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Pathological Spectrum of colorectal cancer in Barak valley with reference to MLH1, MSH2 and MSH6 Assay

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Abstract
Aim: Pathology of Colorectal Cancer (CRC) is associated with environmental factors, food habits, lifestyle as well as genetic causes. Different study revealed sporadic cases of CRC accounting to approximately 60% of CRC with Microsatellite Instability observed in 7 to 20% of all sporadic cases. This study was aimed to determine the pathological spectrum of CRC in Barak valley (constitute district Silchar, Karimganj and Hailagandhi) and secondly to correlate the histopathological spectrum with MSI marker protein MLH1, MSH2 and MSH6 by Immunohistochemistry (IHC).
Materials and Methods: A total of 54 CRC cases were assessed in the Department of Pathology out of all colectomy specimen and /or biopsy sample collected from the Department of Surgery, during the period from July 2013 to June 2014. IHC was done in paraffin embedded tissue containing carcinoma in total 32 cases with MLH1, MSH2 and MSH6.
Results: The mean age of CRC was 49.31 yrs with male to female ratio of 2:1. Rectum was the most common site of CRC (55.55%) and colon cases together constituted 44.45%. Histopathologically adenocarcinoma constituted 90.74% of which moderately differentiated constituting 53.70%. IHC detected 6 out of 32 cases showing loss of expression for one or more of MLH1, MSH2 and MSH6 (18.75%). Tumors with loss of expression for MLH1 was significantly associated with right sided CRC ($p = 0.00363$) and with poorly differentiated, mucinous and signet ring cell carcinoma by morphology ($p < 0.0001$).
Conclusion: 18.75% of sporadic CRC showed loss of expression of MMR protein. Use of IHC can add to determine cases with different clinical and morphological characteristics for further prognosis.

Keywords: CRC Colorectal cancer, IHC Immunohistochemistry, MMR Mismatch Repair, MSI Microsatellite Instability, Adenocarcinoma, Histopathological

1. Introduction

The incidence of colorectal cancer has been rising dramatically with changing lifestyle, economic development and industrialization. The highest incidence rates among both men and women are reported in Eastern European countries with lowest rates found in Asia [7].

The risk of developing CRC increases with age. Diagnosis is rare before the age of 40 years, and incidence begins to increase significantly after age 50 yrs. More than 90% of colorectal carcinoma seems to occur in people aged 50yrs or older [8]. The onset of familial and hereditary form of cancer occurs at much earlier age, in less than 40 yrs [9].

Various studies on different aspect of colorectal cancer are done, however in this part of our country few studies have been carried out. In our institution, in recent times no such study on colorectal cancer has been done. This study was done in the Department of Pathology in association with Department of Surgery to see the pathological spectrum of colorectal carcinoma through histopathological examination of biopsy sample and colectomy specimen collected from patient admitted in Surgery Department, Silchar Medical College, being the only one referral hospital in the Barak Valley. A special emphasis was given to correlate the different pathological aspects of colorectal cancer with immunohistochemistry for MLH1, MSH2 and MSH6 assay.

2. Materials and methods

The present work was a hospital based descriptive study conducted in the Department of Pathology, Silchar Medical College with the help of Department of Surgery, Silchar Medical College, Silchar from July 2013 to June 2014. Colectomy specimen from operated patient and biopsy samples were collected which were processed and studied. 54 cases of colorectal carcinoma diagnosed by routine histopathological examination were included in the study. Nonepithelial tumors, benign epithelial tumor and anal cancers were excluded.

Parameters Studied

I. Detailed clinical history and routine investigations after taking consent from the patients. II. Hospital records of the patients. III. Macroscopic examination of the colorectal carcinoma. Macroscopic growth type were recorded as per colonoscopy, sigmoidoscopy reports and by gross examination and classified as proliferative, ulceroproliferative and annular type. IV. Location of colorectal cancer were recorded by anatomical site as caecum, ascending colon, descending colon, sigmoid colon and rectum. Tumors were classified into right-sided cancers (from the caecum to and

including the splenic flexure), left-sided cancers (located in the descending and the sigmoid colon), and rectal cancers (rectal lesions). V. Microscopic examination of colectomy and biopsy tissues. VI. Immunohistochemistry on paraffin embedded tissue in histopathological diagnosed colorectal carcinoma in unselected patients.

Analysis of Data: All data were collected, compiled and subjected to suitable stastical analysis. For statistical analysis multiple logistic regression were performed, with the help of software Jmp 10 of SAS 9.3 and SPSS 15.0.

IHC Interpretation: Normal colonic mucosal epithelial cells and lymphocytes were reactive to MLH1, MSH2 and MSH6 marker protein and served as internal control. Loss of expression was recorded when all malignant cells showed absent nuclear staining or when less than 10% of tumour cells showed positive nuclear staining. Nuclear staining of more than 10% of tumour cells was considered positive for protein expression. Tumours with loss of expression of one or more of MLH1, MSH2 and MSH6 protein were considered to be tumours with mismatch repair defects (MMR-d) while tumours with intact expression for one of three proteins were considered to be non MMR-d tumours.

3. Results

The highest incidence of colorectal cancer in the study was in the age group above 55years accounting for 40.74% of cases of the total. 31.48% of the patients are in the age group 40-55yrs and 27.78 % were below 40 yrs. The youngest patients was a 19 years female while the oldest patient was a 78 years male. Male accounted for 66.67 % of the total cases while female corresponded to 33.33% of the total cases (Figure 2). The male to female ratio is 2:1.

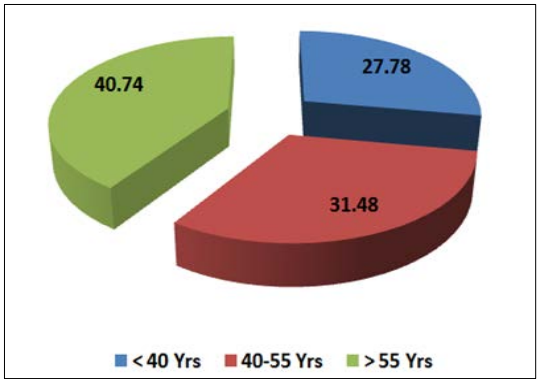


Fig 1: Pie diagram showing distribution of subjects.

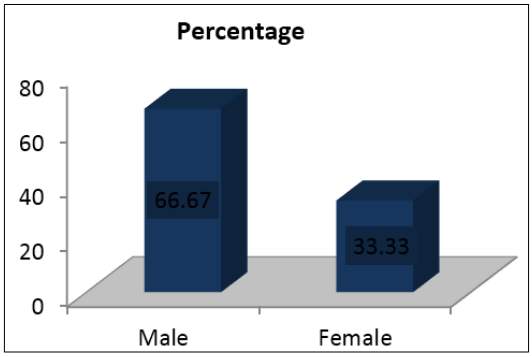


Fig 2: Bar diagram showing sex distribution.

The mean age of developing colorectal cancer was found to be 51.28 yrs in male (Table 1). The same in case of female was 45.39 yrs. The pooled average age of CRC for male and female together was estimated 49.31 yrs.

Table 1: Distribution of Mean age of cases

Sex	Frequency	%	Minimum	Maximum	Mean	±SD
Female	18	33.3%	19	72	45.39	16.332
Male	36	66.7%	21	78	51.28	15.248
Total	54	100.0%	19	78	49.31	15.714

Histological examination of different types of CRC, showed highest frequency of Adenocarcinoma, total 90.74% among all variants. Among Adenocarcinoma, moderately differentiated carcinoma constitute the maximum number of cases (53.70%) and well differentiated and poorly differentiated type constitute 25.93% and 11.11% respectively. The other type we found include 2 cases of mucinous carcinoma and 1 cases each were amounted to signet ring cell, small cell and squamous cell carcinoma (Table 2).

Table 2: Table showing distribution of CRC

Carcinoma Type	Frequency	Percentage (%)
Adenocarcinoma well differentiated	14	25.93
Adenocarcinoma moderately differentiated	29	53.70
Adenocarcinoma poorly differentiated	6	11.11
Mucinous carcinoma	2	3.70
Signet-ring carcinoma	1	1.85
Squamous cell carcinoma	1	1.85
Small cell carcinoma	1	1.85

Macroscopic examination of different types of colorectal cancer revealed maximum of ulceroproliferative type of growth (44.44%) while Proliferative and annular variant of growth constituted 29.63% and 25.93%, respectively (Table 3).

Table 3: Distribution of Macroscopic type of CRC

Macroscopic type	Frequency	Percentage
Annular	14	25.93
Proliferative	16	29.63
Ulceroproliferative	24	44.44

Different location of Colorectal cancer cases showed rectum as the most common site of tumor (n=30, 55.55%), followed by Sigmoid colon, ascending colon, caecum and the least common site observed in the descending colon. In both male and female the maximum number of colorectal cancer were found to be located in the rectum irrespective of age. The frequency of colon cancer was more in the left colon (total n-11, 30.6%) than in the right colon (8.4 %) in men. However, in females the frequency of colon cancer was more in right colon (n-9, 50.0%) than in the left colon (n-1, 5.6%) and according to Pearson's chi-square test P value is 0.005, considered significant. (Table 4)

Table 4: Distribution of location of CRC

Location	Male		Female	
	n	%	n	%
Caecum	1	2.8	5	27.8
Ascending colon	2	5.6	4	22.2
Descending colon	2	5.6	1	5.6
Sigmoid	9	25.0	0	0.0
Rectum	22	61.0	8	44.4
Total	36	100.0	18	100.0

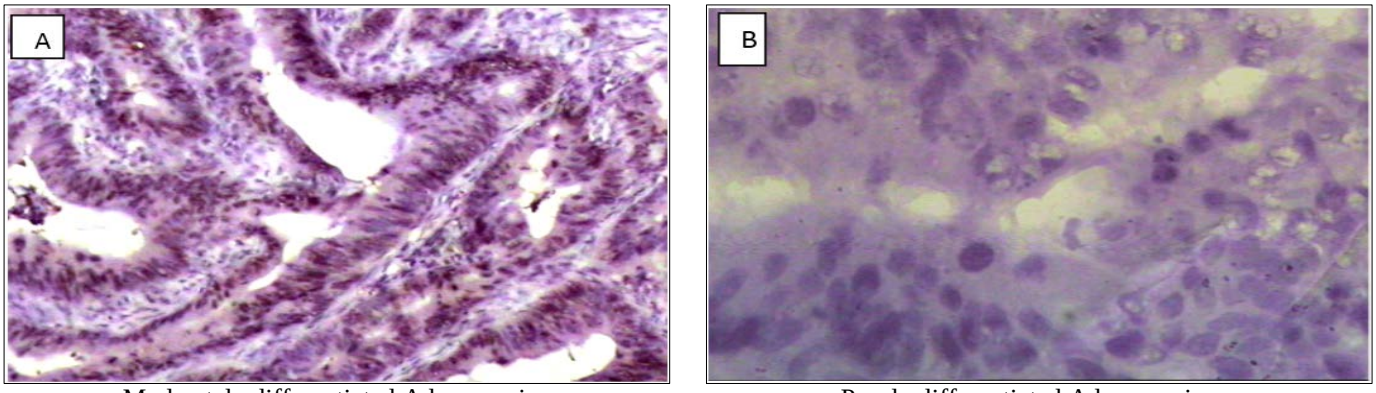
IHC Results

Of the 32 cases, 18.75% (6 cases) CRC showed loss of staining for one or more of the MMR protein. Among the 6 cases that show abnormal nuclear staining, 4 cases showed isolated loss of MLH1staining (66.66%), 1 case showed loss of staining for both MLH1and MSH2 and 1 case showed loss of staining for both MSH2 and MSH6. None of the case showed loss of staining in all the three mismatched repair protein. Overall loss of MLH1 protein expression was found in 15.625%, loss of MSH2 was found in 6.25 % and MSH6 was found in 3.125% (Table 5).

Table 5: Distribution of MLH1, MSH2 and MSH6 in CRC.

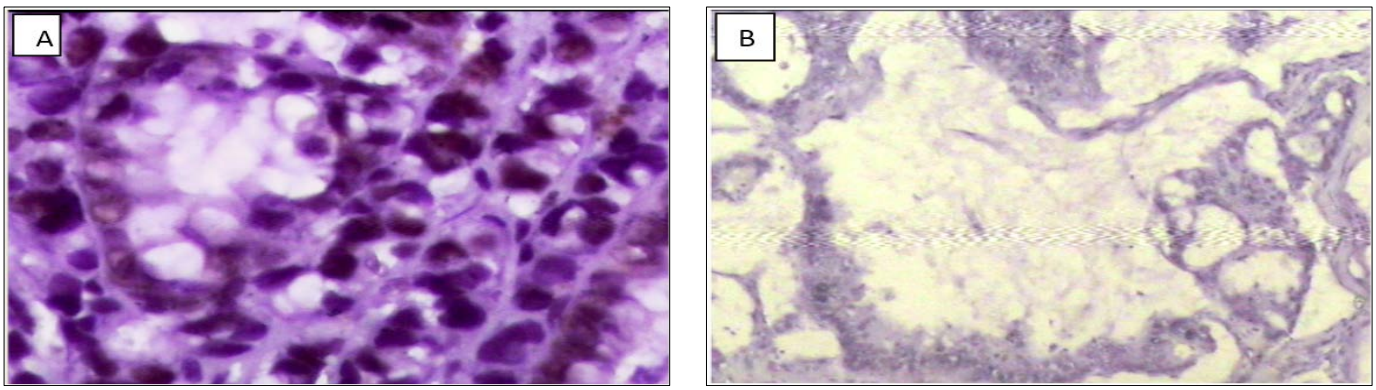
Antigen	Positive		Negative		Total
	Frequency	%	Frequency	%	
MLH1	27	84.375	5	15.625	32(100%)
MSH2	30	93.75	2	6.25	
MSH6	31	96.875	1	3.125	

In our study, tumor with MMR protein defect did not show any association with sex, age and with macroscopic type of growth than that of tumors with intact MMR protein expression. Tumors with loss of expression of MLH1 protein was significantly located on right side of colon (p=0.0363). There was also significant association of loss of expression of MLH1 and MSH2 protein with poorly differentiated, mucinous and signet ring cell carcinoma (p< 0.001) by morphology (Table 6). Also there was significant association of loss of expression of MSH2 and MSH6 together in MMR-d tumors (p <0.001) but not between MLH1 to MSH2 and between MLH1 to MSH6 loss of expression in MMR-d tumor.



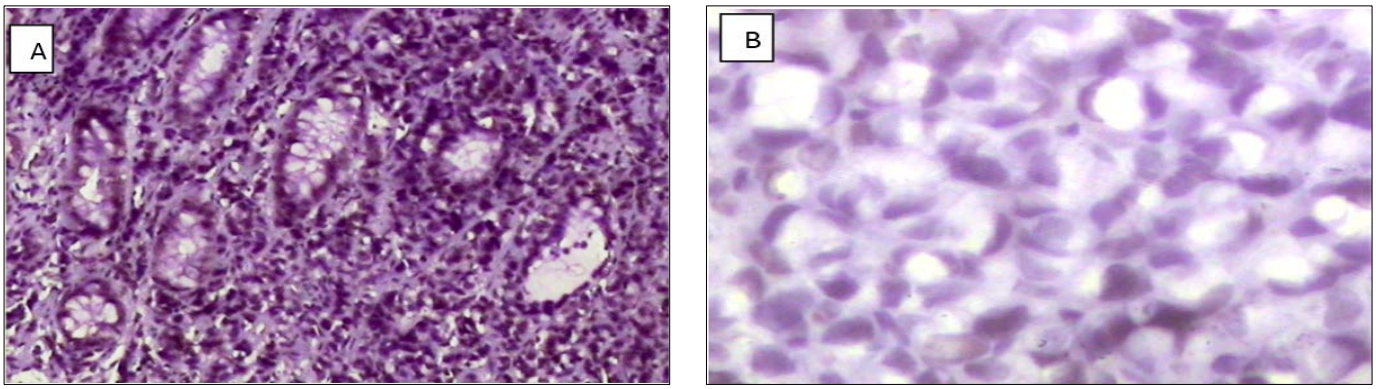
Moderately differentiated Adenocarcinoma — Poorly differentiated Adenocarcinoma

Fig 3: IHC showing positive (A) and negative (B) expression of MLH1 antigen.



Moderately differentiated Adenocarcinoma — Mucinous Carcinoma

Fig 4: IHC showing positive (A) and negative (B) expression of MSH2 antigen



Moderately differentiated Adenocarcinoma, Signet ring cell carcinoma

Fig 5: IHC showing positive (A) and negative (B) expression of MSH6

Table 6: Distribution of Expression of MLH1, MSH2 and MSH6 protein

		MLH1			MSH2			MSH6		
		+ve	-ve	P value	+ve	-ve	P value	+ve	-ve	P value
Age group (yrs)	<40	8	1	0.358	8	1	0.682	8	1	0.267
	40- 55	6	0		6	0		6	0	
	>55	13	4		16	1		17	0	
Sex	Female	9	3	0.258	11	1	0.706	11	1	0.19
	Male	16	2		19	1		20	0	
Macroscopic Growth	Annular	4	1	0.409	4	1	0.259	5	0	0.423
	Proliferative	9	3		11	1		11	1	
	Ulceroproliferative	14	1		15	0		15	0	
Anatomic site	Caecum	6	0	0.0363*	5	1	0.834	5	1	0.268
	Ascending Colon	2	3		5	0		6	0	
	Descending Colon	3	0		3	0		3	0	
	Sigmoid	6	0		6	0		6	0	
	Rectum	10	2		11	1		11	0	
Histological type Adenocarcinoma	Well differentiated	5	0	0.001*	5	0	0.001*	5	0	0.346
	Moderately differentiated	18	0		18	0		18	0	
	Poorly differentiate	4	2		5	1		5	1	
	Mucinous Carcinoma	0	2		2	0		2	0	
	Signet ring Carcinoma	0	1		0	1		1	0	

*Significant

4. Discussion

Colorectal cancer is mainly a disease of the elderly although different studies have documented distinct difference in disease. Maximum incidence of CRC in the present study was observed in age group above 55 years (40.74%). Peak incidence was in the 6th decade of life. In the present study male to female ratio is 2:1 which was in accordance with male preponderance reported in literature [10, 11, 12]. Rectal cancer (55.55%) were found to be more in our study overall than colon cases (44.45%) which was consistent with studies of other authors [11, 12, 13, 14]. Overall adenocarcinoma constitute 90.74% of all the histological types of CRC cases and this was in concordance with other various studies [10, 11, 15, 16].

There were 6 cases (18.75%) with loss of one or more MMR defect protein. This result appeared to be the same as previously reported in other literature [17, 18, 19, 20]. We found 5 cases (15.65%) with loss of expression of MLH1, 2 cases (6.25%) with loss of MSH2 protein and loss of MSH6 protein expression was found in 1cases (3.13%) in our study. Pooja Malhotra *et al.* [21] identified a loss of hMLH1 expression in 4(13.3%) and loss of hMSH2 expression in 2 (6.6%) in 30 Indian CRC patients. Parkin IJ *et al.* [22] in there study of the 402 sporadic CRC cases, found immunohistochemical analysis of 35cases (8.7%) with loss of expression of hMLH1, 19 (4.7%) with loss of expression of hMSH2, and three cases (0.7%) with loss of expression of both proteins.

In our study we found no significant relation of location of CRC with respect to age and sex in MMR–defective tumor. Joon-Joon Khoo [23] also found no statistical difference for patients with mismatch repair defect tumours when compared with patients with intact tumours with regards to the gender. Giovanni lanza *et al.* [24] found that MLH1 negative carcinoma occurred more often in women (62.5%) than MSH2 negative (41.7%) and MLH1/MSH2 positive (45.5%) but difference was not statistically significant. In addition MLH1- negative carcinoma developed less frequently in patients aging < 50yrs than did MLH1/MSH2 positive (p= not significant) and MSH2- negative tumor (p=0.005).

In our study, there was significant association of location of tumors with loss of expression for MLH1 to the right side of colon (p=0.0363). However no association was found between loss of expression of MSH2 and MSH6 with respect to location in MMR-d tumor. Joon-Joon Khoo *et al.* [23]

showed a significant predilection of MMR-d colorectal carcinomas to the right side of the colon ($p< 0.001$). Patrick Joost *et al.* [25] found significant correlation of proximal localisation of tumor with MMR deficiency.

Our study did not found any correlation with MMR-d tumors with its macroscopic type of growth. The identification of an expanding growth pattern in MMR defective CRC is found in various studies. Messerini *et al.* [26] studied the growth appearance of tumours found in MMR-d colon cancers. They noted that there was a significant correlation between mismatch repair deficient tumours and exophytic growth.

There was significant correlation between MLH1 and MSH2 defective tumor with histological type ($p< 0.001$) like poorly differentiated tumor, mucinous and signet ring cell tumors in our study. Gurjeet Kaur *et al.* [11] also found similar correlation for MMR-d tumor with poorly differentiated carcinoma, mucinous and signet cell cancer. Arai *et al.* [27] in there study found that the prevelance of absent hMLH1 expression was higher in poorly differentiated adenocarcinoma (63%) and mucinous carcinoma (43%) than in well (8%) and moderately (12%) differentiated adenocarcinoma.

Our study also revealed that there is significant association of loss of expression of MSH2 and MSH6 together in MMR-d CRC ($p<0.001$). However this was not the same with loss of expression of MLH1 and MSH2 together or between loss of MLH1 and MSH6 together in MSI CRC. Boland *et al.* [28] also found that if MSH2 is absent, there is no DNA MMR activity and this lead to loss of MSH6 together.

Evidence from various studies demonstrated that in sporadic cases hypermethylation of the hMLH1 promoter is the major cause of the MMR defect. In general, about 85 percent of sporadic colorectal cancers possess normal MMR function, whereas 15 percent have defective MMR function [29, 30]. In our study, five subjects (15.65%) had absent MLH1 expression, a characteristic feature in sporadic CRC had been detected.

5. Conclusion

Spectrum of CRC in Barak Valley was consistent with most data in our country. Application of IHC with MLH1, MSH2 and MSH6 can be used as an important parameters for further histopathological correlation.

6. Acknowledgement

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