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Original Research Article

MMR status of colorectal carcinomas at a tertiary cancer care centre in Central India

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ABSTRACT

Background: Microsatellite instability (MSI) and mismatch repair (MMR) deficiency are important biomarkers in colorectal cancer (CRC) that have clinical implications for patient management and treatment. MMR deficiency can lead to MSI, which is characterized by alterations in the length of microsatellite DNA sequences. Clinical applications of MMR deficiency in colorectal adenocarcinoma are as a prognostic marker as well as Predictive marker for adjuvant chemotherapy and immunotherapy.
Objectives: To assess the MMR status of colorectal adenocarcinomas.
Materials and Methods: This was a retrospective study of 127 colorectal adenocarcinomas cases. All the samples were either biopsies or surgically resected tumor tissues from patients of Carcinoma Colon and rectum. Immunohistochemistry was performed on Roche Ventana Benchmark XT autostainer.
Results: MLH-1, PMS2, MSH2 and MSH6 expression was retained in the tumor cells in 106 cases and it was lost in 21 cases. Among various clinicopathological variables, tumor site and AJCC pStage were found to be significantly associated with dMMR tumors.
Conclusion: Patients of colorectal adenocarcinomas were benefited by MMR testing as it influenced the adjuvant treatment in all Stage IIA cases as well as all metastatic cases. Incorporating MMR testing into routine clinical practice can help optimize patient management and treatment strategies in CRC.

1. Introduction

Colon cancer is the third most common cancer in men (663000 cases, 10.0% of all cancer cases) and the second most common in women worldwide (571000 cases, 9.4%). Colon adenocarcinoma ranks 8^{th} in Indian males and 9^{th} in Indian females.[1]

One of the molecular genetic pathways that underlies colon carcinogenesis is the microsatellite instability (MSI) mutational pathway. MSI results from inactivation of mismatch repair (MMR) genes.[2,3]

MMR stands for DNA mismatch repair, which is a crucial process for maintaining the integrity of the genome. In colorectal cancer, the standard of care includes status of mismatch repair in the pathological assessment as part of the pretreatment protocol.[4]

One of the two standard reference methods are currently recommended for the detection of mismatch repair deficieny (dMMR)/microsatellite instability (MSI) in Colorectal adenocarcinoma: testing for MSI using polymerase chain reaction (PCR), according to international criteria, and screening for loss of MMR protein expression using immunohistochemistry with antibodies directed against MLH1, MSH2, MSH6 and PMS2. These methods are equally valid as the initial screening test for dMMR/MSI in CRC.[5] On the basis of the test results, the resectable as well as non resectable colon carcinomas are classified as

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proficient (MMR proficient) or deficient (MMR deficient). This classification has important implications for prognosis and treatment strategies:

1. To identify individuals with Lynch syndrome.
2. To inform use of immunotherapy in patients with metastatic disease.
3. To inform decisions for patients with stage II disease.

(Patients with stage II MSI-H/MMR deficient tumors may have a good prognosis and do not benefit from 5-FU adjuvant therapy, and adjuvant therapy should not be given to patients with low-risk stage II MSI-H/MMR deficient tumors).[4]

MMR-proficient (pMMR) colorectal cancers, have a functional DNA mismatch repair system. These tumors are typically characterized by the correct repair of errors that occur during DNA replication. MMR-proficient tumors are less likely to have high levels of microsatellite instability (MSI-H), which is a hallmark of MMR deficiency.

MMR deficient (dMMR) colorectal cancers have mutations or epigenetic changes that impair the function of the DNA mismatch repair system. This can lead to the accumulation of errors in the DNA, particularly in regions known as microsatellites, resulting in high levels of microsatellite instability (MSI-H).[6]

The determination of MMR status is often done through immunohistochemistry (IHC) testing to assess the expression of MMR proteins (e.g., MLH1, MSH2, MSH6, and PMS2) or through polymerase chain reaction (PCR) testing to evaluate microsatellite instability. (IHC for MMR and DNA analysis for MSI are different assays and measure different biological effects caused by dMMR function).

Few studies have been reported on MMR status of colon carcinoma from India.[2,7–10]

2. Materials and Methods

This was a retrospective, observational study done in patients of Colonic adenocarcinoma diagnosed at our Institute. The period of study was from January 18 till December 23 (6 years).

The surgically resected specimens received in the department of Pathology were examined by gross dissection according to the CAP protocol[11] and representative sections submitted for histopathology examination. Pathological staging was reported.

The specimens received as Biopsy tissue were processed and sections submitted for histopathology examination. One tumor section was selected for IHC staining for four markers – MLH1, PMS2, MSH2 and MSH 6.

The antibodies used were MLH-1 (Roche, M1, Mouse monoclonal), PMS2 (Roche, A16-4, Mouse monoclonal), MSH2 (Roche,G219-1129, mouse monoclonal), MSH6 (Roche, SP93, Rabbit monoclonal). The staining was performed on Roche Ventana Benchmark XT autostainer (Roche, Switzerland) and the appropriate protocol was followed.

The results were interpreted as:

MMR proficient – intact nuclear staining in at least 1% of tumor cells for all four markers.

MMR deficient - loss/absence of staining in nuclei of tumor cells for one or more than one or all the four markers.

3. Observations

Total number of colorectal adenocarcinoma cases in the present study was 127. The mean age of the patients was 56.94 years while the M:F ratio was 1.7:1.

The primary tumor site was Colon in 70 and Rectum in 57 cases. The common clinical presentations were blood in stools, pain in abdomen, loss of appetite, lump in abdomen, loss of weight and fatigue. Histopathological diagnosis was Well differentiated adenocarcinoma- 22 (17.32%), moderately differentiated adenocarcinoma - 80 (62.90%) and poorly differentiated adenocarcinoma- 25 (19.68%). (Table 1)

Out of 127 cases, MMR proficient cases were 106 (83.46%) (Figure 1) and MMR deficient cases were 21(16.53%) (Figure 2).

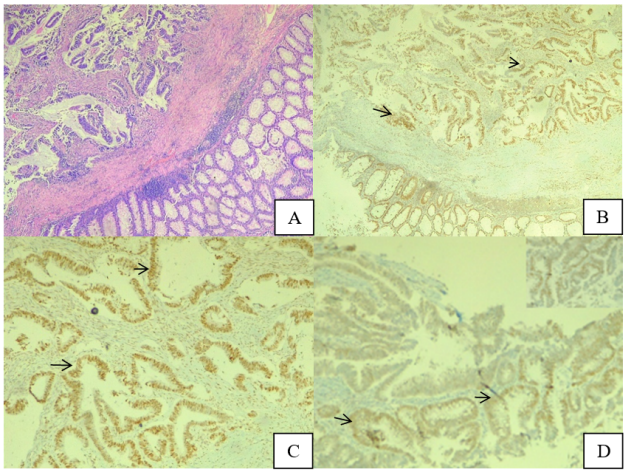

Figure 1: pMMR colonic adenocarcinoma. (**A**) Section of colonic mucosa with adenocarcinoma; (**B**) Intact, retained nuclear staining for MMR proteins (MLH1, MSH2, MSH6); (**C**) Intact, retained nuclear staining for MMR proteins (MLH1, MSH2, MSH6); (**D**) Weaker intensity of PMS2 protein stain. (A-H & E stain, X10, B-IHC, X10, C-IHC, X40, D-IHC, X10, Inset- IHC, X40)

Amongst the MMR deficient cases 8 were biopsies and 13 were Surgical resections.

Amongst 13 resected CRCs, 11 had p stage T2N0 (Stage IIA) and 2 had T3N0 (Stage I).

MMR proficient CRCs were 106, out of which 63 were biopsies, amongst these, 8 biopsies were from Metastatic sites and Stage IVA and IVB at presentation.

Table 1: Association of dMMR and pMMR CRC with clinicopathological factors

		dMMR	Percent	pMMR	Percent	Total	P value
Age	25-55	11	18.97	47	81.03	58	0.499
	55-85	10	14.49	59	85.51	69	
Gender	Females	5	10.64	42	89.36	47	0.170
	Males	16	20	64	80	80	
Site	Colon	16	22.86	54	77.14	70	0.034
	Rectum	5	8.77	52	91.23	57	
Grade	I	5	21.73	17	77.27	22	
	II	13	16.25	67	83.75	80	NA
	III	4	16	21	84	25	

Table 2: Comparison of dMMR cases across different Indian studies

Study	dMMR patients in CRC	pMMR patients in CRC	Total patients
Present study	21 (16.67%)	106	127
Kale et al	41 (13.85%)	255	296
Sharma et al	18 (32.72%	37	55
Arora et al	15 (48.1%)	27	42
Malik et al	17 (11.33%)	133	150

Table 3: Association of dMMR and pMMR CRC with AJCC stages

	Biopsy	Resection	Stage I	IIA	IIB	IIIA	IIIB	IIIC	IVA/B/C
dMMR	8	13	2	11(84.6%)	0	0	0	0	0
pMMR	63	43	7	14(27.45%)	2	3	9	8	8

Table 4: Frequency of different patterns of MMR proteins loss

MLH1 and PMS2 loss	10
Isolated loss of any one	4
MSH2 and MSH6 loss	4
All four loss	3
Total	**21**

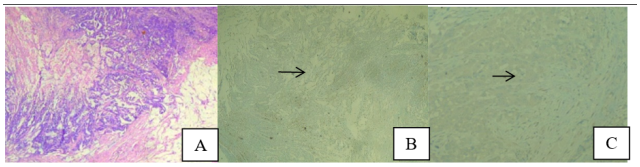

Figure 2: dMMR colonic adenocarcinoma. (**A**) Section of colon adenocarcinoma. (**B**) Absence of nuclear staining for MMR proteins (MLH1, PMS2, MSH2, MSH6). (**C**) Absence of nuclear staining for MMR proteins (MLH1, PMS2, MSH2, MSH6). Note positive internal control of stromal cells. (A-H & E stain, X10, B-IHC, X10, C- IHC, X40)

43 were surgical resections, out of which, 34 were primary resections, and 7 were post NACT surgeries (Table 3)

The various patterns of MMR deficient cases were: MLH1 and PMS2 loss (10), all four loss (3), MSH2 and MSH 6 loss (4), isolated MSH 2 loss (1), isolated MSH 6 loss (1), isolated MLH1 loss (1), isolated PMS2 loss (1). (Table 4)

The database was created in MS Excel by Windows and the statistical software IBM SPSS version 21 was used to analyse the observations.

4. Discussion

Microsatellite instability (MSI) and Mismatch repair (MMR) are important biomarkers in Colorectal adenocarcinoma (CRC). MMR deficiency can lead to MSI, which is characterised by alterations in the length of microsatellite DNA sequences. MMR deficiency has prognostic as well as predictive implications in CRC management.

The present study consisted of 127 cases.

The association of age, gender, site and grade of adenocarcinoma with the MMR status is depicted in Table 1.

Overall, age, gender and grade don't appear to influence proficiency significantly, but the site of the tumor (colon vs. rectum) does seem to have an impact, with colon site showing a higher proportion in dMMR patients.

In this study, 21 cases out of 126 were dMMR (16.67%). This is comparable to other studies from India viz.13.85%

(Kale et al.),[7] 32.7% (Sharma et al.),[8] 35.71% (Arora et al.)[9] and 11.3% (Malik et al.).[10] (Table 2)

Amongst the 21 dMMR patients, 13 (61.90%) were surgically resected and staged as I and IIA. Thus, following the NCCN guidelines, these patients may be spared adjuvant chemotherapy.[4]

Remaining 8 (38.09%) cases were unresectable and were managed with appropriate chemotherapy.

In the follow up of these unresectable dMMR cases, the option of second line immunotherapy can be kept whenever they show progression.

As can be seen from Table 3, stage IIA was more common in dMMR cases (84.6%) as compared to pMMR cases (27.45%). The translational effect of MMR testing is most relevant in stage II as it decides whether adjuvant treatment is given or not.[2]

Thus, in the present study, adjuvant treatment was avoided in 11 patients with stage IIA who were dMMR. pMMR cases more commonly presented with a higher stage – out of 43 resected cases, 22 were IIB/IIIA/B/C. These cases were give adjuvant chemotherapy as per protocol. Moreover, adjuvant chemotherapy was indicated in 14 stage IIA cases who were pMMR. 8 pMMR patients were metastatic at presentation and the option of immunotherapy would not be considered as beneficial in them.[4]

As can be seen from Table 4 the different patterns observed in the present study are somewhat different from other reported studies.[7,12–14] However, due to less number of cases, the association with clinicopathological factors could not be established.

Different patterns have been described in the loss of MMR proteins and also varying significance has been shown by association with mutation analysis.[12]

dMMR patients should be followed up by Mutational analysis for MSI status. This serves to screen for patients and close family members for Lynch syndrome. However, due to cost constraints, in the present study, mutational analysis could not be done in these cases. This is a limitation of the study.

All the cases of CRC are being followed up at the institute for recording their Progression free survival (PFS) as well as Overall survival (OS).

5. Conclusion

In the present study, dMMR colorectal adenocarcinoma cases were 21 and pMMR cases were 106. All patients of colorectal adenocarcinoma benefit significantly by testing for MMR proteins in the tumor tissue.

6. Limitation

A five-year follow up of all patients would be required to assess the difference in progression free survival as well as overall survival between the MMR deficient and MMR proficient patients.

7. Source of Funding

None.

8. Conflict of Interest

None.

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