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CLINICO-HISTOPATHOLOGICAL FEATURES OF COLONIC ADENOCARCINOMA IN YOUNG (<50YEARS) PATIENTS WITH SPECIAL REFERENCE TO MICROSATELLITE INSTABILITY STATUS

Pallavi Singh[1], Kapil Sharma*, Reshu Tewari, Vatsala Misra[1], Manisha Dwivedi, S P Misra

Department of Gastroenterology and [1] Department of Pathology, Moti Lal Nehru Medical College, Allahabad, UP, INDIA

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Cororectal Carcinoma, Micro satellite instability

Abstract

Aims. To study the clinic-histopathological features and Microsatellite instability (MSI) status of colorectal carcinoma (CRC) in patients < 50 years of age.

Methods. Sixty-one cases of CRC were studied in detail. Complete history, clinical details, colonoscopic findings and gross and microscopic features were noted. Histological type, grade of tumour and histological features like tumour infiltrating lymphocyte (TIL), Crohn like lymphoid aggregates and peritumoral lymphocytes were noted. Immunohistochemistry for MSH-2 and MLH-1 was done in 30 cases of <50 years of age.

Results. Proximal location, mucinous and signet ring cell carcinoma were more common in young (<50 years of age) as compared to elders (<50 years of age) (47.3%, 19.5% and 16.7% vs. 17.4%, 8% and 12%). Mucinous evidence (52.7%); TIL (94.4%) and Crohn-like lymphoid aggregates (52.7%) were also common in younger patients. Nineteen were labeled as MSI +ve, of which 13 cases were MSI-H and 6 MSI-L. Out of 19 MSI +ve cases, 2 had +ve family history, 17 cases were labeled as sporadic. MSI +ve cases were proximally located (63.2%), had mucinous (31.5%) and signet ring cell morphology (42.0%), TIL (89.5%) and Crohn-like lymphoid aggregates (73.5%).

Conclusion. Sporadic cases with MSI in young show unique clinicopathologic spectrum. Studies for denovo /somatic germ line mutation may help in further elaboration.

Introduction.

Colorectal cancer (CRC) is a major cause of morbidity and mortality worldwide. CRC is the third most common cancer and the third leading cause of cancer death in both sexes, accounting for approximately 10% of cancer deaths overall.[1] Data from GLOBOCAN 2002 have shown that in South East Asia as a whole, CRC is the third most common malignant disease in both men and women.[2] GLOBOCON 2008 results published in 2010 found that it is the 2nd most common cancer in females.[3]

The population – based incidence of CRC in Indian Cancer Registries have been one of the lowest in the World.[4,5] Over the last two decades, population-based data from the Bombay cancer registry has shown a significant increase in the incidence of colon cancer in both men and women. According to the recent data of GLOBOCAN 2002, the colon cancer has become 8th leading cancer in male with incidence rate of 2.7/100,000/year in India.

Like most cancers, the incidence of CRC is also age and sex-dependent. Both colon and rectal cancers are more common in old age. Interestingly, for reasons that remain largely speculative, 20-30% of colorectal cancer in India occur before 40 years of age and nearly half occur below 55 years. The mean age of colorectal cancer in India has been reported as 45.3 years.[6] It is possible that the younger age of onset has a hereditary basis, and the incidence of hereditary syndromes associated with colorectal cancers (like HNPCC) may be high in India.

Microsatellite Instability (MSI) is defined as a change of any length, due to either insertion or deletion, of repeating units in a microsatellite within a tumour cell when compared to normal tissue, it has been recommend that a panel of five microsatellites should be used as a reference standard (BAT 25, BAT 26, D5S346, D2S123, D17S250) for carcinomas of the large intestine.[7]

If two or more of these markers show MSI, the lesion is classified as high frequency microsatellite instability (MSI-H). If only one marker shows MSI, it is classified as low frequency microsatellite instability (MSI-L). If no markers show MSI it is classified as microsatellite stable (MSS),

MSI-H carcinomas are characteristic of hereditary non-polyposis colorectal cancer syndrome (HNPCC) due to germ line mutation of one of a group of DNA mismatch repair genes followed by somatic inactivation of the other allele.[8] Sporadic MSI-H tumours comprise about 15% colorectal carcinomas HNPCC cancers and sporadic MSI-H cancers share the mutation pathway (DNA instability).[9] The demonstration of DNA microsatellite instability serves as an important biomarker for HNPCC cancers.

Tumour with high level of microsatellite instability (MSI-H) or ras proto-oncogene mutations are more frequently located in the caecum, ascending colon and transverse colon. In >80% HNPCC, germ line mutation in MLH-1 and MSH-2 occur. HNPCC accounts for 2-4% of colorectal cancers. All cancers arising in HNPCC demonstrate the phenomenon of microsatellite instability.
Recently guideline has been proposed for the selection of cases that should be worked up for HNPCC with either immunohistochemistry or microsatellite instability analysis by PCR.

The Amsterdam Criteria [10]were crucial to achieve the original purpose of classifying a family as having HNPCC but their limited sensitivity hampered decisions about which patients should undergo genetic testing.[8]
In order to overcome this limitation, an international workshop on HNPCC hosted by the National Cancer Institute in 1996 outlined a set of recommendations, known as the Bethesda guidelines, for the identification of individuals with HNPCC who should be tested for MSI[11] Recently, a second HNPCC workshop revised these criteria and proposed a new set of recommendations. The revised Bethesda guidelines define the situations warranting work-up for HNPCC[8]

Bethesda guidelines:-

1. CRC in patient <50years
2. Synchronous or metachronous CRC, regardless of age
3. CRC in 1 or more first-degree relative with HNPCC- related tumour, with one of these cancers occurring <50 years.
4. CRC in 2 or more first- of second- degree relatives with HNPCC related tumour, regardless of age
5. CRC with typical MSI-H histology in patients < 50 years

a. Tumour-infiltrating lymphocytes
b. Crohn-like reaction
c. Mucinous / signet ring differentiation
d. Medullary growth pattern

(*HNPCC related tumours include colorectal, endometrial, stomach, small bowel, ovarian, pancreas, ureter, renal pelvis, biliary tract, brain (usually GBM),sebaceous adenoma.

As a large number of colorectal cases are occurring in younger age group and there is lack of proper studies regarding prevalence of HNPCC and/or MSI+ve sporadic cancers in Indian population, the present study was taken to compare the clinic-histopathological features of CRC in patients above and below the 50 years of age group and find out the MSI status of the patients below the 50 years of age.

MATERIAL AND METHODS

Sixty-one cases of colorectal carcinoma were studied in detail. Thirty six cases were <50 years of age and 25 were ≥50 years of age. Complete clinical details, which included age, sex, clinical history, site of lesion, anaemia, change in bowel habits, haematochezia were noted. Personal history (vegetarian and non-vegetarian), past history, family

history of cancer (especially for stomach, endometrium, ovary and renal) was also taken. A thorough colonoscopic examination was done and colonoscopic findings were noted. Gross and microscopic features were noted in detail in surgically resected specimens. Histological type and grade of tumour was noted according to WHO classification. Histological features like peritumoral lymphocytes, tumour infiltrating lymphocyte (TIL) and Crohn like lymphoid aggregate were noted. Peritumoural lymphocytes were defined as cap of chronic inflammatory cells seen in the deep invasive border of the tumour (figure1 A&B), Crohn-like lymphoid aggregate was defined as three or more nodular lymphoid aggregates deep to the advancing tumour margin within a single x40 field (figure1 C-E) and TIL was defined as ≥ 2 TIL/10HPF (×400) on H &E stained slides (Figure 1 F&G).[12]

Immunohistochemistry for MSH-2 and MLH-1 (HRP,Horse Radish Peroxidase, Immunostaining Kit,KP50L Diagnostic Biosystem**)** was done in 30 cases of <50 years of age with CRC. Cases were classified as MSS (when both MSH-2 and MLH-1 were positive), MSI-L (when one of the markers was positive) and MSI-H (when both the markers were negative). Sections from normal colonic mucosa from resected ends were taken as positive controls.

Results

Thirty six cases were <50 years of age and 25 were ≥50 years of age. Mean age (±SD) was 33(±5.62) years and 65(±12.48) years respectively in the two groups. Two cases had history of malignancy (one case each of adenocarcinoma colon and carcinoma stomach) in their first degree relative (2/36; 5.5%) in <50 years of CRC cases but none of the patients in ≥50 years of CRC had a family history. None of the patients in either group had history of familial polyposis coli. Right sided (proximal) lesions were more common in younger group (17/36; 47.3% and 4/25; 17.4% respectively) than elders. Peritumoral lymphocytes(10; 27.8%), Crohn-like lymphoid aggregates (19; 52.7%), TIL (34; 94.4%) and Mucin secretion (22; 52.7%); were more common in younger patients. (Figure 1A-G) In <50 years of CRCs cases, mucinous and signet ring cell carcinoma were found in 7/36 (19.5%) cases and 6/36 (16.7%) cases respectively as compared to 2/25(8%) cases and 3/25(12%) cases respectively in patients above 50 years. (Figure1H&I and Figure 2 A&D; Table1)

MSI STATUS

Immunohistochemistry for MSH-2 and MLH-1 were done in 30 cases. Out of 30 cases, 19 were labeled as MSI +ve, in which 13 cases were MSI-H and 6 MSI-L.Rest of the 11 cases were labeled as MSS. Out of 19 MSI +ve cases, 2 cases had +ve family history rest of the 17 cases had no feature of HNPCC according to modified Bethesda Guidelines and therefore they were labeled as sporadic cancer (17/28; 60.6%). Various clinicopathological parameters were correlated with MSI status. For statistical calculation, MSI-H and MSI-L cases were taken as MSI +ve and MSS were taken as MSI-ve (Table 2).

Mean (SD) age of MSI +ve cases was 38(14.8) years and MSI- ve cases was 49(10.2) years. Majority of MSI +ve cases were proximally located (12/19; 63.2%). Most of the mucinous (6/19; 31.5%) and signet ring cell carcinoma (8/19; 42.0%) were seen in MSI+ve (Loss of immunostaining) cases (figure2B,Cand E-I). Mucinous evidence (16/19; 84.2%), TIL (14/19; 89.5%) and Crohn-like lymphoid aggregates (14/19; 73.5%) were observed more frequently in MSI +ve cases than MSI –ve cases Statistically significant difference were observed in conventional adenocarcinoma (P <0.05) and tumour infiltrating lymphocytes (*P*<0.01) in relation to MSI status.

Discussion

The incidence of cancers of colon and rectum is low in India being 5.9 and 5.3 per 100,000 in males and females respectively compared to 32.9 and 24.4per 100,000 in US. Among the Asian population, Singapore has higher incidence of colorectal carcinoma than the Malays and Indians.[3, 5] 20-30% of colorectal cancer in India occur before 40 years of age, and nearly half occur below 55 years. The mean age of colorectal cancer in India has been reported as 45.3 years.[6] It is possible that the younger age of onset has a hereditary basis, and the incidence of hereditary syndromes associated with colorectal cancers (like HNPCC) may be high in India. Specific histological features have been described in patients with HNPCC according to modified Betheseda guide lines.[8]
In this study, we have evaluated the clinico pathological features of colonic adenocarcinoma in patients <50 years and > 50 years of age group with special reference to New Bethesda guidelines and also tried to assess the MSI

status in patients < 50years of age. Laishram *et al* also compared the two groups and found that CRC in younger age group is more common on right side and has a higher grade as compared to > 50 age group where most of the tumours were well differentiated. The findings were similar to our observations.[14]

The presence of mutations in hMSH-2 and hMLH-1 was observed by the immunohistochemistry. Only 30 cases of CRC were taken for immunohistochemistry due to unavailability of appropriate tissue in six cases. All cases except two had no family history and were considered as sporadic colorectal cancer (CRC). According to the earlier literature approximately 15% of sporadic cancer show MSI positivity.[9] However in present study 60.6% of sporadic cancers had evidence of MSI that is much higher than earlier reports. Hamper *et al* also observed that despite most research being focused on MSI in familial CRC, only 3% of all CRC come from HNPCC and most MSI CRC are sporadic.[15]

Macroscopically sporadic MSI tumors are characteristically proximally located, poorly differentiated and of a mucinous histology with lymphocytic infiltration.[16] Therefore further detailed molecular studies are required to explain high positivity of MSI in North Indian population with sporadic cancers. Out of 19 MSI +ve cases, 12 were proximally located (Right sided) and 7 were distally located (Left sided) Most of the MSI +ve cases belonged to proximal site and the histological features (earlier described) were also same as the HNPCC cases (poorly differentiated histological features, extracellular mucin and signet ring cells Crohn-like lymphoid aggregates and TIL) similar to the observations made by Cinzia *et al*., and Young *et al***.**[16,17] In present study also MSI+ve sporadic CRCs differed from MSI-ve CRCs in marked right sided predominance, tendency, for poorly differentiated histological patterns, formation of extracellular mucin, peritumoral Crohn-like lymphoid reaction and TIL. However a statistically significant difference could only be found in conventional adenocarcinoma **(***P* < 0.025**)** and TIL **(***P* < 0.01**),** which was probably due to small sample size. The clinicopathological features of MSI +ve sporadic cancer were similar to the two cases of colorectal cancer with positive family history and MSI positivity. Frequent proximal colonic involvement (63.2%) in MSI+ve young CRC cases was similar to earlier studies.[9,18,19] The reported histopathological features of colorectal cancers in HNPCC include excess of poorly differentiated carcinomas and mucinous carcinomas in one study[20] and marked peritumoral inflammatory infiltrates in another study.[21] These histopathological features of HNPCC-associated colorectal cancers were also found as characteristic features in our patients with sporadic MSI +ve colorectal carcinomas. Though signet ring-cell carcinomas have been reported to be more common in HNPCC patients,[22] 42% signet ring cell cancers were found in present series. Earlier studies suggested that patients with MSI +ve sporadic cancer have a better prognosis than those with MSI -ve cancers.[19,22] This tendency toward improved prognosis is especially interesting in light of the high prevalence of poorly differentiated carcinomas in this and other series**.**[17, 19, 22] The discrepancy between the aggressive histological features and the more favorable prognosis could relate to the marked peritumoral inflammatory infiltrates representing host response.

Perhaps the numerous mutations that develop in MSI +ve tumours affect not only growth controlling genes but other genes as well, such as those influencing expression of tumour associated antigens that elicit host immune response.
MSI testing by immunostaining has been found to be equivalent and highly cost effective strategies to further select those patients who should be tested for MSH2/MLH1 germ line mutation.[23,24] In our study, absence of protein expression with either MSH-2 or MLH- 1 or both was considered as MSI +ve and normal protein expression for MSH- 2 and MLH-1 were considered as MSI -ve. Similar observations were made in earlier studies that all of the tumors with mutations in either MSH-2 or MLH- 1 were MSI+ve. However, not all of the MSI +ve tumors had a recognizable mutation. There may be other proteins or factors that are involved in the regulation or stabilization of MSH-2 or MLH-1 protein levels. Alterations of these components may influence both the amount of mismatch repair protein and subsequently MSI.[25]

The genetic properties of sporadic CRCs include bialleleic methylation of the MLHI promoter, absence of MLH1 and PM2 protein and frequent mutations in BRAF.[16,26] Van Roon *et al* investigated etiology of *MLH1* promoter methylation in mismatch repair (MMR) mutation-negative early onset MSI-H colon cancer with an aim to achieve additional insight into the etiology of sporadic MSI-H colon cancer in young patients.They observed that this early onset group consists of two sub-groups: those which are CIMP-high and contain a *BRAF* mutation (resembling

sporadic MSI-H in the older age group to a great extent) and those with wild-type *BRAF* and limited methylation in addition to *MLH1* methylation.Although they could not identify a cause for *MLH1* methylation in sporadic MSI-H colon cancer with an age of onset below 50 years, they observed methylation to be almost restricted to the *MLH1* locus in patients without a *BRAF* mutation. They also excluded a role for somatic and germline *GADD45A* mutations in the tumorigenesis of early onset sporadic MSI-H colon cancer.[27]

Because antibody staining is more easily available than DNA analysis in a clinical setting, the use of immunohistochemistry appears to offer a relatively convenient and rapid method for prescreening tumors for defects in the expression of mismatch repair genes. Ultimately, this technique, along with testing for tumor MSI, should help to identify those individuals who may have germ line mutations in the mismatch repair gene complex. However, verification of these findings in a much larger number of patients is necessary. Furthermore, it will also be important to identify the underlying molecular defect in those MSI +ve tumors that have an absence of protein expression but no apparent gene mutations.

From the present study, it was concluded that in North Indian population has a higher percentage of sporadic cases with MSI. Sporadic cases with MSI in young show unique clinicopathologic spectrum. Studies with larger sample size and detailed molecular analysis for some inherited germ line mutation or denovo germ line /somatic mutation are required to further elaborate these changing patterns of sporadic colorectal carcinoma.

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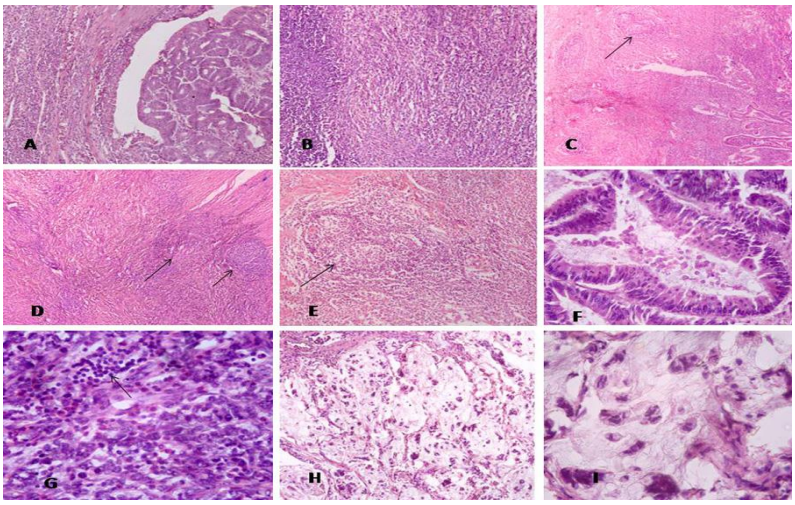
Figure 1: Differentiated adenocarcinoma showing peritumoral lymphocytes



Legend to figures

Figure1-

A A case of well differentiated adenocarcinoma showing peritumoral lymphocytes (H&E x100)
B. A case of poorly differentiated adenocarcinoma showing peritumoral lymphocytes (H&E x100)
C,D &E – Crohn like lymphoid aggregates (arrow) seen in a case of well differentiated adenocarcinoma (H&E x 40,100 & 400 respectively)
F. Tumour infiltrating lymphocytes (TIL) seen in the lining epithelium in a case of well differentiated adenocarcinoma (H&Ex400)
G. Tumour infiltrating lymphocytes (TIL) (arrow) seen in a case of poorly differentiated adenocarcinoma (H&Ex400)
H. A case of mucoid adenocarcinoma showing abundant extracellular mucin (H&E x100)
I. Higher magnification of the same showing small groups of pleomorphic and signet ring cells in a pool of abundant mucin (H&E x400)

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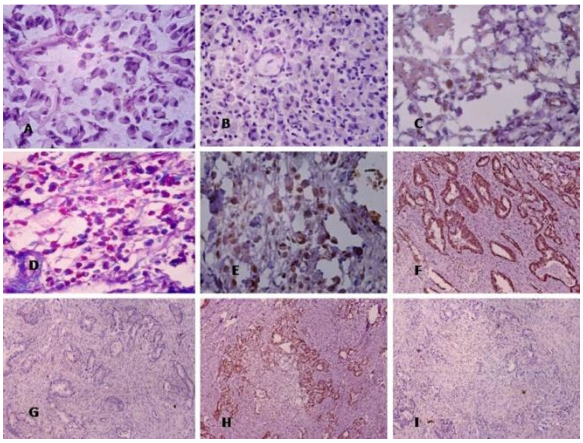
Figure 2: A case of signet ring Carcinoma colon



Legend to figures

Figure 2-

A.A case of signet ring Carcinoma colon (H&E x400)
B. Same case negative for MLH1 immunostaining (x400)
C.Another case showing weak MLH1 positivity (x400)
D.Infiltrating signet ring cells in muscle layer highlighted by PAS staining (PAS-AB pH2.5 x100)
E. Signet ring cells positive for MSH 2 immunostaining (X100)
F Section from a case of well differentiated adenocarcinoma showing positive immunostaining for MSH 2 (x 40)
G. Section from another case negative for MSH 2 (x40)
H&I. Section from a case of moderately differentiated adenocarcinoma showing positive immunostaining for MSH 2 but negative for MLH-1(x 40)

Table 1 - Comparison of clinicopathological data of cases of colorectal carcinoma of <50 years and ≥50 years of age.

S.No.	Parameter	**<50years** n(%) = 36 (59)	**>50 years** n(%)=25 (40.9)	***P***
1.	Mean age ±SD(years)	33±5.62	65±12.48	
2.	Male : Female	1.4:1	1:1.1	
3.	**Site of Lesion** Right side *(proximal to the splenic flexure)* n (%)	17(47.3)	4(16)	<0.01
4.	Left side *(distal to the splenic flexure)* n (%)	19(52.7)	21(84)	<0.01
5.	Family H/o colorectal cancer n (%)	2(5.5)	0(0)	N.S.
6.	Dietary History (Veg : Non Veg)	2:1	3:1	
7.	**Tumour Type**			
	a. Well differentiated adenocarcinoma	7(19.5)	6(24)	N.S.
	b. Moderately differentiated adenocarcinoma	11(30.5)	11(44)	N.S.
	c Poorly differentiated adenocarcinoma	4(11.1)	3(12)	N.S.
	d Mucinous carcinoma	7(19.5)	2(8)	N.S.
	e Signet ring cell carcinoma	6(16.7)	3(12)	N.S.
	f Undifferentiated carcinoma	1(2.7)	0(0)	N.S.
8.	**Growth Pattern**			
	(a) Diffuse	25(69.5)	19(76)	N.S.
	(g) Cribriform	10(27.8)	6(24)	N.S.
	(h) Medullary	1(2.7)	0(0)	N.S.
9.	**Mucinous evidence**			
	(a) Extracellular mucin	14(63.6)	7(70)	N.S.
	(e) Intracellular mucin (Signet ring cells)	8(36.4)	3(30)	N.S.
10.	**Tumour Infiltrating Lymphocyte**	34(94.4)	19(76)	**<0.05**
11.	**Crohnlike lymphoid aggregates**	19(52.7)	14(56)	NS
12.	**Peri tumoral Lymphocytes**	10(27.8)	05(20)	NS

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Table 2 - Comparison of clinicopathological data in the MSI+ve and MSI –ve colorectal carcinoma cases in < 50 years of age

S.No.	Parameter	**MSI +ve** 19 (63.3)	**MSI–ve** 11(36.7)	***P***
1.	**Male : Female**	1.2:1	1.5:1	
2.	**Mean Age ±SD**	38±14.8	49±10.2	
3.	**Site of Lesion** (e) Right side *(proximal to the splenic flexure)* n (%) (f) Left side *(distal to the splenic flexure)* n (%)	 12(63.2) 7(36.8)	 3(27.3) 8(72.7)	 N.S. N.S.
4.	**Tumour type** Adenocarcinoma (W,M,P) Mucinous carcinoma Signet ring cell carcinoma	 5(26.5) 6(31.5) 8(42.0)	 7(63.6) 3(27.3) 1(9.1)	 **<0.025** N.S. N.S.
5.	**Growth Pattern** Diffuse Cribriform Papillary	 16(84.2) 2 (10.5) 1(5.3)	 10(90.9) 1(9.1) 0(0.0)	 N.S. N.S. N.S.
6.	**Mucinous evidence** Extracellular mucin Intra cellular mucin (Signet ring cell)	 7(43.8) 9(56.2)	 2(33.3) 5(45.4)	 N.S. N.S.
7.	**Tumour Infiltrating Lymphocyte**	17(89.5)	5(45.4)	**<0.01**
8.	**Crohn like lymphoid aggregates**	14(73.5)	8(72.7)	N.S.
9.	**Peritumoral lymphocytes**	6(60)	4(40)	NS