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Original Article

Microsatellite Instability in Stage II and III Colorectal Cancer: Patterns and Profile

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Abstract

Introduction: Around 80% of colorectal carcinoma are associated with chromosomal instability while rest of 20% are euploid, possessing defect in mismatch repair system (MMR) quintessential for surveillance and correction of errors introduced into microsatellites. The microsatellite instability (MSI) phenotype has three major clinical applications: prognosis of colorectal cancer (CRC), prediction of response to 5 fluorouracil, and irinotecan, and genetic assessment of Lynch syndrome. **Materials and Methods:** We analyzed all Stage II and Stage III colorectal cancer (CRC) for MSI, who presented at Army Hospital, Research and Referral, New Delhi, from January 2014 to December 2016. Although patients of Stage II CRC were taken throughout the study period, Stage III CRC was included in last 1½ years to compare the prevalence of MSI in these two subsets of patients. **Results:** 26.2% of Stage II and 11.3% of Stage III patients were found to be MSI-high (MSI-H) (P = 0.04). Nineteen (86%) of 22 MSI-H patients were below 30 years of age (P = 0.01). Of 22 MSI-H patients, 18 had right-sided tumors (P = 0.03) and only three patients had rectal tumors. Most common pattern of MSI-H tumors was loss of expression of MLH1 and PMS2, seen in 15 of 16 (88%) of Stage II and three of 6 (50%) of Stage III CRC (P = 0.04). **Conclusion:** We conclude higher prevalence of MSI-H tumors in Stage II, as compared to Stage III CRC, which was demonstrated slightly higher in our study compared to published literature. MSI-H tumors tend to occur with high frequency in younger population, with right-sided colonic tumors, histopathology characterized by mucinous subtype with high prevalence of tumor infiltrating lymphocytes. Loss of expression of two MMR proteins, namely, PMS2 and MLH1 has been identified in most of MSI-H patients of our study, of which 86% were <30 years of age. This is in contrast to observation in previous studies where loss of PMS2 and MLH1 proteins was observed in older (>70 years) patients with MSI-H tumors, and in younger patients, MSI-H status was associated with loss of MLH1, MSH2, and MSH6.

Keywords: *Colorectal carcinoma, microsatellite instability, mismatch repair*

Introduction

Colorectal cancers (CRCs) are the third most common malignancy in men (10.0% of all cancer cases) and the second most common malignancy in women (9.4% of all cancer cases) worldwide.[1] It remains the fourth most common cause of death due to cancer. In India, the annual incidence rates for colon cancer in men are 4.4 per 100000, while that in women is 3.9 per 100000.[2] Around 80% of CRC are associated chromosomal instability, while rest 20% are euploid, defective in mismatch repair (MMR) system quintessential for surveillance and correction of errors introduced into microsatellites. This defective MMR system creates microsatellite instability (MSI), noted in 12%–15% of all CRC, of which 2%–3% are caused due to germline mutational inactivation of MMR genes and rest due to epigenetic mutational inactivation of MMR system. These are classified as MSI-high (MSI-H), where >30% of the microsatellite marker panel is mutated and MSI-low (MSI-L) if <30% of the marker panel is mutated. MMR status should be assessed in all patients who present with Stage II CRC and irrespective of stage, in those fulfilling the Bethesda guidelines as they have both prognostic and therapeutic implications.

The MMR genes are highly conserved from bacteria to humans. Hereditary nonpolyposis colorectal cancer (HNPCC) is a common, autosomal dominant syndrome characterized by early onset (average age at onset <45 years), the development of

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neoplastic lesions in a variety of tissues (e.g., endometrial, gastric, renal, ovarian, and skin), and MSI.[3,4] Cancers with MSI account for approximately 15% of all CRCs (usually MLH1 methylation), and for HNPCC germline mutations, there are three key DNA MMR genes (i.e., MSH2, MLH1 and in attenuated cases, MSH6) that are responsible for these cancers. A few candidate genes (e.g., PMS2 and MLH3) are still awaiting additional validation regarding their role in the etiology of CRCs with MSI.[3,4] MSI has been observed in gastric, endometrial, ovarian, and sebaceous carcinomas as well as glioblastoma and lymphomas apart from CRC.[5] Several population-based studies have reported that the prevalence of MSI-H in CRC ranges from 15% to 20%.[6] MSI is more common among Stage II (~20%) than Stage III (~12%) CRC and is even less frequent among Stage IV CRC (~4%).[7]

Microsatellite instability -high and microsatellite instability -low

MSI is detected by PCR amplification of specific microsatellite repeats. The presence of instability is determined by comparing the length of nucleotide repeats in tumor cells and normal cells. Normal DNA is typically extracted from adjacent normal mucosa.[13] A consensus reference panel, known as the Bethesda panel, established a panel of microsatellite markers with appropriate sensitivity and specificity to diagnose MSI CRC.[14] Three categories of MSI have been established based on the following criteria: MSI-H, indicating instability at two or more loci (or >30% of loci if a larger panel of markers is used); MSI-L, indicating instability at one locus (or in 10%–30% of loci in larger panels), and microsatellite stable (MSS), indicating no loci with instability (or <10% of loci in larger panels).

Diagnosis of microsatellite instability

The principal use of MSI testing in the clinic is to identify patients with Lynch syndrome. Approximately 15% of all colorectal tumors have MSI and 75% to 80% of this group have acquired methylation of MLH1; only ~2% to 3% of all CRCs have germline mutations in one of the MMR genes.[15] MSI identifies MMR deficient CRC with approximately 93% sensitivity; most insensitivity is caused by mutations in MSH6.[16]

Immunohistochemical (IHC) analysis of MMR proteins recently has become a popular alternative to detect MSI. Antibodies against MLH1, MSH2, MSH6, and PMS2 provide insight into the functionality of the MMR system. Lack of expression of one or more of these proteins is diagnostic of deficient MMR. The limitation of IHC is that staining can be heterogeneous throughout the tumor, which affects the sensitivity of the test.

Features and applications of microsatellite instability

CRC displaying MSI-H tends to be right sided and diagnosed at lower pathological stages compared with MSS cancers. Sporadic MSI-H cases are generally diagnosed in the elderly >70 years of age, whereas familial cases in younger <50 years of ages.[17] CRC with MSI-H generally have high-histological grades, mucinous phenotypes with prominent numbers of tumor infiltrating lymphocytes, a lack of necrotic cellular debris within the lumen of the neoplastic glands in the colorectal mucosa (dirty necrosis), and a Crohn's-like host response.[12] The MSI phenotype has three major clinical applications: prognosis of CRC, prediction of response to 5 fluorouracil (5FU) and irinotecan, and genetic assessment of Lynch syndrome.

Sporadic MSI-H CRC cases are more frequently associated with BRAF mutations than in hereditary cases, and are caused by hyper methylation of *MLH*.[8,9] Strong association has been proved in studies between sporadic MSI and the presence of the V600E *BRAF* mutation. *BRAF* mutation profoundly reduces the probability of a diagnosis of Lynch syndrome, though it does not entirely exclude the possibility.[10] *KRAS* mutations, however, are more likely to be observed in MSS (CIN) cancers than MSI tumor. *PTEN*, *a tumor suppressor gene* is not only mutated but also epigenetically silenced with higher frequency in MSI-H tumors.[11]

Prognostic and predictive value of microsatellite instability in colorectal cancer

MSI tumors had a more favorable prognosis and are less prone to lymph node spread and metastasis than MSS tumors. The prognostic value of MSI is more prominent in Stage II than Stage III CRC cases.[18] Tumors with MSI-H have greater numbers of tumor-infiltrating lymphocytes that are activated and cytotoxic;[19] which by itself is an independent factor associated with longer survival.[20] Patients receiving 5FU had no advantage over those who did not receive 5FU and this treatment might even be harmful for Stage II cases displaying MSI-H. Adjuvant combined chemotherapy with a 5FU-based regimen remains the standard of care in patients diagnosed with Stage III disease, regardless of MSI status. MSI-H cells are especially sensitive to irinotecan compared with their proficient counterparts.[21]

Aims and objectives

The aim of this study was to analyze the frequency and clinicopathological characteristics of MSI-H colorectal Stage II and Stage III cancers at a tertiary care center.

Materials and Methods

We analyzed all Stage II and Stage III CRC patients who visited malignant disease treatment center at Army Hospital Research and Referral from January 2014 to December 2016. Data of Stage II CRC were taken throughout the 3 years of study whereas patients of Stage III CRC were included only during the last 1½ years for comparing the relative prevalence of MSI-H between these two subsets of patients. Data were collected in a predesigned computerized format containing

Table 1: Baseline characteristics

Characteristics	*n* (%)
Study population	114
Stage II CRC (*n*=61)	
Right sided	48 (79)
Left sided	13 (21)
Stage III CRC (*n*=53)	
Right sided	32 (60)
Left sided	21 (40)
Colon: Rectal cancers	2.1:1
Number of MSI-H cases	22 (19)
Median age at diagnosis of MSI-H cases (years)	35.2
Sex wise distribution of MSI-H cases (*n*=22)	
Male	18 (80)
Female	4 (20)
Stage wise distribution of MSI-H cases	
Stage II (*n*=16)	
Right sided	12 (75)
Left sided	4 (25)
Stage III (*n*=6)	
Right sided	4 (67)
Left sided	2 (33)
Site wise distribution of MSI-H cases	
Colon cancer (*n*=19)	
Stage II	14 (74)
Stage III	5 (26)
Rectal cancer (*n*=3)	
Stage II	2 (67)
Stage III	1 (33)
Positive family history of colorectal cancer in MSI-H cases	5 (23)
Incidence of synchronous tumors in MSI-H cases	4 (18)
Histology in MSI-H cases	
Mucinous adenocarcinoma	14 (64)
Well-differentiated adenocarcinoma	4 (18)
Moderately differentiated adenocarcinoma	4 (18)
Tumor-infiltrating lymphocytes	15 (68)
Pattern of loss of MMR proteins (*n*=22)	
MLH1/PMS2	18 (82)
MLH1/MSH2	4 (18)
Stagewise distribution of MMR protein losses	
Stage II (*n*=16)	
MLH1/PMS2	15 (88)
MSH2/MSH6	1 (12)
Stage III (*n*=6)	
MLH1/PMS2	3 (50)
MSH2/MSH6	3 (50)

CRC – Colorectal cancers; MSI-H – Microsatellite instability-high; MMR – Mismatch repair system

details about patient characteristics, risk factors, clinical presentation, imaging findings, tumor stage, histology, MSI, genetic mutation testing, and treatment received.

Results

Of the 114 patients evaluated, 61 had Stage II colorectal tumors (CRC), rest 53 had Stage III CRC [Table 1]. Median age of patients with MSI-H CRC was 35.2 years. Of 22 MSI-H patients, 19 (87%) were <30 years of age ($P = -0.018$) [Table 2]. There was no significant difference between males and females in the prevalence of MSI-H tumors [Table 3]. Of 61 Stage II CRC, 48 (79%) had right-sided tumors and 13 (21%) had left-sided lesions. Sixteen (26.2%) patients had profile of MSI-H tumors in the form of lack of expression of two or more MMR proteins on immunohistochemistry (IHC). Of 16 Stage II MSI-H CRC cases, 12 (75%) were right-sided and rest 4 (25%) of cases were left-sided tumors. Of the sixteen Stage II MSI-H cases, 14 (87%) had colon cancer, and rest two had rectal carcinoma ($P = 0.04$) [Table 4]. Fifty-three patients of Stage III CRC, who presented to this institute for 1½ years, were evaluated for MSI. Six (11.3%) patients tested positive for MSI-H, of which 4 (67%) were right-sided CRC and rest two (33%) were left-sided CRC. Of 6 MSI-H Stage III CRC, five were colonic tumors and one was rectal carcinoma ($P = -0.04$). The incidence of MSI had a striking association with age at CRC diagnosis (87% MSI-H <30 years of age, $P = 0.018$) [Table 5]. The median age at diagnosis of MSI-H case was 35.2 years. Family history of CRC in first- and second-degree relatives was associated with significantly higher occurrence of MSI-H tumors: 23% ($n = 5$) compared with 5% ($n = 4$) in patients with MSI-L/MSS tumors in our study ($P = 0.03$) [Table 6].

Table 2: Incidence of microsatellite instability in various age groups (*P*=0.018)

Age (years)	MSI-H (*n*=22)	MSS/MSI-L (*n*=92)
<30	19	55
>31	3	37

MSI-H – Microsatellite instability-high; MSS – Microsatellite stable; MSI-L – Microsatellite instability-low

Loss of MLH1 and PMS2 proteins was the most common pattern seen in MSI-H CRC. Eighteen (81%) of 22 had loss of MLH1 and PMS2 proteins whereas four had loss of MSH2 and MSH6 as the pattern of MSI-H ($P = 0.017$) [Table 7]. In MSI-H Stage II CRC, loss of MLH1 and PMS2 was seen in 82% of cases ($P < 0.05$), whereas the same pattern was seen in 50% of MSI-H Stage III CRC. On histopathology, mucinous adenocarcinoma was present in 14 (64%) case, whereas rest were well or moderately differentiated adenocarcinoma with no mucinous component identified. Tumor-infiltrating lymphocytes were present in 15 (68%) of cases. None of the MSI-H cases had unfavorable histologic features, namely, lymphovascular or perineural invasion. Patients with MSI-H tumors had an increased incidence of synchronous cancers in colorectum accounting to 20% ($n = 2$), this association in our study was found to be statistically significant ($P < 0.05$).

Discussion

MSI-H is more common among Stage II (~20%) than

Table 3: Sex-wise distribution of microsatellite instability cases (*P*: Not significant)

Sex	MSI-H (*n*=22)	MSI-L/MSS (*n*=92)
Male	18	74
Female	4	18

MSI-H – Microsatellite instability-high; MSS – Micro satellite stable; MSI-L – Microsatellite instability-low

Table 4: Site-wise distribution of cases (*P*=0.04)

Site	MSI-H (*n*=22)	MSI-L/MSS (*n*=92)
Colon	19	59
Rectum	3	33

MSI-H – Microsatellite instability-high; MSS – Micro satellite stable; MSI-L – Microsatellite instability-low

Table 5: Correlation of stage with microsatellite instability tumors (*P*=0.04)

Stage	MSI-H	MSI-L/MSS
II	16	45
III	6	47

MSI-H – Microsatellite instability-high; MSS – Microsatellite stable; MSI-L – Microsatellite instability-low

Table 6: Proportion of microsatellite instability tumors correlated with family history of colorectal cancer (*P*<0.05)

Family history of CRC	MSI-H (*n*=22)	MSI-L/MSS (*n*=92)
Present	5	4
Absent	17	88

MSI-H – Microsatellite instability-high; MSS – Microsatellite stable; MSI-L – Microsatellite instability-low; CRC – Colorectal cancers

Table 7: Correlation of stage with microsatellite instability pattern (*P*=0.017)

Stage	MLH1/PMS2	MSH2/PMS6
II	15	1
III	3	3

Stage III (~12%) CRC and is even less frequent among Stage IV CRC (~4%).[7] We found a slightly higher percentage of patients in Stage II CRC to be MSI-H in our study (26.2%, *n* = 16), whereas incidence of MSI-H in Stage III CRC was found to be similar, i.e., 11.2%, as found in previous studies.[7] Similar to a previous report by Liu *et al*., (1995), we also found that the MSI-H rate in our tumors diminished as age at cancer diagnosis increased. Around 87% of the patients with MSI-H were younger than 30 years. The MSI-H rate fell rapidly for those more than 40 years. The MSI rate decreased even further for those older than 50 years (Chan *et al*., 1999b). In our study, patients aged <40 years had higher incidence of MSI-H tumors, which was higher than those found in the study by Ho *et al*.(70% vs. 43.1%, respectively). Comparing the present and the previous studies, we have proven by statistical analysis that age is an important determinant of the prevalence of MSI-H.

MSI-H tumors characteristically occur lesions in proximal or right colon, with significantly high prevalence of such tumors in colon than rectum. We found 12 (75%) of Stage II CRC occurring in right colon and rest 25% occurring in left colon (two in rectum). Four (67%) of six Stage III CRC were present in right colon, rest 33% in left colon out of which only one was in rectum. This finding in our study was statistically significant (P = 0.04), and commensurate with previous studies.[5]

Recognizable clinicopathological profile of MSI has been established from clinical studies. CRC with MSI-H has high-histological grade, predominant mucinous phenotype, with a significant proportion of cases showing Crohn's-like host response and numerous tumor-infiltrating lymphocytes on histology. In our study, of 22 patients, 14 (60%) had mucinous histology and 68% had tumor-infiltrating lymphocytes. This finding correlates with the study by Greenson *et al*., which found that CRC with MSI-H generally have high-histological grades, mucinous phenotypes with prominent numbers of tumor-infiltrating lymphocytes, a lack of necrotic cellular debris within the lumen of the neoplastic glands in the colorectal mucosa (dirty necrosis), and a Crohn's-like host response.[17]

For the whole group of young patients (<30 years), MSI-H was significantly associated with tumor location at the proximal colon, in accordance with previous results (Lothe *et al*., 1993; Thibodeau *et al*., 1993; Kim *et al*., 1994; Liu *et al*., 1995; Senba *et al*., 1998).

Positive family history of CRC was also an independent predictor for MSI tumor in our young patients. The group of patients who had synchronous cancers in colorectum was too small in our study, to comment on the statistically insignificant association found in relation to MSI-H. All patients tested positive for PMS2 and MLH1 MMR protein losses (n = 10).

In view of smaller study population of Stage II CRC (n = 45), the association of clinical features with loss of MMR proteins cannot be determined with heightened certainty. Even so, the preliminary data suggest that patients with MMR protein losses are younger, have a stronger family history of CRC and a predominantly proximal colon location. However, we could not observe any association of loss of MMR protein expression with synchronous cancers in our study population.

Our dedicated oncology center at Delhi is the referral center for all cases of CRCs over India, serving the beneficiaries of armed forces throughout the nation, with its resource rich setting providing all the appropriate investigations and treatment available for its patients. Although the Stage III CRC patients were included only during last 1½ years of our study, which may not truly depict the prevalence of

Stage III CRC as well as incidence of MSI-H in this subset, but the data genuinely reflect the prevalence of MSI tumors in Stage II CRC patients over 3-year study period.

Loss of expression of two MMR proteins, namely, PMS2 and MLH1, has been identified in 18 (81%) of total 22 MSI-H tumors. Rest four (19%) patients of our study had loss of MSH2 and MSH6 as MSI-H pattern. 82% of Stage II MSI-H and 50% of Stage III MSI-H tumors had loss of MLH1and PMS2 proteins. The majority of CRC with MSI-H have loss of expression of MLH1 and PMS2 protein. The characteristic features of sporadic CRC with MSI-H include the absence of significant familial clustering, absence of MLH1 and PMS2 proteins, and frequent mutation (usually V600E) in BRAF.[22] Although our study commensurate with the most common pattern of MSI-H CRC, but in contrast to observation in previous studies where loss of PMS2 and MLH1 proteins was observed in older (>70 years) patients with MSI-H tumors, and in younger patients, MSI-H status was associated with loss of MLH1, MSH2, and MSH6 (Lynch syndrome).[22,23]

Knowledge of the association of various clinical features with MSI tumors and loss of MMR protein expression may facilitate selection of suitable patients for genetic testing. Besides, such an association may have an impact on the clinical management of young CRC patients and their families. Of the exceptionally young patients (<30 years) and the patients aged 30–49 years with proximal cancers, about half will have MSI tumors and may harbor germline losses in MMR protein expression. Therefore, total abdominal colectomy should be the surgical treatment of choice for these patients in view of their high chance of metachronous CRC development. In addition, first-degree relatives of these patients should be offered CRC screening. Similar clinical management should also be considered for a young CRC patient who has a strong family history of CRC or a personal history of metachronous cancer. For patients with proven germline loss of MMR protein expression, their family members should be offered predictive genetic testing.[15]

In conclusion, MSI occurs in a significant proportion of CRCs in patients <50 years old. Young ages at CRC diagnosis, proximal tumor location, and family history of CRC were independent predictors of MSI status in our patients.

Conclusion

The MSI phenotype has three major clinical applications: prognosis of CRC, prediction of response to 5FU and irinotecan, and genetic assessment of Lynch syndrome. MSI-H tumors are more prominent in Stage II CRC, with characteristic features involving proximal or right colon, predominant mucinous histology, presence of tumor infiltrating lymphocytes. Our study commensurate with all characteristic findings, but we found the prevalence of MSI-H in Stage II CRC slightly higher than previous studies and also the most common MSI-H pattern (loss of MLH1 and PMS2) was predominant in younger age group, in contrast to previous studies which demonstrated this pattern in elder age groups.

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Conflicts of interest

There are no conflicts of interest.

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