MEDWIN PUBLISHERS
Committed to Create Value for Researchers

Clinical Pathology & Research Journal
ISSN: 2642-6145

Expression of DNA Mismatch Repair Proteins for Microsatellite Instability in Endometrial Endometrioid Adenocarcinoma and its Association with Tumor Grade and Myometrial Invasion

Ara I[1]*, Islam S[2], Begum F[3], Khanam KF[4], Chowdhury FH[5] and Sabikunnahar S[6]

[1]Department of Pathology, Sir Salimullah Medical College, Bangladesh
[2]Department of Cardiology, National Institute of Cardiovascular Diseases & Hospital, Bangladesh
[3]Department of Pathology, Bangladesh Medical University, Bangladesh
[4]Department of Pathology, Bangladesh Medical University, Bangladesh
[5]Department of Clinical Pathology, Dhaka Medical College and Hospital, Bangladesh
[6]Bangladesh Medical College, Bangladesh

***Corresponding author:** Ismot Ara, Assistant Professor, Department of Pathology, Sir Salimullah Medical College, Mitford Dhaka, Bangladesh

Research Article
Volume 10 Issue 1
Received Date: August 18, 2026
Published Date: August 25, 2026
DOI: 10.23880/cprj-16000223

Abstract

Background: Endometrial carcinoma is one of the most common gynecologic malignancies worldwide, with increasing incidence and distinct molecular subtypes that influence prognosis and treatment response. DNA mismatch repair (MMR) deficiency plays a significant role in the pathogenesis of endometrioid endometrial carcinoma (EEC). Evaluation of MMR proteins PMS2, MLH1, MSH2 and MSH6 provides important diagnostic and prognostic information, including possible association with Lynch syndrome.

Objective: To assess the expression pattern of MMR proteins (PMS2, MLH1, MSH2, MSH6) and their association with histopathological characteristics, tumor grade and myometrial invasion.

Methods: This cross-sectional observational study was conducted in the Department of Pathology, BSMMU. A total of 50 histologically confirmed cases of endometrioid endometrial adenocarcinoma were included. Resected uterine specimens were evaluated for histopathological features including tumor grade, morphological pattern and depth of myometrial invasion. Immunohistochemistry was performed to assess expression of PMS2, MLH1, MSH2 and MSH6. Loss of any MMR protein expression was considered DNA mismatch repair deficient (dMMR), while intact expression of all four proteins was classified as proficient (pMMR). Data were analyzed using Fisher's exact test and independent t-test.

Results: Among 50 cases, 19 (38%) showed dMMR status, while 31 (62%) were pMMR. Loss of PMS2, MLH1, MSH2 and MSH6 was observed in 24%, 28%, 24% and 22% of cases, respectively. The mean age of patients was 53.56 ± 15.86 years. Gross examination frequently revealed infiltrative tumor pattern (52%) and microscopically, 88% showed complex glandular architecture. Grade II tumors constituted 62% of cases and 66% had superficial myometrial invasion. dMMR tumors demonstrated significant association with grade-II tumor and superficial myometrial invasion.

Conclusion: A substantial proportion (38%) of endometrioid endometrial carcinoma cases demonstrated dMMR status. Identification of MMR deficiency is clinically important for recognizing potential Lynch syndrome cases and guiding therapeutic decisions. Routine immunohistochemical screening of all EEC cases using the four MMR antibodies is strongly recommended for accurate molecular classification and improved patient management.

CC BY

Keywords: Intracranial Ancient Schwannoma; Cerebellopontine Angle Tumors; Pathology; Vestibular Schwannoma

Abbreviations

MMR: Mismatch Repair; MSI: Microsatellite Instability; EEC: Endometrioid Endometrial Carcinoma; EEA: Endometrioid Endometrial Adenocarcinoma; dMMR: Mismatch Repair Deficient; pMMR: Mismatch Repair Proficient; PMS2: Postmeiotic Segregation Increased 2; MLH1: MutL Homolog 1; MSH2: MutS Homolog 2; MSH6: MutS Homolog 6; IHC: Immunohistochemistry; EC: Endometrial Carcinoma; TCGA: The Cancer Genome Atlas; POLE: DNA Polymerase Epsilon; IRB: Institutional Review Board; SPSS: Statistical Package for the Social Sciences; SD: Standard Deviation; NOS: Not Otherwise Specified; PCR: Polymerase Chain Reaction; NGS: Next-Generation Sequencing; BRAF: B-Raf Proto-Oncogene; PD-L1: Programmed Death-Ligand 1; GOG: Gynecologic Oncology Group; NRG: National Research Group.

Introduction

Endometrial carcinoma (EC), commonly referred to as uterine cancer, is one of the most prevalent gynecological malignancies affecting women worldwide and accounts for approximately 7% of all female cancers [1-3]. It originates from the endometrium, the inner lining of the uterus, and is generally diagnosed at an early stage, resulting in favorable survival outcomes for most patients. However, a subset of tumors demonstrates aggressive biological behavior and is associated with poor prognosis. Traditionally, endometrial carcinoma has been classified into two major clinicopathological categories. Type I tumors are estrogen-dependent, predominantly endometrioid in histology, usually low grade (Grade 1–2) and associated with a favorable prognosis. In contrast, Type II tumors are estrogen-independent, commonly high grade (Grade 3) or non-endometrioid in histology and are characterized by a more aggressive clinical course and poorer outcomes [4]. Advances in molecular pathology have significantly improved the understanding of EC pathogenesis.

In 2013, The Cancer Genome Atlas (TCGA) Research Network proposed a molecular classification system that categorizes EC into four prognostic subgroups: (i) POLE ultra-mutated tumors characterized by pathogenic variants in the exonuclease domain of DNA polymerase epsilon (POLE); (ii) microsatellite instability (MSI)-hypermutated tumors; (iii) copy-number low tumors with relatively low mutational burden and (iv) copy-number high tumors frequently harboring TP53 mutations. This integrated molecular classification provides superior prognostic stratification and offers opportunities for personalized management of endometrial carcinoma patients. One of the most important molecular pathways implicated in EC development is the DNA mismatch repair (MMR) system [5]. The MMR pathway is a highly conserved cellular mechanism responsible for correcting DNA replication errors, including mismatched nucleotides and insertion-deletion loops. Defects in this pathway lead to the accumulation of genetic mutations and genomic instability, thereby promoting carcinogenesis [5].

The principal MMR proteins include MutL homolog 1 (MLH1), Postmeiotic Segregation Increased 2 (PMS2), MutS homolog 2 (MSH2) and MutS homolog 6 (MSH6). MLH1 forms a heterodimer with PMS2, while MSH2 pairs with MSH6. These complexes work cooperatively to detect and repair replication-associated DNA mismatches, ensuring genomic integrity [6]. Loss of function of these proteins results in microsatellite instability (MSI), a hallmark of MMR deficiency. Immunohistochemistry (IHC) for MLH1, PMS2, MSH2 and MSH6 has become a standard method for assessing MMR status in EC. Loss of expression of MLH1/PMS2 and/or MSH2/MSH6 is frequently observed in MMR-deficient tumors and serves as a reliable surrogate marker for MSI [7].

Such abnormalities are particularly common in patients with Lynch syndrome, an inherited cancer predisposition syndrome caused by germline mutations in MMR genes. Resnick, et al. [8] reported MLH1 and MSH2 mutations in 57% of Lynch syndrome-associated EC cases, while Sjursen, et al. [9] identified PMS2 and MSH6 mutations in 17% of affected patients. Several studies have demonstrated the prognostic and therapeutic significance of MMR deficiency. Goodfellow, et al. [10] observed improved prognosis among MMR-deficient tumors, whereas Schrader, et al. [11] confirmed the high diagnostic accuracy of MMR IHC in identifying Lynch syndrome-associated EC.

Furthermore, Bosse, et al. [12] reported enrichment of immune-related genetic alterations in MMR-deficient tumors, supporting the role of immunotherapy in these patients. Therefore, assessment of MMR protein expression is increasingly recommended as part of universal screening in newly diagnosed endometrial carcinoma patients. Evaluating the prevalence and patterns of MLH1/PMS2 and MSH2/MSH6 loss may provide valuable prognostic information and identify patients who could benefit from targeted therapeutic approaches.

Methodology

This cross-sectional observational study was conducted in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from June 2022 to June 2024. The study population comprised

Copyright© Ara I, et al.

histopathologically confirmed cases of endometrial endometrioid adenocarcinoma of the uterus. A purposive sampling technique was employed for participant selection. The sample size was calculated using the formula $n = Z^2pq/d^2$, where Z = 1.96 at a 95% confidence level, p = 0.15 (expected prevalence of MMR protein loss in endometrial carcinoma), q = 0.85 and d = 0.10. The calculated sample size was 48.93; therefore, 50 cases were included in the study. Patients with histopathologically confirmed endometrial endometrioid adenocarcinoma in resected specimens were enrolled. Cases that had received preoperative chemotherapy, radiotherapy, or hormonal therapy, histological subtypes other than endometrioid adenocarcinoma and endometrial curettage specimens were excluded.

Histopathological variables included tumor grade (Grade 1, Grade 2 and Grade 3) and depth of myometrial invasion. Immunohistochemical variables included the expression status of mismatch repair (MMR) proteins MLH1, PMS2, MSH2 and MSH6. Ethical approval was obtained from the Institutional Review Board (IRB) of BSMMU before commencement of the study. Written informed consent was obtained from all participants or their legal guardians. Paraffin-embedded tissue blocks and relevant clinical information were collected from the Department of Pathology. Histopathological slides were reviewed for tumor morphology, grade, lymphovascular invasion and depth of myometrial invasion. Immunohistochemistry (IHC) was performed using monoclonal antibodies against MLH1, PMS2, MSH2 and MSH6 according to standard laboratory protocols.

Normal proliferating cells, including fibroblasts, lymphocytes and normal epithelial cells, served as internal positive controls. Loss of staining was considered valid only when internal controls demonstrated appropriate nuclear staining. The IHC results were interpreted according to the recommendations of the College of American Pathologists, using the terms “intact” or “lost” expression. Preserved expression was defined as unequivocal nuclear staining in more than 10% of tumor cells with staining intensity comparable to internal controls. Relevant clinical, histopathological and immunohistochemical data were recorded in a predesigned case record form. Data were processed and analyzed using SPSS version 26.0. Continuous variables were expressed as mean ± standard deviation and compared using Student’s t-test. Categorical variables were analyzed using Pearson’s chi-square test. A p-value of less than 0.05 was considered statistically significant at a 95% confidence interval.

Results

All the 50 cases were categorized into two age groups where samples from cases less than 50 years old belonged to ‘<50-year age’ group whereas that of cases 50-years old belonged to the group ‘≥50-year-old’. Age of the study population varied from 42 years to 67 years with mean 53.56±5.86 (SD) Table 1.

Variables	Frequency (n)	Percentage (%)
Menopausal status		
Pre-menopausal	10	20
Post-menopausal	40	80
Parity		
Nulliparous	4	8
Parous	46	92

Table 1: Distribution of the reproductive History of the Study Cases (n=50).

Figure 1 illustrates the distribution of the study population by age 43 (86.00%) cases were in “Age > 50 years” group while 7 (14.00%) cases were in “Age < 50 years” group.

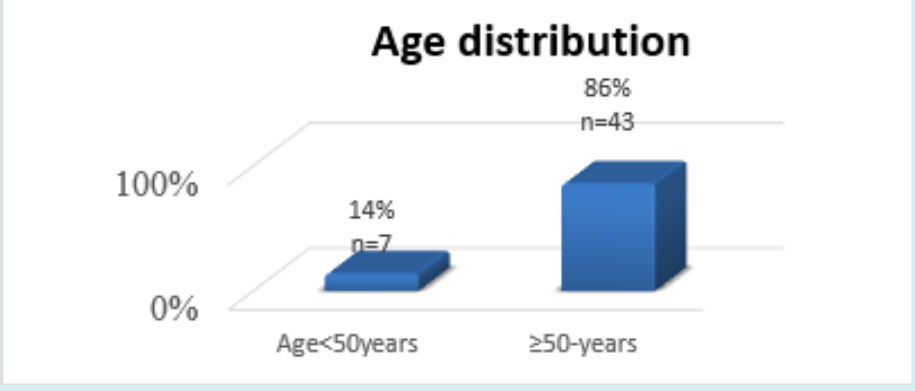


Figure 1: Distribution of the Study Cases by Age (n=50).

Copyright© Ara I, et al.

However, family history of cancer and history of extra uterine malignancy were taken from patient/patient's attendants and also searched from hospital records. Only one case had positive family history of uterine cancer among the 50 studied cases. None of the cases showed history of extra uterine cancer.

Figure 2 show the patterns of loss of expression of PMS2, MLH1, MSH2 & MSH6 MMR proteins among the study cases. It was noticeable that loss of immunohistochemical expression of PMS2 occurred in 12 (24%), MLH1 14 (28%), MSH2 12 (24%) & MSH6 11 (22%). The distribution of the study cases by loss of selected proteins (PMS2, MLH1, MSH2 & MSH6) is depicted in Figure 2.

Distribution of loss of expression of mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) (n=50).

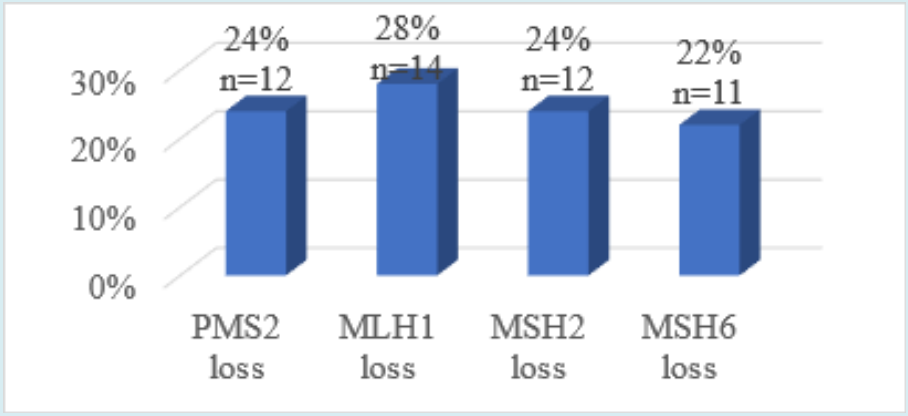


Figure 2: Distribution of loss of expression of mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) (n=50).

Figure 3 show the patterns of intact of expression PMS2, MLH1, MSH2 & MSH6 MMR proteins among the study cases. It was observed that intact of immunohistochemical expression of PMS2 occurred in 38 (76%), MLH1 occurred in 36 (72%), MSH2 occurred in 38 (76%) & MSH6 occurred in 39 (78%). The distribution of the study cases by intact of expression of selected proteins (PMS2, MLHI, and MSH2 & MSH6) is depicted in Figure 3.

Distribution of intact expression of mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) (n=50).

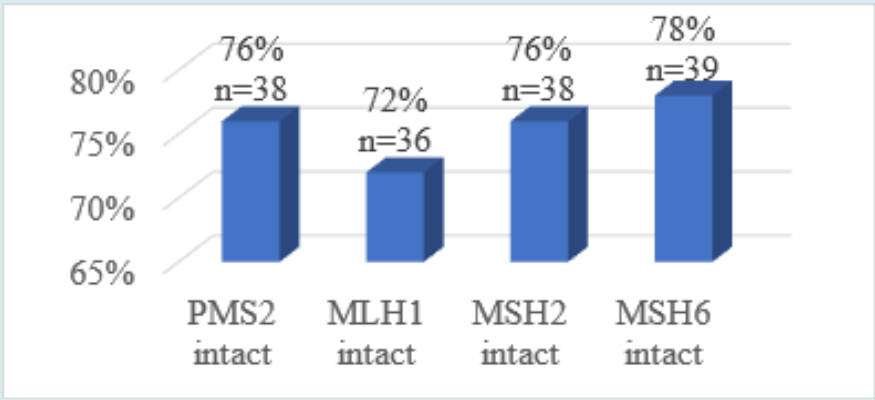


Figure 3: Distribution of Intact Expression of Mismatch Repair Protein (MLHI, PMS2, MSH2 And MSH6) (n=50).

Out of 50 cases, 19 (38%) cases had loss of expression of MMR protein, while rest 31(62%) cases had intact expression of MMR protein. The distribution of the study cases by MMR protein MMR proficient and MMR deficient is depicted in Figure 4.

Copyright© Ara I, et al.

Distribution of the Study Cases by Microsatellite Instability (n=50).

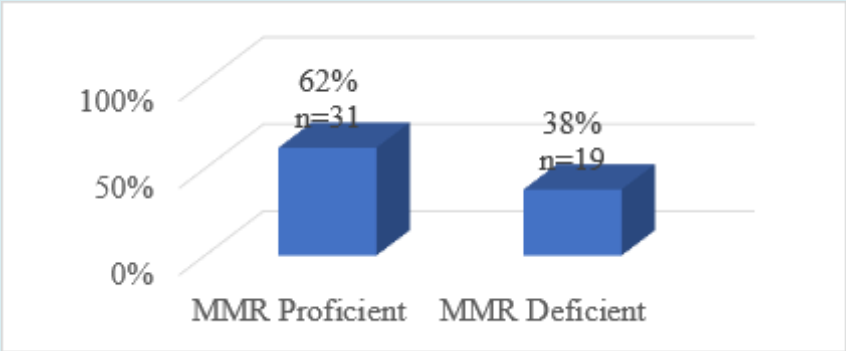


Figure 4: Distribution of the study cases by MMR protein (n=50) MMR protein proficient 31 (62%), MMR protein deficient 19(38%) cases out of 50 cases.

The patterns of loss of expression PMS2, MLH1, MSH2 & MSH6 MMR proteins were observed among the selected cases. It was noticeable that loss of immunohistochemical expression of MLH1 & PMS2 occurred in 6 (12%), MSH2 & MSH6 occurred in 4 (8%) loss of all four protein 4 (8%) only MSH6 3(6%) only MLH1 2(4%). The pattern of distribution of the study cases by loss of selected proteins (PMS2, MLH1, MSH2 & MSH6) is depicted in Figure 5.

The Pattern of Distribution Of Loss Of Expression of Mismatch Repair Protein (MLH1, PMS2, MSH2 and MSH6) (n=19).

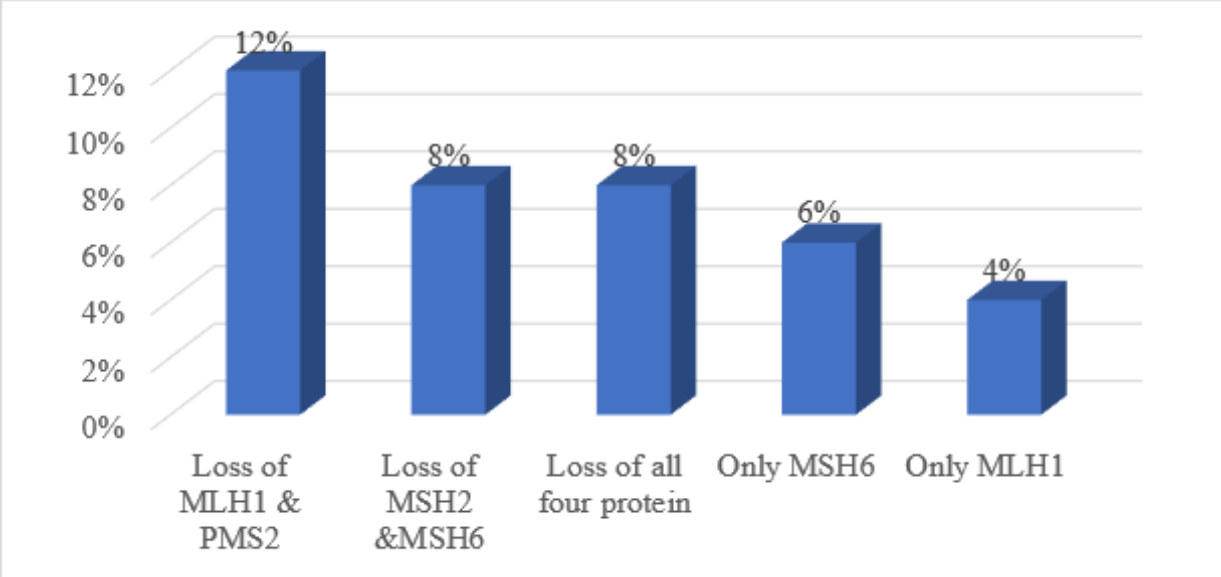


Figure 5: Pattern of Distribution of loss of expression of mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) (n=19).

In our study, only one variants of adenocarcinoma were observed; which is endometrioid endometrial adenocarcinoma. Among 50 selected cases majority of the cases had grade-2 (62.00%) tumor followed by grade-1 (24.00%) tumor while 7 (14.00%) cases had grade-3 tumor in Figure 6.

Copyright© Ara I, et al.

Distribution of the Study Cases by Tumor Grade

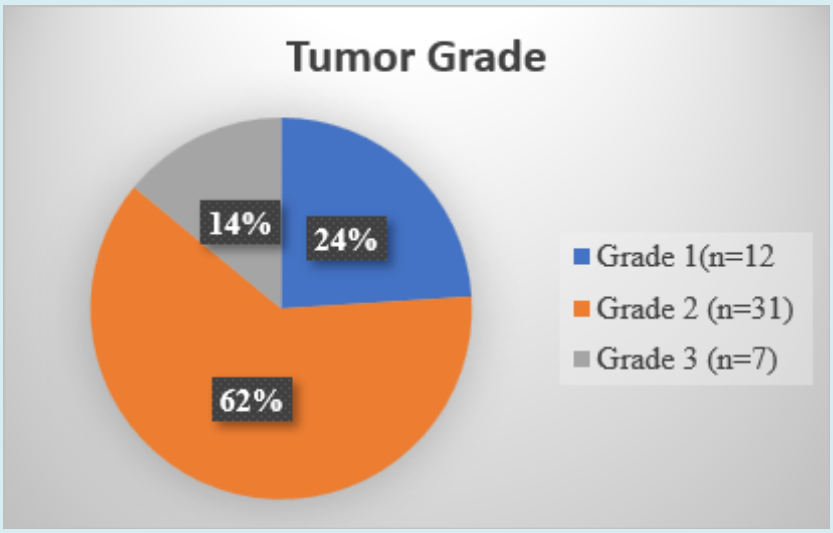


Figure 6: Distribution of the study cases by Tumor Grade (n=50).

Tumors were observed for pathological depth of invasion and the most common were superficial invasion 33 (66.00%) cases while 17 (34.00%) were in deep invasion. The distributions of the study cases by myometrial invasion are illustrated in Figure 7.

Distribution of the Study Cases by Myometrial Invasion.

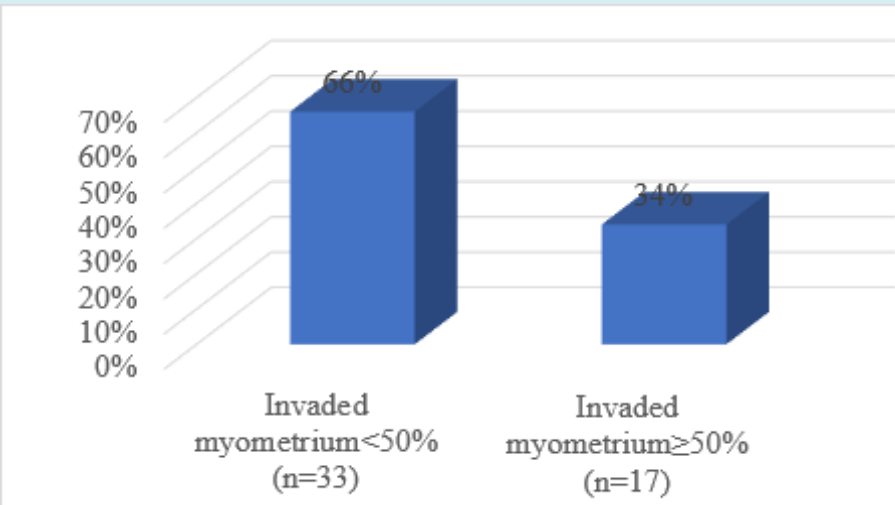


Figure 7: Distribution of the study cases by Myometrial invasion (n=50).

Distribution of the study patient by tumor site is illustrated in Figure 8. Among 50 cases, 24 (48%) tumour had involved both anterior and posterior wall. However, 14(28%) cases had anterior wall tumor and 12(24%) had posterior wall tumor.

Copyright© Ara I, et al.

Distribution of the Study Cases by Tumor Site (n=50).

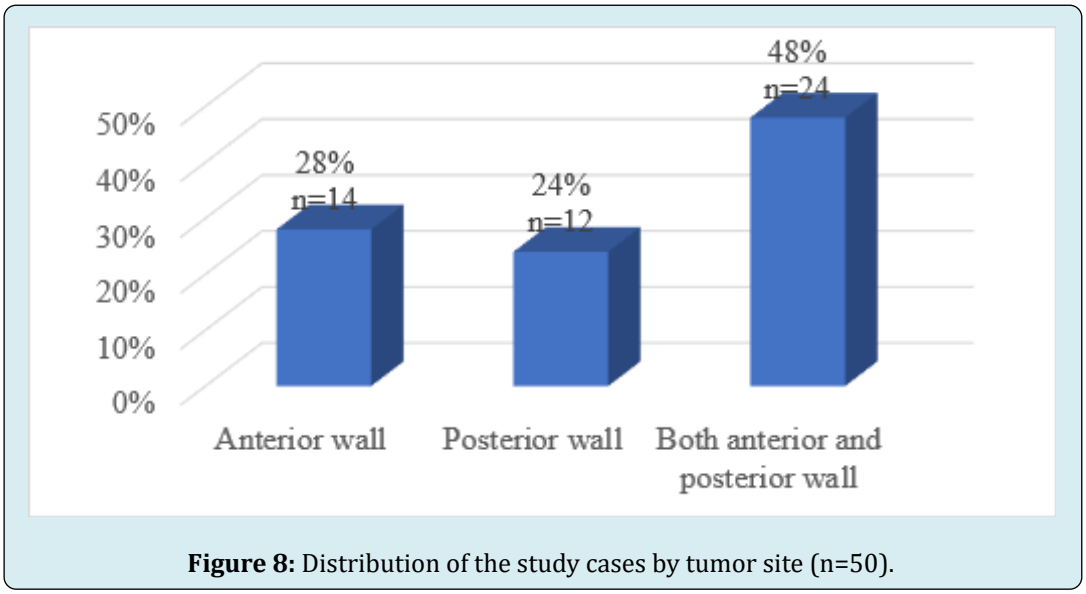


Figure 8: Distribution of the study cases by tumor site (n=50).

Distribution of the study patient by Gross morphology is illustrated in Figure 9. 26 (52.00%) cases was in "Infiltrative" group while 24 (48.00%) was in "Exophytic" group according to Gross morphology.

Distribution of the Study Cases by Gross Morphology (n=50).

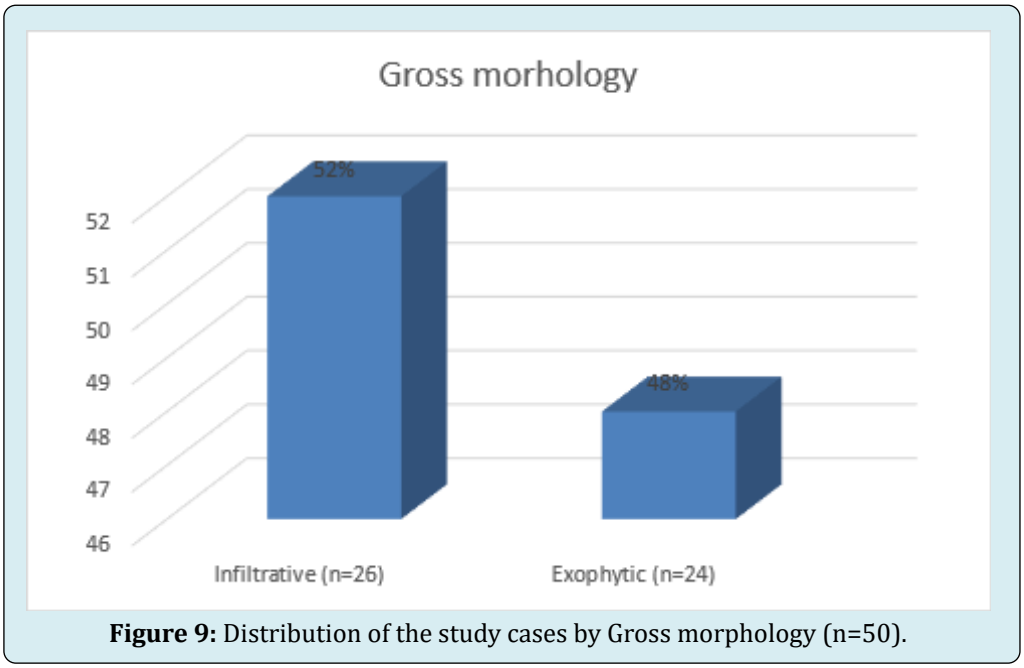


Figure 9: Distribution of the study cases by Gross morphology (n=50).

Distribution of histopathological findings of the study cases by tumor morphology is illustrated in Table 2. Microscopic features of the selected cases were evaluated. Endometrioid carcinoma, NOS was the most common flowed by endometrioid carcinoma with squamous differentiation. According to histopathological findings 21(42%) had endometrrioid carcinoma NOS, 15(30%) had endometrioid carcinoma with squamous differentiation, 6(12%) had endometrioid carcinoma with secretory differentiation, 04 (8%) had endometrioid carcinoma with villoglandular variant, 04 (8%) had endometrioid carcinoma with mucinous differentiation.

Copyright© Ara I, et al.

Variables	Frequency (N)	Percentage (%)
Endometrrioid Carcinoma NOS	21	42%
Endometrioid Carcinoma with Squamous differentiation	15	30%
Endometrioid Carcinoma with Secretory differentiation	6	12%
Endometrioid Carcinoma with Villoglandular Variant	4	8%
Endometrioid Carcinoma with Mucinous differentiation	4	8%

Table 2: Distribution of Histopathological Type of Study Cases (n=50).

Distribution of Histopathological Findings the Study Cases (n=50).

Distribution of the study cases by tumor morphology is illustrated in Table 3. Microscopic features of the selected cases were evaluated. Complex glandular pattern was the most common followed by villoglandular pattern. According to histopathological findings, 44(88.00%) had Complex glandular, 6(12.00%) had Villoglandular, 12(24.00%) had Cibriform, 21(42.00%) had Papillary among the respondents.

Variables	Frequency (n)	Percentage (%)
Complex Glandular		
Yes	44	88
No	6	12
Villoglandular		
Yes	6	12
No	44	88
Cibriform		
Yes	12	24
No	38	76
Papillary		
Yes	21	42
No	29	58

Table 3: Distribution of Histopathological Findings the Study Cases (n=50).

Association of loss of expression of mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) with tumor grading Among loss of Microsatellite instability expression, 52.6% cases had grade 2 and grade 1 tumor. While, 67.7% had grade 2 and 19.4% had grade 3 tumor among intact Microsatellite instability expression. So there was significant association found between grading of tumor and Microsatellite instability expression. 42.1% had However, 63.89% of intact MLHI MMR cases and 57.14% of loss MLHI MMR cases had grade-2 tumnor, while no cases had grade 3 tumor among loss of MLHI MMR expression (p>0.05). Also, 63.16% of intact PMS2 MMR cases and 58.33% of loss PMS2 MMR cases had grade-2 tumor while 60.53% of intact PMS2 MMR cases. 63.16% of intact MSH2 MMR cases and 58.33% of loss MSH2 MMR cases had grade-2 tumor and 61.54% of intact MSH6 MMR cases and 63.64% of loss MSH6 MMR cases had grade-2 tumor. Details of asso ciation are shown in Table 4.

Tumor grading	Microsatellite instability			MLHI MMR protein expression			PMS2 MMR protein expression			MSH2 MMR protein expression			MSH6 MMR protein expression		
	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value
Grade 1	4(12.9)	8(42.1)		6(16.67)	6(42.86)		7(18.42)	5(41.67)		8(21.05)	4(33.33)		9(23.08)	3(27.27)	
Grade 2	21(67.7)	10(52.6)	0.043	23(63.89)	8(57.14)	0.059	24(63.16)	7(58.33)	0.117	24(63.16)	7(58.33)	0.616	24(61.54)	7(63.64)	0.856
Grade 3	6(19.4)	1(5.3)		7(19.44)	0(0.00)		7(18.42)	0(0.00)		6(15.79)	1(8.33)		6(15.38)	1(9.09)	

Table 4: Association of Loss of Expression of Mismatch Repair Protein (MLHI, PMS2, MSH2 and MSH6) with Tumor Grading (n=50).

Copyright© Ara I, et al.

P-value was Determined by Pearson Chi-square Test and Percentages were Shown over Columns

Association of loss of expression of mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) with myometrial invasion. According to myometrial invasion, among intact Microsatellite instability 41.9% had more than 50% myometrial invasion, while among loss of expression 21.1% had more than 50% myometrial invasion and 78.9% had less 50% myometrial invasion. However, no significant association was found among Microsatellite instability and myometrial invasion. Also, in Table 4, 58.33% of intact MLH1 MMR cases and 85.71% of loss MLH1 MMR cases had less 50% myometrial invasion. Among intact PMS2 MMR cases 60.53% and 83.33% of loss PMS2 MMR cases had less 50% myometrial invasion. Furthermore 65.79% of intact MSH2 MMR cases and 66.67% of loss MSH2 MMR cases had invaded myometrium<50% and 66.67% of intact MSH6 MMR cases and 63.64% of loss MSH6 MMR cases had less 50% myometrial invasion. Though, no significant association was found between loss of expression of mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) with myometrial invasion. Details of association is shown in Table 5.

Myometrial invasion	Microsatellite instability			MLHI MMR protein expression			PMS2 MMR protein expression			MSH2 MMR protein expression			MSH6 MMR protein expression		
	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value
Invaded	18	15	0.130	21	12	0.066	23	10	0.146	25	8	0.955	26	7	0.851
Myometrium<50%	(58.10	(78.9)		(58.33)	(85.71)		(60.53)	(83.33)		(65.79)	(66.67)		(66.67)	(63.64)	
Invaded	13	4		15	2		15	2		13	4		13	4	
myometrium ≥50%	(41.9)	(21.1)		(41.67)	(14.29)		(39.47)	(16.67)		(34.21)	(33.33)		(33.33)	(36.36)	

Table 5: Association of Loss of Expression of Mismatch Repair Protein (MLH1, PMS2, MSH2 And MSH6) with Myometrial Invasion Myometrial Invasion(n=50).

P-value was Determined by Pearson Chi-square Test and Percentages were Shown over Columns

Among loss of MSI expression of cases, 52.6% had exophytic morphology and 47.4% had infiltrative morphology. However, among intact MSI expression, 51.6% had exophytic and 48.4% had infiltrative. There was no significant association between gross morphology and MSI. Also, there was no significant association between loss of expression of mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) with gross morphology. Among loss of MLH1 MMR protein 42.86% had exophytic morphology and 57.14% had infiltrative morphology. Again among PMS2 MMR protein, 41.67% had exophytic morphology and 58.33% had infiltrative morphology. Furthermore, among loss of MSH2 MMR protein, 58.33% had exophytic morphology and 41.67% had infiltrative morphology and among loss of MSH6 MMR expression, 63.64% had exophytic morphology and 36.36% had infiltrative morphology. Details of association is shown in Table 6.

Gross morphology	Microsatellite instability			MLHI MMR protein expression			PMS2 MMR protein expression			MSH2 MMR protein expression			MSH6 MMR protein expression		
	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value
Exophytic	16(51.6)	10(52.6)	0.944	20(55.56)	6(42.86)	0.42	21(55.56)	5(41.67)	0.411	19(50.00)	7(58.33)	0.614	19(48.72)	7(63.64)	0.42
Infiltrative	15(48.4)	9(47.4)		16(44.44)	8(57.14)		17(44.74)	7(58.33)		19(50.00)	5(41.67)		20(51.28)	4(36.36)	

Table 6: Association of Loss of Expression of Mismatch Repair Protein (MLH1, PMS2, MSH2 And MSH6) with Gross Morphology (n=50).

P-value was Determined by Pearson Chi-square Test and Percentages were Shown over Columns

Among loss of Microsatellite instability, 42.1% had both anterior and posterior wall tumor, while, 36.8% had posterior wall tumor and 21.1% had anterior wall. However, there was no significant association found between Microsatellite instability and tumor site. Furthermore, loss of PMS2 MMR expression had 33.3% of anterior wall, posterior wall and both anterior and posterior wall tumor respectively. Also, among loss MLH1 expression, 35.7% had both posterior and anterior wall tumor while, among intact MLH1 expression,

Copyright© Ara I, et al.

52.8% had both anterior and posterior wall tumor. There was no significant association between MLH1 and PMS2 expression with tumor site. Although, MSH2 and MHS6 had significant association with tumor site. Among loss of MSH2 expression, 50% had posterior wall tumor, while 41.7% had both posterior wall and anterior wall tumor. Also, among loss of MSH6 expression, 54.5% had posterior wall tumor and 36.4% had both posterior and anterior wall tumor. Details of association is shown in Table 7.

Tumor Site	Microsatellite Instability			Mlhi Mmr Protein Expression			Pms2 Mmr Protein Expression			Msh2 Mmr Protein Expression			Msh6 Mmr Protein Expression		
	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value	Intact	Loss	p-value
Anterior wall	10(32.3)	4(21.1)	0.24	10(27.8)	4(28.6)	0.422	10(26.3)	4(33.3)	0.486	13(34.2)	1(8.3)	0.034	13(33.3)	1(9.1)	0.021
Posterior wall	5(16.1)	7(36.8)		7(19.4)	5(35.7)		8(21.1)	4(33.3)		6(15.8)	6(50)		6(54.5	6(54.5)	
Both anterior and posterior wall	16(51.6)	8(42.1)		19(52.8)	5(35.7)		20(52.6)	4(33.3)		19(50)	5(41.7)		4(36.4)	4(36.4)	

Table 7: Association of Loss of Expression of Mismatch Repair Protein (MLH1, PMS2, MSH2 And MSH6) with Tumor Site (n-50).

Discussion

Endometrial cancer is a common type of cancer that affects the uterus and its molecular classification has gained importance for new therapeutic approaches [13,14]. The study presented a comprehensive analysis of DNA mismatch repair (MMR) protein expression in endometrial endometrioid adenocarcinoma (EEA) and its association with tumor grade and myometrial invasion, providing significant insights into the biological behavior of this cancer type. The patient group ranged in age from 42 years to 67 years, with a mean of around 53.56 years. Notably, a larger proportion of cases (86%) were above the age of 50, implying that EEC may impact at postmenopausal age range. This was also found in Yoshida, et al. [15] where the cases had a median age of 56 years (28 to 77 years) with endometrial endometrioid carcinoma. Also, this study found 80% cases had postmenopausal condition, which was consistent with earlier research demonstrating that endometrial cancer can develop in pre-menopausal women, but it is more likely after menopause [16].

Parity is an established protective factor against endometrial cancer, therefore the large proportion of parous women in this group is somewhat controversial. Similar study found no significant difference of MMR protein proficiency in nulliparous or multiparous and in premenopausal women [16]. Families that have Lynch syndrome which might include colon cancer, endometrial cancer and other types of cancer, have more instances of cancer than expected. However, only one cases had positive family history of endometrial carcinoma in this study, which does not provide any significance in this study results. Histologically, the majority of tumors were complex glandular (88%), followed by villoglandular (12%), cribriform (24%) and papillary (42%). In Ismael, et al. [17] the most of the cases were endometrioid adenocarcinomas (80%) including conventional endometrioid carcinoma (66.7%), endometrioid with squamoid differentiation (8.3%) and endometrioid villoglandular subtype (5%). This distribution of histological subtypes provides a detailed perspective of the tumor morphology within the cohort, which could influence treatment responses and prognostic outcomes. Mismatch repair proteins play a crucial role in maintaining genomic stability by correcting DNA replication errors.

Loss of MMR protein function leads to microsatellite instability (MSI), which is implicated in various cancers, including EEA. The study reports the expression status of four key MMR proteins: PMS2, MSH6, MLH1 and MSH2. Following this study, 38% cases had loss of microsatellite instability. Among four mismatch protein expression showed, 38 (76%) cases had intact PMS2 MMR protein while 12 (24.00%) cases had loss PMS2 MMR protein. Also, 39 (78%) cases had intact MSH6 MMR protein while 11 (22.00 %) cases had loss MSH6 MMR protein. Then in this study, 36 (72%) cases had intact MLHI MMR protein while 14 (28.00%) cases had loss MLH1 MMR protein and 38 (78%) cases had intact MSH2 MMR protein while 12 (22.00%) cases had loss MSH2 MMR protein. In the study of Brincat, et al. [18] 31% of the overall EEC cases were deficient for one or more MMR-proteins, among them dual loss of the MLH1-PMS2 protein heterodimer was the most common deficiency, occurring in 24.5% of cases and loss of MSH2-MSH6 protein expression represented in 3.2% of MMR-deficient cases.

This findings in Brincat, et al. [18] coincided without results. However, MSH6 protein expression was significantly lower than expression for MSH2, even when both were overall considered positive by immunohistochemistry. This was probably due to absence of previous detailed characterized of mismatch repair protein. Still, the loss of these proteins is crucial for understanding the tumor's molecular profile and potential responsiveness to immunotherapy, as MSI-

Copyright© Ara I, et al.

high tumors often respond better to immune checkpoint inhibitors [19]. Following this study, the study reports a predominance of grade-2 tumors (62%), followed by grade-1 (24%) and grade-3 (14%). This grading is essential as it correlates with the aggressiveness of the tumor and overall prognosis. Also, in this study When correlating MMR protein expression with tumor grade and myometrial invasion, mismatch MMR proteins MLH1, PMS2, MSH2, MSH6, were found more in grade 2 tumor and more had superficial invasion (Invaded myometrium<50%), though statistically no significant association was found, which was also strongly performed by Ismael, et al. [17].

However, the significant association between MSI status and tumor grading underscores the potential role of MSI as a prognostic marker in tumor characterization. Tumors with intact MSI are more likely to be of higher grade, suggesting a potential link to more aggressive tumor behavior. This finding aligns with previous research indicating that MSI status can impact the biological behavior of tumors, influencing treatment outcomes and survival rates. Some similar studies showed either mismatch MMR protein had significant relation with tumor grade or myometrial invasion [16,20,21]. Yet these studies lack of individual protein expression and relation with tumor grading and myometrial invasion extent. Nevertheless, this finding could inform treatment strategies, indicating that cases with MMR protein loss may have a relatively better prognosis due to less aggressive tumor characteristics.

Additionally, the extent of myometrial invasion is a critical prognostic factor. The cohort had a higher proportion of cases (66%) with superficial invasion (stage-S) compared to those with deep invasion (stage-D, 34%). In the case of microsatellite instability, the data indicated that 41.9% of those with intact MSI exhibited more than 50% myometrial invasion, whereas only 21.1% of those with loss of expression showed similar levels of invasion. Conversely, a significant majority (78.9%) of the MSI loss group had less than 50% myometrial invasion. These findings suggest a potential trend where loss of MSI might correlate with a lower extent of myometrial invasion. However, the lack of statistical significance implies that other factors may be at play and the relationship between MSI status and myometrial invasion is not straightforward. Another study found 15% (7/50) of microsatellite instability in high endometrial carcinomas showed isolated loss of MSH6 [22].

This was also found in other studies that the sharp contrast underscores the role of MMR deficiency in driving MSI and suggests that testing for MMR protein loss could be a reliable surrogate marker for MSI [23-25]. Given that MSI-high status is associated with better responses to certain immunotherapies, these cases might benefit from targeted treatments, enhancing personalized medicine approaches. Most tumors (48%) were located in the both posterior and anterior wall, while, 28% were anterior wall and 24% was posterior wall tumor, suggesting a possible predilection for the posterior endometrium in EEA. Similar study found expression of all of the MMR proteins had no statistical relation with location of tumor in uterus [26].

Gross morphology was evenly splited between exophytic (52%) and infiltrative (48%) types. The association between MSI and gross morphology revealed that exophytic growth was more common in intact MMR protein cases compared to those with loss of MMR proteins. This relationship could reflect underlying biological differences in tumor growth patterns based on MMR status. Only except for MSH2 and MSH6 MMR protein for tumor wall (<0.05), where both proteins loss of expression were significantly more found in posterior wall tumor. This study provides a detailed analysis of the expression of MMR proteins and their association with clinicopathological features in EEA. The findings suggest that MMR protein loss is associated with MSI and may correlate with less aggressive tumor behavior, as indicated by the higher rates of superficial myometrial invasion. As superficial myometrial invasion has good prognosis.

Conclusion

The findings of this study underscore the significant association between DNA mismatch repair (MMR) protein deficiencies and the clinical features of endometrial carcinoma. The loss of PMS2, MLH1, MSH2 and MSH6 proteins is linked with higher rates of microsatellite instability and specific tumor characteristics, including grade-2 tumors and superficial myometrial invasion. These insights highlight the importance of MMR status in the molecular characterization of endometrial carcinoma and suggest that MMR protein expression could serve as a valuable biomarker for prognosis and potential therapeutic targeting in this disease. However, further multicenter study with longer follow up is recommended to corroborate my research findings.

Limitations and Recommendations

A notable limitation of the present study was the lack of advanced molecular diagnostic facilities, including BRAF mutation testing, polymerase chain reaction (PCR)-based microsatellite instability analysis, and next-generation sequencing (NGS), which restricted comprehensive molecular characterization of mismatch repair (MMR) deficient tumors. Despite these limitations, the findings have important clinical implications. As 38% of endometrioid carcinoma cases demonstrated loss of MMR protein expression, universal screening for MMR deficiency in all newly diagnosed endometrioid carcinoma patients is

Copyright© Ara I, et al.

recommended to identify individuals who may benefit from personalized treatment strategies. Patients exhibiting MMR protein loss should undergo further molecular evaluation, including assessment of PD-L1 expression, to determine their suitability for immunotherapy. Furthermore, the establishment of structured surveillance programs for patients with identified MMR deficiencies is recommended to facilitate monitoring for microsatellite instability, enable early detection of disease progression or recurrence, and improve long-term clinical outcomes.

References:

1. Haydu DC, Black JD, Schwab CL, English DP, Santin AD, et al. (2016) An update on the current pharmacotherapy for endometrial cancer. Expert opinion on pharmacotherapy 17(4): 489-499.

2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, et al (2014) Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver and pancreas cancers in the United States. Cancer research 74(11): 2913-2921.

3. Siegel RL, Miller KD, Jemal A (2019) Cancer statistics 2019. CA: a cancer journal for clinicians 69(1): 7-34.

4. Bokhman JV (1983) Gynecologic oncology 15(1): 10-17.

5. Garg K, Shih K, Barakat R, Zhou Q, Iasonos A, et al. (2009) Endometrial carcinomas in women aged 40 years and younger: tumors associated with loss of DNA mismatch repair proteins comprise a distinct clinicopathologic subset. The American journal of surgical pathology 33(12): 1869-1877.

6. Li GM (2008) Mechanisms and functions of DNA mismatch repair. Cell research 18(1): 85-98.

7. Salem ME, Bodor JN, Puccini A, Xiu J, Goldberg RM, et al. (2020) Relationship between MLH1, PMS2, MSH2 and MSH6 gene-specific alterations and tumor mutational burden in 1057 microsatellite instability-high solid tumors. International journal of cancer 147(10): 2948-2956.

8. Resnick KE, Hampel H, Fishel R, Cohn DE (2009) Current and emerging trends in Lynch syndrome identification in women with endometrial cancer. Gynecologic oncology 114(1): 128-134.

9. Sjursen W, Haukanes BI, Grindedal EM, Aarset H, Stormorken A, et al. (2010) Current status of BRCA1, BRCA2 and Lynch syndrome germline mutation in Norway. Familial Cancer 9(4): 555-563.

10. Goodfellow PJ, Billingsley CC, Lankes HA, Ali S, Cohn DE, et al. (2015) Combined microsatellite instability, MLH1 methylation analysis and immunohistochemistry for Lynch syndrome screening in endometrial cancers from GOG210: an NRG Oncology and Gynecologic Oncology Group Study. Journal of Clinical Oncology 33(36): 4301-4308.

11. Schrader KA (2013) Characterization of hereditary cancer syndromes.

12. Bosse T, Nout RA, Alpine MJN, Conechy MMK, Britton H, et al. (2018) Molecular classification of grade 3 endometrioid endometrial cancers identifies distinct prognostic subgroups. The American journal of surgical pathology 42(5): 561-568.

13. Kim J, Kong JK, Yang W, Cho H, Chay DB, et al. (2018) DNA mismatch repair protein immunohistochemistry and MLH1 promotor methylation testing for practical molecular classification and the prediction of prognosis in endometrial cancer. Cancers 10(9): 1-15.

14. Yang, Y, Wu SF, Bao W (2024) Molecular subtypes of endometrial cancer: Implications for adjuvant treatment strategies. International Journal of Gynecology and Obstetrics 164(2): 436-459.

15. Yoshida, H, Takigawa W, Kato KM, Nishikawa T, Shiraishi K, et al. (2023) Mismatch Repair Protein Expression in Endometrial Cancer: Assessing Concordance and Unveiling Pitfalls in Two Different Immunohistochemistry Assays. Journal of Personalized Medicine 13(8): 1-12.

16. Chaowiwatkun K, Trongwongsa T, Rodpenpear N, Nutthachote P (2023) Comparison of Tissue Mismatch Repair Protein Deficiency between Early and Advanced-Stage Endometrial Cancer. Asian Pacific Journal of Cancer Prevention 24(1): 345-351.

17. Ismael NEHS, Naguib HM, Talaat SM, Bakry RF (2020) Mismatch repair proteins (Mlh1, msh2, msh6 and pms2) immunohistochemical expression and microsatellite instability in endometrial carcinoma. Open Access Macedonian Journal of Medical Sciences 8(A): 306-310.

18. Brincat MR, Buhagiar T, Falzon S, Ariff S, Bugeja S, et al. (2021) Mismatch repair protein expression defects in endometrioid endometrial adenocarcinoma. European Journal of Gynaecological Oncology 42(2): 265-277.

19. Tandon N, Hudgens C, Fellman B, Tetzlaff MT, Broaddus RR, et al. (2020) Variable Expression of MSH6 in Endometrial Carcinomas with Intact Mismatch Repair and with MLH1 Loss Due to MLH1 Methylation. International Journal of Gynecological Pathology 39(6):

Copyright© Ara I, et al.

507-513.

20. Freitas DD, Aguiar FN, Anton C, Almeida DDC, Bacchi CE, et al. (2023) Clinicopathological characteristics of endometrial carcinomas according to DNA mismatch repair protein status. Heliyon 9(6): 1-11.

21. Mwafy S, Elanwar N, Eid A (2020b) Mismatch repair status in Endometrioid type of Endometrial Carcinoma: association with clinicopathological parameters. International Journal of Cancer and Biomedical Research 4(3): 209-215.

22. Wu X, Snir O, Rottmann, D, Wong S, Buza N (2019) Minimal microsatellite shift in microsatellite instability high endometrial cancer: a significant pitfall in diagnostic interpretation. Modern Pathology 32(5): 650-658.

23. Rabban JT, Calkins SM, Karnezis AN, Grenert JP, Blanco A, et al. (2014) Association of tumor morphology with mismatch-repair protein status in older endometrial cancer patients: Implications for universal versus selective screening strategies for lynch syndrome. American Journal of Surgical Pathology 38(6): 793-800.

24. Sharma A, Kamboj M, Panaych A, Gupta G, Pasricha S, et al. (2020) Assessment of mismatch repair protein expression by immunohistochemistry in endometrial carcinomas with clinicopathological correlation: A study from Indian Tertiary Cancer Care Centre. International Journal of Molecular and Immuno Oncology 5(3): 101-107.

25. Atilgan AO, Akcay YE, Ozen O, Reyhan ANH, Ayhan A, et al. (2023) Microsatellite Instability Status and the Expression of p16 and Cyclin D1 Proteins in Uterine Adenosarcoma and Their Clinicopathological Significance. Turk Patoloji Dergisi 39(1): 31-41.

26. Mehdizadeh B, Gharib M, Jafarian AH, Afzalaghaee M, Shandiz FH, et al. (2022) Prevalence of DNA Mismatch Repair Deficiency in Endometrial Cancer Using Immunohistochemistry. International Journal of Cancer Management 15(8): 1-7.

Copyright© Ara I, et al.