DOI: 10.7759/cureus.42781

Review began 07/31/2023 Review ended 07/31/2023 Published 08/01/2023

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# Mismatch Repair Deficient (dMMR) Colorectal Carcinoma in a Pakistani Cohort: Association With Clinical and Pathological Parameters

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### **Abstract**

#### Introduction

Microsatellite instability (MSI) is an important pathway in colorectal carcinoma (CRC) pathogenesis. MSI occurs due to mutations in mismatch repair (MMR) genes that include MutL protein homolog 1 (MLH1), postmeiotic segregation increased 2 (PMS2), MutS homolog 2 (MSH2), and MutS homolog 6 (MSH6). CRC with MSI is termed MMR deficient (dMMR) CRC. Conversely, CRC with intact MMR genes is called microsatellite stable (MSS) or MMR proficient (pMMR). In this study, we compared the clinicopathological features of dMMR CRC with pMMR CRC.

#### **Methods**

It was a retrospective study conducted in the Department of Histopathology, Liaquat National Hospital, Karachi, Pakistan, from March 2020 to February 2022, over a duration of two years. Biopsy-proven cases of CRC with upfront surgical resection were included in the study. Microscopic examination was performed to evaluate tumor type, grade, and extent of invasion, presence of necrosis, perineural invasion (PNI), lymphovascular invasion (LVI), peritumoral lymphocytes (PTL), intratumoral lymphocytes (ITL), and nodal metastasis. Immunohistochemical staining was performed using antibodies, namely, MLH1, PMS2, MSH2, and MSH6. Any loss of nuclear expression in tumor cells was termed dMMR or microsatellite instable, whereas the intact nuclear expression in tumor cells was labeled as MSS or pMMR.

#### Results

A total of 135 cases of CRC were included in the study. The mean age at diagnosis was  $46.76 \pm 17.74$  years, with female predominance (60.7%). The loss of MLH1, PMS2, MSH2, and MSH6 expression was noted in 39.3%, 34.1%, 17.8%, and 16.3% cases, respectively. Overall, 59.3% of CRCs were pMMR, while 40.7% were dMMR. A significant association of MMR status was noted with respect to age, PNI, LVI, tumor grade, tumor (T) and nodal (N) stage, mucinous differentiation, and ITL. dMMR CRC was significantly above 50 years than pMMR CRC. The frequency of PNI and LVI was lower in dMMR CRC than in pMMR CRC. Conversely, the higher grade (grade 3) and higher T-stage (T4) were associated with dMMR CRC. Alternatively, the frequency of higher N stage (N2b) was more commonly seen in pMMR CRC. Moreover, mucinous differentiation and ITL were significantly associated with dMMR CRC.

#### Conclusion

A significant proportion of CRC patients in our population demonstrated dMMR status. dMMR CRC had a higher histological grade with a higher frequency of mucinous differentiation and higher T-stage. Conversely, the presence of LVI, PNI, and higher N stages were associated with pMMR CRC.

Categories: Pathology, Gastroenterology, Oncology

**Keywords:** colorectal carcinoma, msh2, mlh1, pmmr, dmmr, mismatch repair, msh6, pms2, microsatellite instability, biomarkers

# Introduction

Microsatellites are short tandem repeats of deoxyribonucleic acid (DNA) sequences. Mutations introduced in microsatellites during DNA synthesis are repaired by mismatch repair (MMR) genes. These genes and their coded proteins include MutL protein homolog 1 (MLH1), postmeiotic segregation increased 2 (PMS2), MutS homolog 2 (MSH2), and MutS homolog 6 (MSH6). When defects are present in MMR genes, mutations are

introduced during DNA synthesis, resulting in microsatellite instability (MSI) [1]. Many human cancers are associated with MSI [2-4]. MSI is one of the major pathways implicated in colorectal carcinoma (CRC) pathogenesis. CRC with MSI is termed MMR deficient (dMMR) CRC. Conversely, CRC with intact MMR genes is called microsatellite stable (MSS) or MMR proficient (pMMR) [5,6].

Approximately 10-15% of CRCs are dMMR, among which 80% are sporadic owing to MLH1 promoter hypermethylation or v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations, whereas 20% are due to autosomal dominant germline mutations, termed Lynch syndrome (LS) [7]. MSI screening for CRC is initially performed through immunohistochemical (IHC) analysis for MLH1, PMS2, MSH2, and MSH6. The loss of one or two markers mandates MLH1 promoter methylation status and BRAF mutation analysis. The absence of BRAF mutation and MLH1 promoter hypermethylation prompts next-generation sequencing for germline mutations. Previous studies have shown that dMMR CRC is associated with certain histological features, such as mucinous histology, peritumoral lymphocytes (PTL), and intratumoral lymphocytes (ITL) [6]. CRC in the Pakistani population was found to be at a higher tumor (T) and nodal (N) stage than western CRC [8,9]. Clinicopathological features of dMMR CRC have not been widely studied in our population; therefore, we conducted this IHC-based MSI analysis in CRC to better understand the pathogenesis and pathological features of dMMR CRC.

#### **Materials And Methods**

It was a retrospective study conducted in the Department of Histopathology, Liaquat National Hospital, Karachi, Pakistan, from March 2020 to February 2022, over a duration of two years. Biopsy-proven cases of CRC with upfront surgical resection were included in the study. Cases with incomplete clinical or pathological records were excluded. Similarly, CRC with neoadjuvant chemotherapy before surgical resection or systemic metastasis at the time of diagnosis was excluded from the analysis. Institutional Review Board (IRB) approval was not needed as it was a retrospective study, and the institution don't mandate IRB approval for retrospective studies.

Surgical specimens were received in the histopathology laboratory, followed by overnight fixation. Gross dimensions of the tumor were recorded, and representative sections were submitted from the tumor along with adjacent normal mucosa and surgical resection margins. Lymph nodes were dissected from the mesenteric tissue and submitted for microscopic examination. Haematoxylin and eosin-stained microscopic sections were examined for tumor type, grade, and extent of invasion, presence of necrosis, perineural invasion (PNI), lymphovascular invasion (LVI), T- and N-stage, PTL, ITL, and nodal metastasis. Histological tumor typing was performed according to the World Health Organization (WHO) classification of the tumors of the digestive tract. Mucinous differentiation was labelled when there were extracellar mucin pools. whereas signet ring differentiation was called when tumor cells show intracytoplasmic mucin vacuole pushing the nucleus to the periphery. Tumors showing more then 50% mucinous and signet ring differentiation were termed mucinous and signet ring adenocarcinoma, respectively. Tumors were graded according to the proportion of gland formation. Well-differentiated/grade 1 tumors had more than 90% gland formation, whereas grade 2 and grade 3 tumors were labeled when tumor showed 6-50% and less than 50% gland formation, respectively. T and N staging was performed according to the American Joint Committee on Cancer (AJCC) staging system. PTL and ITL were categorized into none, mild to moderate, and marked according to the College of American Pathologist's (CAP) guidelines. PTL is labelled when tumor periphery showed lymphoid follicles with germinal center formation. ITL corresponds to lymphocytes within tumor cells. More than three lymphocytes per high-power field were required for marked ITL categorization.

#### Immunohistochemical analysis

IHC staining was performed using antibodies, namely, MLH1, PMS2, MSH2, and MSH6 antibodies on representative tumor blocks that contained adjacent normal mucosa for the documentation of internal positive control. Any loss of nuclear expression in tumor cells was termed dMMR, whereas intact nuclear expression in all tumor cells was labeled as MSS or pMMR [10].

#### Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (Released 2019; IBM Corp., Armonk, NY). The mean was calculated for patient age, while frequencies and percentages were calculated for other clinicopathological variables. Chi-square and Fisher's exact tests were applied to determine the association of various clinicopathological features with MMR status. A p-value of <0.05 was considered significant.

## Results

A total of 135 cases of CRC were included in the study. The mean age at diagnosis was  $46.76 \pm 17.74$  years, with female predominance (60.7%). Rectosigmoid was the most common tumor location (67.4%), followed by cecum and splenic flexure (11.9%). The most common tumor type was adenocarcinoma, not otherwise specified (65.2), followed by mucinous carcinoma (24.4%). The most common tumor grade was grade 2 (67.4%), while PNI and LVI were present in 33.3% and 23.7% cases, respectively. Nodal metastasis was seen

in 66.7% of cases, with 52.6% demonstrating perinodal extension. Signet ring and mucinous differentiation were present in 18.5% and 36.3% cases, respectively, while marked PTL and ITL were noted in 11.1% and 17.8% cases, respectively. The majority of tumors were at T-stage T3 (71.1%) and N-stage N0 (33.3%). Overall, 59.3% CRCs were pMMR, while, 40.7% were dMMR, as shown in Table 1.

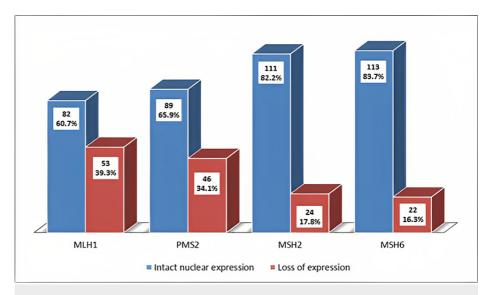
Clinicopathological features	Values
Age (years)	
Mean ± SD	46.76 ± 17.74
Age groups	
(50 years, n (%)	72 (53.3)
-50 years, n (%)	63 (46.7)
Gender Control of the	
Male, n (%)	53 (39.3)
Female, n (%)	82 (60.7)
aterality	
Right, n (%)	23 (17)
eft, n (%)	112 (83)
Tumor location	
Cecum, n (%)	16 (11.9)
Ascending colon, n (%)	5 (3.7)
fransverse colon, n (%)	6 (4.4)
Recto-sigmoid, n (%)	91 (67.4)
Descending colon, n (%)	1 (0.7)
Splenic flexure, n (%)	16 (11.9)
Histological features	
Perineural invasion	
Present, n (%)	45 (33.3)
Absent, n (%)	90 (66.7)
ymphovascular invasion	
Present, n (%)	32 (23.7)
Absent, n (%)	103 (76.3)
Pre-existing polyp	
Present, n (%)	8 (5.9)
Absent, n (%)	127 (94.1)
Tumor type	
Adenocarcinoma, NOS, n (%)	88 (65.2)
ducinous adenocarcinoma, n (%)	33 (24.4)
Medullary carcinoma, n (%)	9 (6.7)
Signet ring adenocarcinoma, n (%)	5 (3.7)
Tumor differentiation/grade	
Well differentiated/grade 1, n (%)	5 (3.7)

Moderately differentiated/grade 2, n (%)	91 (67.4)
Poorly differentiated/grade 3, n (%)	39 (28.9)
T-stage	
T2, n (%)	11 (8.1)
T3, n (%)	96 (71.1)
T4, n (%)	28 (20.7)
Nodal metastasis	
Present, n (%)	90 (66.7)
Absent, n (%)	45 (33.3)
N-stage	
NO, n (%)	45 (33.3)
N1, n (%)	32 (23.7)
N2a, n (%)	32 (23.7)
N2b, n (%)	26 (19.3)
Perinodal extension	
Present, n (%)	71 (52.6)
Absent, n (%)	64 (47.4)
Signet ring differentiation	
Present, n (%)	25 (18.5)
Absent, n (%)	110 (81.5)
Mucinous differentiation	
Present, n (%)	49 (36.3)
Absent, n (%)	86 (63.7)
Peritumoral lymphocytes	
None, n (%)	104 (77)
Mild to moderate, n (%)	16 (11.9)
Marked, n (%)	15 (11.1)
Intratumoral lymphocytes	
None, n (%)	72 (53.3)
Mild to moderate, n (%)	39 (28.9)
Marked, n (%)	24 (17.8)
Overall MMR status	
pMMR	80 (59.3)
dMMR	55 (40.7)

# TABLE 1: Clinicopathological features of the population under study

SD: standard deviation; NOS: not otherwise specified; T: tumor; N: nodal; MMR: mismatch repair; pMMR: mismatch repair proficient; dMMR: mismatch repair deficient

The loss of MLH1, PMS2, MSH2, and MSH6 expression was noted in 39.3%, 34.1%, 17.8% and 16.3% cases, respectively, as shown in Figure  $\it 1$ .



# FIGURE 1: Mismatch repair protein expression by immunohistochemistry

MLH1: MutL protein homolog 1; PMS2: postmeiotic segregation increased 2; MSH2: MutS homolog 2; MSH6: MutS homolog 6

Table 2 shows the association of the MMR status of CRC with clinicopathological variables. A significant association of MMR status was noted with respect to age, PNI, LVI, tumor grade, T and N-stage, mucinous differentiation, and ITL. dMMR CRC was significantly above 50 years than pMMR CRC. The frequency of PNI and LVI was lower in dMMR CRC than in pMMR CRC. Conversely, the higher grade (grade 3) and higher T-stage (T4) were associated with dMMR CRC. Alternatively, the frequency of higher N-stage (N2b) was more commonly seen in pMMR CRC. Moreover, mucinous differentiation and ITL were significantly associated with dMMR CRC.

	Values	Values	
Clinicopathological features	Mismatch repair status		p-value
	pMMR	dMMR	
Gender*			
Male, n (%)	35 (45)	17 (30.9)	0.099
Female, n (%)	44 (55)	38 (69.1)	0.000
Age groups*			
≤50 years, n (%)	52 (65)	20 (36.4)	0.001**
>50 years, n (%)	28 (35)	35 (63.6)	5.551
Laterality*			
Right, n (%)	10 (12.5)	13 (23.6)	0.091
Left, n (%)	70 (87.5)	42 (76.4)	0.091
Location***			
Cecum, n (%)	9 (11.3)	7 (12.7)	
Ascending colon, n (%)	2 (2.5)	3 (5.5)	
Transverse colon, n (%)	3 (3.8)	3 (5.5)	0.004
Recto-sigmoid, n (%)	60 (75)	31 (56.4)	0.094
Descending colon, n (%)	1 (1.3)	0 (0)	

Priority (Priority (Prio	Splenic flexure, n (%)	5 (6.3)	11 (20)	
### 1950	Histopathological features			
### Part	Perineural invasion*			
### 1990年の	Present, n (%)	34 (42.5)	11 (20)	
Page 17 (1975) (197	Absent, n (%)	46 (57.5)	44 (80)	0.006**
Marcia (Si	Lymphovascular invasion*			
### 1979	Present, n (%)	27 (33.8)	5 (9.1)	
Proof. (2)         10 (8)         (8) (1)         200           Abunta (2)         (7 (6.3)         (8) (1)         200           Trans (1)         (7 (6.3)         (8) (1)         (8) (1)           Modicial strongerium, (2)         (8) (8)         (8) (2)         (8)           Boding varione, (2)         (9) (1)         (1)         (1)           Sport (8) (8) (8) (8) (8)         (8) (1)         (8) (1)         (8) (1)           Sport (8) (8) (8) (8) (8) (8) (8) (8) (8)         (8) (1)         (8) (1)         (8) (1)         (8) (1)           Trans (2)         (8) (8) (8) (8) (8) (8) (8) (8)         (8) (1)	Absent, n (%)	53 (66.3)	50 (90.9)	0.001**
Autonotion   77,844   50,800   200	Pre-existing polyp***			
Asact (中) (1705年) (	Present, n (%)	3 (3.8)	5 (9.1)	
Accordance MOS, π(%)         50 (68)         30 (60)         4 (62 7)           Maciona administration, π(%)         15 (18.8)         18 (62 7)           Modifiery concisions, π(%)         10 (0)         11 (10)           Signal ring and administration (%)         2 (2.5)         3 (55)           Tomor administration (%)         0 (0)         5 (6.7)           Mediate relation (%)         10 (7.3)         30 (24.5)         20 (20.4)           Modifier (%)         10 (7.3)         30 (24.5)         20 (20.4)           Power, all relationship (%)         10 (7.3)         30 (24.5)         20 (20.4)           Power, all relationship (%)         10 (7.2)         30 (24.5)         20 (20.4)           T-ranger**         2 (8)         10 (7.2)         20 (20.4)           Table (%)         10 (12.3)         30 (24.5)         20 (20.4)           Table (%)         10 (12.3)         30 (24.5)         20 (20.4)           Nation (%)         2 (10.2)         2 (10.2)         2 (10.2)           <	Absent, n (%)	77 (96.3)	50 (90.9)	0.270
Moderous aefrocuentoms. n (h) 1081 1081 1082 1082 1083 1083 1083 1083 1083 1083 1083 1083	Tumor type***			
Machadry outcomes (10)   11.50   11.	Adenocarcinoma, NOS, n (%)	55 (68.8)	33 (60)	
Mediany carcinoma. (べ) (10 (10 (10 (10 (10 (10 (10 (10 (10 (10	Mucinous adenocarcinoma, n (%)	15 (18.8)	18 (32.7)	0.066
Transport of differentiation by solution  Fine of the differentiation  Fine of the differentiation by solution  Fine of t	Medullary carcinoma, n (%)	8 (10)	1 (1.8)	U.U00
Well differentiated grade 1, n (h)         5 (P.1)         2 (P.1)           Modurately differentiated grade 2, n (k)         6 (P6.3)         30 (64.5)         0000**           Pourly differentiated grade 2, n (k)         10 (23.6)         20 (68.6)         0000**           T-dags***         T-dags***         T (12.7)         0000**           T2, n (h)         4 (5)         30 (64.5)         0000**           T4, n (h)         10 (12.5)         40 (82.7)         0000**           Notal mutataliza*         T         <	Signet ring cell adenocarcinoma, n (%)	2 (2.5)	3 (5.5)	
Manufaction of the content delignate 2, n (%)   10 (20)   20 (34)   20 (3	Tumor differentiation/grade***			
Peorly differentiatelgrade 3. n (%)  7 categor**  72. n (%)  73. n (%)  74. n (%)  75. n (%)  76.	Well differentiated/grade 1, n (%)	0 (0)	5 (9.1)	
T-dags "	Moderately differentiated/grade 2, n (%)	61 (76.3)	30 (54.5)	0.003**
T2. n (%)       4 (5)       7 (127)       4 (5)       4 (5)       5 (127)       4 (5)       4 (5)       5 (127)       4 (5)       4 (5)       5 (127)       4 (5)       4 (5)       5 (127)       4 (5)       4 (5)       4 (5)       4 (5)       4 (5)       4 (5)       4 (5)       4 (5)       4 (5)       4 (5)       4 (6)<	Poorly differentiated/grade 3, n (%)	19 (23.8)	20 (36.4)	
T3, n (%)       66 (82.5)       30 (84.5)       0.002**         T4, n (%)       10 (12.5)       18 (82.7)       18 (82.7)         No. n (%)       56 (70)       34 (61.8)       322         Absent, n (%)       24 (90)       21 (38.2)       322         No. n (%)       24 (90)       21 (38.2)       44 (76.4)         N2. n (%)       24 (90)       21 (38.2)       44 (76.4)         N2. n (%)       24 (90)       21 (38.2)       44 (76.4)         N2. n (%)       24 (80.8)       3 (55.9)       4001**         N2. n (%)       23 (28.8)       3 (55.9)       4001**         Persont, n (%)       28 (90.9)       27 (48.1)       47 (49.1)         Absent, n (%)       37 (46.3)       27 (49.1)       47 (49.1)         Sport ring differentiation*       12 (15)       13 (21.6)       42 (76.4)         Persont, n (%)       68 (85)       42 (76.4)       42 (76.4)         Mucirous differentiation*       19 (23.8)       30 (54.5)       44 (76.4)	T-stage***			
T4, n(%)       10 (12.5)       18 (32.7)         Nodal metastasia*       ***********************************	T2, n (%)	4 (5)	7 (12.7)	
Nodal metastasis*         Present, n (%)         34 (81.8)         0.322           Absent, n (%)         24 (30)         21 (38.2)	T3, n (%)	66 (82.5)	30 (54.5)	0.002**
Present, n (%)       34 (61.8)       0.322         Absent, n (%)       24 (30)       21 (38.2)         N-stage*	T4, n (%)	10 (12.5)	18 (32.7)	
Absent, n (%) 24 (30) 21 (38.2) 0.322  N-stage*  N0, n (%) 24 (30) 21 (38.2)  N1, n (%) 21 (38.2)  N2a, n (%) 11 (20)  N2a, n (%) 22 (28.8) 31 (5.5)  Periodal extension*  Present, n (%) 43 (33.8) 28 (50.9) 0.745  Absent, n (%) 37 (48.3) 27 (49.1) 0.745  Signet ring differentiation*  Present, n (%) 12 (15) 13 (23.6) 0.204  Absent, n (%) 86 (85) 42 (76.4)  Mucinous differentiation*  Present, n (%) 80 (50.9) 0.745  Absent, n (%) 10 (23.8) 30 (53.8) 0.204	Nodal metastasis*			
Absent, n (%)         24 (30)         21 (38.2)           N-stage*         ν         ν           N0, n (%)         24 (30)         21 (38.2)           N1, n (%)         21 (28.3)         11 (20)           N2ω, n (%)         12 (15)         20 (36.4)           Perindal extension*         23 (28.8)         3 (5.5)           Present, n (%)         43 (38.8)         28 (50.9)         745           Absent, n (%)         37 (46.3)         27 (48.1)         745           Signet ring differentiation*         12 (15)         13 (23.6)         744           Absent, n (%)         48 (85)         42 (76.4)         76.4           Mucinous differentiation*         19 (23.8)         30 (54.5)         14 (10.2)	Present, n (%)	56 (70)	34 (61.8)	0.222
N0, n (%) 24 (30) 21 (38.2)  N1, n (%) 21 (26.3) 11 (20) 0.001**  N2a, n (%) 12 (15) 20 (36.4)  Perinodal extension*  Present, n (%) 37 (46.3) 27 (49.1)  Signet ring differentiation*  Present, n (%) 12 (15) 13 (23.6)  Absent, n (%) 12 (15) 13 (23.6)  Determined the control of	Absent, n (%)	24 (30)	21 (38.2)	0.322
N1, n (%)       21 (26.3)       11 (20)         N2a, n (%)       12 (15)       20 (38.4)         N2b, n (%)       23 (28.8)       3 (5.5)         Perinodal extension*       ****         Present, n (%)       43 (53.8)       28 (50.9)       0,745         Absent, n (%)       37 (46.3)       27 (49.1)       ****          Present, n (%)       12 (15)       13 (23.6)       0,204         Absent, n (%)       68 (85)       42 (76.4)       0,204         Mucinous differentiation*       19 (23.8)       30 (54.5)       ***	N-stage*			
N2a, n (%)       12 (15)       20 (36.4)         N2b, n (%)       23 (28.8)       3 (5.5)         Perinodal extension*       ****         Present, n (%)       43 (53.8)       28 (50.9)       0.745         Absent, n (%)       37 (46.3)       27 (49.1)       0.745         Present, n (%)       12 (15)       13 (23.6)       0.204         Absent, n (%)       68 (85)       42 (76.4)       0.204         Mucinous differentiation*       19 (23.8)       30 (54.5)       ***	NO, n (%)	24 (30)	21 (38.2)	
N2a, n (%)       12 (15)       20 (36.4)         N2b, n (%)       23 (28.8)       3 (5.5)         Perinodal extension*       Present, n (%)       43 (53.8)       28 (50.9)       0.745         Absent, n (%)       37 (46.3)       27 (49.1)       12 (15)       13 (23.6)       2.044         Absent, n (%)       68 (85)       42 (76.4)       0.204         Mucinous differentiation*       19 (23.8)       30 )54.5)       10 (36.4)       10 (36.4)	N1. n (%)	21 (26.3)	11 (20)	0.001**
Perinodal extension*       43 (53.8)       28 (50.9)       0.745         Absent, n (%)       37 (46.3)       27 (49.1)         Signet ring differentiation*       12 (15)       13 (23.6)         Present, n (%)       68 (85)       42 (76.4)         Mucinous differentiation*         Present, n (%)       19 (23.8)       30 )54.5)	N2a, n (%)	12 (15)	20 (36.4)	2.50
Present, n (%)       43 (53.8)       28 (50.9)       0.745         Absent, n (%)       37 (46.3)       27 (49.1)       0.745         Signet ring differentiation*       12 (15)       13 (23.6)       0.204         Absent, n (%)       68 (85)       42 (76.4)       0.204         Mucinous differentiation*       19 (23.8)       30 (54.5)       30 (54.5)	N2b, n (%)	23 (28.8)	3 (5.5)	
Absent, n (%) 37 (46.3) 27 (49.1) 0.745  Signet ring differentiation* 12 (15) 13 (23.6)  Absent, n (%) 68 (85) 42 (76.4) 0.204  Mucinous differentiation*  Present, n (%) 19 (23.8) 30 (54.5)	Perinodal extension*			
Absent, n (%)       37 (46.3)       27 (49.1)         Signet ring differentiation*	Present, n (%)	43 (53.8)	28 (50.9)	0.745
Present, n (%)     12 (15)     13 (23.6)       Absent, n (%)     68 (85)     42 (76.4)       Mucinous differentiation*       Present, n (%)     19 (23.8)     30 )54.5)	Absent, n (%)	37 (46.3)	27 (49.1)	
Absent, n (%) 68 (85) 42 (76.4) 0.204  Mucinous differentiation*  Present, n (%) 30 (54.5)	Signet ring differentiation*			
Absent, n (%)       68 (85)       42 (76.4)         Mucinous differentiation*       Present, n (%)       19 (23.8)       30 )54.5)	Present, n (%)	12 (15)	13 (23.6)	0.204
Present, n (%) 19 (23.8) 30 )54.5)	Absent, n (%)	68 (85)	42 (76.4)	
	Mucinous differentiation*			
	Present, n (%)	19 (23.8)	30 )54.5)	<0.001**

Absent, n (%)	61 (76.3)	25 (45.5)	
Peritumoral lymphocytes*			
None, n (%)	61 (76.3)	43 (78.2)	
Mild to moderate, n (%)	10 (12.5)	6 (10.9)	0.957
Marked, n (%)	9 (11.3)	6 (10.9)	
Intratumoral lymphocytes*			
None, n (%)	53 (66.3)	19 (34.5)	
Mild to moderate, n (%)	17 (21.3)	22 (40)	0.001**
Marked, n (%)	10 (12.5)	14 (25.5)	

# TABLE 2: Comparison of clinicopathological features of colorectal carcinoma with mismatch repair status

pMMR: mismatch repair proficient; dMMR: mismatch repair deficient; NOS: not otherwise specified; T: tumor; N: nodal

\*Chi-square test was applied, \*\*p-value significant as < 0.05, \*\*\*Fisher's exact test was applied.

Table 3 shows the association of MLH1 expression with clinicopathological features. A significant association was noted with respect to gender, age, LVI, tumor type, grade, T and N-stage, mucinous differentiation, and ITL. Loss of MLH1 expression was significantly more frequent in females with older age (>50 years). MLH1 loss of expression was also associated with higher grade, mucinous differentiation, and higher T-stage. Moreover, the frequency of LVI in MLH1-deficient tumors was less frequent, whereas marked ITL was more commonly seen in MLH1-deficient tumors.

	Values		
Clinicopathological features	MLH1 expression		p-value
	Intact expression/MLH1 procifient	Loss of expression/MLH1 deficient	
Gender*			
Male, n (%)	38 (46.3)	15 (28.3)	0.036**
Female, n (%)	44 (53.7)	38 (71.7)	0.030
Age groups*			
≤50 years, n (%)	51 (62.2)	21 (39.6)	0.010**
>50 years, n (%)	31 (37.8)	32 (60.4)	0.010
Laterality*			
Right, n (%)	12 (14.6)	11 (20.8)	0.356
Left, n (%)	70 (85.4)	42 (79.2)	0.356
Location***			
Cecum, n (%)	11 (13.4)	5 (9.4)	
Ascending colon, n (%)	2 (2.4)	3 (5.7)	
Transverse colon, n (%)	3 (3.7)	3 (5.7)	0.228
Recto-sigmoid, n (%)	59 (72)	32 (60.4)	0.220
Descending colon, n (%)	1 (1.2)	0 (0)	
Splenic flexure, n (%)	6 (7.3)	10 (18.9)	
Histopathological features			

Perineural invasion*			
Present, n (%)	32 (39)	13 (24.5)	
Absent, n (%)	50 (61)	40 (75.5)	0.081
Lymphovascular invasion*			
Present, n (%)	27 (32.9)	5 (9.4)	
Absent, n (%)	55 (67.1)	48 (90.6)	0.002**
Pre-existing polyp***			
Present, n (%)	4 (4.9)	4 (7.5)	
Absent, n (%)	78 (95.1)	49 (92.5)	0.711
Tumor type***			
Adenocarcinoma, NOS, n (%)	58 (70.7)	30 (56.6)	
Mucinous adenocarcinoma, n (%)	13 (15.9)	20 (37.7)	
Medullary carcinoma, n (%)	9 (11)	0 (0)	0.001 **
Signet ring adenocarcinoma, n (%)	2 (2.4)	3 (5.7)	
Tumor differentiation/grade***			
Well differentiated/grade 1, n (%)	0 (0)	5 (9.4)	
Moderately differentiated/grade 2, n (%)	62 (75.6)	29 (54.7)	0.003**
Poorly differentiated/grade 3, n (%)	20 (24.4)	19 (35.8)	
T-stage***			
T2, n (%)	5 (6.1)	6 (11.3)	
T3, n (%)	67 (81.7)	29 (54.7)	0.003**
T4, n (%)	10 (12.2)	18 (34)	
Nodal metastasis*			
Present, n (%)	57 (69.5)	33 (62.3)	0.000
Absent, n (%)	25 (30.5)	20 (37.7)	0.383
N-stage*			
N0, n (%)	25 (30.5)	20 (37.7)	
N1, n (%)	22 (26.8)	10 (18.9)	0.004**
N2a, n (%)	12 (14.6)	20 (37.7)	0.001**
N2b, n (%)	23 (28)	3 (5.7)	
Perinodal extension*			
Present, n (%)	43 (52.4)	28 (52.8)	0.005
Absent, n (%)	39 (47.6)	25 (47.2)	0.965
Signet ring differentiation*			
Present, n (%)	12 (14.6)	13 (24.5)	0.149
Absent, n (%)	70 (85.4)	40 (75.5)	0.148
Mucinous differentiation*			
Present, n (%)	18 (22)	31 (58.5)	*0.004F*
Absent, n (%)	64 (78)	22 (41.5)	<0.001**
Peritumoral lymphocytes*			

None, n (%)	61 (74.4)	43 (81.1)	
Mild to moderate, n (%)	10 (12.2)	6 (11.3)	0.547
Marked, n (%)	11 (13.4)	4 (7.5)	
Intratumoral lymphocytes*			
None, n (%)	55 (67.1)	17 (32.1)	
Mild to moderate, n (%)	17 (20.7)	22 (41.5)	<0.001**
Marked, n (%)	10 (12.2)	14 (26.4)	

# TABLE 3: Association of MutL protein homolog 1 (MLH1) expression with clinicopathological features

MLH1: MutL protein homolog 1; NOS: not otherwise specified; T: tumor; N: nodal

\*Chi-square test was applied, \*\*p-value significant as < 0.05, \*\*\*Fisher's exact test was applied.

A significant expression of PMS2 was noted with PNI, LVI, tumor type, grade, T and N-stage, mucinous differentiation, and ITL. The frequency of PNI and LVI in PMS2-deficient tumors was significantly low, whereas PMS2-deficient tumors had higher grade and T-stage. Moreover, the loss of PMS2 was associated with marked ITL and mucinous differentiation, as shown in Table 4.

	Values		
Clinicopathological features	PMS2	PMS2	
	Intact expression/PMS2 proficient	Loss of expression/PMS2 deficient	
Gender*			
Male, n (%)	40 (44.9)	13 (28.3)	0.060
Female, n (%)	49 (55.1)	33 (71.7)	
Age groups*			
≤50 years, n (%)	51 (57.3)	21 (45.7)	0.198
>50 years, n (%)	38 (42.7)	25 (54.3)	
Laterality*			
Right, n (%)	16 (18)	7 (15.2)	0.686
Left, n (%)	73 (82)	39 (84.8)	
Location**			
Cecum, n (%)	13 (14.6)	3 (6.5)	
Ascending colon, n (%)	4 (4.5)	1 (2.2)	
Transverse colon, n (%)	3 (3.4)	3 (6.5)	0.086
Recto-sigmoid, n (%)	62 (69.7)	29 (63)	
Descending colon, n (%)	1 (1.1)	0 (0)	
Splenic flexure, n (%)	6 (6.7)	10 (21.7)	
Histopathological features			
Perineural invasion*			
Present, n (%)	37 (41.6)	8 (17.4)	0.005***
Absent, n (%)	52 (58.4)	38 (82.6)	

Lymphovascular invasion*			
Present, n (%)	32 (36)	0 (0)	
Absent, n (%)	57 (64)	46 (100)	<0.001***
Pre-existing polyp**			
Present, n (%)	6 (6.7)	2 (4.3)	
Absent, n (%)	83 (93.3)	44 (95.7)	0.716
Tumor type**			
Adenocarcinoma, NOS, n (%)	60 (67.4)	28 (60.9)	
Mucinous adenocarcinoma, n (%)	15 (16.9)	18 (39.1)	
Medullary carcinoma, n (%)	9 (10.1)	0 (0)	0.003***
Signet ring adenocarcinoma, n (%)	5 (5.6)	0 (0)	
Tumor differentiation/grade**			
Well differentiated/grade 1, n (%)	0 (0)	5 (10.9)	
Moderately differentiated/grade 2, n (%)	66 (74.2)	25 (54.3)	0.002***
Poorly differentiated/grade 3, n (%)	23 (25.8)	16 (34.8)	
T-stage**			
T2, n (%)	5 (5.6)	6 (13)	
T3, n (%)	71 (79.8)	25 (54.3)	0.009***
T4, n (%)	13 (14.6)	15 (32.6)	
Nodal metastasis*			
Present, n (%)	64 (71.9)	26 (56.5)	0.072
Absent, n (%)	25 (28.1)	20 (43.5)	0.072
N-stage*			
N0, n (%)	25 (28.1)	20 (43.5)	
N1, n (%)	22 (24.7)	10 (21.7)	<0.001***
N2a, n (%)	16 (18)	16 (34.8)	5.501
N2b, n (%)	26 (29.2)	0 (0)	
Perinodal extension*			
Present, n (%)	50 (56.2)	21 (45.7)	0.246
Absent, n (%)	39 (43.8)	25 (54.3)	
Signet ring differentiation*			
Present, n (%)	15 (16.9)	10 (21.7)	0.489
Absent, n (%)	74 (83.1)	36 (78.3)	
Mucinous differentiation*			
Present, n (%)	20 (22.5)	29 (63)	<0.001***
Absent, n (%)	69 (77.5)	17 (37)	
Peritumoral lymphocytes*			
None, n (%)	65 (73)	39 (84.8)	
Mild to moderate, n (%)	13 (14.6)	3 (6.5)	0.275

Marked, n (%)	11 (12.4)	4 (8.7)	
Intratumoral lymphocytes*			
None, n (%)	62 (69.7)	10 (21.7)	
Mild to moderate, n (%)	17 (19.1)	22 (47.8)	<0.001***
Marked, n (%)	10 (11.2)	14 (30.4)	

# TABLE 4: Association of postmeiotic segregation increased 2 (PMS2) expression with clinicopathological features

PMS2: postmeiotic segregation increased 2; NOS: not otherwise specified; T: tumor; N: nodal

\*Chi-square test was applied. \*\*Fisher's exact test was applied. \*\*\*p-value significant as < 0.05.

Table 5 depicts the association of MSH2 expression with clinicopathological parameters. A significant association was noted with LVI, tumor grade, nodal metastasis, T and N stage, perinodal extension, mucinous and signet ring differentiation, and ITL. MSH2-proficient tumors had a higher frequency of LVI, nodal metastasis, perinodal extension, and signet ring differentiation. Conversely, MSH2-deficient tumors were significantly associated with higher grade and T-stage, mucinous differentiation, and ITL.

Values		
MSH2	MSH2	
Intact expression/MSH2 proficient	Loss of expression/MSH2 deficient	
44 (39.6)	9 (37.5)	0.846
67 (60.4)	15 (62.5)	3.3.13
60 (54.1)	12 (50)	0.718
51 (45.9)	12 (50)	
18 (16.2)	5 (20.8)	0.559
93 (83.8)	19 (79.2)	
14 (12.6)	2 (8.3)	
5 (4.5)	0 (0)	
3 (2.7)	3 (12.5)	0.300
76 (68.5)	15 (62.5)	
1 (0.9)	0 (0)	
12 (10.8)	4 (16.7)	
40 (36)	5 (20.8)	0.152
71 (64)	19 (79.2)	
	MSH2 Intact expression/MSH2 proficient  44 (39.6) 67 (60.4)  60 (54.1) 51 (45.9)  18 (16.2) 93 (83.8)  14 (12.6) 5 (4.5) 3 (2.7) 76 (68.5) 1 (0.9) 12 (10.8)	MSH2 Intact expression/MSH2 proficient  44 (39.6) 9 (37.5) 67 (60.4) 15 (62.5)  60 (54.1) 12 (50) 18 (16.2) 5 (20.8) 93 (83.8) 19 (79.2)  14 (12.6) 2 (8.3) 5 (4.5) 0 (0) 3 (2.7) 76 (88.5) 1 (9.9) 1 (10.9) 1 (10.9) 1 (10.8) 4 (16.7)

Absent, n (%)	79 (71.2)	24 (100)	0.003***
Pre-existing polyp**			
Present, n (%)	7 (6.3)	1 (4.2)	
Absent, n (%)	104 (93.7)	23 (95.8)	1.000
Tumor type**			
Adenocarcinoma, NOS, n (%)	76 (68.5)	12 (50)	
Mucinous adenocarcinoma, n (%)	22 (19.8)	11 (45.8)	
Medullary carcinoma, n (%)	8 (7.2)	1 (4.2)	0.065
Signet ring adenocarcinoma, n (%)	5 (4.5)	0 (0)	
Tumor differentiation/grade**			
Well differentiated/grade 1, n (%)	2 (1.8)	3 (12.5)	
Moderately differentiated/grade 2, n (%)	80 (72.1)	11 (45.8)	0.011***
Poorly differentiated/grade 3, n (%)	29 (26.1)	10 (41.7)	
T-stage**			
T2, n (%)	7 (6.3)	4 (16.7)	
T3, n (%)	88 (79.3)	8 (33.3)	<0.001***
T4, n (%)	16 (14.4)	12 (50)	
Nodal metastasis*			
Present, n (%)	83 (74.8)	7 (29.2)	
Absent, n (%)	28 (25.2)	17 (70.8)	<0.001***
N-stage*			
N0, n (%)	28 (25.2)	17 (70.8)	
N1, n (%)	28 (25.2)	4 (16.7)	
N2a, n (%)	29 (26.1)	3 (12.5)	<0.001***
N2b, n (%)	26 (23.4)	0 (0)	
Perinodal extension*			
Present, n (%)	68 (61.3)	3 (12.5)	
Absent, n (%)	43 (38.7)	21 (87.5)	<0.001***
Signet ring differentiation**			
Present, n (%)	25 (22.5)	0 (0)	0.007***
Absent, n (%)	86 (77.5)	24 (100)	0.007
Mucinous differentiation*			
Present, n (%)	31 (27.9)	18 (75)	<0.001**
Absent, n (%)	80 (72.1)	6 (25)	2.55
Peritumoral lymphocytes**			
None, n (%)	85 (76.6)	19 (79.2)	
Mild to moderate, n (%)	13 (11.7)	3 (12.5)	1.000
Marked, n (%)	13 (11.7)	2 (8.3)	
Intratumoral lymphocytes**			

None, n (%)	70 (63.1)	2 (8.3)	
Mild to moderate, n (%)	27 (24.3)	12 (50)	<0.001***
Marked, n (%)	14 (12.6)	10 (41.7)	

# TABLE 5: Association of MutS homolog 2 (MSH2) expression with clinicopathological features

MSH2: MutS homolog 2; NOS: not otherwise specified; T: tumor; N: nodal

\*Chi-square test was applied. \*\*Fisher's exact test was applied. \*\*\*p-value significant as < 0.05.

Table 6 presents the association of MSH6 expression with clinical and pathological features. A significant association of MSH 6 expression was noted with PNI, LVI, nodal metastasis, tumor grade, T and N-stage, perinodal extension, signet ring and mucinous differentiation, and ITL. MSH6-proficient tumors had a higher frequency of PNI, LVI, signet ring differentiation, nodal metastasis, and perinodal extension. Alternatively, MSH6-deficient tumors had higher grade and T-stage, higher frequency of mucinous differentiation, and marked ITL.

	Values		
Clinicopathological features	MSH6	MSH6	
	Intact expression/MSH6 proficient	Loss of expression/MSH6 deficient	
Gender*			
Male, n (%)	46 (40.7)	7 (31.8)	0.435
emale, n (%)	67 (59.3)	15 (68.2)	
Age groups*			
550 years, n (%)	62 (54.9)	10 (45.5)	0.418
r50 years, n (%)	51 (45.1)	12 (54.5)	0.110
aterality**			
Right, n (%)	18 (15.9)	5 (22.7)	0.534
.eft, n (%)	95 (84.1)	17 (77.3)	0.504
.ocation**			
Cecum, n (%)	14 (12.4)	2 (9.1)	
Ascending colon, n (%)	5 (4.4)	0 (0)	
Fransverse colon, n (%)	3 (2.7)	3 (13.6)	0.214
Recto-sigmoid, n (%)	78 (69)	13 (59.1)	0.214
Descending colon, n (%)	1 (0.9)	0 (0)	
Splenic flexure, n (%)	12 (10.6)	4 (18.2)	
Histopathological features			
Perineural invasion*			
Present, n (%)	42 (37.2)	3 (13.6)	0.032***
Absent, n (%)	71 (62.8)	19 (86.4)	0.032
ymphovascular invasion*			
Present, n (%)	32 (28.3)	0 (0)	
Absent, n (%)	81 (71.7)	22 (100)	0.004***

Position	Pre-existing polyp**			
Manuform	Present, n (%)	7 (6.2)	1 (4.5)	4 000
### 1987년	Absent, n (%)	106 (93.8)	21 (95.5)	1.000
Manufact (1974)	Tumor type**			
1987年   19	Adenocarcinoma, NOS, n (%)	76 (67.3)	12 (54.5)	
	Mucinous adenocarcinoma, n (%)	24 (21.2)	9 (40.9)	0.054
Horse discontanty profession of the continuous profession of the contin	Medullary carcinoma, n (%)	8 (7.1)	1 (4.5)	0.251
Moderate production (1998)         2119         3150         4000         <	Signet ring adenocarcinoma, n (%)	5 (4.4)	0 (0)	
Nonething differential significant (10)         10,720         96,85         60,95           Prody differential significant (10)         29,057         10,955         10,955           Transport         7,920         4(192)         40,900         10,900           Tare (11)         90,920         4(192)         40,900         10,900           Tare (12)         10,162         12,900         10,900         10,900           Note (12)         30,053         10,900         10,900         10,900           Note (12)         30,053         10,900         10,900         10,900           Name (12)         30,053         10,900         10,900         10,900           Name (12)         30,053         10,900         10,900         10,900         10,900           Name (12)         30,053         10,900	Tumor differentiation/grade**			
Poly of disconsideral pole A in (%)         20,871         10,465         10,412         1	Well differentiated/grade 1, n (%)	2 (1.8)	3 (13.6)	
Table 1987         1982         4 1823         4 1823         4 1824         4 1	Moderately differentiated/grade 2, n (%)	82 (72.6)	9 (40.9)	0.003***
Τε π(τ)         (8/2)         4 1422         4 00011           Τε π(τ)         50 0730         6 0233         4 00011           Τε π(τ)         16 (42)         12 (40)         12 (40)           Nondimensions*         10 0000         7 (10)         4 0000           Admit n (5)         30 0330         7 (10)         4 0000           Admit n (5)         30 0850         10 0000         10 0000           No. n (5)         20 0850         10 0000         10 0000           No. n (5)         20 0850         3 (13)         20 0000           No. n (5)         20 0850         3 (13)         20 0000           No. n (7)         20 0227         3 (13)         20 0000           Personal n (7)         4 (20)         3 (13)         20 0000           Personal n (7)         4 (20)         3 (13)         20 0000           Personal n (7)         4 (20)         3 (20)         20 0000           Personal n (7)         4 (20)         3 (20)         20 0000           Personal n (7)         4 (20)         3 (20)         20 0000           Molecular n (8)         4 (20)         3 (20)         20 0000           Molecular n (8)         4 (20)         3 (20)	Poorly differentiated/grade 3, n (%)	29 (25.7)	10 (45.5)	
Τα (τ)         10 (40)         60/23         60/24         60/24           Τα (τ)         16 (42)         12 (43)         60/24         60/24           Τα (τ)         16 (42)         12 (43)         10 (43)         10 (43)           Νοια (τ)         20 (23)         10 (43)         1	T-stage**			
Τα (α)         [4 (4)         [5 (4	T2, n (%)	7 (6.2)	4 (18.2)	
Neart. n (%)	T3, n (%)	90 (79.6)	6 (27.3)	<0.001***
Present, η(γ)         81 73.51         71.81         71.81         70.00011	T4, n (%)	16 (14.2)	12 (54.5)	
Absent, n (%)         30 08 5)         56 08 2)         2001***           N-chage*         90 08 5)         56 08 2)         2001***           NCA, n (%)         20 08 3         56 08 2)         2001***           NCA, n (%)         20 08 7)         31 18 6         2001***           NCA, n (%)         20 08 7)         31 18 6         2001***           Personal adentison**         ****         ****         40 001****           Personal n (%)         46 08 22         31 18 6         40 001****           Absent, n (%)         26 02 1)         90 64         40 001****           Personal n (%)         26 02 1)         0 (0)         0 01****           Absent, n (%)         26 02 1)         0 (0)         0 01****           Absent, n (%)         80 77 9         20 100***         0 01****           Absent, n (%)         40 000***         40 000***         0 00***           Absent, n (%)         80 70 20         60 72 3         0 00***           Absent, n (%)         40 70 73         0 00 00***         0 00***           Absent, n (%)         40 70 73         0 00 00***         0 00 00***           Absent, n (%)         40 70 73         0 00 00***         0 00 00***	Nodal metastasis*			
Assent, n (%)         2008.5         168.2         1 <td>Present, n (%)</td> <td>83 (73.5)</td> <td>7 (31.8)</td> <td></td>	Present, n (%)	83 (73.5)	7 (31.8)	
No. n (%) 15 (86.2)  No. n (%) 26 (24.8) 4 (16.2)  No. n (%) 26 (25.7) 3 (13.6)  No. n (%) 26 (25.7)  No. n (%) 26 (25.7)  Periotical administrativo*  Present, n (%) 45 (80.8) 19 (80.4)  Persent, n (%) 26 (27.1)  No. n (%) 27 (77.1)  No. n (%) 37 (37.1)  No. n	Absent, n (%)	30 (26.5)	15 (68.2)	<0.001***
Ni, n (s)         26/43         4 (8.2)         <	N-stage*			
N2a, n (%)         29 (25.7)         3 (13.6)         10 (14.7) <t< td=""><td>N0, n (%)</td><td>30 (26.5)</td><td>15 (68.2)</td><td></td></t<>	N0, n (%)	30 (26.5)	15 (68.2)	
N2a. n (%)         26 (25 7)         3 (3.6)           N2b. n (%)         26 (23)         (0)           Perindul adensisir*           Present, n (%)         88 (80.2)         3 (3.6)         40001***           Absont, n (%)         45 (30.8)         10 (84.4)         40001***           Signet ring differentiation**         25 (22.1)         0 (0)         40001***           Absont, n (%)         88 (77.9)         20 (100)         40001***           Absont, n (%)         33 (29.2)         16 (72.7)         40001***           Absont, n (%)         90 (70.8)         6 (27.3)         40001***           Perturnarul lymphocytes**         10 (77.3)         40001***           Note, n (%)         3 (11.5)         3 (18.6)         40001***           Mild to moderate, n (%)         3 (11.5)         2 (8.1)           Multicultural lymphocytes**         1 (8.9)         2 (8.1)	N1, n (%)	28 (24.8)	4 (18.2)	
Perindal adension*         Perindal adension*         Control (%)         68 (80.2)         3 (13.6)         4 (20.0)	N2a, n (%)	29 (25.7)	3 (13.6)	0.001***
Present, n (%)         68 (60.2)         3 (13.6)         40001***           Absent, n (%)         45 (39.8)         19 (86.4)         1000           Signet fring differentiation**         V         0 (0)         1000           Absent, n (%)         88 (77.9)         22 (100)         1013***           Absent, n (%)         33 (39.2)         16 (72.7)         0001***           Absent, n (%)         80 (70.8)         6 (27.3)         0001***           Pertumoral lymphocytes**         17 (77.3)         17 (77.3)           Mid to moderate, n (%)         13 (11.5)         3 (13.6)         2 (9.1)           Marked, n (%)         13 (11.5)         2 (9.1)         17 (77.3)           None, n (%)         15 (11.5)         2 (9.1)         17 (77.3)           Marked, n (%)         13 (11.5)         2 (9.1)         17 (77.3)           None, n (%)         15 (11.5)         2 (9.1)         17 (77.3)           None, n (%)         15 (11.5)         2 (9.1)         17 (77.3)           None, n (%)         15 (11.5)         2 (9.1)         17 (77.3)           None, n (%)         15 (11.5)         2 (9.1)         17 (77.3)           None, n (%)         15 (11.5)         17 (77.5)         17 (77.5) <td>N2b, n (%)</td> <td>26 (23)</td> <td>0 (0)</td> <td></td>	N2b, n (%)	26 (23)	0 (0)	
Absent, n (%)         45 (93.8)         19 (86.4)         40001***           Signet ring differentiation*         25 (22.1)         0 (0)         74           Absent, n (%)         88 (77.9)         22 (100)         74           Mucirous differentiation*         50 (27.7)         40.001***           Absent, n (%)         80 (70.8)         6 (27.3)         40.001***           Pentumoral lymphocytes**         87 (77)         17 (77.3)         40.001***           Mid to moderate, n (%)         13 (11.5)         3 (13.6)         0.922           Marked, n (%)         13 (11.5)         2 (9.1)         11 (11.5)           None, n (%)         2 (9.1)         11 (11.5)         11 (11.5)           None, n (%)         2 (9.1)         11 (11.5)         11 (11.5)	Perinodal extension*			
Absent, n (%)         45 (39.8)         19 (86.4)         1 (19.6)         1 (19.6)         1 (19.6)         1 (19.6)         1 (19.6)         1 (19.6)         1 (19.6)         2 (20.1)         0 (0)         1 (19.6)         2 (19.0)         1 (19.6)         2 (19.0)         1 (19.6)         2 (19.0)         1 (19.6)         2 (19.0)         1 (19.6)         2 (19.0)         1 (19.6)         2 (19.0)	Present, n (%)	68 (60.2)	3 (13.6)	
Present, n (%)         25 (22.1)         0 (0)           Absent, n (%)         22 (100)         0.013***           Mucinous differentiation*         ************************************	Absent, n (%)	45 (39.8)	19 (86.4)	<0.001***
Absent, n(%) 88(77.9) 22(100)  Mucinous differentiation*  Present, n(%) 33(29.2) 16(72.7)  Absent, n(%) 80(70.8) 62(73.)  Peritumoral lymphocytes**  None, n(%) 17(77.3)  Mild to moderate, n(%) 13(11.5) 13(11.5) 2(9.1)  Intratumoral lymphocytes**  None, n(%) 2(9.1)  Intratumoral lymphocytes**  None, n(%) 2(9.1)	Signet ring differentiation**			
Absent, n (%)         88 (7.9)         22 (100)           Mucinous differentiation*         Present, n (%)         33 (29.2)         16 (72.7)         40.001***           Absent, n (%)         80 (70.8)         6 (27.3)	Present, n (%)	25 (22.1)	0 (0)	
Present, n(%)         33 (29.2)         16 (72.7)         40.001***	Absent, n (%)	88 (77.9)	22 (100)	0.013***
Absent, n (%) 80 (70.8) 6(27.3) (20.01)***  Peritumoral lymphocytes**  None, n (%) 13 (11.5) (20.01) (	Mucinous differentiation*			
Absent, n (%)         80 (70.8)         6(27.3)         Image: Companie of the companie of t	Present, n (%)	33 (29.2)	16 (72.7)	
None, n (%) 87 (77) 17 (77.3)  Mild to moderate, n (%) 13 (11.5) 3 (13.6) 2.922  Marked, n (%) 2 (9.1)  Intratumoral lymphocytes**  70 (61.9) 2 (9.1)	Absent, n (%)	80 (70.8)	6 (27.3)	<0.001***
Mild to moderate, n (%)       13 (11.5)       3 (13.6)       0.922         Marked, n (%)       13 (11.5)       2 (9.1)         Intratumoral lymphocytes**       70 (81.9)       2 (9.1)	Peritumoral lymphocytes**			
Marked, n (%) 13 (11.5) 2 (9.1)  Intratumoral lymphocytes**  None, n (%) 70 (61.9) 2 (9.1)	None, n (%)	87 (77)	17 (77.3)	
Intratumoral lymphocytes**  None, n (%) 70 (61.9) 2 (9.1)	Mild to moderate, n (%)	13 (11.5)	3 (13.6)	0.922
None, n (%) 70 (61.9) 2 (9.1)	Marked, n (%)	13 (11.5)	2 (9.1)	
	Intratumoral lymphocytes**			
Mild to moderate, n (%) 27 (23.9) 12 (54.5) <0.001**	None, n (%)	70 (61.9)	2 (9.1)	
	Mild to moderate, n (%)	27 (23.9)	12 (54.5)	<0.001**

Marked, n (%)	16 (14.2)	8 (36.4)

#### TABLE 6: Association of MutS homolog 6 (MSH6) expression with clinicopathological features

MSH6: MutS homolog 6; NOS: not otherwise specified; T: tumor; N: nodal

## **Discussion**

We found a relatively high dMMR CRC in our population (40.7%). MLH1 was the most frequently deficient MMR marker in our cohort of cases, followed by PMS2, MSH2, and MSH6. dMMR CRCs were associated with mucinous histology and ITL. With respect to prognostic parameters, dMMR CRCs were associated with higher grade and T-stage, whereas the frequency of PNI, LVI, and higher N-stage was lower.

There has been an interest in dMMR CRC recently owing to differences in prognosis and the role of immunotherapy. dMMR CRC can be due to germline mutations owing to LS or sporadic BRAF mutations or MLH1 promoter hypermethylation. Errors introduced during DNA replication are corrected by the function of two pairs of genes: MLH1 and PMS; and MSH2 and MSH6. MLH is needed for the proper function of PMS2, whereas MSH2 is required for MSH6 functioning. IHC analysis for MLH1, PMS2, MSH2, and MSH6 is initially indicated and specific staining combinations predict sporadic vs. familial dMMR CRC [1,5].

The frequency of dMMR CRC varies in different studies. A Chinese study involving 133 cases of CRC revealed a loss of expression of MLH1, MSH2, MSH6, and PMS2 in 55.6%, 33.8%, 41.4%, and 57.9% cases, respectively. They found a significant association of dMMR status with age, gender, and tumor site, whereas no significant association was noted with T- and N-stages [11]. Conversely, Liang et al. [12] in a study including 61 CRC patients concluded that there was a significant association of dMMR status with age (<55 years), female gender, location (right colon), tumor size (>5 cm), T-stage (T4), high grade, and mucinous differentiation. We also found a significant association of dMMR CRC with T4 stage, higher tumor grade, and mucinous differentiation, however, the association of dMMR CRC with younger age was not established in our study. Similarly, Liang et al. [12] did not find any association of dMMR status with LVI, as noted in our study. Sacdalan et al. [13] also reported aggressive histological features (poor differentiation, mucinous histology) of dMMR CRC in Filipino patients. Concordant with our findings, mucinous and poor differentiation were found in dMMR CRC [14].

Other histological features of dMMR CRC include the presence of immune cell infiltration (PTL and ITL). PTL corresponds to a Crohn's-like inflammatory reaction at the periphery of the tumor, whereas ITL is the presence of inflammatory cells within the cancer cells. This unique feature of dMMR CRC was attributed to elevated mutational burden and neoantigen overload that results in an immune reaction against tumor cells [15]. We found a significant association of dMMR CRC with ITL, whereas no significant association was established with PTL.

Despite aggressive histological features, there is a survival benefit in CRC with dMMR status. Previous studies have reported better overall survival in dMMR CRC than pMMR CRC, especially in early-stage CRC [16]. We did not evaluate survival in our study but found a significant association of dMMR status with lack of PNI, LVI, and lower N-stage signifying prognostically better pathological features in dMMR CRC.

Another significance of dMMR status is the predictive role of immunotherapy. There is a promising role of immunotherapy in dMMR metastatic CRC, while its role in pMMR CRC is being sought [17]. We did not evaluate the predictive value of dMMR status in our study.

### Limitations

The main limitation of our study was the lack of clinical follow-up to evaluate the overall and disease-free survival of dMMR CRC. Second, molecular studies for BRAF, MLH1 promoter hypermethylation, and next-generation sequencing for germline mutations for LS were not performed. Additionally, there is an increasing interest in the role of immunotherapy in dMMR CRC that we did not evaluate in our study. Moreover, this was a single-center study; therefore, the sample size was also limited.

#### **Conclusions**

In this study, we evaluated dMMR status in CRC and found that a significant proportion of CRC cases were dMMR in our study population. We noted that poor differentiation (high grade) and mucinous histology were associated with dMMR CRC. Moreover, dMMR CRC was of higher T-stage than pMMR in our study. Conversely, dMMR CRC was associated with better prognostic features, such as lower N-stage and lower frequency of PNI and LVI. Due to these distinctive features of dMMR CRC, evaluating the dMMR status in CRC is of utmost importance. Our study was limited because we did not evaluate the predictive value of

dMMR status in CRC, and the frequency of germline mutations with dMMR CRC was not sought in our study.

#### **Additional Information**

#### **Disclosures**

**Human subjects:** Consent was obtained or waived by all participants in this study. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

### **Acknowledgements**

AAH, UB, RR, FF, and RK made substantial contributions to the conception and design of the work and the interpretation/analysis of the data, along with the drafting of the work. UAM, SZ, ARK, SS, and MI were involved in the acquisition/analysis of data, along with the drafting of the work. They made substantial contributions to the study design. All authors approved the final version of the manuscript.

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