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Original Article

Pathology Section

Association of Clinicopathological Profile and Immunohistochemical Expression of KRAS and BRAF in Colorectal Carcinoma: A Cross-sectional Study

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ABSTRACT

Introduction: Colorectal Carcinoma (CRC) is a multi-step process that occurs due to the accumulation of several genetic alterations. The most important alterations are related to the rat sarcoma viral oncogene homolog (RAS) and Rapidly Accelerated Fibrosarcoma (RAF), which have been implicated as key intermediates in the RAS-mediated signalling cascade. However, the levels of Kristen Rat Sarcoma Virus (KRAS) and v-raf Murine Sarcoma Viral Oncogene Homolog (BRAF) protein expression and their prognostic evaluation in CRC patients remain unknown.

Aim: To investigate the immunohistochemical expression of KRAS and BRAF proteins in CRC.

Materials and Methods: The present institutional-based cross-sectional observational study was conducted in a tertiary care centre in West Bengal, specifically in the Department of Pathology in collaboration with the Department of Surgery at Murshidabad Medical College and Hospital, Berhampore, West Bengal, India. A total of 26 CRC cases were enrolled in the present study, received over a period of one and a half years from January 2021 to June 2022. The parameters studied included demographic and clinical information of the patients, histopathological findings, pathological grade and stage of carcinoma, and immunohistochemical findings for KRAS and BRAF. For statistical analysis, data were entered into Microsoft (MS) Excel. Descriptive measures such as mean±Standard Deviation (SD), range, and percentage were used. The Chi-square test was used to determine the significance of the study.

Results: A total of biopsy-proven CRC specimens were studied, consisting of 17 male patients (65.38%) with a male-to-female ratio of 1.9:1. The most common age group involved was 51-60 years (38%). Conventional adenocarcinoma accounted for the majority of cases (85%), with mucinous carcinoma comprising the remaining 15%. Among the 26 cases, 15 (58%) showed KRAS positivity, which was significantly associated with tumour grade and stage. Most of the cases were BRAF-negative. Out of the 21 cases where either KRAS or BRAF or both were positive, 20 cases showed high T stage (T3 and T4) and/or metastatic lesions (p-value 0.001). All four cases that were negative for both BRAF and KRAS belonged to the low T stage.

Conclusion: A significant correlation was observed between the expression of KRAS and high-grade, high pathological Tumour, Node, Metastasis (TNM) T stage (T3 and T4) CRC. Therefore, KRAS Immunohistochemistry (IHC) biomarkers should be included in the standard diagnostic protocol for colorectal cancer, as they help identify KRAS-positive CRC cases that are resistant to targeted immunotherapy.

Keywords: Immunohistochemistry, Kristen rat sarcoma virus, V-raf murine sarcoma viral oncogene homolog

INTRODUCTION

The CRC is one of the deadliest cancers and the leading cause of cancer-related deaths in developed countries. It stands as the third most common malignancy in men and the second most common in women worldwide [1]. In India, CRC occupies the fifth most common position following breast, cervix/uterus, oral cavity, and lung cancers [2]. The dietary factors most associated with an increased risk of CRC are low consumption of unabsorbable vegetables and fibre, as well as a high intake of refined fats and carbohydrates. Smoking, alcohol intake, and increased body weight also increase the risk of cancer. With each unit increase in Body Mass Index (BMI), the risk for CRC increases by 2-3%. Patients with type 2 diabetes mellitus also have an increased risk of colorectal cancer [3].

The KRAS protein belongs to the large superfamily of guanine Guanosine-50-Triphosphate (GTP) and guanine Guanosine-50-Diphosphate (GDP) binding proteins and plays a powerful downstream effector role in the Epidermal Growth Factor Receptor (EGFR) transduction cascade. Somatic KRAS mutations are detected in about 40% of CRC patients and lead to an abnormal affinity of KRAS for GTP, resulting in permanent activation of the transduction cascade. BRAF, a member of the RAS/RAF family, encodes a serine-threonine protein kinase involved in the Mitogen-Activated Protein Kinase (MAPK) signalling cascade. BRAF acts as a direct effector of RAS and promotes tumour growth, proliferation, and survival through the activation of Mitogen-activated Protein Kinase (MAPK/ERK kinase). Current researchers mostly focus on the theory of heterogeneity of CRC, which highlights the differences in the KRAS mutational status between primary and metastatic tumours [4].

The IHC is a less time-consuming and less expensive alternate procedure to identify genetic mutations. The levels of KRAS and BRAF protein expression and their prognostic evaluation in CRC patients remain unknown. Some studies have found that KRAS mutation in colorectal cancer is associated with a poor response to EGFR inhibitors Cetuximab and Panitumumab and resistance to chemotherapy [5]. The aim of the present study was to analyse the clinical and histopathological features of CRC and identify the occurrence of BRAF and KRAS mutations in CRC patients through immunohistochemical studies, helping to redefine targeted therapy and chemotherapy. Although molecular studies have been carried

out on KRAS and BRAF in CRC, very few studies have highlighted the importance of IHC in identifying the expression of KRAS and BRAF [6,7]. The present study is the first of its kind in eastern India to highlight the importance of immunomarkers (BRAF and KRAS) in CRC.

To determine the clinical and epidemiological profile and immunohistochemical expression of KRAS and BRAF in CRC at a tertiary care hospital in West Bengal and to estimate the distribution of KRAS and BRAF expression among CRC patients through IHC.

MATERIALS AND METHODS

An Institutional-based cross-sectional observational study was conducted in a tertiary care centre in West Bengal in the Department of Pathology in collaboration with the Department of Surgery at Murshidabad Medical College and Hospital, Berhampore, West Bengal, India. The study spanned one and a half years, from January 2021 to June 2022. Ethical clearance was obtained from the Institutional Ethical Committee (IEC no- MSD/MCH/PR/2376/2020, dated 21/12/2020), and informed consent was obtained from the study population.

Inclusion and Exclusion criteria: The study included all patients clinically and histologically confirmed to have Colorectal Cancer (CRC) who attended the Outpatient Department (OPD) or were admitted to Murshidabad Medical College and Hospital during the study period. Trained histopathologists conducted the reporting, and tissue samples from all cases that fulfilled the inclusion and exclusion criteria were sent to the Pathology Department for routine histopathological examination.

Study Procedure

All tissue samples were collected in 10% buffered formalin and processed for routine histopathological examination. Grossing and reporting of specimens suggestive of colorectal adenocarcinoma were conducted according to the College of American Pathologists (CAP) protocol [8]. Histopathological diagnosis was made by cutting 5-micrometer thick sections from formalin-fixed paraffin-embedded blocks and staining them with Haematoxylin and Eosin (H&E).

The study considered epidemiological and clinical parameters of the patients, histopathological findings, pathological grade and stage of carcinoma, and immunohistochemical findings. The classification of histological type was based on the TNM stage of CRC as advocated by the International Agency for Research on Cancer and World Health Organisation (WHO) 2019 [9].

Immunohistochemistry (IHC): Samples that tested positive for colon cancer by histopathology underwent further analysis using IHC markers. The positivity of IHC expression was reported using a standard procedure and scoring pattern. The clone for KRAS Rabbit Monoclonal Antibody was 2J13, and for BRAF V600E Rabbit Monoclonal Antibody, the clone used was RM8. The study examined the association of KRAS and BRAF expression with respect to histological type, grade, and stage of cancer. For IHC staining, 3 µm thick sections from formalin-fixed paraffin-embedded tissues were taken on poly-L-lysine-coated slides. IHC staining was performed manually using a rabbit monoclonal antibody, following the steps mentioned in the supplied kit. Cytoplasmic IHC staining of KRAS protein was subjectively scored under a light microscope, and the percentage of stained tumour cells (brown colour) was expressed using established criteria as follows: 3+ when most cells (>50%) were strongly stained, 2+ when 25-50% of cells were moderately stained, 1+ when the staining was focal (<25%) and weak, and no stained cells were considered negative or 0 [6].

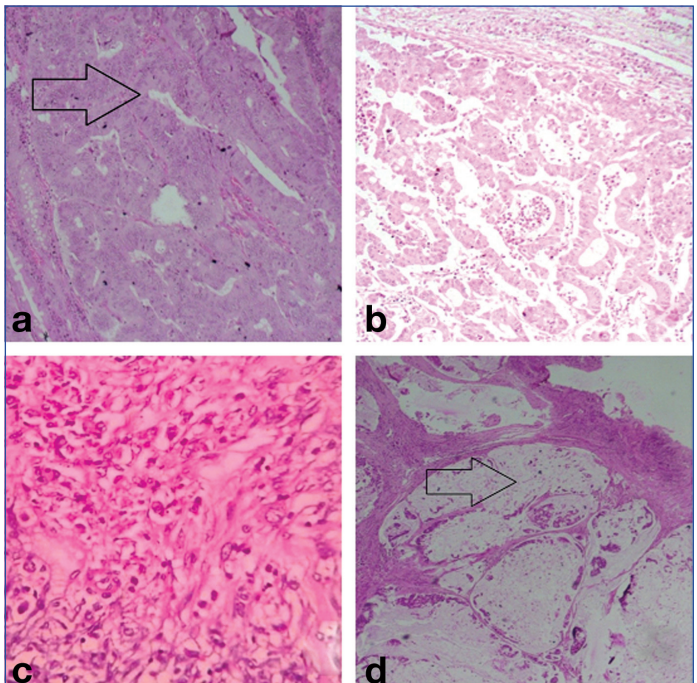
Cytoplasmic IHC staining of the BRAF protein was subjectively scored under a light microscope, and the percentage of stained tumour cells (brown colour) was expressed based on previously established criteria as follows: 3+ for strong staining, 2+ for moderate staining, and 1+ for weak staining. The staining intensity of the anti-BRAF V600E (VE1) antibody in tumour cells was recorded on a 0-3 scale. A score of 3+ was assigned for strong cytoplasmic staining when more than 50% of cells were stained intensely, 2+ for medium cytoplasmic staining when 31-50% of cells were stained, and 1+ for weak cytoplasmic staining when 11-30% of cells were stained. The absence of staining or less than 10% of stained cells was scored as 0. Additionally, any nuclear staining and the percentage of tumour cells stained positive with the anti-BRAF V600E (VE1) antibody were recorded. Positive BRAF V600E staining criteria included unequivocal, diffuse, uniform cytoplasmic staining with an intensity of 1 or higher in the majority of malignant cells. Cases were considered negative for BRAF V600E mutation if they showed no staining or weak, cytoplasmic, non granular, uniform staining [10].

STATISTICAL ANALYSIS

For statistical analysis, the data were entered into MS excel. For descriptive purposes, the mean±SD, range, and percentage were used. The Chi-square test was used to determine the significance of the study using Statistical Package for Social Sciences (SPSS) version 20.0. The significance level was set at a p-value <0.05.

RESULTS

Epidemiological and histological profile: The majority of 22 cases (84.61%) were conventional adenocarcinoma, while 4 cases (15.38%) were mucinous carcinoma. It was observed that the majority of cases were males, accounting for 65.38%, while females constituted 34.63% of the cases. The male-to-female ratio was 1.9:1. The most common age group affected was 51-60 years (38%), followed by 61-70 years (23%). The mean age at presentation was 53.69 years. The highest risk factor for Colorectal Cancer (CRC) in the present study was cigarette smoking (43.31%), followed by obesity (30.76%), alcohol consumption (19.23%), and a family history (7.69%) of carcinoma. The majority of people were non vegetarian (80.77%), while the rest were vegetarian. CRCs mostly occurred in the rectum with 11 cases (42.30%), followed by the ascending colon with 9 cases (34.62%). Gross examination revealed that the majority of tumours showed ulceroproliferative growth (57%), followed by infiltrative growth in 23% of cases. Among the 22 cases of adenocarcinoma, the majority were moderately differentiated adenocarcinoma (63.64%), 31.81% were well-differentiated adenocarcinoma, and 4.55% were poorly-differentiated adenocarcinoma [Table/Fig-1a-d]. Most cases



[Table/Fig-1]: Microphotograph of: (a) Well-differentiated adenocarcinoma. (H&E 400X). Arrow showing glandular formation; (b) Moderately differentiated adenocarcinoma, (H&E 400X); (c) Poorly differentiated adenocarcinoma. (H&E 400X); and (d) Mucinous adenocarcinoma (H&E 100X) Arrow showing extracellular mucin pool.

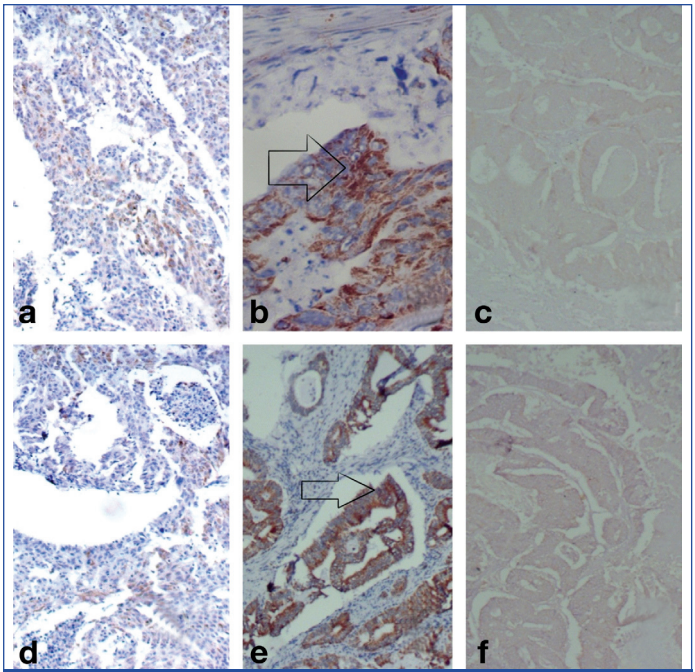
belonged to the T3 stage (69%), followed by T2 (19%). Thirteen cases showed lymphovascular invasion, and three cases showed perineural invasion. Lymph nodes of 11 cases (42.31%) exhibited metastatic carcinomatous deposits, while 6 cases (23.08%) showed reactive hyperplasia. Nodal status could not be assessed in approximately 9 cases (34.61%).

KRAS

In the present study, it was observed that the majority of 15 cases (58%) were KRAS positive, and 11 cases (42%) were KRAS negative [Table/Fig-2]. Six cases (23%) had a score of 3+, 8 cases (31%) had a score of 2+, and 1 case (4%) had a score of 1+ [Table/Fig-3a-d]. Out of a total of 15 KRAS-positive CRC cases, 13 cases were G2 and G3, moderately and poorly differentiated adenocarcinoma (87%), whereas out of a total of seven KRAS-negative adenocarcinoma cases, two were G2 and G3 (29%). There was a significant association between the expression of KRAS and moderately and poorly differentiated adenocarcinoma, i.e., Grade-2 and 3 tumours (p-value 0.006) [Table/Fig-4]. Out of a total of 15 KRAS-positive cases, 14 cases showed high T staging (T3 and T4), accounting for around 93%. However, out of a total of 11 KRAS-negative cases, six cases showed high T staging (54%). The association between KRAS expression and high T stage was found to be statistically significant with a p-value of 0.02 [Table/Fig-5].

Mutation	No. of cases	Percent (%)
KRAS		
Detected	15	57.69
Not detected	11	42.31
Total	26	100
BRAF		
Detected	10	38.46
Not detected	16	61.54
Total	26	100

[Table/Fig-2]: Distribution of KRAS F and BRAF mutations in Colorectal Carcinoma (CRC) cases. KRAS mutation is seen in 58% of CRC and BRAF mutation is seen in 38% of CRC cases.



[Table/Fig-3]: Histopathological and immunohistochemical examination of colorectal cancers. Upper panel: HP microphotographs of: (a) Moderately differentiated adenocarcinoma- KRAS immunostained (score 2 +) (400X); (b) Well-differentiated adenocarcinoma- KRAS immunostained (arrow showing score 3 +) (400X); (c) Moderately differentiated adenocarcinoma- KRAS immunostained (negative); (d) Left: moderately differentiated adenocarcinoma- BRAF immunostained (score 2+) (400X); (e) well-differentiated adenocarcinoma- BRAF immunostained (arrow showing score 3+) (400X); and (f) moderately differentiated adenocarcinoma- BRAF immunostained (negative) (400X).

Subtype	KRAS positive	KRAS negative	Statistical significance
Grade 1 (WD)*	2	5	Z=7.4, p=0.006 significant
Grade 2+Grade 3 (Moderately and poorly differentiated)*	13	2	
Subtype	**BRAF positive**	**BRAF negative**	**Statistical significance**
Grade1 (WD)	3	4	Z=0.57, p=0.44, not significant
Grade 2+Grade 3 (MD and PD)	4	11	

[Table/Fig-4]: Distribution of the moderately and poorly differentiated carcinoma combined with well-differentiated carcinoma in KRAS and BRAF positive and negative cases.
*WD: Well differentiated; **MD: Moderately differentiated; #PD: Poorly Differentiated; #G: Grade 1, 2, and 3

Aggressiveness of tumours	KRAS and BRAF anyone/ both positive	KRAS and BRAF both negative
T3 + T4 + all metastatic lesions	20	1
Rest of T1 and T2 tumours	1	4

[Table/Fig-5]: Schematic chart depicting the distribution of all aggressive CRC and lower-stage lesions (T1 and T2 combined) in KRAS and BRAF anyone/both positive group and both negative groups. The Chi-square statistic was 14.7 and the p-value was 0.001.

BRAF

It was observed that 10 cases (38.46%) showed BRAF positivity, while 16 cases (61.54%) were negative for BRAF [Table/Fig-3]. In [Table/Fig-2], 4 cases (15%) showed a score of 3+, 5 cases (19%) showed 2+, and 1 case (4%) showed a score of 1+. Among the 10 BRAF-positive cases, 3 cases showed mucinous histology (30%), whereas among the 16 BRAF-negative cases, only 1 case showed mucinous histology (6%). There was no significant association found between BRAF positivity and mucinous or non mucinous carcinoma (p-value 0.26). Out of a total of 7 BRAF-positive conventional adenocarcinoma cases, 4 cases were G2 and G3, moderately and poorly differentiated adenocarcinoma (57%). Among the 15 BRAF-negative conventional adenocarcinoma cases, 11 were G2 and G3 adenocarcinoma (73%). There was no significant correlation between the expression of BRAF and the histological grading of tumours.

Out of the total 26 cases of CRC, only 4 cases showed both KRAS and BRAF positivity (15%) [Table/Fig-6]. Among the 10 BRAF-positive cases, 9 cases (90%) showed high T staging (T3 and T4). However, out of the 16 BRAF-negative cases, 11 cases (68.75%) showed high T staging [Table/Fig-5]. The association between BRAF expression and the high T stage is not statistically significant, with a p-value of 0.21. No association between KRAS and BRAF expression was observed in CRC. Out of a total of 21 cases where KRAS and BRAF were positive in anyone/both, 20 cases showed high T stage (T3 and T4) and/or metastatic lesions. The association between them is statistically significant, with a p-value of 0.001.

Subtype	KRAS positive	KRAS negative
BRAF positive	4	6
BRAF negative	11	5

[Table/Fig-6]: Distribution of BRAF mutations in KRAS positive and negative cases.

DISCUSSION

Rectum was the site with the highest occurrence of carcinoma, accounting for 11 cases (42.3%) in the present study. This finding is consistent with other studies by Patra T et al., where 46.2% of carcinomas occurred in the rectum, and Hajmanoochehri F et al., where 55% were rectal carcinomas [11,12]. Regarding the histological subtypes of Colorectal Cancer (CRC), the present study observed that adenocarcinoma-usual type accounted for 84.6% and mucinous type accounted for 15.4%. This is in line with a study by Hajmanoochehri F et al., where the majority of cases included the conventional type of adenocarcinoma [12].

BRAF plays a crucial role in activating the RAS/RAF/MAPK/ERK signalling cascade, which regulates cellular growth, proliferation, differentiation, apoptosis, and cell survival [13,14]. There are approximately 30 different BRAF mutations, with the V600E mutation being the most common [15]. Both of these mutations are oncogenic driver mutations responsible for initiating and maintaining tumours [16,17]. In the present study, it was observed that 15 cases (58%) were KRAS positive, while 11 cases (42%) were KRAS negative. This is consistent with findings by Payandeh M et al., and Dinu D et al., which showed KRAS mutation positivity in 30-50% of CRC cases [17,18].

Of the 15 KRAS-positive cases, 14 cases showed high T staging (T3 and T4), accounting for approximately 93%. However, out of the 11 KRAS-negative cases, six cases showed high T staging (54%). The association between KRAS and high T-stage CRC was found to be statistically significant with a p-value of 0.02. According to Dinu D et al., KRAS mutation is associated with poor survival and increased tumour aggressiveness, specifically with higher T-stage in CRC [18]. This finding aligns with authors observations. However, Ogino S et al., stated that there is no significant difference in survival rates between KRAS-mutated and wild-type CRC [19]. In the present study, it was observed that 10 cases (38%) were BRAF positive, while 16 cases (62%) were BRAF negative. However, most studies to date, such as Barras D et al., (2015), have shown that BRAF mutation is found in approximately 10% of CRC cases [20]. This does not align with the present study. Grassi E et al., also demonstrated that the incidence of BRAF mutation is around 8-12% in colorectal cancer patients [21].

Out of a total of 10 BRAF-positive cases, 3 cases showed mucinous histology (30%). Among the 16 BRAF-negative cases, only 1 case showed mucinous histology (6%). The association between BRAF positivity and mucinous histology was not statistically significant (p-value=0.1), but a noticeable trend was evident from the results. Caputo F et al., also pointed out that BRAF mutation is associated with female sex, advanced age, proximal colon involvement, poorly differentiated tumours, and mucinous histology [22]. Among the total of 10 BRAF-positive cases, the proximal colon was involved in six cases (60%), while the distal colon including the rectum was involved in four cases. However, out of the 14 BRAF-negative cases, the proximal colon was involved in six cases (43%). Although the difference is not statistically significant, there is a clear predilection for BRAF-positive cases to involve the proximal colon. Thiel A and Ristimäki A stated that BRAF mutation is associated with proximal colon involvement and higher-grade carcinoma [23]. Missiaglia E et al., also commented that proximal colon cancers often exhibit mucinous histology and express BRAF mutation [24]. This finding somewhat supports the present study results. Tanaka H et al., stated that BRAF is mutated in most right-sided colon cancers, and mucinous histology is common in them [25].

Out of the total of 10 BRAF-positive cases, nine cases showed high T staging (T3 and T4), which is around 90%. Conversely, out of the 16 BRAF-negative cases, 11 cases showed high T staging, approximately 68%. However, the association between BRAF mutation and high T staging is not statistically significant, with a p-value of 0.21. According to Caputo F et al., BRAF mutations are often associated with advanced stages of CRC [22]. This is in accordance with the present study. Yuan ZX et al., observed that the frequency of BRAF mutation is higher in stages 3 and 4 compared to stages 1 and 2 [26]. These findings correlate with the present study results.

Out of a total of 21 cases where KRAS and BRAF were positive, 20 cases showed either high T stage (T3 and T4) or metastatic lesions (95%). In contrast, out of a total of five cases where both KRAS and BRAF were negative, only one case showed a high T stage or metastatic lesion (20%). The association is significant (p-value=0.001). Combining the KRAS and BRAF mutation status, if anyone or both come positive, it is highly suspicious that the patient has an aggressive lesion in terms of higher tumour stages. Conversely, if both come negative, it is likely that the patient does not have an aggressive tumour, possibly T1/T2 lesions, and is non metastatic in nature. Therefore, combining both KRAS and BRAF testing can better diagnose aggressive/invasive tumours and predict possible poor outcomes or poor responses to therapy.

Out of a total of 15 KRAS-positive CRC cases, 13 cases were Grade-2/3 adenocarcinoma (87%), whereas out of a total of seven KRAS-negative adenocarcinoma cases, two were Grade-2/3 tumours (29%). The association between KRAS mutation and combined moderately and poorly differentiated adenocarcinoma is highly significant (p-value=0.006). Hence, tumour grading can be predicted from the mutational status and prognosis as well.

Among the 10 BRAF-positive cases, lymphovascular invasion was found in six cases (60%), whereas out of the 16 BRAF-negative cases, lymphovascular invasion was seen in seven cases (43%). Out of the total 15 KRAS-positive cases, lymphovascular invasion was seen in eight cases (53%), whereas out of a total of 11 KRAS-negative cases, lymphovascular invasion was seen in five cases (45%). This also supports authors findings. Guo TA et al., stated that BRAF mutation is associated with more lymphovascular invasion, poor differentiation, and positive tumour deposit [27]. KRAS mutations at codon 13 are more prone to metastasize to more than two organs, leading to higher recurrence rates and lower survival rates, according to Pereira AA et al., [28]. These findings support the present research. Pereira AA et al., identified 494 mCRC patients, of which 202 (41%) had tumours with KRAS mutations. They found that KRAS mutations were associated with a twofold greater odds of developing lung metastases during the disease course in patients with liver-limited metastatic CRC at diagnosis (72% vs. 56%, p=0.007). Lung metastasis was more likely to develop in patients whose tumours had a KRAS mutation compared to those without a KRAS mutation. This finding may influence decision-making regarding surgical resection of metastatic disease. Modest DP et al., also reported a similar finding in 2011 [29]. This pooled analysis suggests that metastatic RC is a heterogeneous disease, which appears to be defined by KRAS mutations of the tumour.

Out of the total 26 cases, four cases showed both KRAS and BRAF positivity (15%). Previously, KRAS and BRAF were thought to be mutually exclusive. However, concomitant mutation has now been identified in multiple cases, although the clinical significance is yet to be established, as per the opinion of Midthun L et al., [30]. Sahin IH et al., mentioned that concomitant KRAS and BRAF mutation is rarely found (0.001%). Larki P et al., stated that KRAS and BRAF are associated with more severe disease when present together, so BRAF testing is highly advisable when the tumour is already KRAS positive [31]. According to them, the presence of both mutations simultaneously signifies the polyclonal nature of the tumour cells and heterogeneous tumour biology. It also indicates an increased incidence of transmural invasion in the tumours.

Based on the study findings, authors suggest that both KRAS and BRAF mutations should be tested in all pre/postoperative biopsies, surgically resected specimens, and metastatic lesions. This will enable authors to diagnose aggressive tumours, identify polyclonal and biologically heterogeneous lesions, and make more informed decisions regarding chemotherapy.

Limitation(s)

The present study included only a small population (26 patients) who attended a tertiary care hospital. The duration of the present study was 18 months, which involved data collection, immunohistochemistry, and data analysis. Therefore, authors faced time constraints in reaching a larger sample size. In several aspects, authors were unable to establish a statistically significant association

due to the small sample size. Hence, a multicentre trial involving a larger sample size and a representative study population is needed to obtain conclusive results, allowing for the generalisation of the prognostic significance to the population.

CONCLUSION(S)

The immunohistochemical expression of KRAS was positive in the majority of the cases, showing a significant correlation between KRAS expression and both moderately and poorly differentiated carcinoma (combined G2 and G3) as well as a high pathological TNM T stage (T3 and T4) in CRC. The most prevalent cytoplasmic staining pattern in both KRAS-positive and BRAF-positive CRC is 2+. If either KRAS or BRAF (or both) is positive in CRC cases, there is a high chance of aggressive tumours, indicated by high TNM T stage (T3 and T4) and/or metastatic disease. KRAS IHC biomarkers should be included in the standard diagnostic protocol for colorectal cancer, as they help screen for KRAS-positive CRC cases that are resistant to targeted immunotherapy. Appropriate chemotherapy can then be initiated based on these findings.

REFERENCES

[1] Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol. 2012;3(3):153-73.
[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer. 2015;136(5):E359-86.
[3] Guraya SY. Association of type 2 diabetes mellitus and the risk of colorectal cancer: A meta-analysis and systematic review. World Journal of Gastroenterology. 2015;21(19):6026-31.
[4] Notarangelo M. Exploiting extracellular vesicles for ultrasensitive detection of cancer biomarkers from liquid biopsies.
[5] De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: A retrospective consortium analysis. The Lancet Oncology. 2010;11(8):753-62.
[6] Elsabah MT, Adel I. Immunohistochemical assay for detection of K-ras protein expression in metastatic colorectal cancer. Journal of the Egyptian National Cancer Institute. 2013;25(1):51-56.
[7] Estrella JS, Tetzlaff MT, Bassett Jr RL, Patel KP, Williams MD, Curry JL, et al. Assessment of BRAF V600E status in colorectal carcinoma: Tissue-specific discordances between immunohistochemistry and sequencing. Molecular Cancer Therapeutics. 2015;14(12):2887-95.
[8] Tsong WH, Koh WP, Yuan JM, Wang R, Sun CL, Yu MC. Cigarettes and alcohol in relation to colorectal cancer: The Singapore Chinese Health Study. British Journal of Cancer. 2007;96(5):821-27.
[9] International Agency for Research on Cancer. WHO Classification of Tumours Editorial Board. Digestive System Tumours. 2019.
[10] Dvorak K, Higgins A, Palting J, Cohen M, Brunhoeber P. Immunohistochemistry with anti-BRAF V600E (VE1) mouse monoclonal antibody is a sensitive method for detection of the BRAF V600E mutation in colon cancer: Evaluation of 120 cases with and without KRAS mutation and literature review. Pathology & Oncology Research. 2019;25(1):349-59.
[11] Patra T, Mandal S, Alam N, Murmu N. Cliniopathological trends of colorectal carcinoma patients in a tertiary cancer center in eastern India. Clinical Epidemiology and Global Health. 2018;6:39-43.
[12] Hajmanoochehri F, Asefzadeh S, Kazemifar AM. Clinicopathological features of colon adenocarcinoma in Qazvin, Iran: A 16-year study. Asian Pacific Journal of Cancer prevention. 2014;15(2):951-55.
[13] Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM. Cancer Genome Project; Jones, CM; Marshall, CJ. Springer C, Barford D, Marais R. Mechanism of Activation of the RAF-ERK Signaling Pathway by Oncogenic Mutations of B-RAF, Cell. 2004;116(6):855-67.
[14] Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, et al. The role of BRAF V600 mutation in melanoma. J Transl Med. 2012;10(1):01-09.
[15] Clarke CN, Kopetz ES. BRAF mutant colorectal cancer as a distinct subset of colorectal cancer: Clinical characteristics, clinical behavior, and response to targeted therapies. Journal of Gastrointestinal Oncology. 2015;6(6):660-67.
[16] Han CB, Li F, Ma JT, Zou HW. Concordant KRAS mutations in primary and metastatic colorectal cancer tissue specimens: A meta-analysis and systematic review. Cancer Investigation. 2012;30(10):741-47.
[17] Payandeh M, Amirifard N, Sadeghi M, Shazad B, Farshchian N, Sadeghi E, et al. The prevalence of KRAS mutation in colorectal cancer patients in the Iranian population: A systematic review and meta-analysis study. Biomedical Research and Therapy. 2017;4(10):1693-704.
[18] Dinu D, Dobre M, Panaitescu E, Bîrlă R, Iosif C, Hoara, et al. Prognostic significance of KRAS gene mutations in colorectal cancer– A preliminary study. Journal of Medicine and Life. 2014;7(4):581-58.
[19] Ogino S, Meyerhardt JA, Irahara N, Niedzwiecki D, Hollis D, Saltz LB, et al. KRAS mutation in stage III colon cancer and clinical outcome following intergroup trial CALGB 89803. Clinical Cancer Research. 2009;15(23):7322-29.
[20] Barras D. BRAF mutation in colorectal cancer: An update: supplementary issue: biomarkers for colon cancer. Biomarkers in Cancer. 2015;7:BIC-S25248.
[21] Grassi E, Corbelli J, Papiani G, Barbera MA, Gazzaneo F, Tamberi S. Current therapeutic strategies in BRAF-Mutant metastatic colorectal cancer. Front Oncol. 2021:11:01-06.
[22] Caputo F, Santini C, Bardasi C, Cerma K, Casadei-Gardini A, Spallanzani A, et al. BRAF-Mutated Colorectal Cancer: Clinical and Molecular Insights. International Journal of Molecular Sciences. 2019;20(21):5369.
[23] Thiel A, Ristimäki A. Toward a molecular classification of colorectal cancer: The role of BRAF. Frontiers in Oncology. 2013;3:281.
[24] Missiaglia E, Jacobs B, D'ario G, Di Narzo AF, Soneson C, Budinska E, et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. Annals of Oncology. 2014;25(10):1995-2001.
[25] Tanaka H, Deng G, Matsuzaki K, Kakar S, Kim GE, Miura S, et al. BRAF mutation, CpG island methylator phenotype and microsatellite instability occur more frequently and concordantly in mucinous than non-mucinous colorectal cancer. International Journal of Cancer. 2006;118(11):2765-71.
[26] Yuan ZX, Wang XY, Qin QY, Chen DF, Zhong QH, Wang L, et al. The prognostic role of BRAF mutation in metastatic colorectal cancer receiving anti-EGFR monoclonal antibodies: A meta-analysis. PLoS one. 2013;8(6):e65995.
[27] Guo TA, Wu YC, Tan C, Jin YT, Sheng WQ, Cai SJ, et al. Clinicopathologic features and prognostic value of KRAS, NRAS and BRAF mutations and DNA mismatch repair status: A single-center retrospective study of 1,834 Chinese patients with Stage I–IV colorectal cancer. International Journal of Cancer. 2019;145(6):1625-34.
[28] Pereira AA, Rego JF, Morris V, Overman MJ, Eng C, Garrett CR, et al. Association between KRAS mutation and lung metastasis in advanced colorectal cancer. British Journal of Cancer. 2015;112(3):424-28.
[29] Modest DP, Stintzing S, Laubender RP, Neumann J, Jung A, Giessen C, et al. Clinical characterization of patients with metastatic colorectal cancer depending on the KRAS status. Anti-Cancer Drugs. 2011;22(9):913-18.
[30] Midthun L, Shaheen S, Deisch J, Senthil M, Tsai J, Hsueh T. Concomitant KRAS and BRAF mutations in colorectal cancer. Journal of Gastrointest Oncol. 2019;10(3):577-81.
[31] Larki P, Gharib E, Taleghani MY, Khorshidi F, Nazemalhosseini-Mojarad E, Aghdaei HA. Co-existence of KRAS and BRAF mutations in colorectal cancer: A case report supporting the concept of tumoural heterogeneity. Cell Journal (Yakhteh). 2017;19(Suppl 1):113-17.

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