International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2021; 4(3): 39-45
Received: 07-05-2021
Accepted: 15-06-2021

Dr. Ajay Shanker Sharma
INHS Patanjali Hospital
Karwar, Naval Base Karwar,
Karnataka, India

Corresponding Author:
Dr. Ajay Shanker Sharma
INHS Patanjali Hospital
Karwar, Naval Base Karwar,
Karnataka, India

Value of histopathology in predicting microsatellite instability in colorectal cancer

Dr. Ajay Shanker Sharma

DOI: https://doi.org/10.33545/pathol.2021.v4.i3a.388

Abstract
Background: Identifying colorectal cancers (CRCs) with high levels of microsatellite instability (MSI-H) is clinically important. Few studies reported the role of histology in identifying MSI-H colorectal carcinomas. Hence the present study was undertaken to assess the value of histopathology in predicting MSI-H and its correlation with histopathologic prognostic features in CRC.
Methods: Total 100 histo-pathologically confirmed cases of CRC were enrolled in the study. Tumour's histopathological typing, staging, and sizing was conducted based on WHO criteria. Tumour grade, extent of mucin production, tumour growth pattern and presence of a Crohn's like inflammatory infiltrate were determined. For immunohistochemistry (IHC) we used monoclonal antibodies (ES05 or 25D12).
Results: Among 100 tumour samples, loss of MLH1 or MSH2 expression was detected in 13(13%) cases (MSI tumours) while 87(87%) tumours showed normal expression for MLH1 and MSH2 immuno-reactivity (MSS tumours). In terms of MSI/MSS status of tumour samples, statistical analysis showed that abnormal MMR protein expression was associated with tumour site, side of colon with cancer, size of tumour, tumour infiltrating lymphocytes and crohn's like inflammatory infiltrate. Some histopathological and clinical features that appeared highly specific but much less sensitive in predicting MSI include stage, and some features were more sensitive and less specific which include tumour differentiation, CLR, TIL, dirty necrosis and pushing margin.
Conclusion: MSI is an important prognostic factor in CRC and an important predictive factor of CRC chemotherapeutic treatment and outcome efficacy. Also result of the study is not consistent in describing various histopathological and clinical features in predicting MSI, CRC.

Keywords: colorectal cancers; microsatellite instability; histopathology; immunohistochemistry; inflammatory infiltrate; chemotherapeutic

Introduction
Colorectal cancer (CRC) is the third most common cancer in men (10.0% of the total cancers) and the second in women (9.4% of the total cases) worldwide. Incidence rates in India are about 2 to 8 per 100,000 with men at a higher risk compared to women (1.4:1) [1, 2]. CRC is a heterogeneous disease with multiple known prognostic factors and many still being investigated. Sporadic colorectal carcinoma is major ly originates from chromosomal instability involving adenomatous polyposis coli and wingless type signalling pathway genes. The tumour is classified as MSI-high (MSI-H), if size alterations or shifts are observed in at least two of the five microsatellite markers or else is classified as MSI-low (MSI-L). If none of the markers show instability the tumour exhibits a microsatellite stable (MSS) phenotype. Most colorectal adenocarcinoma cases with hereditary nonpolyposis colorectal cancer (HNPCC) have occurrence of high frequency which tends to be diploid. The precursor lesion for sporadic MSI-H CRC is likely to be a sessile serrated adenoma (SSA) instead of conventional polypoid adenoma accounting for up to 17.5% of proximal CRCs. The MSI-H tumours have a propensity to occur in females and localizing the proximal colon. Histologic heterogeneity and a prominent inflammatory reaction at the advancing edge of the tumour (Crohn's-like reaction) are more likely to be MSI-H. It shows enhanced immunologic response with marked lymphocytic infiltration, presenting at a lower stage with a less aggressive course with a good response to certain adjuvant chemotherapy. Behaviour of MSI-L tumors MSI-L tumors show a similar behaviour to that of MSS tumors, creating an uncertainty regarding its clinical and biologic significance [3, 4].

MSI is characterized by a defect in any of the mismatch repair (MMR) genes, (MLH1, MSH2, PMS1, PMS2, GTBP/MSH6) that leads to incapacity to recognise and repair errors that occur during DNA replication. In human, nine genes with MMR function have been identified, of which five have particular clinical interest because they may be mutated in families with HNPCC (the relative frequency in parenthesis): MLH1 (49%), PMS1 (0.3%), PMS2 (2%), MSH2 (38%), and MSH6 (9%). The loss of DNA MMR activity accelerates the rate of accumulation of mutations in other genes involved in apoptosis and growth control that predispose to a more rapid adenoma-to-carcinoma transition [5]. However, in HNPCC, a germ-line mutation in any of the MMR genes (usually in hMLH1 or hMSH2) is accompanied by allelic loss that inactivates the MMR gene. Whereas an epigenetic event involving hypermethylation of the promoter region of MLH1 gene causes transcriptional silencing of gene. Furthermore, in sporadic tumours with MSI-H phenotype, a very high proportion of all tumours have methylation of the hMLH1 promoter. Therefore, hMLH1 or hMSH2 are either mutated or methylated in the vast majority of MSI-H tumours identified to date [6].

Monoclonal antibodies to the protein products of both hMLH1 and hMSH2 are now available. This technique is far less labour intensive than traditional MSI testing, and the results can be available to inform clinical decisions within 24 hours [7]. The present study was undertaken to assess the utility of IHC in identifying MSI patients using monoclonal antibody to MLH1 and MSH2. Also identify histopathologic features that are more commonly seen in microsatellite instable colorectal carcinoma and find out the correlation of IHC finding with histopathologic features.

Material and Methods

This retrospective study was conducted in 100 histo-pathologically confirmed cases of CRC and only resection specimens showing invasive adenocarcinoma of the colon and rectum were accepted for analysis. A prior ethical clearance was obtained from Institutional Ethical Committee. The cases included both Inpatients and Outpatients receiving treatment from our institute and were referred from various parts of the country. Patients with positive family history of colorectal cancer and recurrent / metastatic cases, adenomas with "intra-mucosal carcinoma or carcinoma in-situ" were excluded from the study. Demographic and clinico-pathological data of each patient including family history of colorectal carcinoma, location, grade and stage and type of tumour were collected from patient's medical records and verbal medical history.

Histopatholological analysis of tumours

Tumour's histopathological typing, staging, and sizing was conducted based on WHO criteria. Whereas criteria like grading, extent of mucin production, tumor's growth pattern, presence of inflammatory infiltrates, intraepithelial lymphocytes or peritumoural lymphocytes was determined without knowledge of mismatch repair status.

(a) Tumour Grade: Tumours were given a single grade of differentiation (well, moderate, or poor) based on the criteria of Jass and colleagues with minor modification.

(b) Mucinous differentiation: Tumours showing>50% areas as extracellular mucins were classified as mucinous else were classified as focal mucinous differentiation.

(c) Signet ring cells: Tumour cells with intra-cytoplasmic mucin vacuoles were designated as signet ring cells.

(d) Histologic heterogeneity: Tumours with at least two distinct growth patterns were classified as showing histological heterogeneity.

(e) Growth pattern of tumour at advancing edge: The advancing edge of the tumour was examined at low power to determine whether the tumour grew with a pushing/expansile pattern or an infiltrative pattern. If the advancing edge of the tumour was not present, this field was coded as unknown.

(f) Tumour necrosis: Tumours were assessed for the presence or absence of dirty or garland necrosis. If only a rare focus of necrosis was present (< 10%) then the tumour was classified as negative. Large geographic areas of necrosis (infarcted tumour) were not included.

(g) Prominent Crohn's-like host response: The advancing edge of the tumour was assessed. Prominent reaction was defined as a minimum of three lymphoid aggregates per section. Tumors with an absent advancing edge were graded as unknown.

(h) Tumour infiltrating lymphocytes (TIL):- TILs were identified on H and E stained sections as small blue mononuclear cells that typically had a halo around them. Only cells infiltrating between tumour cells were counted. Care was taken not to count apoptotic cells. The tumour was scanned at low powerto look for the area with the most TILs (which was often the more superficial region of a deeply invasive carcinoma). Intraepithelial lymphocytes were identified and classified as conspicuous.

TNM Staging was used to classify the tumours. Care was taken not to count apoptotic cells. The tumour was scanned at low power to look for the area with the most TILs. The tumour growth pattern was interpreted as infiltrative or expansile. Presence of residual adenomatous tissue, around the cancer, and distant colonic polyps (hyper -plastic, adenomatous, or serrated) was noted.

Immunohistochemistry

We used in monoclonal antibodies against MLH1 and MSH2 as given in Table 1. One paraffin embedded tissue block from each resected bowel specimen containing carcinoma and preferably adjacent non-neoplastic colon were selected. 4 μm thick tissue sections were cut. After cutting IHC procedure was completed. Normal colorectal tissue adjacent to the carcinoma was used as the positive control. Loss of expression was recorded when nuclear staining was observed in normal tissue but not in adjacent malignant cells.

Table 1: Details of Immunohistochemistry Antibodies

Antigen	Antibody clone	Dilution of concentrated antibody	Source
hMLH1	ES05	1/200	Dako, Glostrup, Denmark
hMSH2	25D12	1/400	Thermo Fischer scientific Anatomical Pathology. USA.

Data analysis

Data was being presented in numbers, percentages and mean ± SD. For categorical variables Chi square test were applied and for continuous variables Student's t test and one way analysis of variance (ANOVA) were done as per the nature of data. For statistical significance p value was considered at 5% level (p value < 0.05). The collected data were analysed using SPSS, Version 20.0.

Observations and Results

Total 100 cases were selected for the study; of them 58 (58%) were males and 42 (42%) females with male to female ratio of 1.38:1. The results of molecular testing for MSI status were summarized in figure 1. Overall 100 tumour samples, loss of MLH1 or MSH2 (Image I-a, b) expression was detected in 13 (13%) cases and those tumour were classified as MSI tumours. In these tumours normal MLH1 and MSH2 expression was noted in normal colon, (Image I-c, d). 87 tumours (87%) showed normal expression for MLH1 and MSH2 immuno-reactivity and classified as MSS colorectal tumours, (Image I e and f). Out of 13 MSI tumours, 10 carcinomas showed complete loss of MLH1 expression (76.92%) and normal (23.07%) immune-reactivity. Three tumours showed complete loss of MSH2 expression and normal immuno-reactivity for MLH1. There was no MSI tumour showing lack of both MLH1 and MSH2 expression.

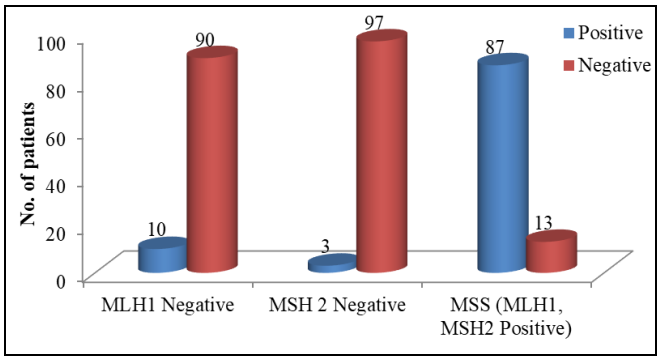


Fig 1: MLH1, MSH2 and MSS status in CRC Tumours

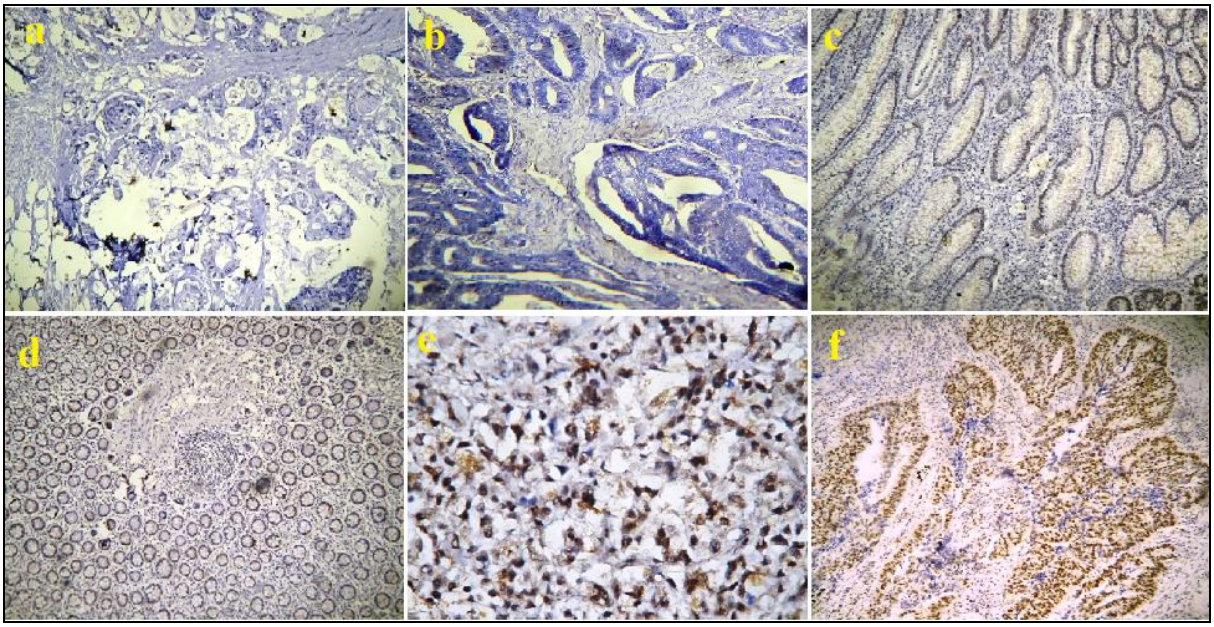


Image 1: a) Mucinous Adenocarcinoma Showing Loss of MLH1 Expression, **b)** Colorectal carcinoma showing loss of MSH2 expression, **c)** MLH1 positive IHC pattern in normal colon, **d)** MSH2 positive IHC pattern in normal colon, **e)** MLH1 positive signet ring cell carcinoma, **f)** Colorectal carcinoma showing MSH2 expression

The most common age group of patients was in sixth and seventh decade (59%) with mean age of 56 years, ranged from 32 to 94 years. Most of the patients among MSI and MSS tumours had age of 50-70 years (61.5% and 58.62% respectively). Both for MLH1 and MSH2 male patient were more affected than female. Age and sex distribution of patient in MSI and MSS tumours are shown in table 2.

Table 2: Distribution of age and sex of patient in MSI and MSS tumours

Variable distribution		Status		Total	p-value
		MSS	MSI		
Age groups	< 50 Yrs	17 (19.54%)	1 (7.69%)	18 (18%)	0.525
	50-70 Yrs	51 (58.62%)	8 (61.53%)	59 (59%)	
	>70 Yrs	19 (21.83%)	4 (30.76%)	23 (23%)	
Sex	Male	51 (58.62%)	7 (53.84%)	58 (58%)	0.745
	Female	36 (41.37%)	6 (46.15%)	42 (42%)	

The commonest site involved by colorectal carcinoma was distal colon and anal canal (56%). Dimensions of the tumours were ranging from 2 cm to 16.5 cm with the average of 7.6cm. Maximum of MSI and MSS tumours were 4-7 cm in size and most of the cases were classified as moderately differentiated adenocarcinoma. The predominant group of tumours in this study belonged to stage III. Maximum number of tumours had inconspicuous lymphocytic infiltration, inconspicuous Crohn-like lymphoid reaction and inconspicuous pushing margin. 15% tumour showed dirty necrosis as shown in table 3.

Table 3: Clinico-pathological data of the patient in MSI and MSS Tumours

Variable distribution		Status		Total	P value
		MSS	MSI		
Toumour site	Proximal colon	34 (39.1%)	10 (76.9%)	44 (44%)	0.01
	Distal colon / Rectum	53 (60.9%)	3 (23.1%)	56 (56%)	
Tumour size	< 4cm	10 (11.49%)	2 (15.4%)	12 (12%)	0.029
	4-7 cm	67(77.01%)	6 (46.2%)	73 (73%)	
	>7 cm	10(11.49%)	5 (38.4%)	15 (15%)	
Tumour Type	Poorly differentiated adenocarcinoma	10 (11.49%)	2 (15.4%)	12 (12%)	0.62
	Moderately differentiated adenocarcinoma.	46 (52.87%)	4 (30.8%)	50 (50%)	
	Well differentiated adenocarcinoma	10 (11.49%)	3 (23.1%)	13 (13%)	
	Mucinous adenocarcinoma	15 (17.24%)	3 (23.1%)	18 (18%)	
	Signet ring cell adenocarcinoma	6 (6.89%)	1 (7.7%)	7 (7%)	
Grade of Tumor	Poorly differentiated	10 (11.49%)	2 (15.4%)	12 (12%)	0.434
	Moderately differentiated,	67 (77.01%)	8 (61.53%)	75 (75%)	
	Well differentiated	10 (11.49%)	3 (23.1%)	13 (13%)	
Staging of Tumor	Stage I	20 (22.98%)	1 (7.7%)	21 (21%)	0.48
	Stage II	30 (34.48%)	4 (30.76%)	34 (34%)	
	Stage III	30 (34.48%)	7 (53.84%)	37 (37%)	
	Stage IV	7 (8.04%)	1 (7.7%)	8 (8%)	
Tumour infiltrating lymphocytes	Marked	12 (13.79%)	5 (38.46%)	17 (17%)	0.027
	Inconspicuous	75 (86.20%)	8 (61.53%)	83 (83%)	
Crohn's like infla-mematory reaction	Conspicuous	9 (10.34%)	4 (30.76%)	13 (13%)	0.041
	Inconspicuous	78 (89.65%)	9 (69.23%)	87 (87%)	
Tumours with Pushing margin	Present	7 (8.04%)	3 (23.07%)	10 (10%)	0.092
	Absent	80 (91.95%)	10 (76.92%)	90 (90%)	
Dirty necrosis	Present	12 (13.79%)	3 (23.07%)	15 (15%)	0.382
	Not seen	75 (86.20%)	10 (76.92%)	85 (85%)	

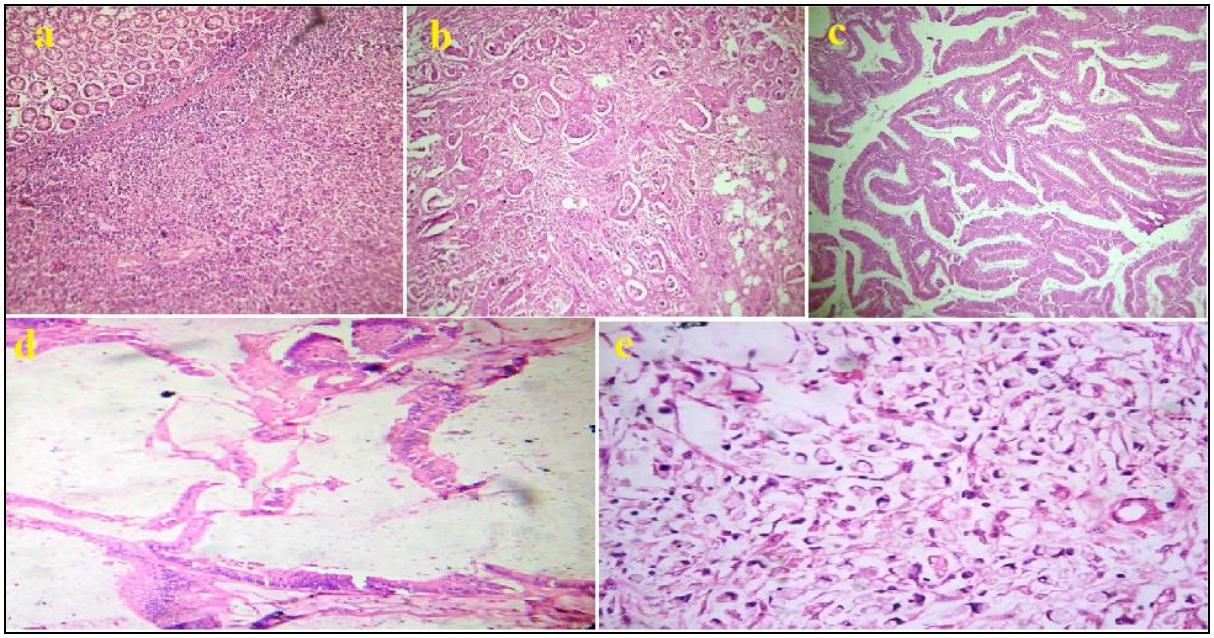


Image 2: a) Poorly differentiated colorectal carcinoma showing adjacent normal mucosa, **b)** Moderately differentiated colorectal carcinoma, **c)** Well differentiated colorectal adenocarcinoma, **d)** Mucinous adenocarcinoma showing pools of mucin with islands of tumour cells, **e)** Signet ring cell carcinoma

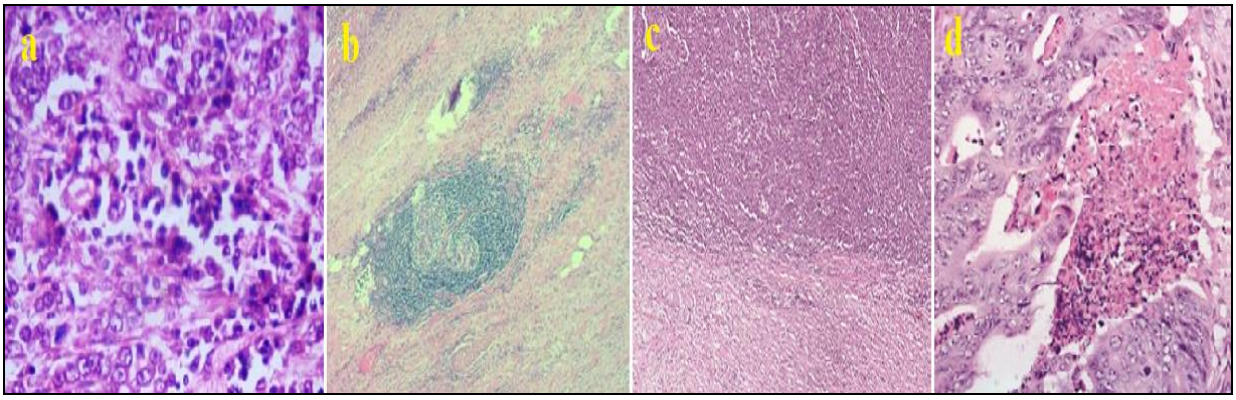


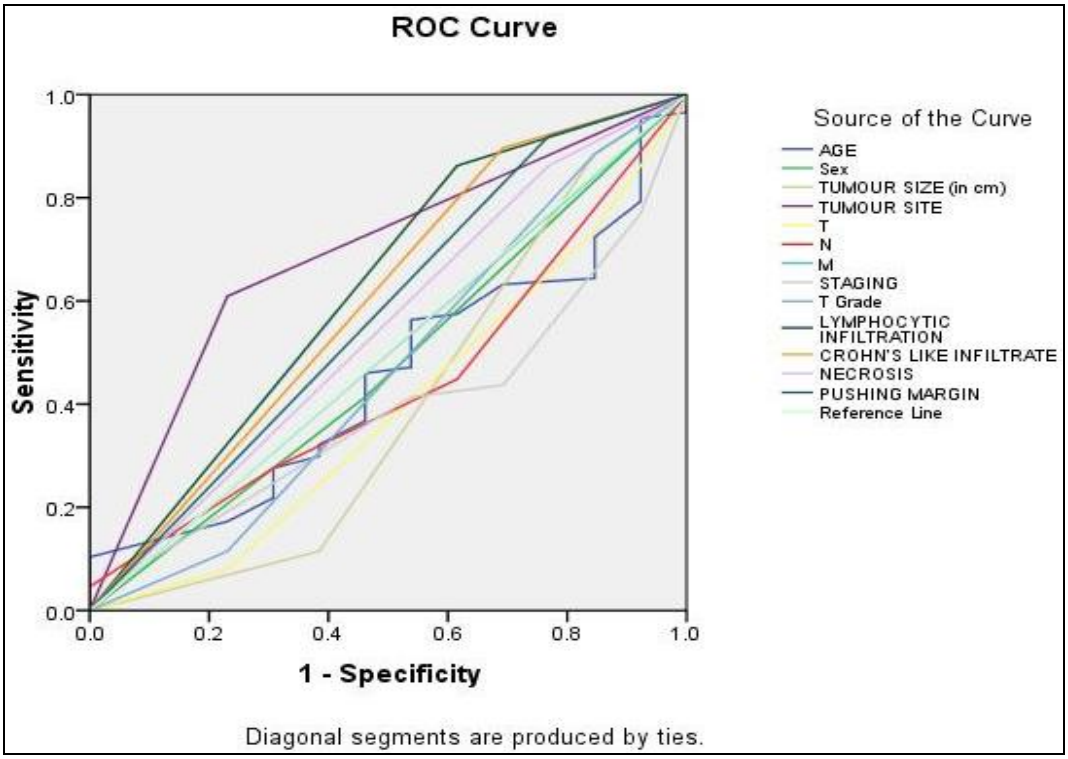
Image 3: a): Tumour Infiltrating Lymphocytes in CRC, **b)** Crohn's like Inflammatory Reaction, **c)** Pushing Border in Colorectal Carcinoma, **d)** Dirty Necrosis in Colorectal Carcinoma

Result of sensitivity and specificity of all variables studied are given in table-4 and ROC curve no-1 as below.

Table 4: Sensitivity and Specificity of Variables

Test Result Variables	Positive if Greater Than or Equal To	Sensitivity	1 -Specificity
Age	0	1	1
	1.5	0.92	0.923
	2.5	0.805	0.923
	3.5	0.471	0.538
	4.5	0.218	0.308
	6	0	0
Sex	0	1	1
	1.5	0.414	0.462
	3	0	0
Tumour Grade	0	1	1
	1.5	0.885	0.846
	2.5	0.115	0.231
	4	0	0
Tumour Stage	0	1	1
	1.5	0.77	0.923
	2.5	0.425	0.615
	3.5	0.08	0.077
	5	0	0
Tumour site	0	1	1
	1.5	0.609	0.231
	3	0	0
Tumour size (in cm)	0	1	1
	1.5	0.885	0.846
	2.5	0.115	0.385
	4	0	0
Tumour infiltrating lymphocytes	0	1	1
	1.5	0.862	0.615
	3	0	0
Crohn's like inflammatory reaction	0	1	1
	1.5	0.897	0.692
	3	0	0
Necrosis	0	1	1
	1.5	0.862	0.769
	3	0	0
Pushing margin	0	1	1
	1.5	0.92	0.769
	3	0	0

There was a significant difference in this TIL between MSI cases and MSS cases ($P < 0.027$). A ROC characteristic curve was constructed for TIL/10 HPF as below. The area under the curve was 0.62, indicating that TIL counts showed sensitivity in discriminating MSI-H cases. When using a cut-point of 1.5, the sensitivity was 86.2% and the specificity was 61.5%. Also for ROC curve for CLR, there was a significant difference in between MSI cases and non-MSS cases ($P < 0.04$). Area under ROC curve is .60. When using a cut-point of 1.5, the sensitivity was 89.7% and the specificity was 69.5%, (Curve-1).



Curve 1: ROC Curve for Various Variables in Our Study

Discussion

In the present study, we have examined the frequency and significance of MSI in a population with apparently sporadic CRC with some possibly harbouring germline mutations. MSI tumours associated with apparent somatic loss of MLH1 were more common in the elderly and in men. The utility of histology may be further amplified by other easily applicable techniques, such as IHC to detect loss of DNA mismatch repair gene products. Clinical information also helps in identifying MSI tumours. In sporadic cases, older age of onset, right-sided location, and less advanced stage raise the likely hood of MSI tumours. Results on the role of histology in predicting MSI discussed was mostly based on mixed HNPCC and sporadic patient and very less studied in separately for sporadic and familial case. Previous studies using such patient populations assumed that HNPCC-associated MSI tumours and sporadic MSI-H tumours have similar morphology.

In terms of MSI/MSS status of tumour samples, statistical analysis showed that abnormal MMR protein expression was associated with tumour site, side of colon with cancer, size of tumour, tumour infiltrating lymphocytes and crohn's like inflammatory infiltrate. There was no association between abnormal MMR gene protein expression and patient age, tumour type, tumour size, grade stage necrosis and pushing margin. The result of various other clinico-pathological studies [8-14] done on MSI, CRC and their results are compared with present study. While the result of our study indicated is not consistent in describing various histopathological and clinical features in predicting MSI, CRC. This difference may be due to biased patient selection, (bias are higher in our study because it consists of personals and relatives of Indian Armed Forces who are not representative of general population) and difference in sensitivity and specificity between various technique in assessing MSI and technical and nontechnical errors. Some features that appeared in our study are highly specific but much less sensitive in predicting MSI and these include stage, and some feature are more sensitive and less specific which, include tumour differentiation, CLR, TIL, dirty necrosis and pushing margin.

Moreover, histology stands today as a first-line screening tool for identification of MSI, CRC and its prognosis. It may not be used as a substitute for a sophisticated method for establishing MSI status, owing to lack of specific identification features and considerable inter-observer variability. The less desirable sensitivity of TIL/10 HPF when using a cut-point of 5 observed in this study (80.5%) differed from that reported by Smyrk *et al.* [15] (93%). The biological and clinical implications of MSI in cancer continue to develop. The clinic-pathological, prognostic, genetic, epigenetic and therapeutic characteristics of MSI, CRC are now becoming clear, but they still remain to be fully clarified. In various studies, there are no clear cut indications that prove significance of histopathology in predicting microsatellite instability in CRC. Histology is and should always support by more sensitive and specific test such as PCR amplification of microsatellite repeats, Hybridization Probe Melting Point Analysis and polyacrylamide gel analysis to diagnose MSI status of tumour. Due to lack of specific histological identification features and considerable variability in the interpretation of certain morphologic findings due to observer variability, the histopathologic differences may not be sufficient to impact on their value in predicting MSI status, but they underline the possibility of different pathogenic pathways.

Conclusion

MSI is an important prognostic factor in CRC and an important predictive factor of CRC chemotherapeutic treatment and outcome efficacy. Various clinical trials have shown contradictory findings in different chemotherapeutic settings, adjuvant and palliative; therefore MSI is going to be the object of the future research. The future of cancer treatment is in the individualized therapy based on molecular characteristics of the tumour.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-2917.
2. National Cancer Registry Programme. Accessed at: http://www.icmr.nic.in/ncrp/cancer _reg. html on November 4, 2010.
3. Lanza G, Gafa R, Maestri I, Santini A, Matteuzzi M, Cavazzini L. Immunohistochemical pattern of MLH1/MSH2 expression is related to clinical and pathological features in colorectal adenocarcinomas with microsatellite instability. Mod Pathol 2002;15(7):741-749.
4. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010;138(6):2073-2087.
5. Shia J, Ellis NA, Klimstra DS. The utility of immunohistochemical detection of DNA mismatch repair gene proteins. Virchows Arch 2004;445(5):431-41.
6. Goodenberger M, Lindor NM. Lynch syndrome and MYH associated polyposis: review and testing strategy. J Clin Gastroenterol 2011;45:488-500.
7. Lindor NM, Burgart LJ, Leontovich O, Goldberg RM, Cunningham JM, Sargent DJ *et al*. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. J Clin Oncol 2002;20:1043-1048.
8. Alexander J, Watanabe T, Wu TT, Rashid A, Li S, Hamilton SR. Histopathological identification of colon cancer with microsatellite instability. Am J Pathol 2001;158:527-535.
9. Benatti P, Gafà R, Barana D *et al*. MSI and Colorectal Cancer Prognosis. Clin Cancer Res 2005;11:8332-8340.
10. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. Am J Pathol 1994;145:148-156.
11. Lanza G, Gafà R, Maestri I, Santini A, Matteuzzi M, Cavazzini L. IHC Pattern of MLH1/MSH2 Expression Is Related to Clinical and Pathological Features in Colorectal Adenocarcinomas with Microsatellite Instability. Mod Pathol 2002;15(7):741-749.
12. Halvarsson B, Anderson H, DomanskaK, Lindmark G, Nilbert M. Clinicopathologic Factors Identify Sporadic Mismatch Repair–Defective Colon Cancers. Am J Clin Pathol 2008;13(2):238-244.
13. Ward R, Meagher A, Tomlinson I, O'Connor T, Norrie M, Wu R *et al*. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. Gut 2001;48:821-829.
14. Moghbeli M, Moaven O, Dadkhah E, Farzadnia M, Roshan NM, Bahar MM. High frequency of microsatellite instability in sporadic colorectal cancer patients in Iran. Gen and Mol Res 2011;10(4):3520-3529.
15. Smyrk TC, Watson P, Kaul K, Lynch. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer 2001;91:2417-2422.