Original Article

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Pathology Section

Comparison of Clinical and Histopathological Parameters amongst Microsatellite Unstable and Microsatellite Stable Cases of Colorectal Carcinomas in an Indian Setting

DIVYA SHELLY[1], KV VINU BALRAAM[2], REENA BHARADWAJ[3], C BHARANI[4]

ABSTRACT

Introduction: In this era of prognosis based medicine, it is important to identify microsatellite unstable Colorectal Cancers (CRCs) as they offer good prospects to the patient and they respond poorly to 5-fluorouracil and platinum based chemotherapeutic regime.

Aim: To find out the prevalence of Microsatellite Instability-High (MSI-H) in CRC, to identify clinicopathological features associated with Microsatellite Instability (MSI) and assess the value of surgical pathology in predicting MSI-H.

Materials and Methods: The present study was a case-control study conducted in a tertiary care centre of Pune in Western India from January 2013 to December 2020. Thirty-five CRCs deficient in Mismatch Repair (MMR) proteins contrasted with 206 Microsatellite Stable (MSS) CRCs were studied and analysed for a given set of clinical and histopathological parameters to find out any correlation between the occurrence of microsatellite unstable tumours and these variables were presented as percentages.

Results: In the present study, the prevalence rate of MSI-H was found to be 14.5% and the statistical analysis was carried out using the software Statistical Package for the Social Sciences (SPSS) version 27.0. Univariate analysis revealed that right-sided/proximal location of tumours, age at diagnosis less than 50 years, no lymph node deposits (N_0 disease), presence of Tumour Infiltrating Lymphocytes (TILs), peri-tumoural reaction, mucinous component, increased stromal plasma cells, histological heterogeneity, signet ring/medullary component and Crohn-like reaction were all statistically significant predictors of microsatellite instability (p-value <0.05). Multivariate analysis of these significant parameters revealed right-sided location of tumours, age at diagnosis less than 50 years, N_0 disease, and presence of TILs, increased stromal plasma cells, histological heterogeneity and Crohn-like reaction to be independent predictors.

Conclusion: Clinical parameters and histological evaluation is handy in screening for the MSI-H colorectal carcinomas. This would go a long way in selecting the patients who will require confirmatory molecular testing and thus precluding the need of Immunohistochemistry (IHC), which will be helpful in day-to-day practice as it is uncomplicated, cost-effective and easy to replicate.

Keywords: Deoxyribonucleic acid mismatch repair, Immunohistochemistry, Microsatellite instability

INTRODUCTION

The CRC are amongst the most common malignant neoplasms in industrialised countries and are currently the third most common cancer taking into account both men and women in the United States and also ranks third in mortality among cancers [1]. In the Asian continent, the incidence rates of this malignancy vary in different provinces, which are lower in the South Asian countries and are on the higher side in the developed countries like Japan [2]. Population grounded time trend studies indicate a rising fashion in the occurrence of CRC in India [3].

Approximately, 15% of sporadic CRCs demonstrate MMR deficiency, primarily because of methylation of the MutL Homolog 1 (MLH1) promoter [4]. CRCs with MMR deficiency manifest MSI-H. IHC can be used to screen for loss of MMR proteins, whereas Polymerase Chain Reaction (PCR) based methods can accurately diagnose MSI. The remainder of the cases are MSS cases.

In addition to young age at diagnosis and proximal location, particular histological features are associated with MSI. Some of these include mucinous, signet ring cell or medullary differentiation; TILs or a Crohn-like reaction at the advancing edge of the tumour; an expansile border; poor differentiation; and marked intratumoural heterogeneity [5,6].

Identifying MSI CRCs are clinically important as they are usually associated with a better prognosis and also, they play the role of being a surrogate marker of resistance to chemotherapeutic agents like platinum based compounds and 5-fluorouracil (5-FU) [7-10]. Many studies have revealed that tumour histology is a better predictor of MSI status than personal and family history of CRC [5,6].

In the modern era, the role of a pathologist is not just limited to giving a histopathological diagnosis. Use of ancillary techniques in the form of IHC and molecular tests have not only helped in better understanding of the biological behaviour of malignancies but have also opened doors for superior prognostication and advancement in management of tumours like development of specific targeted therapies [11,12].

There have been quite a number of studies in the western literature relating the histopathological parameters with the microsatellite instability. However, data is limited in the Indian setup. Hence, this study was undertaken to correlate the deficiency of MMR proteins in tumour with their certain histopathological parameters and thereby to assess the value of histopathology in predicting MSI-H.

MATERIALS AND METHODS

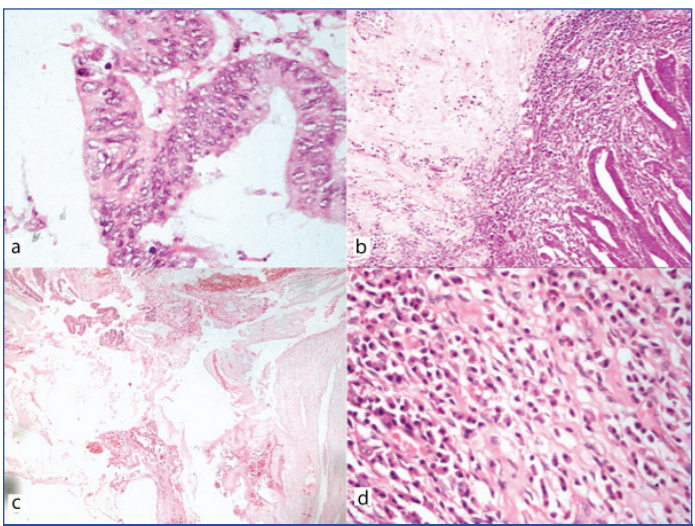
This was a case-control study conducted in the Department of Pathology of a tertiary care hospital in Western India in collaboration with the Department of Surgical Oncology and Gastrointestinal Surgery from the year 2016 to 2020. Ethical clearance was sought and informed consent was taken from the patients whose specimens were received postundertaking of the study (prospective cases) vide the ethical clearance number MUHS/PG-T/E1/FL42/2849/2016. Consent couldn't be taken for the cases retrieved from archives/ database (retrospective cases). Sample was collected both

prospectively and retrospectively. A total of 35 CRCs which were deficient in Deoxyribonucleic Acid (DNA) mismatch (MMR) proteins and 206 MSS cases of CRC were studied. MMR protein deficient cases are here designated as cases and MSS cases of CRC are designated as controls. Secondary tumours, non adenocarcinomas, recurrence and postchemotherapy specimens were excluded from this study. Only resection specimens were included in the index study. The possibility of occurrence of synchronous/metachronous as well as history of extracolonic cancers couldn't be taken into account as most of the data pertinent to studies were taken retrospectively with the help of stored paraffin blocks and archived database.

The database was thoroughly searched backwards with the help of the registers manually as well as online database dating back to the year 2013. The study was taken ahead prospectively till at least 35 MMR protein deficient samples were included by using a panel of IHC tests. Following IHC, they were divided into MMR protein deficient (cases) and MSS cases (controls). The immunostains used were anti-MLH1 (PathnSitu mouse monoclonal antibody clone GM011; Isotype IgG1), anti-MSH2 (PathnSitu rabbit monoclonal antibody clone RED2; Isotype rabbit IgG), anti-MSH6 (PathnSitu rabbit monoclonal antibody clone EP49; Isotype rabbit IgG) and anti-PMS2 (PathnSitu rabbit monoclonal antibody EP51; Isotype rabbit IgG).

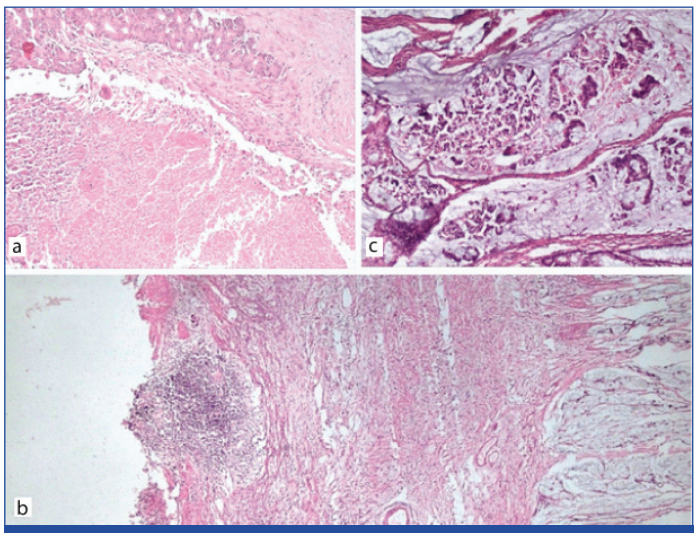
The IHC parameters were scored as either positive or negative. Strong nuclear positivity was considered positive, even if present focally. Loss of expression of even one of the IHC marker was considered to be MMR deficient case in the present study whereas samples with retained expression of all the markers were termed MSS. Care was taken to ensure that the internal controls (reactive lymphocytes and/or normal colonic mucosa) have worked properly before labelling a particular marker as not being expressed. After segregating into MMR deficient and MSS CRCs, the representative Haematoxylin and Eosin (H&E) slides were assessed for the following parameters:

1. The TILs were seen as small blue cells with a halo around them within the neoplastic glands or cells. The tumour was scanned in low power (100x) and then five consecutive fields were counted in high power (400x) and an average was calculated. More than 2 TILs/hpf was considered positive [Table/Fig-1a].
2. Peri-tumoural reaction, defined as a distinct band of lymphocytes at the advancing edge of the tumour in the subserosa or mesenteric fat [Table/Fig-1b].
3. Mucinous component- Any amount of extracellular dissecting mucin was taken as positive in the current study [Table/Fig-1c].



[Table/Fig-1]: a) Lymphocytes infiltrating the neoplastic glands which are surrounded by a halo (H&E 400x); b) Peri-tumoural reaction surrounding the invading glands at the advancing edge (H&E 100x); c) Mucinous component in colorectal adenocarcinoma (H&E 100x); d) Increased stromal plasma cells accounting for more than 25% of the immune cells (H&E 400x).

4. Increased stromal plasma cells- This was considered to be present when the amount of plasma cells infiltrate made up at least 25% of the population of the inflammatory cells [Table/Fig-1d].
5. Histological heterogeneity- Malignancies with two distinct set of patterns of growth were considered to be histologically heterogeneous. However, this terminology excluded co-existing mucinous and non mucinous areas.
6. Dirty luminal necrosis [Table/Fig-2a].
7. Crohn-like reaction- Presence of at least three well-formed lymphoid aggregates at the advancing edge of the tumour within one section was considered as positive [Table/Fig-2b].
8. Tubular component- Tubular component was defined as presence of tubular adenoma like areas in the vicinity of the malignancy.
9. Signet ring- Tumour cells exhibiting intra-cytoplasmic vacuoles with an eccentrically pushed hyperchromatic atypical nucleus was defined as signet ring cell [Table/Fig-2c].



[Table/Fig-2]: a) Neoplastic glands showing central dirty necrosis (H&E 100x); b) Crohn-like reaction in the form of lymphoid follicles at the advancing edge of the tumour; c) Infiltrating signet ring cells lying in extracellular pools of mucin.

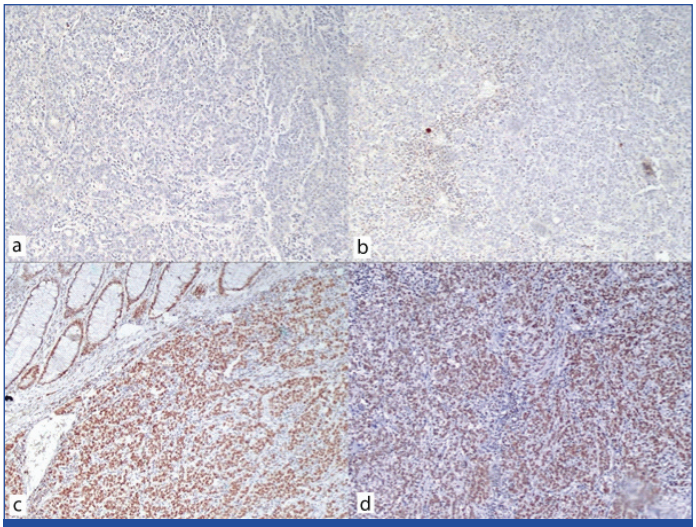
STATISTICAL ANALYSIS

The results were charted on the software SPSS version 27.0 and then compared for MMR deficient case groups and MSS control groups. Chi-square test was used for univariate analysis of the variables used in this study. Multivariate analysis was done using binomial logistic regression on parameters which were statistically significant on univariate analysis to find out the independent predictors of microsatellite instability. Spearman rank-order correlation was used to measure the extent to which the variables tend to change together describing the strength and the direction of relationship between the categorical variables.

RESULTS

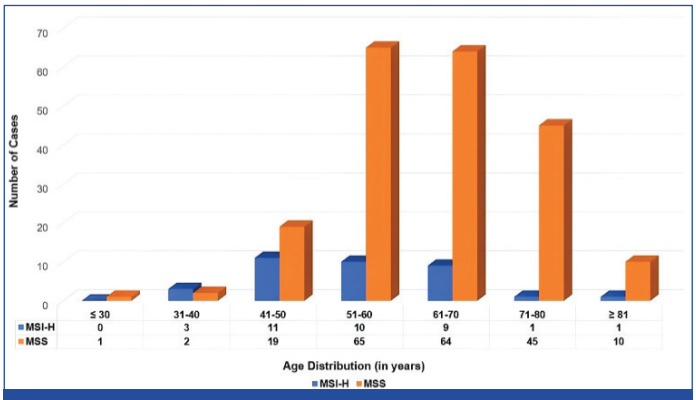
A total of 241 resection specimens were studied which included 35 MMR deficient case group and 206 MSS control group. The prevalence rate of MSI/deficient MMR proteins in the present study came out to be 14.52%. The 35 cases which were MMR protein deficient showed loss of MLH1 and PMS2 in 30 cases (85.7%), loss of MSH2 and MSH6 in two cases (5.7%) and loss of all four markers in the remaining three cases [Table/Fig-3].

The age of the patients ranged from 30-85 years (mean-62 years) with most of them in the age group 51-80 years (194/241 cases; 80.5%). In the cases, the age range included from as low as 32 years to a maximum age of 83 years with the average age being 55.4 years. Among the controls, the age range was from 30-85 years with the mean age at diagnosis being 63.2 years. The age distribution of the cases and controls are illustrated in [Table/Fig-4].



[Table/Fig-3]: a) Tumour cells showing loss of MLH1 (Internal control positive for the marker - Lymphocytes); b) Tumour cells showing loss of PMS2 (Internal control positive for the marker - Scattered lymphocytes); c) Tumour cells showing MSH2 expression (Positive internal control - Normal colonic mucosa); d) Tumour cells along with intervening reactive lymphocytes (Positive internal control) showing MSH6 expression.

The present study showed a male preponderance (1.98:1) and did not show any statistical significance in terms of association with occurrence of MMR protein deficiency.



[Table/Fig-4]: Age distribution comparison of MMR Protein Deficient (MSI-H) and MSS tumours.

Of the 241 CRCs, 98 (40.7%) were located on the right-side of colon. Of the 35 cases groups, 27 (77.2%) were in the right-side of colon. Around 9% of the total samples studied were poorly differentiated in which the percentage rose to 14.3% when it came to MSI-H tumours. There was no significant difference between grade of tumour in cases and controls in the present study.

Of the total 241 samples studied, 107 were node negative (N_0 disease) on histopathology. Within the cohort of cases, 28 (80%) were node negative and on histopathological evaluation, 21 (60%) had significant TILs of >2/hpf, 28 (80%) had prominent peri-tumoural reaction, 18 (51.4%) had extracellular dissecting mucin component, 26 (74.3%) had increased stromal plasma cells, 11 (31.4%) of them exhibited intra-tumoural histological heterogeneity, 27 (77.1%) had intraluminal dirty necrosis, 7 (20%) had signet ring morphology, 30 (85.7%) had prominent crohn-like reaction at the advancing edge of the tumour while only a minority (2/35 cases; 5.7%) had co-located tubular adenoma. The manifestation of MMR protein deficiency was significantly associated with all the factors mentioned barring presence/absence of dirty necrosis and co-existing tubular adenoma [Table/Fig-5].

Univariate analysis of the clinical and histopathological parameters revealed that right-sided/proximal location of tumours, age at diagnosis less than 50, no lymph node deposits (No disease), presence of TILs, peri-tumoural reaction, mucinous component, increased stromal plasma cells, histological heterogeneity, signet ring component and crohn-like reaction were all statistically significant predictors of MMR protein deficiency [Table/Fig-5].

Feature	MMR protein deficient tumours (n=35)	MSS tumours (n=206)	Total with feature	Odds ratio (95% CI)	Chi-square statistic	p-value
1. Anatomic site						
Right	27	71	98	6.42 (2.77-14.86)	22.5826	<0.001*
Left	08	135	143			
2. Age at diagnosis						
<50 years	14	22	36	5.58 (2.49-12.51)	20.2412	<0.001*
>50 years	21	184	205			
3. Sex						
Female	12	69	81	1.04 (0.49-2.21)	0.0084	0.927
Male	23	137	160			
4. Grade						
Poorly differentiated	05	17	22	1.85 (0.64-5.4)	1.3128	0.252
Well and moderately differentiated	30	189	219			
5. Lymph node status						
No node involved	28	79	107	6.43 (2.68-15.42)	21.0234	<0.001*
Node involved	07	127	134			
6. Tumour infiltrating lymphocytes						
Yes	21	29	50	9.16 (4.19-20.01)	38.3704	<0.001*
No	14	177	191			
7. Peri-tumoural reaction						
Yes	28	108	136	3.63 (1.52-8.68)	9.2509	0.002*
No	07	98	105			
8. Mucinous component						
Yes	18	64	82	2.35 (1.14-4.85)	5.5249	0.0187*
No	17	142	159			
9. Increased stromal plasma cells						
Yes	26	104	130	2.83 (1.27-6.34)	6.821	0.009*
No	09	102	111			
10. Histological heterogeneity						
Yes	11	10	21	8.98 (3.46-23.36)	26.5602	<0.001*
No	24	196	220			
11. Dirty necrosis						
No	08	27	35	1.96 (0.81-4.77)	2.2912	0.130
Yes	27	179	206			
12. Signet ring/Medullary component						
Yes	07	05	12	10.05 (2.99-33.83)	19.5262	<0.001*
No	28	201	229			
13. Crohn-like reaction						
Yes	30	111	141	5.14 (1.92-13.76)	12.4861	<0.001*
No	05	95	100			
14. Tubular component						
Yes	02	17	19	0.67 (0.15-3.05)	0.2654	0.606
No	33	189	222			

[Table/Fig-5]: Univariate analysis of all the variables in MMR protein deficient and MSS colorectal carcinomas.
*Statistically significant

Multivariate analysis of these significant parameters using binomial logistic regression analysis revealed N_0 disease, TILs, increased stromal plasma cells, histological heterogeneity and Crohn-like reaction to be independent predictors of the occurrence of MMR deficiency in the present study. Right-sided tumour age at diagnosis less than 50 years was the clinical parameters which were independent predictors of MMR protein deficiency [Table/Fig-6].

The sensitivity, specificity and predictive values of these independent predictors have been analysed and summarised in [Table/Fig-7].

Variables	B	S.E	Wald Chi-square test	Degrees of freedom (d.f)	Exp (B)	p-value
Right-sided anatomical location	2.007	0.626	10.292	1	7.442	0.001*
Age at diagnosis <50 years of age	1.314	0.584	5.053	1	3.720	0.025*
N_0 disease	-1.727	0.666	6.732	1	0.178	0.009*
Tumour infiltrating lymphocytes	2.243	0.579	14.993	1	9.426	0.001*
Peri-tumoural reaction	1.040	0.608	2.928	1	2.829	0.087
Mucinous component	0.876	0.557	2.474	1	2.402	0.116
Increased stromal plasma cells	1.382	0.601	5.292	1	3.984	0.021*
Histological heterogeneity	2.695	0.954	7.974	1	14.811	0.005*
Signet ring component	1.057	0.995	1.127	1	2.877	0.289
Crohn-like reaction	1.398	0.700	3.989	1	4.049	0.046*
Constant	-5.398	1.420	14.443	1	0.005	<0.001

[Table/Fig-6]: Multivariate analysis of statistically significant variables by using binomial logistic regression.
*Statistically significant

The parameters of sensitivity and specificity are more useful for the physicians in determining as to whether the patients are true/false negatives/positives, whereas the Positive and Negative Predictive Values (PPV and NPV) are more useful for the patients as it will tell the odds of having or not having a disease if one is tested positive or negative using that variable. As per the present analysis, the variables proximal tumours (77.14%), N_0 disease (80%), increased stromal plasma cells (74.29%) and crohn-like reaction (85.71%) had higher sensitivity while age at diagnosis <50 years (89.32%), TILs (85.92%) and intra-tumoural histological heterogeneity (95.15%) were more specific for diagnosing MMR protein deficiency. In the present study, all the seven clinico-pathological parameters had >89% NPV meaning absence of these parameters will more or less exclude MMR protein deficiency in patients of CRCs.

Variable	Sensitivity	Specificity	PPV	NPV
Right sided anatomical location	77.14%	65.53%	27.55%	94.41%
Age at diagnosis <50 years of age	40%	89.32%	38.88%	89.76%
N_0 disease	80%	61.65%	26.16%	94.78%
Tumour infiltrating lymphocytes	60%	85.92%	41.99%	92.67%
Increased stromal plasma cells	74.29%	49.51%	20%	91.89%
Histological heterogeneity	31.43%	95.15%	52.38%	89.09%
Crohn-like reaction	85.71%	46.12%	21.27%	95%

[Table/Fig-7]: Sensitivity, Specificity and Predictive values of the clinico-pathological parameters in predicting mismatch repair protein deficiency.
PPV: Positive predictive value; NPV: Negative predictive value

Spearman rank-order correlation was used to review the strength and direction of relationship between the categorical variables evaluated in the index study [Table/Fig-8]. The statistically significant correlation was found out between the following:

a. Proximal location of the tumour with signet ring component (+0.160) and Crohn-like reaction (+0.131).
b. Age at diagnosis <50 years with N_0 lymph node status (-0.141), TILs (+0.159) and histological heterogeneity (+0.159).
c. Grade of the tumour and histological heterogeneity (-0.157).

DISCUSSION

Extending the application of the ancillary techniques to the tumours of the colon and rectum has led to a tremendous increase in the existing plethora of knowledge on this topic. This, in turn, has given us a greater insight into the tumour biology of these colorectal adenocarcinomas.

The majority of MSI-H tumours have absent expression of MLH1 and PMS2 which was what it was found in present study as well [13-15]. Studies have been conducted across the globe to find out the association of histopathological features with the manifestation of MMR deficiency. Some have gone on to design some pathology-based models as well to predict the likelihood of the tumour being microsatellite unstable by studying the histopathology of the tumour [5,6,16].

Microsatellite instability associated CRCs in general have a favourable outcome as far as longevity is concerned, which has been confirmed in various researches [17-19]. They are also resistant to 5-fluorouracil, 6-thioguanine and platinum-based compounds [7-10].

The literature was reviewed to compare the results obtained in the present study to those by other investigators across the world which disclosed two studies conducted on the very similar lines one of which by Greenson JK et al., developed logistic regression-based models helping in prediction of MSI from clinical and histopathological parameters whereas the other by Hyde A et al., developed and validated prediction models which incorporated clinicopathological variables in forecasting MSI in CRC [5,6].

Apart from these, other interesting research articles were reviewed. Alexander J et al., and Halvarsson B et al., in their studies independently brought out that histopathological evaluation like tumour features and host immune response can be used to prioritise sporadic colon cancer [20,21]. Ward R et al., evaluated the clinical significance of MSI phenotype in sporadic CRC, and investigated methods for effective identification of MSI tumours in routine surgical pathology practice while Lanza G et al., specifically evaluated the prognostic magnitude of MMR status in a large cohort of stage II and III CRCs [22,23].

Literature reported around 15% of all sporadic cases to be MSI positive [4-6]. In the present study, 241 consecutive sample specimen tissue blocks were studied, out of which 35 samples were deficient in DNA MMR proteins. This comes to around 14.5% which is very much comparable to the prevalence rate derived from studies conducted by Greenson JK et al., and Hyde A et al., who studied a total of 1649 and 710 CRCs respectively [5,6].

In the index study, 98 (40.7%) of the total samples studied originated in the proximal colon. And out of the 35 cases groups, 27 (77.2%) were in the proximal part of colon. In a study conducted by Hyde A et al., right-sided carcinomas constituted 42.1% of all and amongst the MSI-H carcinomas, 60.3% were right-sided in anatomical location. About 9% of the total samples evaluated were poorly differentiated on histology whereas this proportion rose to 14.3% when it came to MSI-H tumours in index study. This figure was comparable with the study by Hyde A et al., which had 10.7% poorly differentiated carcinomas while the fraction of poorly differentiated CRCs in MSI tumours was 16.2% [6].

The study conducted on similar lines by Greenson JK et al., had found TILs, well/poorly differentiated tumour, age <50 years at diagnosis, crohn-like reaction, right-sided location, absence of dirty necrosis and extracellular mucinous component to be significantly associated with the occurrence of MSI-H [5]. In another study by Hyde A et al., they found TILs, anatomical site, peri-tumoural reaction, mucinous component, increased stromal plasma cells, intra-tumoural histological heterogeneity, absence of dirty necrosis, age at diagnosis <50 years, signet/medullary component, crohn-like reaction and female gender to significantly associated with the occurrence of MSI-H CRCs [6]. They did not find any association between occurrence of MSI-H with the grade of the tumour, angiogenesis or co-existing tubular adenoma. These two studies closely resemble the index study, however with subtle differences

Spearman's rho (2-tailed correlation)		Anatomical site	Age at diagnosis <50 years	Sex	Grade of tumour	Lymph node status	Tumour infiltrating lymphocytes	Peri tumoural reaction	Mucinous component	Increased stromal plasma cells	Histological heterogeneity	Dirty necrosis	Signet ring component	Crohn-like reaction	Tubular component
Anatomical site	Correlation coefficient	1.000	0.103	-0.055	-0.031	-0.076	-0.181**	0.097	0.101	0.019	0.074	-0.018	**0.160***	**0.131***	-0.023
	p-value		0.110	0.397	0.633	0.238	0.005	0.133	0.118	0.766	0.254	0.776	0.013	0.042	0.725
Age at diagnosis <50 years	Correlation coefficient	0.103	1.000	0.027	-0.029	**-0.141***	**0.159***	0.016	0.018	-0.010	**0.159***	-0.059	0.065	0.093	-0.123
	p-value	0.110	-	0.676	0.656	0.029	0.014	0.804	0.776	0.880	0.013	0.366	0.318	0.150	0.057
Sex	Correlation coefficient	-0.055	0.027	1.000	-0.073	0.018	0.017	-0.058	-0.027	0.030	0.033	0.006	0.042	-0.029	0.045
	p-value	0.397	0.676		0.259	0.777	0.788	0.368	0.680	0.645	0.611	0.927	0.519	0.658	0.485
Grade of tumour	Correlation coefficient	-0.031	-0.029	-0.073	1.000	-0.051	0.056	-0.046	0.015	-0.033	**-0.157***	-0.049	0.006	0.025	0.039
	p-value	0.633	0.656	0.259		0.428	0.390	0.477	0.820	0.613	0.014	0.450	0.922	0.694	0.544
Lymph node status	Correlation coefficient	-0.076	**-0.141***	0.018	-0.051	1.000	-0.181**	-0.078	-0.010	-0.072	-0.050	0.082	-0.103	-0.193**	0.106
	p-value	0.238	0.029	0.777	0.428		0.005	0.229	0.872	0.267	0.443	0.204	0.112	0.003	0.099
Tumour infiltrating lymphocytes	Correlation coefficient	0.181**	**0.159***	0.017	0.056	-0.181**	1.000	0.099	0.108	0.042	0.060	-0.080	0.024	0.099	-0.074
	p-value	0.005	0.014	0.788	0.390	0.005		0.126	0.095	0.520	0.357	0.219	0.711	0.127	0.254
Peri-tumoural reaction	Correlation coefficient	0.097	0.016	-0.058	-0.046	-0.078	0.099	1.000	0.030	-0.090	0.123	0.018	0.047	0.177**	0.009
	p-value	0.133	0.804	0.368	0.477	0.229	0.126		0.638	0.164	0.056	0.783	0.465	0.006	0.894
Mucinous component	Correlation coefficient	0.101	0.018	-0.027	0.015	-0.010	0.108	0.030	1.000	0.049	0.089	-0.027	0.117	0.018	0.017
	p-value	0.118	0.776	0.680	0.820	0.872	0.095	0.638		0.452	0.170	0.675	0.069	0.778	0.788
Increased stromal plasma cells	Correlation coefficient	0.019	-0.010	0.030	-0.033	-0.072	0.042	-0.090	0.049	1.000	0.049	-0.026	-0.018	0.067	0.054
	p-value	0.766	0.880	0.645	0.613	0.267	0.520	0.164	0.452		0.446	0.683	0.780	0.303	0.403
Histological heterogeneity	Correlation coefficient	0.074	**0.159***	0.033	**-0.157***	-0.050	0.060	0.123	0.089	0.049	1.000	-0.123	0.470**	-0.009	-0.036
	p-value	0.254	0.013	0.611	0.014	0.443	0.357	0.056	0.170	0.446		0.056	0.000	0.895	0.580
Dirty necrosis	Correlation coefficient	-0.018	-0.059	0.006	-0.049	0.082	-0.080	0.018	-0.027	-0.026	-0.123	1.000	-0.231**	0.011	-0.011
	p-value	0.776	0.366	0.927	0.450	0.204	0.219	0.783	0.675	0.683	0.056		0.000	0.860	0.871
Signet ring component	Correlation coefficient	**0.160***	0.065	0.042	0.006	-0.103	0.024	0.047	0.117	-0.018	0.470**	-0.231**	1.000	0.077	0.004
	p-value	0.013	0.318	0.519	0.922	0.112	0.711	0.465	0.069	0.780	0.000	0.000		0.236	0.953
Crohn-like reaction	Correlation coefficient	**0.131***	0.093	-0.029	0.025	-0.193**	0.099	0.177**	0.018	0.067	-0.009	0.011	0.077	1.000	0.121
	p-value	0.042	0.150	0.658	0.694	0.003	0.127	0.006	0.778	0.303	0.895	0.860	0.236		0.060
Tubular component	Correlation coefficient	-0.023	-0.123	0.045	0.039	0.106	-0.074	0.009	0.017	0.054	-0.036	-0.011	0.004	0.121	1.000
	p-value	0.725	0.057	0.485	0.544	0.099	0.254	0.894	0.788	0.403	0.580	0.871	0.953	0.060	

[Table/Fig-8]: Spearman rank-order correlation between the variables.
(* - Correlation is statistically significant at the 0.05 level)

as in there was no association with the presence or absence of dirty necrosis or gender in the index study with the incidence of MMR protein deficient CRCs unlike other studies [5,6].

The multivariate analysis corroborates well with the study by Greenson JK et al., who found TILs, well/poor differentiation, age <50 years, crohn-like reaction, proximal location, lack of dirty necrosis and mucinous component to be independent predictors of MSI-H in their research work [5].

Study by Greenson JK et al., revealed that lack of dirty necrosis, mucinous component and well/poor differentiation were more specific and proximal location of the tumour was more sensitive for identifying MSI-H. Crohn-like reaction revealed intermediate sensitivity whereas TILs were equally sensitive and specific for identifying MSI-H. The study pointed out that all of these variables had a higher NPV as seen in present work [5].

There were other variables which were found to be significantly related to the occurrence of MMR protein deficient tumours like poor differentiation, lack of dirty necrosis and female sex in the studies mentioned under reference but these variables were not significantly associated with the outcome under analysis in present study [5,6].

The differences could be attributed to the fact that the patient clientele in the present setup includes mainly the government employees and their dependant members of the family which may or may not be representative of the entire population. Also, the point to be taken into account is the ethnic and genetic differences in the population studied as, in the literature review, authors could only come across the studies done on the western world population. Thus, in this case, the comparison of data may not be entirely appropriate.

Limitation(s)

The spectrum of the present study is immense. However, the sample size in a larger amount would have added a greater weightage to the study and would have given better insight in delineating the correlation. Another constraint was the population included in the study (western India) which may not accurately be generalised to the entire gamut. Hence, the outcome of the present study is to be viewed within the scaffold of the above limitations.

CONCLUSION(S)

The CRCs are amongst the common malignancies of the gastrointestinal tract causing tremendous amount of morbidity and mortality among the sufferers. With the advancement in the understanding of the pathology and evolution of the disease, more and more targeted approach has come in light with regards to the management of the disease. Although MSI analysis by molecular studies is the first approach as far as detection of microsatellite instabilities associated CRCs are concerned, there is an alternative modality to suspect MSI. IHC methods can be used as a reliable tool in work up for MSI. MSI IHC markers (antibodies to the MMR proteins) are now easily accessible in most of the laboratories.

To conclude, authors would like to reaffirm that evaluation of histopathological parameters is handy in suspecting MSI-H colorectal carcinomas. Employing this method as an initial screening tool will reduce the costs of applying IHC panel on all the cases of CRCs and in addition, it will also be complimentary to the clinical input of family history for applying Amsterdam II criteria and Revised Bethesda Guidelines which will make a candidate more suitable for molecular testing thus making the overall process very much sensitive. In the experience of the present study, the authors feel that these clinical and histopathological attributes can form an important foundation and base for suspecting and finally identifying correctly the patients who are harbouring MSI-H tumours. This evaluation being unsophisticated, frugal and reproducible will hence be easy to employ in routine clinical scenario.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Pathology, INHS Asvini, Colaba, Mumbai, Maharashtra, India.
2. Graded Specialist, Department of Pathology, Military Hospital, Roorkee, Uttarakhand, India.
3. Consultant, Department of Pathology, Apollo Hospitals, New Delhi, India.
4. Senior Resident, Department of Pathology, Seth GS Medical College, Mumbai, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. KV Vinu Balraam,
Graded Specialist, Department of Pathology, Military Hospital Roorkee,
Roorkee Cantonment, Haridwar District-247667, Uttarakhand, India.
E-mail: vbalraam@gmail.com

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