Mismatch repair protein expression in colorectal adenocarcinoma patients

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Abstract

Background: Colorectal cancer is the third most common cancer worldwide. Microsatellite instability (MSI) results from the functional deficiency of one of the mismatch repair (MMR) proteins, which can be identified using immunohistochemistry. Determining MMR status is crucial for clinicians in diagnosing, prognosticating, and making chemotherapy decisions for CRC patients. **Objectives**: (1) To assess the expression status of MMR proteins using immunohistochemistry. (2) To evaluate the association between MMR expression and certain clinicopathological features in colorectal adenocarcinoma. **Materials and methods**: A cross-sectional descriptive study was carried out on 81 colorectal cancer patients who were examined and treated at Hue University of Medicine and Pharmacy Hospital. The study was conducted from 04/2024 to 03/2025. **Results:** Deficient mismatch repair (dMMR) rate was 16%, proficient mismatch repair (pMMR) rate accounted for 84%. The tumors with dMMR status were significantly associated with right-sided location, tumor size larger than 5 cm, and moderate to poor differentiation ($p < 0.05$). There was no association between dMMR status and age, gender, histological subtype, T stage, lymph node metastasis, or disease stage. **Conclusion:** Testing for MMR status should be done for all patients with colorectal cancer, particularly those with right-sided tumors greater than 5 cm in size and high-grade histology.

Keywords*: colorectal cancer, microsatellite instability, immunohistochemistry, mismatch repair protein.*

1. INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent malignancy worldwide, following lung and breast cancer. In Vietnam, according to 2022 statistical data, CRC ranks fourth after liver, lung, and breast cancer, with an increasing incidence trend [1]. Three major mechanisms leading to CRC have been described, among which microsatellite instability (MSI) is observed in 15% of CRC cases. MSI results from the functional deficiency of one of the mismatch repair (MMR) proteins (including MLH1, MSH2, MSH6 and PMS2) and can be identified using immunohistochemistry (IHC). MSI can lead to sporadic CRC and Lynch syndrome. Moreover, dysfunction of the MMR system has been associated with an elevated risk of malignancies in other organ systems. Identifying MMR deficiency holds significant clinical implications, aiding in diagnosis, prognostication, and therapeutic decision-making, particularly in guiding chemotherapy strategies for CRC [2]. To date, no study investigating MMR deficiency in colorectal cancer has been conducted in the Central Highlands region of Vietnam. Therefore, this study aims to: (1) To assess the expression status of mismatch repair (MMR) proteins in CRC using immunohistochemistry. (2) To evaluate the association between MMR expression and certain clinical and histopathological characteristics in CRC.

2. MATERIALS AND METHODS

2.1. Subjects

2.1.1. Inclusion criteria

- Patients who were diagnosed with and underwent surgery for colorectal cancer.
- Postoperative histopathological results confirming primary colorectal adenocarcinoma.
- Availability of sufficient paraffin-embedded tissue samples for immunohistochemical staining with MLH1, MSH2, MSH6, and PMS2 antibodies.

2.1.2. Exclusion criteria

- Patients who received chemotherapy for CRC prior to surgery.

2.2. Research methods

2.2.1. Study design

Cross-sectional descriptive study.

2.2.2. Sample size

- A total of 81 patients diagnosed with colorectal cancer who presented for examination and treatment at Hue University of Medicine and Pharmacy Hospital were recruited from March 2023 to March 2025 (Samples were collected prospectively from April 2024 to March 2025 (55 patients) and retrospectively from March 2023 to March 2024 (26 patients).
- The study was conducted from 04/2024 to 03/2025.

2.2.3. Analytical techniques and Evaluation criteria

Immunohistochemical technique and

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Interpretation criteria

- IHC staining was performed to detect the expression of mismatch repair (MMR) proteins using four monoclonal antibodies: MLH1, PMS2, MSH2, and MSH6 (manufactured by Ventana).

- Slide staining: The IHC staining procedure was performed using the Ventana BenchMark GX automated system, with a total duration of approximately 6 hours.

- Evaluation of IHC staining results:

+ Control slides: included a positive control (co-stained with a tissue sample known to be positive) and a negative control (omitting the primary antibody incubation step during the staining process).

+ Normal (intact) MMR expression was assessed when tumor cell nuclei showed strong positive staining.

+ Loss of MMR expression was defined by tumor cell nuclei exhibiting absent or weak, focal staining, accompanied by the presence of internal positive controls.

+ Loss of expression of at least one of the MMR proteins (MLH1, MSH2, MSH6, and PMS2) was considered indicative of deficient mismatch repair function.

+ If all four MMR proteins exhibited normal expression, it was classified as pMMR [3].

2.3. Data analysis

All collected data were statistically analyzed using SPSS version 26.0. We choose the $p<0.05$ value to find the level of statistical significance.

2.4. Ethical considerations

The data collected were used solely for research purposes. The study was approved by the Biomedical Research Ethics Committee of Hue University of Medicine and Pharmacy.

3. RESULTS

3.1. Mismatch repair (MMR) protein expression

Table 1. Proportion of Mismatch repair protein expression

MMR status	Frequency (n)	Percentage (%)
dMMR	13	16.0
pMMR	68	84.0
Total	81	100

Deficient MMR expression accounted for 16% of cases, while 84% maintained proficient MMR protein expression.

Table 2. Expression patterns of Immunohistochemical markers

Protein expression	MLH1	PMS2	MSH2	MSH6	n	Percentage %
dMMR (n=13)	+	-	+	+	4	4.9
	+	+	+	-	1	1.2
	-	-	+	+	5	6.2
	+	+	-	-	3	3.7
pMMR	+	+	+	+	68	84.0

Note: (+): positive (intact expression); (–): negative (loss of protein expression).

Loss of MLH1-PMS2 expression was the most common pattern (6.2%), followed by isolated loss of PMS2 (4.9%) and concurrent loss of MSH2-MSH6 (3.7%). One case showed isolated loss of MSH6 expression.

3.2. Association between MMR protein expression and clinical and histopathological characteristics in Colorectal Adenocarcinoma

Table 3. Association between MMR status and clinical characteristics

Characteristic	dMMR		pMMR		P
	n	%	n	%	
Age (years)					
< 50	6	46.2	8	11.8	> 0.05
50 - 70	4	30.8	38	55.8	
> 70	3	23.0	22	32.4	

Gender					
Male	7	53.8	39	57.4	> 0.05
Female	6	46.2	29	42.6	
Tumor location					
Right colon	8	61.5	13	19.1	< 0.05
Left colon	4	30.8	25	36.8	
Rectum	1	7.7	30	44.1	
Tumor size					
< 5 cm	3	23.1	50	73.5	< 0.05
≥ 5 cm	10	76.9	18	26.7	

The proportion of patients under 50 years old was higher in the dMMR group compared to the pMMR group (46.2% vs. 11.8%) ($p > 0.05$). In the dMMR group, tumors were predominantly located in the right colon (61.5%), and most tumors were ≥ 5 cm in size (76.9%) ($p < 0.05$).

Table 4. Association between MMR status and histopathological features

Characteristic	dMMR		pMMR		P
	n	%	n	%	
Histological type					
Conventional adenocarcinoma	8	61.5	55	80.8	> 0.05
Mucinous adenocarcinoma	3	23.1	11	16.2	
Adenoma-like adenocarcinoma	1	7.7	1	1.5	
Mixed neuroendocrine - nonneuroendocrine neoplasm	1	7.7	1	1.5	
Tumor differentiation					
Well differentiated	0	0	5	7.4	< 0.05
Moderately differentiated	9	69.2	60	88.2	
Poorly differentiated	4	30.8	3	4.4	
T stage					
T1	0	0	4	5.9	> 0.05
T2	2	15.4	16	23.5	
T3	10	76.9	29	42.7	
T4	1	7.7	19	27.9	
Lymph Node Metastasis					
N0	10	76.9	47	69.1	> 0.05
N1	1	7.7	12	17.6	
N2	2	15.4	9	13.2	
Disease stage					
I	2	15.4	17	25.0	>0.05
II	8	61.5	25	36.7	
III	3	23.1	18	26.5	
IV	0	0	8	11.8	

The conventional adenocarcinoma subtype accounted for the highest proportion of cases in both the dMMR and pMMR groups. Poorly differentiated tumors were more common in the dMMR group ($p < 0.05$). Both dMMR and pMMR tumors were generally deeply invasive, rarely associated with lymph node metastasis,

and most commonly diagnosed at stage II ($p > 0.05$).

4. DISCUSSION

The DNA mismatch repair (MMR) system is one of the key mechanisms responsible for maintaining genomic integrity by correcting base-pairing errors during DNA replication. Deficiency in this system (dMMR) leads to microsatellite instability (MSI) characterized by widespread mutations in short repetitive DNA sequences. Several studies have demonstrated that dMMR/MSI tumors are associated with a better prognosis but show poor response to adjuvant 5-fluorouracil-based chemotherapy. Furthermore, identification of dMMR/MSI status also plays an important role in screening for Lynch syndrome in affected patients.

In this study, the proportion of dMMR was 16.0%, while pMMR accounted for 84.0% (Table 1). These findings were relatively consistent with those reported by Frank A. (2006), who observed a dMMR rate of 18%, and Williams (2020), who reported 26.2% [4], [5]. However, the dMMR rate in our study was lower than that reported by Hashmi A.A. (2017) at 34%, and Nguyen Van Chu (2021) at 30.2% [6], [7].

The results of our analysis showed that the most common pattern of MMR protein loss was the MLH1-PMS2 (6.2%), followed by isolated loss of PMS2 (4.9%), MSH2-MSH6 pair (3.7%), and a single case of isolated MSH6 loss (Table 2). Similarly, Hashmi (2017) reported the highest frequency of MLH1-PMS2 loss at 16% [6]. In a study by Dang Thai Tra (2023), 13 out of 21 cases showed MLH1-PMS2 loss, followed by 5 cases with MSH2-MSH6 loss, 2 cases with isolated MSH6 loss, and 1 case with isolated PMS2 loss [8]. These findings were consistent with domestic and international studies, which have commonly reported MLH1-PMS2 loss as the most frequent pattern. Several studies have suggested that PMS2 loss may occur either in isolation or in combination with MLH1 loss, similar to MSH6 in the MSH2-MSH6 pair. Therefore, many current diagnostic protocols recommend a two-step IHC approach, starting with PMS2 and MSH6 instead of staining all four MMR markers initially.

The proportion of patients under 50 years old was higher in the dMMR group compared to the pMMR group (46.2% vs. 11.8%). In contrast, the proportion of patients over 50 years old was lower in the dMMR group than in the pMMR group (53.8% vs. 88.2%) ($p > 0.05$) (Table 3). Our study revealed findings comparable to those of other studies, in which the proportion of younger patients with dMMR tumors was higher than that of younger patients in the pMMR group, whereas patients over 50 years old more frequently exhibited retained MMR expression than loss of expression [9].

Our results indicated that dMMR status was associated with certain clinical features, including tumor location and size. Specifically, dMMR tumors were predominantly found in the right colon (61.5%), and 76.9% of dMMR tumors measured ≥ 5 cm, with these differences reaching statistical significance ($p < 0.05$). Other studies have also reported that, in colorectal adenocarcinoma, tumors located in the right colon exhibit a higher frequency of dMMR and tend to have an average size greater than 5 cm [8, 9].

Previous studies worldwide have demonstrated a strong association between dMMR/MSI status and the histological subtype of tumors, with a higher prevalence in mucinous adenocarcinoma [4]. However, in our study, conventional colorectal adenocarcinoma was the most common histological subtype in both pMMR and dMMR groups. The discrepancy may be due to our study's limited sample size, highlighting the need for further research with a larger sample to draw more conclusive results.

We observed that dMMR status was most frequently found in moderately differentiated tumors (69.2%), followed by poorly differentiated tumors (30.8%), with no cases detected in well-differentiated tumors (Table 3). There was a significant association between tumor differentiation grade and loss of MMR protein expression ($p < 0.05$). Similar findings were reported by Dang Thai Tra and Nguyen Thi Thanh Mai, indicating that dMMR/MSI status commonly occurs in moderately and poorly differentiated tumors and is rare in well-differentiated ones [8], [9].

Regarding tumor invasion depth, our study, along with other studies, has shown that colorectal tumors often exhibit deep invasion, extending to the subserosal layer, with a large proportion classified as T3 stage [8, 9].

In terms of lymph node metastasis, our study showed that the rate of non-metastatic lymph nodes was 76.9% in dMMR tumors and 69.1% in pMMR tumors, which was quite consistent with the findings of Nguyen Thi Thanh Mai (2021), where the proportion of cases without metastasis was higher than those with metastasis in both dMMR and pMMR groups [9]. However, a study by Nour El reported contrasting results, indicating that in the MSS/MSI-L group, the rate of lymph node metastasis

was higher than without metastasis (58% vs. 42%), while in the MSI-H group, these two rates were equal (p > 0.05) [10]. Thus, the status of lymph node metastasis varies across studies and appears not to be correlated with dMMR/MSI status.

In this study, the majority of colorectal adenocarcinoma cases in the dMMR group were classified as stage II, accounting for 61.5%. The difference in disease stage and MMR protein expression status was not statistically significant (p > 0.05). Similarly, Nguyen Thi Thanh Mai (2021) reported that among 51 colorectal adenocarcinoma patients with MSI, 100% were classified as stage II (p = 0.29) [9].

The identification of dMMR status plays an important role in diagnosis and prognosis, as dMMR tumors are often associated with specific clinicopathological features such as right-sided location, poor differentiation, and larger tumor size. Moreover, dMMR has significant therapeutic implications, particularly in predicting favorable response to immune checkpoint inhibitors.

5. CONCLUSION

Through the study of 81 cases of colorectal adenocarcinoma, using immunohistochemical staining of four markers to assess mismatch repair (MMR) protein expression status, we arrived at the following conclusions:

- The prevalence of mismatch repair deficiency (dMMR) was 16.0%. Among these, loss of the MLH1-PMS2 pair was the most frequent.

- It is necessary to perform a comprehensive evaluation of histological features in all cases of colorectal cancer. For right-sided tumors, tumors larger than 5 cm, and those with high histologic grade, immunohistochemistry using a four-antibody panel (MLH1, PMS2, MSH2 and MSH6) should be performed.

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