MICROSATELLITE INSTABILITY STATUS IN COLORECTAL CARCINOMA: EXPERIENCE OF AN INDIAN TERTIATY CARE HOSPITAL

Pathology

Prasanta Sengupta	MD (PATHOLOGY), Armed Forces Medical College, Pune, Maharashtra, India
Avnit Sidhu*	MD (PATHOLOGY), Command Hospital, Pune, Maharashtra, India *Corresponding Author
Nabeel Azeez K	MD (PATHOLOGY), Armed Forces Medical College, Pune, Maharashtra, India

ABSTRACT

Background: Carcinogenesis in colorectal carcinomas has unravelled numerous molecular pathways with microsatellite instability pathway gaining immense attention because of utility in diagnosis, management and prognosis.
Methods: A retrospective observational study of 34 colorectal carcinoma cases managed at an Indian tertiary care hospital during 2015-2017 was carried out. The relevant clinical data was archived and histopathology sections were analyzed followed by immunohistochemistry. IHC for four MMR markers was performed to assess the MSI status.
Results: Amongst the 34 cases studied, based on IHC only 3 (8.8%) cases had MSI-H and 2 (5.9%) cases had MSI-L. Majority cases 29 (85.3%) cases were microsatellite stable (MSS).
Conclusion: Despite microsatellite instability being detected in only a minor subset of patients, it was a worthy exercise in deciding the future management protocols. The reliability and ease of performing IHC adds further merit to this simple histopathology laboratory tool.

KEYWORDS

microsatellite instability, colorectal carcinoma, IHC

INTRODUCTION

Carcinogenesis in colorectal carcinomas (CRC) has generated great interest worldwide and numerous molecular pathways have unravelled. Amongst the various pathways of carcinogenesis microsatellite instability (MSI) pathway has gained immense attention and has emerged as a useful tool in predicting individual predisposition, prognosis and treatment options. It is now known that deficient mismatch repair (MMR) of DNA leads to microsatellite instability which is recognized by the presence of insertion or deletion mutations in repetitive DNA sequences, called microsatellites ie mononucleotide, dinucleotide or polynucleotide sequence loci. MSI has been reported not just in colorectal carcinoma but even in carcinomas arising at non-colonic sites like endometrium, gastric, small intestine, hepatic, pancreaticobiliary, ureteral, ovarian and brain [1, 2].

Mechanism of MSI has been reported in both hereditary and sporadic CRC cases [3].

MSI is reportedly detected in 15-20 % of sporadic CRC cases and in 3-5% cases of Lynch syndrome [4]. MSI in relation to CRC is due to inactivation of any of the several mismatch repair genes (MMR) namely, MLH1, MSH2, MSH6 or PMS2. Germline mutations in the MMR lead to Lynch syndrome with MLH1 and MSH2 mutations contributing for 40% cases each. While MSH6 and PMS2 mutations are less common accounting for 10% and 5% cases respectively.

On the other hand most sporadic cases are due to MLH1 gene inactivation consequent to promoter hypermethylation [5]. Three distinct MSI phenotypes have been described in sporadic cases ie, microsatellite stable (MSS), MSI with low instability (MSI-L) and MSI with high instability (MSI-H) [6].

MSI-H histology is linked with distinct features like presence of tumors with signet-ring cell or mucinous or medullary pattern, presence of Crohn-like lymphoid reaction and increased tumor infiltrating lymphocytes (TIL) [7]. Additional features include right sided cancers, intra tumoral heterogeneity and absence of dirty necrosis [8, 9, 10].

Studies have shown improved survival rates, lower risk of tumor metastasis and poor response to adjuvant 5-fluorouracil-based chemotherapy in CRC with MSI [11].

Thus the necessity for detecting MSI status in CRC in the current era cannot be understated. We undertook this retrospective study at an Indian tertiary care hospital to correlate the histopathological features with MSI status in CRC cases using immunohistochemistry (IHC) as the first-line-screening tool.

MATERIAL AND METHODS

We retrospectively studied 34 cases of colorectal carcinoma diagnosed at our hospital from 2015 to 2017. Relevant clinical and demographic data was retrieved from the request forms and case records. Histopathology reports and slides were reassessed. From the archival paraffin blocks 3 um sections were restained for Hematoxylin & Eosin (H&E) and revaluated.

In all the cases gross findings including tumor location were noted. Histopathological findings taken into account were tumor type, grade, lymphovascular invasion and lymph node status along with the clinical stage. Additional features to be noted were tumor budding, pushing/infiltrative border, dirty necrosis, TIL, preitumoral lymphocytes and Crohn-like reaction.

Tumor budding was defined as an isolated tumor cell or cluster with less than five tumor cells, observed in the stroma of the actively invasive front. It was observed at high power magnification by conventional H&E stain assisted with pan-cytokeratin IHC.

Pushing tumor border would have round circumscribed configuration under low magnification with clear delineation of the invasive front and host tissue. While an infiltrative border would show dissection of tumor through the anatomic structures of bowel wall with little or absent desmoplastic stromal response. The dissecting tumor glands form irregular clusters or cords of cells with residual host tissue in between giving a pattern of "streaming dissection". Dirty necrosis was to be characterized by luminal necrotic debris [12].

TIL was defined as presence of $\geq$ 2TIL/10 HPF on H&E staining. Crohn-like reaction was defined as $\geq$ 3 nodular lymphoid aggregates deep to the advancing tumor margin within a single x40 field and preitumoral lymphocytes are defined as a cap of chronic inflammatory cells seen in the deep invasive border of the tumor [8]. In addition IHC was also performed for MSI markers on the deparaffinized and rehydrated sections. Heat induced antigen retrieval protocol using tris-EDTA buffer was performed. Ready to use IHC antibody panel (Dako) for MMR included staining for MLH1, MSH2, MSH6 and PMS2. Loss of nuclear staining in tumor cells was considered as indicative of MSI while presence of nuclear staining was considered as MSS. Cases were classified as MSS (when all the markers were positive), MSI-L (when one of the markers was negative) and MSI-H (when two markers were negative). The adjacent non-neoplastic colonic epithelium and stromal inflammatory cells served as internal positive control. The results were analyzed using Fishers exact test.

RESULT

Our retrospective study included 34 cases of CRC, 21 (62%) were males and 13 (38%) were females. Highest number of cases 12 (35%) belonged to the age group of 51-60 years [Figure 1] followed by 9 cases in 61-70 years age bracket. Only 6 cases were less than 50 years of age which included 2 females and 4 males.
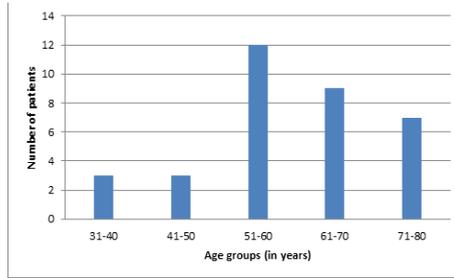

Figure 1. Age distribution of colorectal carcinoma cases

As regards to the tumor location, majority cases were of left sided tumor 21 (62%) with 17 (50%) being located in rectosigmoid and just 4 cases (12%) being located in descending colon. Right sided tumor was seen in 13 (38%) cases only.

Stage II cases were 14 and 07 cases were stage III.

Majority cases were graded as moderately differentiated (19 cases). In patients above 50 years of age, 60.7% (17 cases) were moderately differentiated while those with age less than 50 years include (2/6 cases) 33.3% cases [Table 1].

Table 1 – Comparison of characteristics of study population < 50 years and > 50 years					
Parameter		**Age**		**Total**	**p value**
		< 50 years (n =6)	**>50 years (n =28)**		
Sex	Female	2	10	13	0.65*
	Male	4	18	21	
tumor site	Left	3	18	21	0.65*
	Right	3	10	13	
grade (differentiation)	Moderate	2	17	19	0.33
	Poorly	1	5	6	
	Well	3	6	9	
LVI	Absent	5	21	26	1*
	Present	1	7	8	
lymphocytic response	marked	3	4	7	0.12
	mild	2	11	13	
	moderate	1	13	14	
dirty necrosis	Absent	5	13	18	0.18*
	Present	1	15	16	
TIL	Absent	6	20	26	0.29*
	Present	0	8	8	
Crohn-like reaction	Absent	5	27	32	
	Present	1	1	2	0.33*
Border configuration	infiltrative	1	19	20	0.05*
	pushing	5	9	14	
tumor budding	Absent	5	8	13	0.02*
	Present	1	20	21	
MLH1 staining	No	2	3	5	0.21*
	Yes	4	25	29	
PMS 2 staining	No	2	1	3	0.07*
	Yes	4	27	31	
*Fishers exact test used since > 25% have expected count less than 5					

Based on IHC 3 cases were MSI-H, 2cases were MSI-L and 29 wee MSS. The MSI-H cases included 33 year female with right sided tumor with well differentiated adenocarcinoma on microscopy; the second case was 46 year male with left sided tumor with signet-ring cell morphology and the third case was 53 year male with left sided tumor having medullary morphology.

Lymphovascular invasion was noted in 8 cases (23.5%) however, it did not include the MSI-H and MSI-L cases. Marked lymphocytic response was noted in 7 cases (20%) which included crohn-like

reaction in 2 cases exhibiting MSI-H. Dirty necrosis was noted in 16 cases (47%) and was conspicuously absent in MSI-H and MSI-L cases. An infiltrative tumor border was seen in 20 cases (58.8%) and pushing border was seen in 14 cases (41.2%). Tumor budding was not noted in cases exhibiting MSI.

DISCUSSION

We studied 34 cases of colorectal carcinoma that were diagnosed at our tertiary care hospital during a three year period. In this study we attempted to correlate microscopic findings with the MSI status. IHC was used as the first-line-screening tool to evaluate MMR gene protein expression. We intended to aid patient management protocols and also to identify unsuspected cases of Lynch syndrome using a commonly available laboratory technique. Like other Indian studies male patients outnumbered the female patients. Most tumors were located on the left with rectosigmoid accounting 50% cases and 38% cases were right sided in location. Other Indian studies by Bhagyalakshmi et al and Laishram et al have also reported similar statistics [13, 14].

GLOBOCAN 2018 statistics reveal highest colon cancer incidence rates in parts of Europe and North America while much lower incidence is reported from South and Central Asia including India [15]. In our study 3/34 (8.8%) cases only demonstrated loss of two of the four MSI markers tested qualifying for MSI-H. While western literature reveals that 15 % sporadic are due to defective MMR. We also did not detect a single case of Lynch syndrome. However, literature reports that 2-4 % cases of Lynch syndrome have germline mutations of MSI pathway. Our study is in concordance with the Iranian study by Kheirelseid et al who reported MSI in 9% cases. Other studies have reported relatively higher rates, a Chinese study by Yuan et al reported MSI in 24.3%, Indian study by Dubey et al reported MSI-H in 22.2% cases and Srdjan et al reported MSI-H in 20% cases [16, 17]. We report loss of IHC expression for MSI markers in a minority of cases only, which include 3 cases of MSI-H, 2 cases of MSI-L and 29 MSS cases. Hence, suggesting that MSI pathway is involved in only a small subset of CRC cases amongst our patient cohort. MSI-H cases showed loss of immunostaining for both MLH1 and PMS 2 and had distinct morphology [Figure 2 & 3]. This included two cases which were tumors of right-sided colon occurring in a 33 year female and 56 year male. While the third case was a 46 year male with left sided tumor. Microscopy of these cases included well differentiated adenocarcinoma, medullary carcinoma and signet-ring cell carcinoma respectively. Crohn-like lymphoid reaction and pushing border morphology was noted in two cases of MSI-H phenotype. MSI-L was noted in patients above fifty years with both cases occurring in left side of colon and showing loss of immunostaining for only MLH-1. On microscopy one case was moderately differentiated adenocarcinoma with mucinous morphology and the other case was poorly differentiated adenocarcinoma.
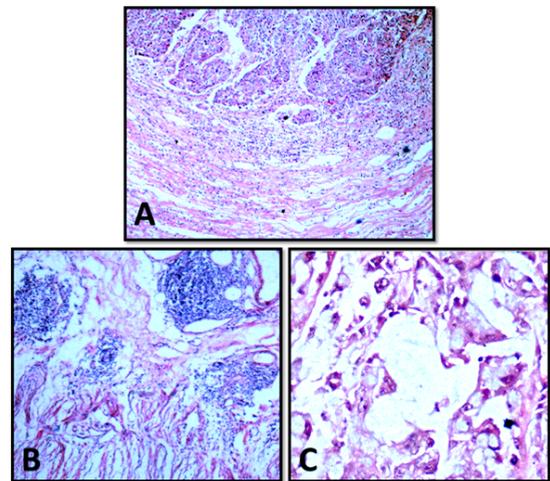

Figure. 2 – Photomicrogaph showing (A) poorly differentiated carcinoma with medullary growth pattern, H&E x40. (B) Crohn-like lymphoid aggregate, H&E x100. (C) Mucinous carcinoma with signet-ring cell morphology, H&E, x100
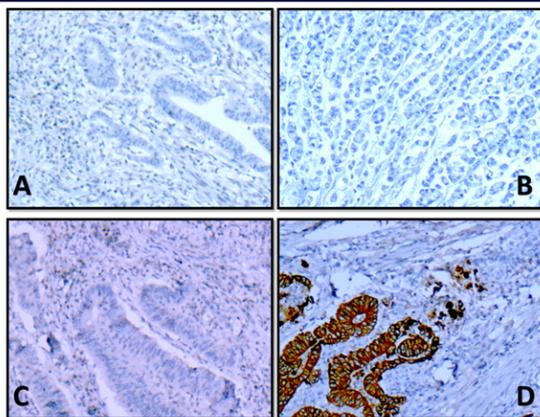
Figure 3. Photomicrographs (A & C) exhibit lack of staining for MLH1 & PMS2 respectively. Expression of MLH1 & PMS2 in stromal inflammatory cells provides internal control, IHC x100. (B) lack of MLH1 staining in signet ring carcinoma, IHC x400 (D) Pancytokeratin highlighting tumor buds at the invasive front, IHC x100.

Our study did not show any significant association between MSI expression and patient parameters with regard to age, gender, anatomical side or morphology. Similar results have been reported in an African study by Duduyeni *et al* as well [18]. A drawback of our study is the smaller sample size from a single institute which may not be representative of the entire Indian population. However, it is still a worthwhile exercise to screen, diagnose and manage cases of colorectal carcinomas exhibiting microsatellite instability in accordance with the recommended guidelines. We did not perform molecular testing in our study but all cases with defective mismatch repair gene should be followed up with molecular testing for optimizing treatment protocols.

CONCLUSION
Immunohistochemistry is a simple, reliable and readily accessible laboratory technique which should undoubtedly be the first line screening tool for MSI detection and MSI screening should be performed in every case of colorectal carcinoma regardless of tumor location or morphology.

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