Original Article

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Pathology Section

Immunophenotypic Evaluation of Mismatch Repair Proteins in Various Solid Tumours: A Cross-sectional Study from a Tertiary Care Cancer Centre in Rajasthan, India

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ABSTRACT

Introduction: Mismatch Repair (MMR) proteins are essential in correcting DeoxyRibo Nucleic Acid (DNA) replication errors, including point mutations, to maintain genetic stability. Among atleast seven MMR proteins in humans, four- MLH1 (MutL homolog 1), MSH2 (MutS homolog 2), MSH6 (MutS homolog 6), and PMS2 (Postmeiotic segregation increased 2) are most significant in cancer biology, particularly in Colorectal Carcinoma (CRC), gastric, endometrial, and ovarian cancers. Deficiencies in these genes can lead to Microsatellite Instability (MSI), which promotes tumourigenesis.

Aim: The present study done to assess the expression of MMR proteins and their association with clinicopathological features in solid tumours like colon, liver, stomach, gall bladder and others.

Materials and Methods: The present retrospective and comparative study was conducted at a North Indian Cancer Centre at Jaipur, Rajasthan, India for three years, 55 cases of various solid tumours were examined for the expression of MMR proteins using immunohistochemistry. The cases were categorised into two groups: proficient (normal expression of MMR proteins, low MSI probability) and deficient (loss of MMR proteins, high MSI probability). The immunophenotypic expression was analysed in relation to clinical and pathological parameters by tabulating the data in Microsoft Excel sheet and statistical analysis was done by using the Statistical Package for Social Sciences (SPSS) statistics software windows version 22.0 released 2013.

Results: Results showed that 8 (14.5%) out of 55 patients had a loss of MMR protein expression. Of these cases, 5 (62.5%) displayed a combined loss of MLH1 and PMS2, while 3 (37.5%) showed a combined loss of MSH2 and MSH6. All cases with MMR deficiency were located proximal to the splenic flexure and exhibited mucinous differentiation along with high levels of Tumour-infiltrating Lymphocytes (TILs). The mean age in the proficient group (59.4 years) was higher compared to the deficient group (54.5 years), with males {5 (62.5%) in MMRd and 25 (53.2%) MMRp} being more commonly affected than females {3 (37.5%) in MMRd and 22 (46.5%) in MMRp} in both groups. Adenocarcinoma was the predominant histological type in both groups {5 (62.5%) in MMRd and 30 (63.8%) in MMRp}.

Conclusion: The study highlights the importance of MMR protein testing, particularly for CRC patients aged around 50 years with low-grade tumours. However, given the limited sample size, larger studies are needed to further explore the relationship between MMR protein deficiencies and clinicopathological features in various cancers.

Keywords: Colorectal carcinoma, Chromosomal instability, Solid tumours

INTRODUCTION

The MSI is a form of genomic instability, arises due to defects in MMR genes, such as MLH1, PMS2, MSH2, and MSH6, which fail to correct replication errors in microsatellite regions, leading to the MSI phenotype. MSI represents a major molecular alteration in CRC [1,2]. Mutations associated with MMR deficiency result in chromosomal alterations, translocations, and the development of MSI, CpG Island Methylator Phenotype (CIMP), and Chromosomal Instability (CIN) [3]. Identifying tumours with MSI is crucial as these patients have a better prognosis and show different responses to chemotherapy [4]. In India, the prevalence of the MSI subtype among all colon cancers is about 30%, which is roughly double that of the Western population, indicating different molecular pathogeneses [5,6]. The objective of the present study was to evaluate the expression of MMR proteins in various solid tumours and to study their relationship with different clinicopathological features. Additionally, a comparative analysis was conducted to evaluate any statistically significant correlation between various parameters and the loss of these proteins.

MATERIALS AND METHODS

The present retrospective, non-randomised, observational, and comparative study was conducted on patients with various solid tumours, either primary or metastatic. The study was carried out at Mahatma Gandhi Medical College Jaipur, Rajasthan, India, over a three-year period from 2021 to 2023. Immunohistochemistry (IHC) for MMR proteins was performed on a Ventana automated platform (Ventana Benchmark Gx). Food and Drug Administration (FDA)-approved antibodies were used for performing IHC for MMR proteins (MLH1 - Clone M1; MSH2 - Clone G219-1129; MSH6- Clone SP93; PMS2- clone A16-4) using an Horseradish Peroxidase (HRP) detection system.

Inclusion criteria:

- All cases of solid cancers (with or without neoadjuvant therapy) in which MMR studies were done as per the clinician request.
- All types of specimen like trucut biopsy, punch biopsy and resection specimen etc.

Exclusion criteria: Solid cancers in which MMR protein IHC was not done.

Study Procedure

All cases in which MMR proteins were analysed by the IHC were listed. There clinical and pathological data (Age, sex, site of tumour, histologic type, histologic grade, TIL, Lymphovascular Invasion (LVI),

T stage, N stage and metastatic status) were retrieved from Medical Record Department of the hospital.

These cases were categorised into two groups: 1) no loss of nuclear expression of MMR proteins (low probability of MSIH, proficient); and 2) loss of nuclear expression of MMR proteins (deficient).

STATISTICAL ANALYSIS

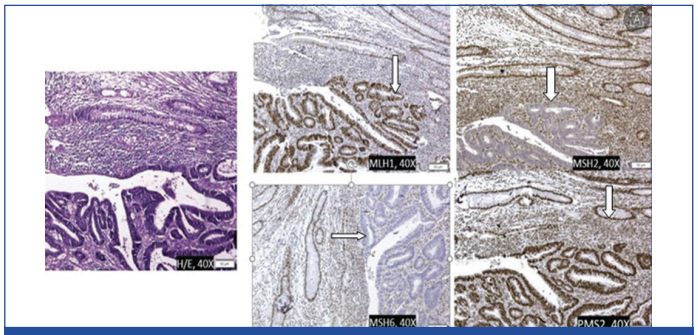
The data was analysed through the SPSS statistics software windows version 22.0 released 2013. Armonk, NY: IBM Corp. Appropriate statistical tests like Pearson's Chi-square test and Mann-Whitney Test were applied to establish the significant association between different variables.

RESULTS

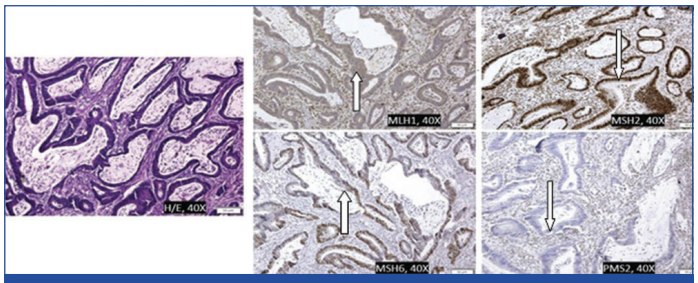
All the demographic and clinical findings for the study population has been listed in [Table/Fig-1]. The present study included 55 cases of various solid tumours, consisting of 30 resection (Colon-15, Stomach-5, Oesophagus-4, Gall bladder-6) and 25 small biopsy specimens. 47 cases showed intact MMR protein expression (proficient group) and loss was found in eight cases (deficient group) [Table/Fig-2]. The most common loss was the combined loss of MLH1 and PMS2, observed in 5 cases (62.5%), and followed by the combined loss of MSH2 and MSH6 in 3 cases (37.5%) [Table/Fig-3]. The distribution and comparison of various clinicopathological parameters including age, sex, site of tumour, histologic type, histologic grade, TIL, LVI, T stage, N stage and metastasis between the two groups are provided in [Table/Fig-1]. However, on statistical analysis no significant association was found between the above parameters and MMR status. Except TIL's (p-value <0.05). High TIL's were significantly associated with MMR protein deficient group.

Variables		Group		p-value
		MMR proficient n=47 n (%)	MMR deficient n=8 n (%)	
Gender	Male	25 (53.2%)	5 (62.5%)	0.625
	Female	22 (46.8%)	3 (37.5%)	
Specimen	Resection	22 (46.8%)	7 (87.5%)	0.033
	Biopsy	25 (53.2%)	1 (12.5%)	
Site	Proximal to spleenic flexure	4 (8.51%)	8 (100.0%)	
	Distal to spleenic flexure	15 (31.91%)	0	
	Liver	2 (4.3%)	0 (0.0%)	
	Stomach	8 (17.0%)	0 (0.0%)	
	Oesophagus	5 (10.6%)	0 (0.0%)	
	Gall bladder	8 (17.0%)	0 (0.0%)	
	Mullerian tract	1 (2.1%)	0 (0.0%)	
	Others	4 (8.5%)	0 (0.0%)	
Tumour type	Primary	39 (83.0%)	8 (100.0%)	0.587
	Metastasis	8 (17.0%)	0 (0.0%)	
Tumour grade differentiation	Well differentiated	20 (44.4%)	4 (50.0%)	<0.597
	Moderatly differentiated	17 (37.8%)	4 (50.0%)	
	Poorly differentiated	8 (17.8%)	0 (0.0%)	
Histological diagnosis	Adenocarcinoma NOS	30 (63.8)	5 (62.5)	0.188
	Adenocarcinoma with mucinous differentiation	0	3 (37.5)	
	Endometriod carcinoma	5 (10.6)	0	
	Squamous cell carcinoma	3 (6.38)	0	
	Metastatic adenocarcinoma	6 (12.8)	0	
	Signet ring cell carcinoma	2 (4.2)	0	
	Serous carcinoma	1 (2.12)	0	
Tumour stage	T1	5 (22.7%)	0 (0.0%)	0.646
	T2	5 (22.7%)	2 (28.6%)	
	T3	8 (36.4%)	4 (57.1%)	
	T4	4 (18.2%)	1 (14.3%)	
Node stage	N0	19 (86.4%)	7 (100.0%)	1.00
	N1	2 (9.1%)	0 (0.0%)	
	N3	1 (4.5%)	0 (0.0%)	
Metastasis stage	M1	1 (9.1%)	0 (0.0%)	0.077
	M0	10 (90.9%)	4 (100.0%)	
TIL (>50%)		5 (10.6)%	5 (62.5)%	<0.05

[Table/Fig-1]: Association of MMR protein with other clinical variables.
TIL: Tumour infiltrating lymphocytes



[Table/Fig-2]: H&E 40x adenocarcinoma colon; intact nuclear staining of MLH1 and PMS2; Loss of nuclear staining of MSH2 and MSH6.
H&E: Haematoxylin and eosin



[Table/Fig-3]: H&E 40X adenocarcinoma colon; intact nuclear staining of MSH2 and MSH6; loss of nuclear staining of MLH1 and PMS2.

On analysis, gender distribution was similar in the groups, with 25 (53.2%) males and 22 (46.8%) females in the MMR proficient group, and 5 (62.5%) males and 3 (37.5%) females in the MMR deficient group (p=0.625). Males were more involved than females in both groups. Mean age of the patient was 54.8 years in deficient group which is slightly lower than proficient group (59 years). In the deficient group, the colon (proximal to the splenic flexure) was the primary site in all eight cases (100%), while in the proficient group, it is seen in only 19 cases (40.4%).

Histopathological examination showed an equal distribution of well 4 (50%) and moderately 4 (50%) differentiated tumours in the deficient group, with no cases found to be poorly differentiated. However, in the proficient group, the majority of cases were well-differentiated (44.4%). In the deficient group, adenocarcinoma NOS was found in 5 (62.5%) cases and adenocarcinoma with mucinous differentiation was found in 3 (37.5%) of cases. Mucinous differentiation was not found in the proficient group, however, this association was not statistically significant (p=0.188). MMR protein-deficient cases were mostly found in the T3 pathological stage 4 (57.14%) without any nodal involvement (N0), followed by T2 2 (28.6%) and T4 1 (14.3%) stages. The proficient group showed a random distribution across various stage.

DISCUSSION

Defects in MMR genes leads to accumulation of mutations which are not repaired which further leads to MSI. Hence, status of MMR protein in tumour cells is directly related to MSI status. These mutations can be sporadic or germline. MMR deficiency is associated with good prognosis and these patients have better stage adjusted survival compared to MSS tumours. The present study showed

loss of MMRp in eight cases (14.54%). As compared to studies by Ismael NE et al., 22 (42.3%) [7], Faghani M et al., 22 (28.9%) [8] and Kumar A et al., 52 (29%), the percentage of MMRp loss was low [9]. While similar results were found by Chauhan S et al., (15.4%) and Singh C et al., 14 (14%) [4,10]. This variability might be due to low cohort size. As per the Western literature, MSIH is found more commonly in sporadic cancers. Right sided colon cancer was the most common site for MSI deficient tumours. In the present study,

present study showed combined loss of MLH1 and PMS2 5 (62.5%) in maximum cases while MSH2 and MSH6 loss was seen in 3 (37.5%). Ismael NE et al., (2007) [7] showed MLH1 and PMS2 combined loss in 7 (30.8%) cases [3]. Kumar A et al., (2018) found combined loss of MSH2+MSH6 seen in 6 (11.5%) cases along with isolated loss of PMS2 in 5 (9.6%) patients [9]. Singh C et al, 2021 also found loss of MSH2+MSH6 in 3 (21.4%) cases [10]. MMR colorectal cancers have predilection for the right colon. In a study by Arora S et al., [2] 10 (66.7%) and Chauhan S et al., [4] 3 (75%) show more incidence of MSI deficient tumours in right-sided colon cancer. The present study also showed similar results. But Kanth VV et al., had more incidence of MSI positive tumours in rectum 37 (40.7%) [5]. MMR deficient colorectal cancers are known to have increased intratumoural and peritumoural lymphocytes. In the present study, MMR deficient cases show significant association with high TIL's (p-value <0.05), similar to Singh C et al., (p-value=0.002) [10]. But is Ismael NE et al., showed no significant association with TILs (p-value is 0.789) [7].

Adenocarcinoma, NOS 5 (62.5%) was the predominant type in deficient group while 3 (37.5%) of deficient cases showed mucinous differentiation. Li C et al., (2020) also found that MSI (p<0.001) is associated with a mucinous histology [11]. As per the Western literature mucinous, medullary and signet ring cell types are more commonly associated with MMR deficiency. There was no case of poor differentiation in the deficient group in the present study. However, as per the literature, MMR deficiency is more commonly associated with poorly differentiated adenocarcinoma. Various studies have shown various association of MMR deficient status to metastasis [12-15]. In the present study, deficient cases show no distant metastasis.

Limitation(s)

The present study had few limitations. Owing to small sample size, the results were variable in some parameters like histological grade etc. Also, patients follow-up of treatment and further survival were not taken which might have added more to the clinical relevance of the results.

CONCLUSION(S)

The present study showed that middle aged males, mucinous histology, moderate differentiation, T3 stage and N0 nodal stage and proximal colon site tumours are more commonly found in MMR protein deficient tumours. Deficient cases were significantly associated with high TIL's. Integrating IHC based MMR protein expression with other clinical and pathological factors allows us to more accurately select patients who will benefit from immune checkpoint inhibitors. Because of the limited number of patients, statistical significance of the association was not established. Hence, larger cohort studies are recommened.

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