Prognostic value of mismatch repair protein expression in unresectable gastric cancer

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Abstract

Background: Gastric cancer (GC) is one of the common types of cancer in Vietnam. Over 50% of GCs are diagnosed at an unresectable stage. Deficiency in mismatch repair proteins (MMR) leading to microsatellite instability (MSI-H) is a crucial prognostic factor currently under investigation in these patients. Therefore, this study aimed to determine the rate of MMR protein expression and its correlation with clinical characteristics, histopathological features, and overall survival in unresectable GC patients. **Materials and methods**: A descriptive case series study on 83 GC patients at unresectable stage, treated at Hue University of Medicine and Pharmacy Hospital and Hue Central Hospital from June 2020 to December 2022. Immunohistochemical staining was utilized to assess MMR protein expression. A deficiency in any MMR protein was considered as deficient mismatch repair proteins (dMMR). Conversely, the expression of all four MMR proteins in tumor cells was defined as proficient mismatch repair proteins (pMMR). **Result:** The dMMR rate was 10.8% and correlated with tumor size > 5 cm (p = 0.026) and well-differentiated tumors (p = 0.012). There was no association between MMR protein expression and tumor location, lymph node metastasis, or histological subtype. The dMMR group showed a significantly improved overall survival compared to the pMMR group, with a median overall survival of 19.1 ± 1.8 months compared to 9.3 ± 0.8 months (p = 0.02). **Conclusions:** There are correlation between MMR protein deficiency and tumor size and differentiation. dMMR GC patients have a better prognosis compared to those with pMMR.

Keywords: *gastric cancer, mismatch repair protein deficiency, immunohistochemistry.*

1. INTRODUCTION

In Vietnam, gastric cancer is one of the most common cancers with the third highest mortality rate [1]. Over 50% of GC patients are diagnosed at an unresectable stage. In these patients, overall survival is typically 10-12 months, with a 5-year survival rate of less than 10% [2 - 5]. Currently, numerous studies worldwide have shown that overall survival and treatment response of patients are not only related to cancer stage but also to molecular biological characteristics of tumors, especially microsatellite instability (MSI-H). MSI-H is caused by a deficiency in mismatch repair proteins (dMMR), including MLH1, PMS2, MSH2, and MSH6, which can be detected by immunohistochemistry with a sensitivity of 91.1% and a specificity of 98.5% [6,7]. MSI-H can occur in Lynch syndrome or sporadic gastric cancer due to non-heritable changes such as methylation of MMR regulatory genes. The correlation between deficient MMR and clinical-pathological factors including older age, distal location of the tumor, histological subtype, fewer lymph node metastases, stage, and overall survival has been reported. Authors Polom K (2018) and Giampieri R. (2015) both identified dMMR as an independent prognostic factor in unresectable gastric cancer patients [4,8]. Patients with dMMR gastric cancer have better overall survival compared to the pMMR group. In Vietnam, the overall survival of unresectable gastric cancer patients is only about 11 months [9, 10]. Therefore, we conducted this study with two objectives: (1) To evaluate MMR protein expression in unresectable gastric cancer patients, and (2) To analyze the correlation between MMR protein expression status with various clinical, paraclinical, and overall survival in unresectable gastric cancer.

2. MATERIALS AND METHODS

2.1. Study design:

We conducted a descriptive case series study, a combination of prospective and retrospective cohorts on 83 patients diagnosed with unresectable gastric cancer by histopathological through endoscopy biopsy at at Hue University of Medicine and Pharmacy Hospital and Hue Central Hospital from June 2020 to December 2022. Patients had sufficient archived tissue for immunohistochemical testing, complete medical records, and follow-up information.

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2.2. Study Variables:

Expression of MLH1, PMS2, MSH2, and MSH6 proteins; association between MMR expression and variables such as age, gender, tumor location, tumor size, lymph metastasis, stage, histological subtype, and overall survival.

2.3. Study Procedure:

All patients diagnosed with unresectable gastric cancer from June 2020 to December 2022 were included.

MMR Immunohistochemistry: Paraffin-block tissue sections were cut and stained with HE and then stained with MLH1, PMS2, MSH2, MSH6 antibodies on the Ventana system from Roche.

Evaluation Criteria: Nuclear staining of normal epithelial cells and lymphocytes was considered positive. Loss of expression of at least one of the four proteins was considered deficient MMR (dMMR). Tumor cells expressing all four proteins to any degree and intensity were considered proficient MMR (pMMR).

Overall Survival Assessment: assessed at 6, 9, and 12 months post-diagnosis.

2.4. Data Analysis:

SPSS 26.0 statistical software. Overall survival was analyzed using Kaplan-Meier method, the Log-rank test for significance ($p < 0.05$), and Cox regression analysis to determine the association between pMMR and prognosis.

3. RESULTS

3.1. The expression of MLH1, MSH2, MSH6, and PMS2 in gastric cancer tissues.

3.1.1. General characteristics:

The average age is 62.3 years, with 72.3% being male. Just 3.6% of tumors are found in the cardia, the majority are found in the antrum (65%). The majority of tumors have a size of ≤ 5 cm (60.2%). 72.3% of patients have regional lymph node metastasis and 97.6% have distant metastasis. Tubular adenocarcinoma is the most common histologic type of unresectable gastric cancer (66.4%), and the intestinal type according to Laurén classification is the most frequently encountered (86.7%), with 50.6% showing poor differentiation.

Table 1. General characteristics

Variable		N	%
Age (mean ± SD)		62.3 ± 13.1	
Gender	Male	60	72.3
	Female	23	27.7
Tumor location	Cardia	3	3.6
	Antrum	54	65
	Other	26	31.4
Tumor size	≤ 5 cm	50	60.2
	> 5 cm	33	39.8
Lymph node metastasis		60	72.3
Stage	IIIC	20	24.1
	IV	63	75.9
Metastasis sites	Abdominal lymph node	49	59
	Peritoneum	40	48.2
	Liver	31	37.3
	Lung	22	26.5
Laurén's classification	Intestinal	72	86.7
	Diffuse	10	12.1
	Mixed	1	1.2
Histologic type	Well	14	16.9
	Moderately	27	32.5
	Poorly	42	50.6
Venous thrombosis		7	8.4

3.1.2. The expression of mismatch repair protein.

The dMMR cases accounted for 10.8% of all unresectable gastric cancer cases. Among these cases, 5 patients do not express both MLH1 and PMS2 markers. One patient does not express both MSH2 and MSH6, and two patients do not express both markers MLH1 and PMS2. One patient did not express PMS2, one patient did not express MSH6.

Table 2. The expression of MMR

Protein	MLH1	PMS2	MSH2	MSH6	n = 83	%
dMMR	–	–	+	+	5	6
	+	+	–	–	1	1.2
	+	+	+	–	1	1.2
	+	–	+	+	2	2.4
pMMR	+	+	+	+	74	89.2

(+): expression; (-) absence.

3.2. The association between protein expression deletion and clinicopathologic features, overall survival of four mismatch repair proteins.

The association between protein expression deletion and clinicopathologic features of four mismatch repair proteins.

Table 3. The association between MMR and clinicopathologic characteristics of unresectable gastric cancer

Clinicopathological finding		dMMR	pMMR	P
Age (yrs)	< 60	4 (11.1%)	32	0.608
	≥ 60	5 (10.6%)	42	
Gender	Male	8 (13.3%)	52	0.433
	Female	1 (4.3%)	22	
Tumor location	Cardia	1 (3.8%)	25	0.467
	Antrum	8 (14.8%)	46	
	Other	0 (0%)	3	
Tumor size	≤ 5 cm	2 (4%)	48	0.026
	> 5 cm	7 (21.2%)	26	
Lymph node metastasis	N0	2 (8.7%)	21	0.521
	N1	7 (11.7%)	53	
Laurén ‘s classification	Intestinal	9 (12.5%)	63	0.636
	Diffuse	0 (0%)	10	
	Mixed	0 (0%)	1	
Histologic type	Well	4 (28.6%)	10	0.012
	Moderately	4 (14.8%)	23	
	Poorly	1 (2.4%)	41	

3.3. The association between MMR protein expression and overall survival

The median overall survival of the 83 patients in the study was 10.4 ± 0.8 months (95% CI: 9.7 - 12.3). Patients with gastric cancer and dMMR had a median overall survival twice as long as the pMMR group (19.1 ± 1.8 months compared to 9.3 ± 0.8 months). This difference was statistically significant with p = 0.02 (< 0.05).

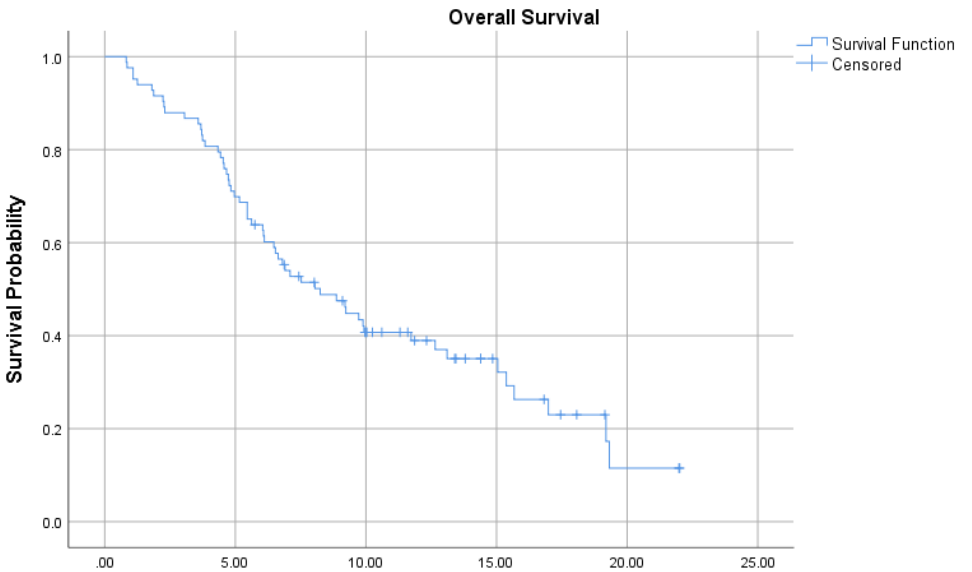

Chart 1. Overall Survival

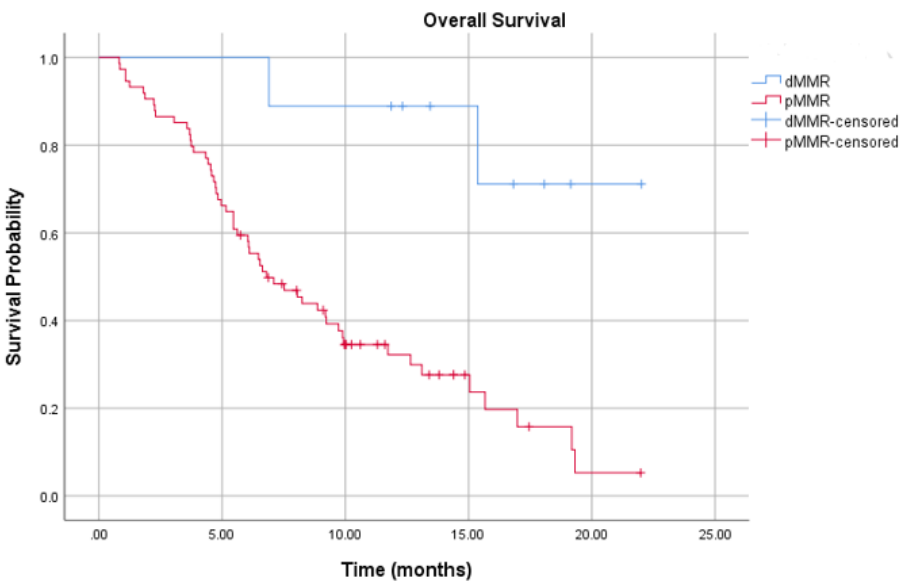

Chart 2. Overall survival for patients stratified according to mismatch repair (MMR) status.

4. DISCUSSION

The average age of patients in the study group was 62.3 ± 13.1 years and the number of men was 2.6 times greater than women. This result is similar to epidemiological statistics and other MMR studies [5,11,12]. Notably, gastric cancer tends to occur more frequently in the age group of ≥ 60 years (56.6%), with a higher prevalence in males than females [5,11]. About 65% of tumors were located in the antrum, which is common in gastric cancer in Vietnam and other developing countries associated with H. pylori infection [13]. Due to the selection of unresectable gastric cancer patients, 72.3% of patients already had regional lymph node metastasis, 97.6% had distant metastasis, and 82.1% had peritoneal metastasis. The most common

sites of distant metastasis were the peritoneum (48.2%), abdominal lymph nodes (43.4%), liver (37.3%), and lungs (26.5%), which aligns with research by Nguyen Minh Phuong (2020) [14]. The majority of tumors were ≤ 5 cm (60.2%), intestinal histopathology according to Laurén (86.7%) and poorly differentiated (50.6%).

In the study of 83 patients with unresectable gastric cancer, the rate of deficient mismatch repair protein (dMMR) expression was 10.8%. The rate of dMMR/MSI-H expression in unresectable gastric cancer according to ESMO is 13%, and in the molecular analysis study by Cristescu (2015), it is 13.2% [7,15]. Among these, loss of MLH1/PMS2 protein expression is the most common in all studies [16–18].

Two studies in Korea by Bae Y.S. (2015) and Seo H.M. (2009) showed a correlation between tumor size larger than 5 cm and deficient MMR protein expression [19,20]. However, other studies worldwide have not shown a clear relationship between MMR/MSI expression and tumor size. Our study results indicated that in the dMMR group, the majority had tumors > 5 cm (77.8%), while in the pMMR group, the majority had tumors ≤ 5 cm (64.9%). The difference in tumor size between the dMMR and pMMR groups was statistically significant with $p < 0.05$, similar to the two studies above.

Several studies worldwide have demonstrated that deficient mismatch repair protein or microsatellite instability (dMMR/MSI-H) is often found in well-differentiated tumors, such as the study by Inada R (2015) in Japan on 489 patients and Yoon Sung Bae (2015) in Korea on 464 patients [21]. Our study (Table 3) also shows similar results, with a higher proportion of well-differentiated tumors in the dMMR group (44.4%) compared to predominantly poorly differentiated tumors in the pMMR group (55.4%). Thus, there is a significant difference in differentiation between the dMMR and pMMR groups with $p < 0.05$.

MMR deficiency is also common in female patients, those under 60 years old, tumors located in the antrum, regional lymph node metastasis, and intestinal-type Laurén histology [8,18]. However, we did not observe these correlations in our study due to the small sample size and the selection of patients who had unresectable tumors, which may not fully reflect the relationship between MMRP and these factors.

In this study, the median overall survival of unresectable gastric cancer was 10.4 ± 0.82 months. Based on single-factor analysis of MMR protein expression with overall survival using Kaplan-Meier analysis showed that the dMMR group had a median overall survival time twice as long as the pMMR group (19.1 ± 1.8 months vs. 9.3 ± 0.8 months). This difference was statistically significant with $p = 0.02$ (< 0.05). This result is quite similar to studies in the world, such as the study of Giampieri R (2015) on 103 patients with unresectable gastric cancer with the median overall survival of the dMMR group was 14.2 months compared to 8 months in the pMMR group, and the dMMR group also reduced the risk of death by 76% (HR = 0.24; 95% CI 0.16 - 0.35; $p < 0.0001$) [4]. A meta-analysis by Polom (2018) combining 21 different studies also showed a significantly reduced risk of death in patients with dMMR/MSI-H gastric cancer compared to pMMR/MSS with HR = 0.69 (95% CI: 0.56 - 8.86) and longer overall survival in the dMMR/MSI-H group compared to pMMR/MSS with $p < 0.05$ [8].

5. CONCLUSION

The dMMR rate in unresectable stage gastric cancer patients was 10.8%. There were correlations between MMR protein deficiency and tumor size and differentiation. Patients with dMMR gastric cancer had a better prognosis compared to those with pMMR gastric cancer.

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