Original Article

Is the Disease Profile in Metastatic Colorectal Cancer Still Driven by the Mutational Parameters as Before? A Tertiary Care Center Study from India in 2020

Abstract

Background: The pathophysiology of colorectal cancer (CRC) is believed to be driven primarily by anomalies in the molecular pathway mechanisms. Mutations in KRAS, NRAS, and BRAF genes are closely associated with tumor differentiation, invasion, and metastasis. These are now recognized as important targets for clinical treatment of metastatic CRC to determine the response to therapy and final prognosis of the disease. The study investigates the relationship of KRAS, NRAS, and BRAF gene mutations in the Indian population in the current era. **Materials and Methods:** A total of 120 patients including 50 patients having metastatic disease, all with proven histological diagnosis of CRC were included. They were followed up for examination of clinical signs and performance status. Ethical approval was obtained from the Ethical Committee at Army Hospital (R and R), New Delhi (IRB No. 91/2016). **Results:** This prospective study shows that the young adults (<45 years) presented with an aggressive biology of disease with advanced disease at presentation and have higher mortality rates due to poor response to therapy. NRAS and BRAF mutations were found mainly with left and right sides, respectively. The right-side CRC had poor prognosis and responses to therapies (P = 0.07 and P = 0.005, respectively). NRAS and BRAF mutations were found mostly in women having comorbidities. Young individuals with a positive family history of CRC must be investigated early for tumor markers for better treatment outcomes. **Conclusion:** This planned investigation affirmed that the Indian populace had more right-side CRC which was metastatic predominantly. RAS and BRAF changes were related essentially with left- and right-side CRCs, respectively. However, both had a poor prognosis and reactions to treatments. NRAS mutation might be a significant marker as it is observed solely in young females with left-side CRC and had a poor prognosis due to an aggressive tumor. BRAF transformations are higher in the Indian populace in contrast to the western information.

Keywords: *BRAF, colorectal cancer, genes, humans, KRAS, mutations, North India, NRAS, prospective studies*

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Introduction

Colorectal cancer (CRC) is a common and lethal disease. It is estimated that approximately 149,500 new cases of large bowel cancer are diagnosed annually in the United States,[1] of which approximately 104,270 arise from the colon and the remainder from the rectum. Approximately 52,980 Americans are expected to die of large bowel cancer each year. Although CRC mortality has been progressively declining since 1990, at a current rate of approximately 1.6%–2.0% per year,[2] it still remains the third most common cause of cancer death in the United States in women and the second leading cause of death in men.

The age-standardized rate for CRC in India is low at 7.2 per 100,000 population in males and 5.1 per 100,000 population in women.[3] In India, the annual incidence rates for colon malignant growth and rectal disease in men are 4.4 and 4.1 per 100,000, individually. The annual incidence rate for colon malignant growth in females is 3.9 per 100,000. Colon malignancy positions eighth and rectal disease positions ninth among men.[4] There were an estimated 14.1 million cancer cases around the world in 2012. Of those cancers, 7.4 million were in men, while 6.7 million were in women.[5] The lifetime risk of developing CRC is approximately 5% and the risk increases with age. Both genetic and epigenetic changes are

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believed to be the common driving force of tumorigenesis in CRC.[5] Most of the CRC patients are >65 years old and record <10% of the all-out CRC found in age underneath 40 years.[6] In the 1980s, it was proposed the adenomacarcinoma hypothesis where in transformation of a normal colorectal epithelium first to an adenoma and over a time converting into an invasive and metastatic tumor. The genetic changes start early in an adenoma and accumulate as it slowly transforms into invasive carcinoma. The factors leading to genetic instability in CRC are chromosomal/microsatellite instability (MSI), CpG island methylator phenotype (CIMP) pathways, and mutations or single-nucleotide polymorphisms in oncogenes.[7] Both gross and point mutations in some tumor oncogenes have been implicated in the development of CRC in every part of the world.[8] The formation of CRC involves major molecular mechanism pathways of which mutations occurring in KRAS, NRAS, and BRAF genes are responsible for tumor differentiation, invasion, and metastasis. Therefore, they are important targets for clinical treatment of mCRC and determinate prognosis.

Despite the growing number of cases of CRC in India, there are only a few studies published from India addressing the mutational analysis of RAS and RAF which are considered as of the proto-oncogene responsible for triggering this disease. The current study aims to evaluate the pathology of CRC and its relationship with mutations in the mixed Indian population of all ages at a tertiary care defense establishment.

Materials and Methods

Study design

This is a prospective study conducted at Army Hospital (R and R), Delhi, which is the largest tertiary care hospital of the Indian armed forces catering to defense personals and their family from all over the nation. All 120 patients (50 patients having metastatic tumor) visiting the hospital with a proven diagnosis of CRC between May 16 to April 18 were included. Patients with histologically proven diagnosis, on treatment, and regular follow-up of any performance status or any age/sex were included. Each patient was given a unique malignant disease treatment center number for identification and follow-up. However, those with the unavailability of histopathological diagnosis, on alternative medication, and uncontrolled comorbidities were excluded. Ethical clearance for the commencement of the investigation was acquired from the Ethical Committee at Army Hospital (RR), New Delhi (IRB No. 91/2016).

Data collection

The demographic data was collected by taking a detailed history of illness, personal habits, and comorbidities. Physical examination was done in all patients to look for clinical signs and assess the performance status. Patients were subjected to tissue diagnosis from the primary site using colonoscopy biopsy or histopathology of the surgical specimen. In certain patients of mCRC pleural fluid aspiration, ascetic fluid analysis or peripheral lymph node sampling was also done.

KRAS, NRAS, and BRAF gene mutations were done in all metastatic CRC patients using real-time-polymerase chain reaction and gene sequencing techniques. The mutation analysis was done to detect KRAS and NRAS (Exon 2, 3, and 4) and exon 15 for BRAF mutation. The analytic sensitivity allows the detection of the mutant clone comprises at least 20% of the total genomic DNA. MSI testing was performed only in a few Stage II patients (due to availability of limited testing facility) who had enough tissue samples using a Ventana detection kit. Future testing of blocks for stage IV disease for MSI is planned as and when the sufficient kits were made available. Cancer stage valuation was done by imaging (contrast-enhanced computed tomography [CT] and/or whole-body-positron-emission tomography/CT).

Statistical analysis

Statistical data analysis was performed using STATA 13 IC, (StataCorp LLC, Texsas, USA) which included preparation of contingencies tables and relation between categorical variables using a Chi-square test. Treatment response was followed using Wilcoxon matched-pairs signed-rank test. $P \leq 0.05$ was considered as statistically significant.

Results

Demographic data

The study included 120 participants (mean age: 51.6 ± 13.8 years) having 71 males and 49 females, distributed into 34.1% and 65.9% with <45 years of age (young adult) and >45 years of age (old adults), respectively. Out of the total patients, 54.2% belonged to rural areas and 45.8% from urban areas, with 93.3% having no familial history. About 28.3% and 81.7% of patients were diagnosed with right- and left-side CRC tumors, respectively. Stage III and IV cancers were most common. Few patients were diagnosed with comorbidities such as diabetes, hypertension, and obesity. The complete demographic data are shown in Table 1.

Diagnosis

The most common symptom at presentation was weight loss (63.3%) which occurred within 3–6 months of diagnosis period and was associated with loss of appetite, followed by rectal bleeding in 59.2% of patients. Anemia was seen in 47% of patients and altered bowel habit (change in frequency and consistency of stools) in 34.2% of patients with associated abdominal pain. Fourteen patients (11.7%) presented with acute intestinal obstruction diagnosed during surgery and 7.5% with perforation.

The occurrence of both metastatic and nonmetastatic cancer was higher in males as compared to females but was not statistically significant (P = 0.6). The right-sided CRC was found to be predominantly metastatic at the onset with 19 cases out of 34 (62%) as compared to the left side which had more nonmetastatic cases (P = 0.05).

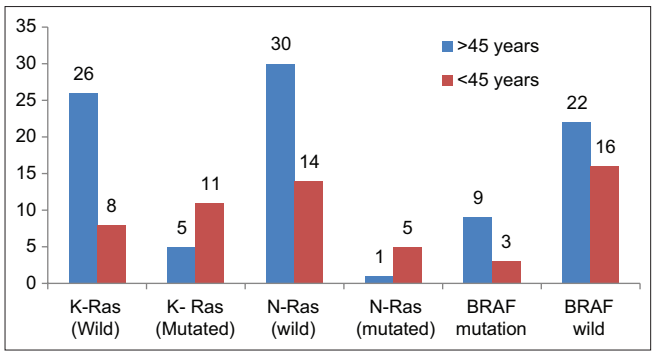

Figure 1: Mutation/wild type in metastatic colorectal cancer

Table 1: Demographic analysis of the patients

General description of the study population (n=120)	
Variable	**Frequency, n (%)**
51.6 years (SD=13.8)	
<25	4 (3.3)
26-45	37 (30.8)
46-60	43 (35.8)
61-75	31 (25.8)
>76	5 (4.2)
Gender	
Male	71 (59.2)
Female	49 (40.8)
Hypertension	
Yes	28 (23.3)
No	92 (76.7)
Diabetes	
Yes	23 (19.2)
No	97 (80.8)
Obesity	
Yes	6 (5)
No	114 (95)
Stage	
I	1 (0.8)
II	10 (8.3)
III	59 (49.2)
IV	50 (41.7)
Histology	
WD adenocarcinoma	19 (15.8)
MD adenocancer	55 (45.8)
PD adenocancer	25 (20.8)
Mucinous adenocancer	14 (11.7)
Signet ring cancer	4 (3.3)
Squamous cell cancer	2 (1.7)
NEC	1 (0.8)

WD: Well-differentiated, MD: Moderately differentiated, PD: Poorly differentiated, SD: Standard deviation, NEC: Neuroendocrine carcinoma

Mutation analysis in metastatic colorectal cancer

Mutation analysis was carried out in all 50 cases of metastatic CRC. KRAS-, NRAS-, and BRAF-mutated and wild-type patients were compared in various subgroups [Figure 1].

KRAS mutation was seen in 16 out of 50 (32%) cases of metastatic CRC, while 34 patients (64%) were wild type. KRAS mutation was predominantly seen in the Young Adults (YA) (<45 years), while the osteoarthritis (>45 years) was KRAS wild type (P = 0.002). In addition, KRAS-mutated patients were associated with unfavorable histology (poorly differentiated adenocarcinoma, mucinous, or signet ring carcinoma), while KRAS wild type was associated with favorable histology (well-differentiated and moderately differentiated adenocarcinoma), showing that mutation had an impact on disease pattern behavior (P = 0.002). There was no statistical difference with respect to site (P = 0.5), sex (P = 0.6), smoking (P = 0.2), duration of symptoms (P = 0.4), place of residence (P = 0.2), occupation (P = 0.9), co-morbidities (P = 0.5), family history (P = 0.3), or type of diet (P = 0.3) between mutated and wild type [Table 1].

NRAS mutation was seen in 6 patients of metastatic CRC which was predominantly seen in young adults. 44 patients were NRAS wild type ,predominantly seen in the old (P = 0.02), which was similar to the trends seen with NRAS mutation in adults. All the NRAS-mutated six cases were seen in the left-side colon (P = 0.07) and 5 (83%) of these cases were females (P = 0.02). There was no statistical difference with respect to smoking (P = 0.2), duration of symptoms (P = 0.4), place of residence (P = 0.9), occupation (P = 0.2), comorbidities (P = 0.2), family history (P = 0.1), histology (P = 0.7), or type of diet (P = 0.3) between K-RAS mutated and wild type [Table 2].

BRAF mutation was seen in 12 out of 50 cases of metastatic CRC, of which 9 cases were seen on the right-side colon (P = 0.005), while left-side colon was predominantly BRAF wild type. Eight cases of mutated BRAF were seen in females (P = 0.04), while males were predominantly wild type. Ten out of the 12 BRAF-mutated individuals had one or more comorbidities (P = 0.02). There was no statistical difference with respect to smoking (P = 0.1), duration of symptoms (P = 0.7), occupation (P = 0.1), family history (P = 0.3), histology (P = 0.3), or type of diet (P = 0.5) between BRAF mutated and wild type.

A total of 10 (8.3%) patients were Stage II and six had high-risk features, thus MSI testing was done in only four patients only (two each MSI high and MSI low) and adjuvant therapy was decided on this basis.

Discussion

With increasing incidences of CRC (more than 1.4 million new cancer cases every year)[6] and

Table 2: Mutation analysis in metastatic colorectal cancer

	Total	KRAS (wild) (*n*=34)	KRAS (mutated) (*n*=16)	*P*	NRAS (wild) (*n*=44)	NRAS (mutated) (*n*=6)	*P*	BRAF mutation (*n*=12)	BRAF wild (*n*=38)	*P*
Age (years)										
>45	31	26	5	0.002	30	1	0.02	9	22	0.3
<45	19	8	11		14	5		3	16	
Site										
Right side	19	14	5	0.5	19	0	0.07	9	10	0.005
Left side	31	20	11		25	6		3	28	
Sex										
Male	31	22	9	0.6	30	1	0.02	4	27	0.04
Female	19	12	7		14	5		8	11	
Rural	25	19	6	0.2	22	3	0.9	8	17	0.3
Urban	25	15	10		22	3		4	21	
Smoker	25	19	6	0.2	24	1	0.2	3	22	0.1
Nonsmoker	25	15	10		20	5		9	16	
<3 months	14	10	4	0.4	11	3	0.4	2	12	0.7
3-6 months	26	19	7		24	2		7	19	
>6 months	10	5	5		9	1		3	7	
Histology										
WD and MD adenoca	26	23	3	0.002	22	4	0.7	8	18	0.3
PD adenocarcinoma and others	24	11	13		22	2		4	20	
Vegetarian	21	16	5	0.3	19	2	0.9	6	15	0.5
Mixed diet	29	18	11		25	4		6	23	
Family history (yes)	5	2	3	0.3	3	2	0.1	0	5	0.3
No	45	32	13		41	4		12	33	
Defense personnel	15	11	4	0.9	13	2	0.2	2	13	0.1
Farmer	15	10	5		15	0		2	13	
Others	20	13	7		16	4		8	12	
Comorbidities										
Yes	25	18	7	0.5	24	1	0.2	10	15	0.02
No	25	16	9		20	5		2	23	

WD: Well-differentiated, MD: Moderately differentiated, PD: Poorly differentiated

having geographical variation in the incidence rates, it is indeed a cancer on the rise. Even though more cases are being reported in developed countries, the mortality rate is still higher in developing countries. This has been blamed repeatedly on limited resources and inadequate health infrastructure. Army hospital (R and R) is the largest tertiary care defense hospital and receives patients from all over India and is therefore the ideal place to study the correlation between the clinic-epidemiological profile and mutational status in mCRC. We observed a high incidence of CRC and an increasing trend in a young population who reside in urban parts of India and eat red meat, similar to the data reported by Sudarshan *et al*., 2003. It has been observed that the "Western Diet" which consists of the high proportion of red meat, rich in fat, low in calcium, and whole-grain fiber accounts for 40%–45% of the diet in the west and only 10%–15% in India, which is one of the possible reasons of emerging CRC.[9] Our data also revealed that the young population having a significant family history of CRC and with diabetes are more prone to metastatic CRC.

The development of CRC may have many aspects. This includes the activation of proto-oncogenes along with the inactivation of tumor suppressor genes. In addition, there is the inactivation of mismatch repair genes which lead to genetic and epigenetic changes in normal intestinal tissues. All these ultimately lead to the formation of tumors.[10] CIMP-H-type CRC has a hypermethylation genotype of CpG island promoter, This leads to gene inactivation of tumor suppressor gene and ultimately leading to CRC. CIMP-L is associated with KRAS mutations and male susceptibility.[11] In our study, the KRAS mutation was observed in 32% of patients with mCRC, similar to the reported data of 30%–50% worldwide.[12] Among the patient population, KRAS mutation was seen predominantly in young adults and was associated with unfavorable histology and higher rates of progressive disease despite treatment based on molecular pathways [Table 2]. The NRAS mutation has been studied only in a handful of studies and its behavior in mCRC is still not completely known. We found that the NRAS mutation was exclusively seen in young females and all were left-sided mCRC [Figure 2]. The similar data was

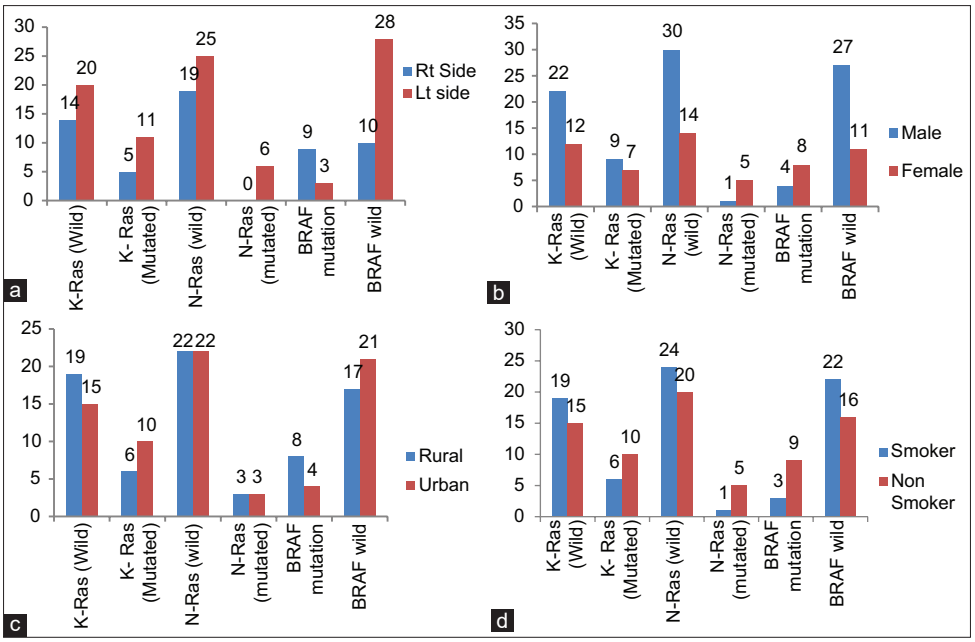

Figure 2: (a) Right- and left-sided mutations in metastatic colorectal cancer. (b) Mutations in males/females in colorectal cancer. (c) Right- and left-sided mutations in metastatic colorectal cancer. (d) Mutations in males/females in colorectal cance

reported by Baran *et al*. that NRAS-mutated lesions are mostly left sided and associated with poorer prognosis.[13] KRAS and NRAS are from the same RAS gene and RAS protein helps in GDP binding at an inactive state, and any mutation in this leads to a perpetual phenotype having continuous growth and differentiation, leading to a cancer phenotype.[14] These young females in our study responded poorly to chemotherapy and 80% of them had progressive disease during or after the first line of chemotherapy. Various studies from the west have reported RAS mutation with a poor prognosis and should be considered an important marker for assessing the aggressiveness of the disease and prognosis in all cases of CRC.

In our study, BRAF mutation was seen in 24% of metastatic CRC cases, which is higher than the reported data (4%–15%) worldwide.[15] The BRAF-mutated mCRC was common on the right-side colon, seen frequently in females and associated with comorbidities such as hypertension, diabetes, or obesity. These patients again like KRAS patients had a poor response to chemotherapy (alone or in combination with anti VEGF agent), with lower rates of partial response and nonattained complete response. All BRAF mutations were V600E in nature, which is similar to the data reported by Yokota *et al*.[16] Targeted therapy for this subset of patients who have a poor prognosis and no directly acting agent should be explored on lines of melanomas and thyroid cancers which also have similar mutations. Similar data were observed by Ogino *et al*., 2012, that BRAF mutations are closely related to KRAS wild type and female susceptibility.[17]

For the diagnosis and treatment of mCRC, more attention should be paid to the molecular typing of malignant tumors and formation mechanism of molecular typing should be further explored. The treatment modalities should be refined to achieve better survival prognosis and therapeutic benefits.

Conclusion

The data from our study at the prime defense tertiary care center showed that young adults with CRC were presented with more aggressive disease forms with unfavorable histology and poor response to therapies, resulting in high mortality. The Indian population had more right-side CRC and these were metastatic predominantly. RAS and BRAF mutations were associated mainly with left- and right-side CRCs, respectively, though both had a poor prognosis and responses to therapies. NRAS mutation which till date has not been studied extensively may be an important indicator as it is seen exclusively in young females with left sided and had poor prognosis aggressive tumor. BRAF mutations are higher in the Indian population as compared to the western population. They were right-side lesion, mostly in women invariably associated with comorbidities such as hypertension, diabetes, and hypothyroidism. BRAF V600E was the most common mutation and an effective targeted agent for the same is the need of the hour to improve outcome in this subset which otherwise has the worst prognosis. Young recruits of the armed forces with a positive family history of CRC and comorbidities should be screened and investigated regularly for the development of CRC with tumor markers, colonoscopy, and mutational analysis during their annual medical examination.

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Conflicts of interest

There are no conflicts of interest.

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