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Expression of MLH-1, PMS-2, MSH-2, MSH-6 markers and histopathological evaluation in colorectal cancer

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Abstract

Background: The clinicopathological characterization of biomarkers to identify microsatellite instability in patients with colon cancers has not been fully studied. This study aims to evaluate the spectrum of histopathology of MLH1, MSH2, MSH6, and PMS2 markers to identify microsatellite instability in patients with colon cancers.

Methods: The study analyzed the association between clinicopathological features and immunohistochemistry MLH-1, MSH-2, PMS-2, & MSH-6 expressions in 36 resected colorectal cancer samples. Patients' data were collected retrospectively, including tumor localization, size, the origin of polyp, mucinous differentiation, tumor stage, lymphovascular and perineural invasion, surgical boundary, and lymph node metastases. The primary antibodies, such as MLH-1, MSH-2, PMS-2, & MSH-6, were tested immunohistochemically.

Results: The number of male cases was higher than female cases, 63.89% vs. 36.11%. The highest incidence (27.78%) of colorectal cancer was seen in the age group of 51-60 years. Tumors were predominantly adenocarcinoma NOS (69.44%). Moderately differentiated tumors (55.56%) were higher in number than poorly differentiated and well-differentiated ones. The highest stage seen was stage III in 50%. 80.56% of cases showed perineural invasion, 27.78% showed lymphovascular invasion, and 52.78% showed nodal metastasis. Right colon placement was associated with mucinous adenocarcinoma and a mucinous component. The ulcerous tumors were quite small, and their pathological tumor stage was higher. Tumors developing from polyps were large in size and had a lower pathological tumor stage. The study found that deletion of MSH-2 & MSH-6 expressions was substantially associated with right-colon placement and poor differentiation. In poorly differentiated adenocarcinomas, mucinous, lymphovascular, and perineural invasions were common. Tumors with mucinous differentiation showed less lymphovascular invasion and lymph node metastases. Perineural invasion was not noted in the pT1 or pT2 tumor stages.

Conclusion: A strong correlation was found between immunohistochemical markers and clinical characteristics of tumors with microsatellite instability. However, these conclusions should be supported by large-scale investigations involving molecular PCR and other approaches.

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Highlights

What is current knowledge?

Loss of expression of immunohistochemical markers such as MLH-1, MSH2, PMS-2, and MSH-6 was associated with poor differentiation and mucinous adenocarcinomas. There was a significant relationship between the cecum and ascending colon location and lower expression of PMS-2 & MSH-6.

What is new here?

The study found that low levels of MSH-2 and MSH-6 were associated with colon location, poor differentiation, and mucinous differentiation. It also found a strong link between MLH-1, MSH-2, PMS-2, and MSH-6 expressions and colorectal cancer and clinical characteristics of tumors with microsatellite instability.

Introduction

Colorectal cancer accounts for 9% of all cancer cases, making it the third most frequent cancer globally and the second leading cause of cancer-related death (1). Several genetic variations have been proposed as prognostic indicators; however, none have been validated for therapeutic application. Evidence suggests that measuring the level of microsatellite instability can provide important prognostic information in this tumor type (2,3).

Microsatellite instability is characterized by substantial fluctuations in the length of short repeating sequences known as microsatellites in tumor DNA. Mismatch repair is a biological post-replication mechanism that maintains DNA balance. Mutations in mismatch repair genes, including MLH1, PMS2, MSH2, and MSH6, lead to MSI colon cancer (4). Mismatch repair protein deficiency occurs in approximately 15-20% of colorectal tumors. Colorectal cancers are classified as high-frequency microsatellite instability (MSI-H: loss of two or more markers) when instability occurs in at least 30% of the loci studied or low-frequency microsatellite instability (MSI-L: Loss of one marker) when less than 30% of the loci are unstable. Microsatellite-stable tumors exhibit no changes in the length of the DNA sequences analyzed. Microsatellites are stable when no markers are lost. Colorectal cancer develops via the microsatellite instability pathway in 12-15% of cases, with 2-5% being inherited (5,6).

MSI-H colorectal carcinomas are less aggressive than common large bowel tumors. In large patient populations, investigations have shown that the MSI-H phenotype confers a survival advantage independent of the cancer stage and other clinical and pathologic factors (7).

MSI-H is caused in most cancers by DNA mismatch repair abnormalities, specifically the inactivation of the MLH1 and MSH2 genes. The immunohistochemical test for MLH1 and MSH2 expression is a simple, inexpensive, and dependable technique to identify most MMR-defective colorectal cancers (8).

In this work, we examined the histopathological spectrum of MLH1, MSH2, MSH6, and PMS2 markers and their prognostic relevance in identifying microsatellite instability in colon cancer patients.

Methods

This retrospective study is based on medical records from the Pathology Department of SVS Medical College Hospital, Mahbubnagar. This study included 36 colorectal cancer patients who underwent resections at our institution between 2019 and 2023. Clinical features and tissue characteristics of the tumors were obtained from the hospital records. The study included 36 consecutive patients with TNM stage II (22%), stage III (53%), and stage III (13%) colorectal adenocarcinoma who had received curative surgical resection. The study comprised patients with malignant colon tumors. Benign colon tumors and inflammatory and metastatic lesions were all excluded.

IHC was used to investigate mucosal samples and surgical resections of colon tumors. Microarrays and immunohistochemistry were used to investigate the expression of mismatch repair proteins such as MLH1, MSH2, MSH6, and PMS2.

Two pathologists analyzed hematoxylin and eosin sections, mucinous components, polyp origins, tumor stages, lymphovascular and perineural invasions, lymphocyte densities, surgical margins, and lymph node metastases.

Tumour staging and histological differentiations were based on the WHO (2010) Classification of Tumours of the Digestive System.

The lymphocyte density in the tumor microenvironment was divided into three categories: 1) stromal lymphocytic infiltration, 2) stromal lymphocyte infiltration and glandular infiltration, and 3) Crohn-like lymphocytic infiltration that occurs independently of lymphocytic infiltration in the tumor.

The presence of a tumor at the surgical margin, either macroscopic or microscopic, was classed as surgical margin positive.

Immunohistochemical analysis:

One of the blocks that depicted the tumor's features was chosen. After determining the tumor's proximity to normal mucosa and lymphocytic infiltration, 4 mm of paraffinized tissue was removed, and several blocks were created. Four-micron-thick slices were cut for IHC examination. The primary antibodies MLH-1, MSH-2, PMS-2, & MSH-6 were employed in the immunohistochemistry study. Nuclear staining in normal mucosa and lymphocytic infiltration served as positive controls. Each tumor was inspected in five regions, and the percentage of labeled tumor cells was divided into 3 categories: negative (<1%), 1-50%, and 51-100% positive.

Results

The highest incidence (27.78%) of colorectal cancer was seen in the age group of 51-60 years, followed by 19.45% in 61-70 years, and 19.45% in the 71-80 years age group. The mean age was 56.5 ± 15.5 years (30-85 years). Males had a higher than female patient population (63.89% vs 36.11%). Tumors were predominantly adenocarcinoma NOS (69.44%) (Table 1). Moderately differentiated tumors (55.56%) were higher in number than poorly differentiated and well-differentiated grading ones. The higher stage was in stage III, which was 50%. 80.56% of cases showed perineural invasion, 27.78% showed lymphovascular invasion, and 52.78% showed nodal metastasis. Colorectal cancer and its distribution cases are illustrated in Table 2.

Table 1. Distribution of colorectal cancer cases according to the tumor size and grading

Characteristics	Frequency	Percentage
Histological type		
Conventional adenocarcinoma	25	69.44%
Mucinous adenocarcinoma	6	16.67%
Signet ring cell carcinoma	3	8.3%
Medullary adenocarcinoma	1	2.7%
Squamous cell carcinoma	1	2.7%
Tumor size		
< 5 cm	11	30.56%
≥ 5cm	25	69.44%
Tumor grading		
Well-differentiated	06	16.67%
Moderately differentiated	20	55.56%
Poorly differentiated	10	27.78%

Table 2. Distribution of colorectal cancer cases according to clinicopathological variables

Variable	Number of cases	Percentage	Variable	Number of cases	Percentage
Age			Mucinous component		
≤ 50	6	16.67	Absent	8	22.22
51-60	10	27.78	Present	28	77.78
61-70	7	19.45	Tumor stage (pT)		
71-80	7	19.45	pT1	1	2.78%
≥ 81	6	16.67	pT2	4	11.11%
Sex			pT3	18	50
Male	23	63.89	pT4a	10	27.78
Female	13	36.11	pT4b	3	8.33
Localization			Lymphovascular invasion		
Cecum	8	22.22	Present	10	27.78
Ascending colon	4	11.11%	Absent	26	72.22
Transverse colon	2	5.4%	Perineural invasion		
Descending colon	1	2.78%	Present	29	80.56
Sigmoid colon	12	33.33%	Absent	7	19.44
Rectosigmoid junction	1	2.78%	Lymphocyte density		
Rectum	8	22.23%	Stromal-lymphocytic- infiltration	7	19.45%
Growth pattern			Glandular- infiltration and stromal- lymphocyte- infiltration	23	63.89%
Ulcerous	7	19.45%	Crohn-like- lymphocytic infiltration irrespective of lymphocytic- infiltration within the tumor	6	16.67%
Ulcerovegetative	23	63.89	Tumor in surgical margin		
Polypoid	6	16.67	Present	3	8.33
Multiple tumors			Absent	33	91.67
Absent	33	91.67	Number of lymph node		
Present	3	8.33	≤ 12	6	16.67%
Accompanying polyp			13-24	12	33.33%
Absent	28	77.78	25-36	10	27.78
Present	8	22.22	37-48	4	11.11%
Arising in polyp			≥ 49	4	11.11%
Absent	33	91.67	Lymph node metastasis (pN)		
Present	3	8.33	pN0	17	47.22%
Differentiation			pN1	8	22.22%
Well	2	5.54			
Moderate	28	77.78	pN2a	4	11.11%
Poor	3	8.33	pN2b	7	19.44%
Mucinous	3	8.33			

In seven cases, there was no staining with MLH-1 or PMS-2. In six cases, MSH-2 & MSH-6 did not stain. Two cases showed only MLH-1 negative. PMS-2 negative was only identified in 3 cases. MSH-2 was positive in the majority of cases (Table 3,4). Immunohistochemical analysis of colorectal cancer samples is depicted in Figure 1, Figure 2, and Figure 3.

Table 3. Expression of MMR proteins in colorectal cancers

MLH1	PMS2	MSH2	MSH6	No of cases	Percentage
-	-	+	+	7	19.44%
+	+	-	-	6	16.67%
-	+	+	+	2	5.56%
+	+	-	+	2	5.56%
+	+	+	-	3	8.31%
+	-	+	+	1	2.78%
+	+	+	+	15	41.67%

Table 4. MSI status based on expression of MMR proteins in colorectal cancer

MSI status	No. of cases	Percentage
MSS	15	41.67%
MSI-L	8	22.22%
MSI-H	13	36.11%
Total	36	100%

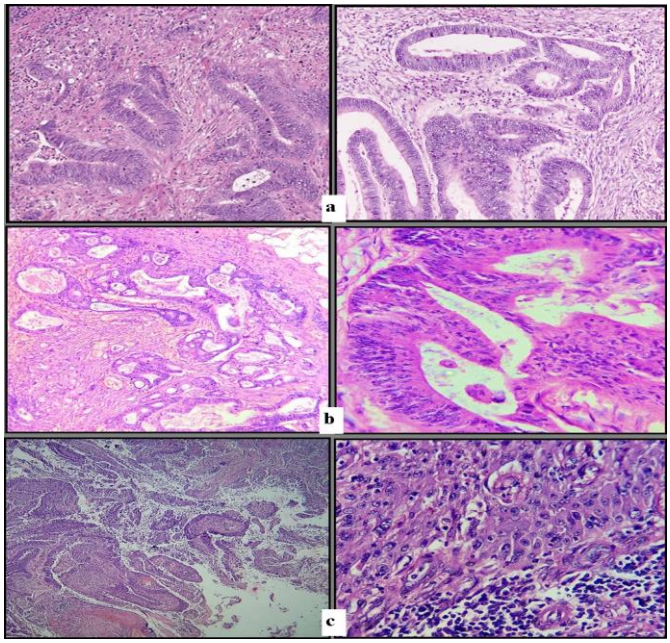

Figure 1. a. Well-differentiated adenocarcinoma, well-formed glands. b. Moderately differentiated adenocarcinoma with 50% to 95% gland development. c. Poorly differentiated adenocarcinoma, less than 50% gland development. Most tumors (Excluding the advancing edge) are composed of sheets of cells without gland development.

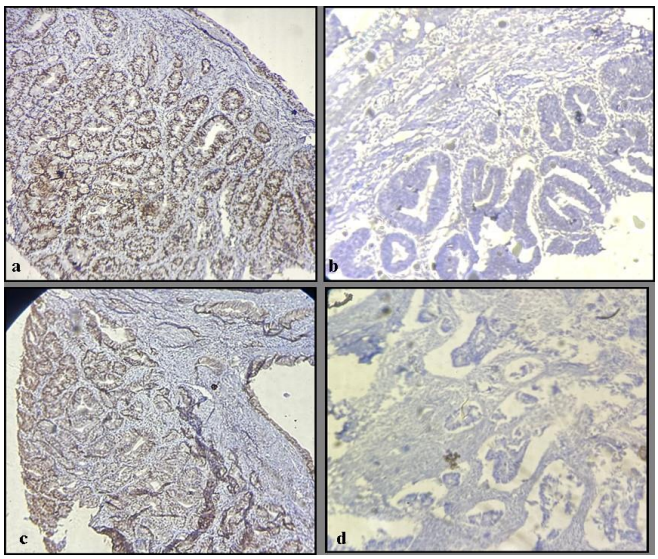

Figure 2. a. MLH1 retained +ve. b. MLH1 deficiency (MLH1 -ve). c. PMS2 was preserved (+ve PMS). d. PMS2 deficit (-ve PMS2).

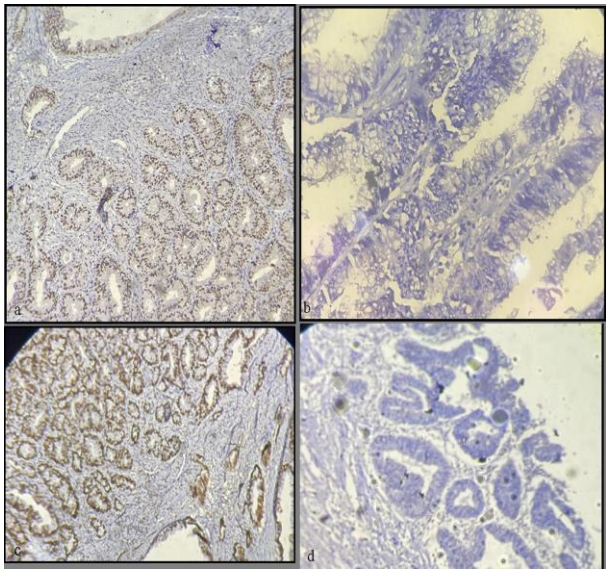

Figure 3. a. MSH2 retained (+ve MSH2). b. MSH2 deficient (-ve MSH2). c. MSH6 retained (+ve MSH6). d. MSH6 deficient (-ve MSH6)

Right colon placement was associated with mucinous adenocarcinoma and a mucinous component. The ulcerous tumors were quite tiny, and their pathological tumor stage was higher. Tumors developing in polyps were large and had a lower pathological tumor stage. In poorly differentiated adenocarcinomas, mucinous, lymphovascular, and perineural invasions were common.

Discussion

The mean age of our study cases was 56.5 years. Due to predisposing circumstances, the incidence of the disease is lower among those under 50. In our study, those aged 51 to 60 comprised 27.78% of colorectal cancer cases. Tumors in the right colon were slightly more common among people aged 51 to 60 than in other age groups.

Colorectal cancer is thought to develop through mechanisms such as chromosomal and microsatellite instability. Microsatellite instability is found in 12% to 15% of colorectal tumors, with 2% to 5% heredity predisposition (9). Tumors induced by microsatellite instability have distinct clinicopathological characteristics, including proximal colon location, mucinous histology, and lymphocyte infiltration.

Due to the complexity and costliness of genetic analysis, it cannot be used to screen for all colorectal malignancies (10,11,12).

We studied the correlation between clinicopathological features and immunohistochemical expressions of MLH-1, PMS-2, MSH-2, & MSH-6 in 36 colorectal cancer resection tissues.

Most malignancies were in the sigmoid colon (33.33%) and rectum (22.23%). Right colon tumors are more likely to have an exophytic growth pattern, be bigger, and be at more advanced stages of development. All left-side tumors were small.

Mucinous adenocarcinomas were detected in young patients, primarily women, with a right colon location, a higher tumor stage, weak differentiation, and advanced lymph node metastases. Our findings revealed a positive correlation between multiple tumor foci and lymph node metastasis, which could be attributable to the tumor advanced stage. Numerous investigations have demonstrated that histological grade, independent of stage, is a major prognostic factor. An advanced tumor grade is associated with increased perineural invasion, peritumoral lymphovascular invasion, lymph node metastases, and tumor invasion (13,14). A significant relationship was noted between high-grade tumors, lymph node metastases, and tumor invasions.

Lymphovascular infiltration was not found in malignant tumors in T1 and T2 stages. The majority of lymphovascular invasion tumors showed lymph node metastases.

A third of tumors without MMR expression were limited to the right colon. According to Chapusot et al., adenocarcinoma in the right colon with poor differentiation has a significant prognostic value for MMR decline (15). In our study, those with negative immunohistochemical markers exhibited right colon location and a poorly differentiated cancer.

No immunohistochemical marker showed a significant correlation with the tumor invasion in our investigation. Our study findings could be supported by large-scale research using molecular approaches.

Conclusion

The right colon had low levels of MSH-2 and MSH-6 expression, which suggests poor differentiation. The study found a strong correlation between immunohistochemical markers and clinicopathological characteristics. Most subjects were male, most between 51 and 60 years old. As the number of patients

in advanced stages increases, cost-effective prognostic markers are becoming increasingly necessary. Development of a four-antibody panel containing the IHC markers MLH1, MSH2, MSH6, and PMS2 is recommended to identify MSI-H patients who may benefit from targeted immunotherapy.

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Ethical statement

The study followed the Declaration of Helsinki, and the subjects provided written informed consent. The Institutional Ethics Committee, SVS Medical College, Mahbubnagar approved the study protocol.

Conflicts of interest

None.

Author contributions

S.A.B.H., S.K., and V.M. analyzed the data; S.A.B.H., P.M.G., A.M., and A.S. conducted the histological analysis; S.K and V.M supervised the work and edited the document. All authors reviewed and approved the final manuscript.

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