cases by MSH2, 14% by MSH6, and 19% of cases by PMS2, respectively.[11] In Mwafy et al study, 80 cases of endometrial carcinoma retrieved, in which 29 (36.3%) carcinomas showed abnormal MMRP expression (11 cases showed isolated MLH1 deficiency (37.93%), 10 cases showed isolated MSH2 deficiency (34.48%), and 8 cases (27.59) showed a combined loss of both proteins), whereas the remaining 51 (63.7%) of cases demonstrated normal MLH1/MSH2 immunoreactivity (MMRP intact)[10], while Hashmi et al study reported that tumors with dMMR status accounted for 44% of total cases, of which 28.5% cases show loss of expression in all markers, 60.7% showed MLH1/PMS2 loss of expression, 7.1% showed MSH2/MSH6 loss of expression and only 3.5% of cases showed isolated MLH1 loss of expression.[8]

In our study, loss of expression of PMS2 was the most common abnormality, followed by MSH2, MSH6 and then MHL1, unlike other studies that showed that MLH1 loss is the most affected in sporadic cases.

Mismatch repair protein expression in this study showed that 32% of patients had deficient mismatch repair protein expression and 68% of patients had retained mismatch repair protein expression. A close reported as compared to Loukovaara et al study, in which among 795 patients diagnosed with endometrial carcinoma, immunohistochemistry confirmed that intact MMR protein expression in 63.9% and MMR deficiency in nearly third of participants (36.1%).[6] Of the 82 cases tested in Jain et al study, 33% of cases showed loss of expression of at least one MMR protein on IHC (MMR deficient) while 67% were found to be retained mismatch repair protein expression.[7] Kato and colleagues in their study reported that among 191 cases evaluated, a total of 76 cases (40%) were judged as MMR-deficient status.[11]

The current study observed that positive deficient mismatch repair protein expression was significantly associated with younger age patients, positive family history; endometrial histological type; FIGO stage IA, FIGO grade I; and TNM stage T1A ($P<0.05$).

Mwafy and colleagues in their study agreed to our results, as observed that MLH1, MSH2 expression, and MMRP status were closely related to some clinicopathologic features (patient's age, histopathological tumor grade, and tumor stage) with a statistically significant relation.[10] On the other hand, results published in Jain et al study contradict the current one, as they observed that deficient mismatch repair protein expression was significantly associated with positive family history and the Uterine segment involved ($P<0.05$) without significant association with age, histology, myometrial invasion, lymph vascular invasion, FIGO Grade, Stage and regional lymph node involvement ($P>0.05$).[7] This was in consistence with Saharti et al study, as reported that there was no significant association between MMRd and lower uterine segment, low-grade differentiation (FIGO 1–2), node metastasis, myometrial involvement, familial history ($P>0.05$). However, a significant association was observed between MMRd and the lymphoepithelial pattern ($p = 0.014$).[11] In Kato et al study, different results observed. There were significant differences between MMR-deficient protein and FIGO stage, histology, and grade of tumor, while there were no significant differences in age, BMI and lymph node dissection ($P>0.05$).[9]

Numerous factors, including demographic characteristics, tumor biology, testing procedures whether Immunohistochemistry or molecular testing, and study designs, methods of defining deficient MMR and Retained MMR, contribute to the varying rates of dMMR and residual MMR protein expression between studies.

DNA mismatch repair gene mutations have been thought to be crucial to tumorigenesis of endometrial cancers. About 20% to 30% of endometrial cancers have loss of MMR function; 3% to 5% of these are attributed to germline mutation, and the remainder arises due to epigenetic methylation of the MLH1 promoter region causing microsatellite instability (MSI).[9] Recent reports suggested that MMR-deficient endometrial cancers are

related with unfavorable outcome in older and younger women.

However, impact of MMR status on prognoses of endometrial cancers has not been determined. Some reports included non-endometrioid cancers and concluded that MMR-deficient endometrial cancers had better prognosis than MMR-retained cases.[12] The importance of MSI evaluation of testing is the therapeutic use of anti-PDL therapy in MSI-associated endometrial carcinoma. Role of immunotherapy is increasing in human cancer which expresses PDL-1. It has been a proposed that MSI-associated endometrial carcinoma have a better response to anti-PDL therapy compared to microsatellite stable endometrial carcinoma.[13]

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