Original Article

DOI: 10.7860/NJLM/2026/85385.2975

Pathology Section

Immunohistochemical Evaluation of BRAF V600E and HER2 Expression in Colorectal Carcinoma and Their Association with Clinicopathological Parameters: A Cross-sectional Study from a Tertiary Care Centre in Eastern India

NAKKA VAISHNAVI[1], RINA BARMAN[2], TARAK BANIK[3]

ABSTRACT

Introduction: Colorectal Carcinoma (CRC) is the third most common cancer in the world according to Global Cancer Observatory (GLOBOCAN) statistics, 2022. CRC's phenotypic and molecular comprehensive characterisation represents a key step, with diagnostic and prognostic value assessment of BRAF V600E {B-Raf proto-oncogene, serine/threonine kinase (VAL600GLU)} and Human Epidermal Growth Factor Receptor 2 (HER2) expression using Immunohistochemistry (IHC) can aid in understanding tumour characteristics in routine diagnostic practice.

Aim: The aim of the present study was to evaluate the immunohistochemical expression of BRAF V600E and HER2 in CRC and their association with clinicopathological parameters.

Materials and Methods: The present observational cross-sectional study was conducted in the Department of Pathology, RG Kar Medical College and Hospital, Kolkata, West Bengal, India, over an 18-month period (July 2023 to December 2024) on 72 diagnosed cases of CRC. Clinicopathological parameters were recorded. IHC was performed using mutation specific monoclonal antibody for BRAF V600E (clone IHC600, GenomeMe, Canada) and HER2 clone EP3 Dygnova, India). Results were analysed using Statistical Package for the Social Sciences (SPSS) 27.0 (SPSS, Inc., Chicago, IL, USA) and carried out Chi-square and Fisher`s-exact tests, with p-value <0.05 was considered statistically significant.

Results: Out of 72 cases of CRC, BRAF V600E expression was positive in 16 cases (22.2%), equivocal in 7 cases (9.7%) and negative in 49 cases (68.1%). BRAF V600E positivity showed a statistically significant association with poor differentiation (p=0.027), advanced stage (p=0.0086), lymph node metastasis (p=0.0055), Lympho-Vascular Invasion (LVSI) (p=0.037) and mucinous histology (p=0.0001). HER2 expression was positive in three cases (4.17%), borderline in four cases (5.56%) and negative in 65 cases (90.27%). HER2 expression showed a significant association only with tumour grade (p=0.009).

Conclusion: BRAF V600E expression is significantly associated with aggressive clinicopathological features in CRC, while HER2 expression showed limited association. Immunohistochemistry serves as a useful screening tool for molecular alterations, however confirmatory molecular testing and long-term follow-up studies are required to establish prognostic significance.

Keywords: Adenocarcinoma, Human epidermal growth factor receptor 2, Pathological staging, Protein expression, Tumour grading

INTRODUCTION

The CRC is the third most common cancer, yet it is the second in terms of mortality worldwide [1]. In India, CRC ranks sixth most common cancer but seventh in terms of mortality. It is fourth most common cancer in males and fifth most common in females [1]. The CRC is a complex and molecularly heterogenous, characterised by different genomic landscapes [2,3]. CRC phenotypic and molecular comprehensive characterisation represents a key step, with diagnostic, prognostic and predictive value both in localised and in metastatic settings [4,5].

The BRAF gene encodes a serine/threonine kinase that functions as a key component of the Mitogen-Activated Protein Kinase (MAPK) signalling pathway, which governs fundamental cellular processes such as proliferation, differentiation, migration, survival, and angiogenesis. Aberrant activation of this pathway is a major contributor to tumour development and progression. The most common BRAF mutation (90%) in CRC is a Cytosine-Thymine-Guanine → Cytosine-Adenine-Guanine (CTG → CAG) transversion at residue 1799 (T1799A), leading to an amino acid substitution from valine to glutamic acid at codon 600 (p.V600E) in the exon 15 which is classified as a Class-I BRAF mutation [6,7]. The other non-V600E mutations that are encountered in CRC are G469A, L597V (Class II BRAF mutations) as well as D594N, G466V, D594G, D594A, D594H (Class III BRAF mutations) [7].

HER2 gene, which is located on chromosome 17q21, is a tyrosine kinase receptor and encodes for a 185-kDa transmembrane protein. HER2 gene amplification plays a pivotal role in tumour growth and metastasis [8].

The HER2 is a well-characterised oncogenic driver and an established therapeutic target in several malignancies, including breast and gastric cancers. In metastatic Colorectal Carcinoma (mCRC), HER2 alterations comprising gene mutations and amplifications occur in approximately 3-5% of cases. Initially recognised for its association with resistance to anti-Epidermal Growth Factor Receptor (EGFR) therapies, HER2 has more recently gained attention as a clinically actionable target, providing new opportunities for targeted therapeutic interventions in mCRC [9]. The present study aimed to evaluate the immunohistochemical expression of BRAF V600E and HER2 in CRC and their association with clinicopathological parameters.

Study Objectives:

- To study the histopathological spectrum of CRC with respect to tumour type, grade and stage.
- To assess the immunohistochemical expression of BRAF V600E and HER2 in CRC.
- To analyse the association of BRAF V600E and HER2 expression with clinicopathological parameters.

MATERIALS AND METHODS

The present observational cross-sectional study was conducted in the Department of Pathology, RG Kar Medical College and Hospital, Kolkata, West Bengal, India, over a period of 18 months from July 2023 to December 2024. The study was approved by the Institutional Ethical Committee of RG KAR Medical College and Hospital (IEC clearance letter memo no. RKC/865 dated June 14, 2023).

Sample size calculation: A total of 72 histologically confirmed cases of adenocarcinoma of the colon and rectum were included over a period of 18 months (July 2023 to December 2024). Sample size is calculated using the formula:

$N= \{(Z\alpha/2)^2 \times P \times Q\}/L2$

Where, N=number of cases to be studied;

$(Z\alpha/2)$ is a constant=Standard normal deviate (taking confidence interval of 95%), value equals to 1.96;

P=Anticipated or estimated proportion of the disease according to GLOBOCAN data, value equals to 4.9% [10];

Q=(100-P), value equals to 95.1;

L=Absolute precision of the study being conducted, it is taken as 5;

N=(3.8416×4.9×95.1)/25=71.6 ~ 72 cases.

Inclusion criteria: All surgically resected specimen of CRC which were colonoscopic biopsy proven were included in the study.

Exclusion criteria: Cases with prior chemotherapy or radiotherapy, tissue biopsies, inflammatory conditions and colorectal tumours other than carcinomas (lymphomas and sarcomas) and patients who didn't wish to participate.

Study Procedure

Parameters included: Age, gender, tumour site, tumour size, histological grade, depth of invasion, tumour stage, histopathological type, lymph node status, lympho-vascular invasion.

Histopathological evaluation: After receiving specimens, we examined it grossly with measurement of size, shape, outer surface, cut surface and then fixed it in 10% buffered neutral formalin overnight. Then during grossing, sections from the fixed tumour proper were taken. Paraffin blocks were prepared using routine histopathological techniques. Thin sections (5 µ thick) were taken on the slide and staining was done with routine Haematoxylin & Eosin (H&E) stain. Light microscopic examination was done & result was noted. Histopathological type, grading and American Joint Committee on Cancer (AJCC) staging, 8th edition [11] were noted.

Immunohistochemistry: IHC was performed using commercially available monoclonal antibodies for BRAFV600E and HER2.

BRAFV600E- Clone- IHC600, (GenomeMe, Canada)

HER2- Clone - EP3 (Dygnova, India)

Immunohistochemistry was performed using mutation specific monoclonal antibody for BRAF V600E (clone IHC600), which detects the mutant BRAF V600E protein and serves as screening marker for underlying mutation.

Sections were cut from selected blocks on poly-L-lysine coated slides. After deparaffinisation, rehydration, and antigen retrieval were done by microwave method. It was followed by perox-blocking and protein blocking. Then primary antibody was added followed by secondary antibody and Horseradish Peroxidase (HRP) label which were subsequently followed by 3,3'-Diaminobenzidine (DAB) visualisation and Haematoxylin counterstaining. With each batch appropriate controls were also run. A known HER2 positive case of Invasive Breast Carcinoma and papillary thyroid carcinoma known to harbour BRAF V600E mutation were used as positive controls, and a negative control was achieved by omitting the primary antibody.

Scoring: BRAF V600E immunohistochemical expression was scored microscopically according to following scoring system [12].

- Cases were considered as positive when there was diffuse (>80% of tumour cells) cytoplasmic staining.
- Cases were considered as equivocal if there was nuclear staining in the tumour cells in addition to cytoplasmic staining and ambiguous staining.
- Cases were considered as negative when there was no staining or faint staining of tumour cells.

HER2 immunohistochemical expression was evaluated using a semi-quantitative scoring system based on the intensity and completeness of membranous staining in tumour cells, according to the Trastuzumab for Gastric Cancer (ToGA) criteria [Table/Fig-1] [13].

Score to report	HER2 protein overexpression assessment	Staining pattern
0	Negative	No staining or membrane staining in less than 10% of tumour cells
1+	Negative	Faint/barely visible membrane staining in at least 10% of cells or staining in parts of their membrane
2+	Borderline	Weak to moderate complete, basolateral, or lateral membrane staining in at least 10% of tumour cells
3+	Positive	Strong complete or basolateral membrane staining in at least 10% of tumour cells

[Table/Fig-1]: IHC scoring method for HER2 expression (ToGA criteria) [13].

STATISTICAL ANALYSIS

Results were compiled and analysed with SPSS 27.0 (SPSS, Inc., Chicago, IL, USA). For comparison of the frequencies among groups, Chi-square test and Fisher-exact test were used. A p-value <0.05 was considered statistically significant.

RESULTS

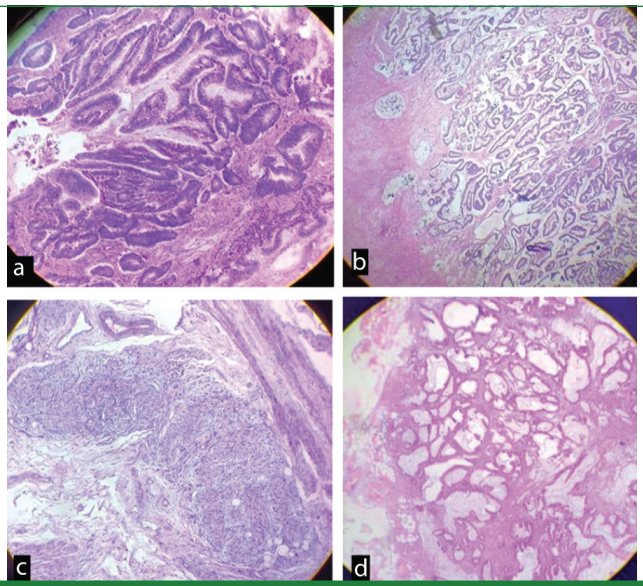
The study included 72 cases (n=72) of CRC aged between 29 years to 78 years with mean age of 55.5 years, a median age of 54.5 and maximum number of patients were in the age group 51-60 years (n=23; 31.96%). An increase in female patients was found in this study with male to female ratio being 1:1.25 [Table/Fig-2]. Most tumours were located in the right colon (n=30; 41.6%) and the commonest size of the tumour was less than 5cm (n=42; 58%) [Table/Fig-2].

Characteristics	Frequency (n)	Percentage (%)
Age (years)		
<50	24	33.4%
>50	48	66.6%
Range- 29 to 78 years		
Mean age- 55.5		
Median age- 54.5		
Gender		
Male	32	44.4%
Female	40	55.6%
Growth site		
Right-sided	30	41.6%
Left-sided	24	33.4%
Rectum	18	25%

Tumour size		
<5 cm	42	58%
5-10 cm	28	39%
>10 cm	2	3%
Tumour grade		
Grade 1	14	19%
Grade 2	50	69%
Grade 3	8	12%
Depth of invasion		
T1	3	4.16%
T2	20	27.78%
T3	42	58.34%
T4a	5	6.94%
T4b	2	2.78%
AJCC Stage, 8th edition		
I	20	27.7%
II	26	36.1%
III	25	34.7%
IV	1	1.5%
Lympho-vascular invasion		
Positive	26	36.1%
Negative	46	63.9%
Histopathological subtype		
Adenocarcinoma NOS	65	90.2%
Mucinous adenocarcinoma	6	8.3%
Signet ring cell carcinoma	1	1.5%

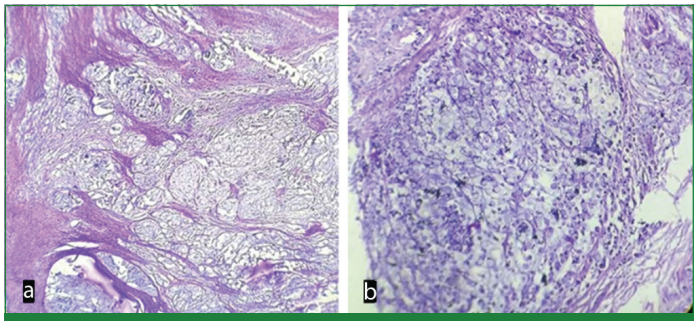
[Table/Fig-2]: Clinicopathological characteristics.
NOS: Not otherwise specified

In the present study, 69% of cases were moderately differentiated (n=50), 19% were well differentiated (n=14), and 12% were poorly differentiated (n=8) [Table/Fig-2,3]. The most frequent pattern of depth of invasion observed was invasion through the muscularis propria into peri-colorectal tissues (n=42; 58.34%), followed by invasion limited to muscularis propria (n=20; 27.78%) [Table/Fig-2]. Regarding nodal status, 46 cases showed no lymph node involvement (63.9%) and rest of the 26 cases showed lymph node involvement (36.1%) [Table/Fig-2,3]. In terms of tumour staging, Stage II was most common (n=26; 36.1%), followed by Stage III (n=25; 34.7%), Stage I (n=20; 27.7%), and Stage IV (n=1; 1.5%) [Table/Fig-2].



[Table/Fig-3]: a) Well to moderately differentiated CRC (H&E, x100); b) Moderately differentiated CRC (H&E, x100); c) Poorly differentiated CRC (H&E, x100); d) Lymph node metastasis (H&E, x100).

Histologically, the predominant tumour type was adenocarcinoma, Not Otherwise Specified (NOS) (n=65; 90.2%), followed by mucinous adenocarcinoma (n=6; 8.3%) and signet-ring cell carcinoma (n=1; 1.5%) [Table/Fig-2,4]. The majority of cases were negative for LVSI (n=46; 63.9%) [Table/Fig-2].

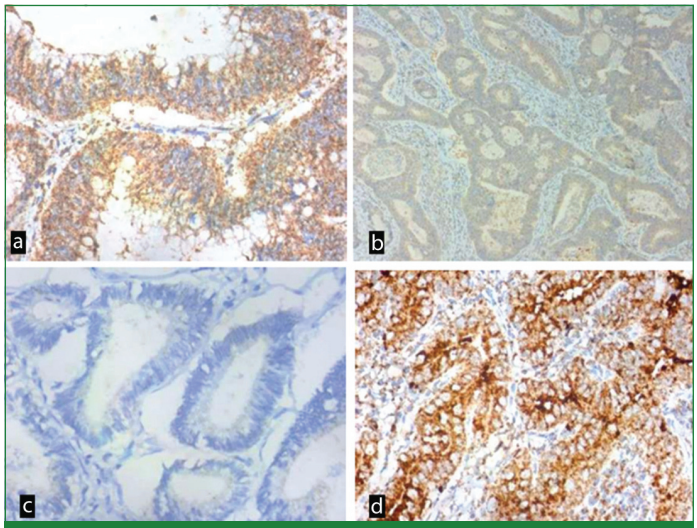


[Table/Fig-4]: (a) Mucinous adenocarcinoma (H&E, x100); (b) Signet cell adenocarcinoma (H&E, x400).

BRAF V600E expression: In the present study, among 72 known cases of CRC, 16 (22.22%) cases were positive, 7 (9.72%) cases were equivocal and 49 (68.06%) cases were negative for BRAF V600E immunohistochemical expression [Table/Fig-5,6].

BRAF V600E IHC expression	Frequency (n)	Percentage (%)
Positive	16	22.22%
Equivocal	7	9.72%
Negative	49	68.06%
Total	72	100%

[Table/Fig-5]: Distribution of BRAF V600E immunohistochemical expression in CRC cases.



[Table/Fig-6]: BRAF V600E IHC results: a) Positive (IHC, x100); b) Equivocal (IHC, x100); c) Negative (IHC, x400); d) positive control – papillary thyroid carcinoma, known BRAF positive (IHC, x200).

A statistically significant association was found between BRAFV600E immunohistochemical expression and tumour grade (p=0.027). BRAF V600E immunohistochemical positivity was significantly higher in poorly differentiated tumours [Table/Fig-7]. There was a significant association between BRAF V600E expression and depth of invasion (p=0.035), lymph node involvement (p=0.0055), tumour stage (p=0.0086) and lympho-vascular invasion (p=0.037) [Table/Fig-7]. In the present study, we also found significant association between BRAF V600E expression and histopathological type (p-value=0.0001). Five out of six cases (83.3%) of mucinous adenocarcinoma showed BRAF V600E positive immunohistochemical expression [Table/Fig-7].

No statistically significant association was found between BRAF V600E expression and age (p=0.75), gender (p=0.434), tumour size (p=0.694) and tumour site (p=0.221) [Table/Fig-6]. Although

statistically not significant, majority of the BRAF V600E positive expression tumours are seen in >50 years age group, males, <5 cm tumour size and right-sided colon tumours in the present study [Table/Fig-7]. Seven cases (9.7%) which showed equivocal BRAF V600E immunostaining were not subjected to further molecular confirmation.

Clinicopathological characteristics	Frequency (n)	BRAF V600E IHC positive (n=16)	Equivocal (n=7)	BRAF V600E IHC negative (n=49)	p-value
Age					
<50 years	24	2 (12.5%)	5 (71.4%)	17 (34.7%)	0.75
>50 years	48	14 (87.5%)	2 (28.6%)	32 (65.3%)	
Gender					
Male	32	9 (56.25%)	2 (28.57%)	21 (42.86%)	0.434
Female	40	7 (43.75%)	5 (71.43%)	28 (57.14%)	
Tumour size					
<5 cm	42	9 (56.25%)	3 (42.86%)	30 (61.22%)	0.694
5-10 cm	28	7 (43.75%)	4 (57.14%)	17 (34.7%)	
>10 cm	2	0	0 (0%)	2 (4.08%)	
Tumour site					
Right-sided	30	10 (62.5%)	3 (42.85%)	17 (34.69%)	0.221
Left-sided	24	4 (25%)	1 (14.29%)	19 (38.78%)	
Rectum	18	2 (12.5%)	3 (42.86%)	13 (26.53%)	
Tumour grade					
Grade 1	14	2 (12.5%)	2 (28.6%)	10 (20.41%)	0.027*
Grade 2	50	8 (50%)	4 (57.1%)	38 (77.55%)	
Grade 3	8	6 (37.5%)	1 (14.3%)	1 (2.04%)	
Depth of invasion					
T1	3	1 (6.25%)	1 (14.28%)	1 (2.04%)	0.035*
T2	20	3 (18.75%)	0 (0%)	17 (34.7%)	
T3	42	10 (62.5%)	3 (42.86%)	29 (59.18%)	
T4	7	2 (12.5%)	3 (42.86%)	2 (4.08%)	
Lymph node involvement					
Positive	26	11 (68.75%)	3 (42.86%)	12 (24.49%)	0.0055*
Negative	46	5 (31.25%)	4 (57.14%)	37 (75.51%)	
AJCC staging, 8th edition					
I	20	1 (6.25%)	1 (14.29%)	18 (36.73%)	0.0086*
II	26	4 (25%)	2 (28.57%)	20 (40.82%)	
III&IV	26	11(68.75%)	4 (57.14%)	11 (22.45%)	
Histopathological type					
Adenocarcinoma NOS	65	11 (68.75%)	6 (85.71%)	48 (97.96%)	0.0001*
Mucinous adenocarcinoma	6	5 (31.25%)	0 (0%)	1 (2.04%)	
Signet ring cell carcinoma	1	0 (0%)	1 (14.29%)	0 (0%)	
Lympho-vascular invasion					
Positive	26	9 (56.3%)	4 (57.1%)	13 (26.5%)	0.037*
Negative	46	7 (43.7%)	3 (42.9%)	36 (73.5%)	

[Table/Fig-7]: Association of BRAF V600E immunohistochemical expression with clinicopathological characteristics.
(Percentages are calculated within BRAF expression groups; *p-value = <0.05: significant association)

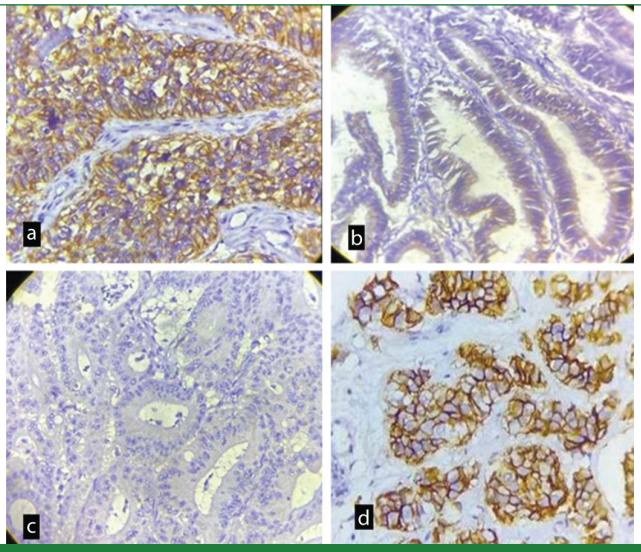
HER2 expression: In the present study, HER2 expression was positive in three cases (4.17%), borderline in four cases (5.56%) and negative in 65 cases (90.27%) of CRC [Table/Fig-8,9].

A statistically significant association was noted between HER2 expression and pathological grade (p-value=0.009), with HER2 positivity noted in well to moderately differentiated tumours [Table/Fig-10].

However, HER2 expression did not show any significant association with Age (p-value=0.088), gender (p-value=0.4), tumour site (p-value=0.8), tumour size (p-value=0.42), depth of invasion (p-value=0.13), lymph node involvement (p-value=0.233), tumour stage (p-value=0.12), histopathological type (p-value=0.457) and LVSI (p-value=0.5) [Table/Fig-10].

HER2 expression	Frequency (n)	Percentage (%)
Positive	3	4.17%
Borderline	4	5.56%
Negative	65	90.27%
Total	72	100%

[Table/Fig-8]: Distribution of HER2 expression in Colorectal Carcinoma (CRC) cases.



[Table/Fig-9]: HER2 IHC results: a) Positive (IHC, x400); b) Borderline (IHC, x100); c) Negative (IHC, x100); d) Positive control - invasive breast carcinoma, known positive for HER2 (IHC, x200).

Clinicopathological characteristics	Frequency (n)	HER2 positive (n=3)	Borderline (n=4)	HER2 negative (n=65)	p-value
Age					
<50 years	24	0 (0%)	0 (0%)	24 (36.9%)	0.088
>50 years	48	3 (100%)	4 (100%)	41 (63.1%)	
Gender					
Male	32	2 (66.7%)	1 (25%)	29 (44.6%)	0.4
Female	40	1 (33.3%)	3 (75%)	36 (55.4%)	
Tumour size					
<5 cm	42	1 (33.3%)	2 (50%)	39 (60%)	0.42
5-10 cm	28	2 (66.7%)	2 (50%)	24 (36.9%)	
>10 cm	2	0 (0%)	0 (0%)	2 (3.1%)	
Tumour site					
Right-sided	30	1 (33.3%)	1 (25%)	28 (43.1%)	0.8
Left-sided	24	1 (33.3%)	2 (50%)	21 (32.3%)	
Rectum	18	1 (33.3%)	1 (25%)	16 (24.6%)	
Tumour grade					
Grade 1	14	1 (33.3%)	1 (25%)	12 (18.5%)	0.009*
Grade 2	50	2 (66.7%)	3 (75%)	45 (69.2%)	
Grade 3	8	0 (0%)	0 (0%)	8 (12.3%)	
Depth of invasion					
T1	3	0 (0%)	0 (0%)	3 (4.61%)	0.13
T2	20	0 (0%)	1 (25%)	19 (29.23%)	
T3	42	2 (66.7%)	3 (75%)	37 (56.92%)	
T4	7	1 (33.3%)	0 (0%)	6 (9.24%)	
Lymph node involvement					
Positive	26	1 (33.3%)	0 (0%)	25 (38.5%)	0.233
Negative	46	2 (66.7%)	4 (100%)	40 (61.5%)	
AJCC staging, 8th edition					

I&II	46	2 (66.7%)	4 (100%)	40 (61.5%)	0.12
III&IV	26	1 (33.3%)	0 (0%)	25 (38.5%)	
Histopathological type					
Adenocarcinoma NOS	65	3 (100%)	4 (100%)	58 (89.2%)	0.457
Mucinous adenocarcinoma	6	0 (0%)	0 (0%)	6 (9.2%)	
Signet ring cell carcinoma	1	0 (0%)	0 (0%)	1 (1.6%)	
Lympho-vascular invasion					
Positive	26	1 (33.3%)	0 (0%)	25 (38.5%)	0.5
Negative	46	2 (66.7%)	4 (100%)	40 (61.5%)	

[Table/Fig-10]: Association of HER2 immunohistochemical expression with clinicopathological characteristics.
(Percentages are calculated within HER2 expression groups; *p-value = <0.05: significant association)

No confirmatory HER2 Fluorescence In Situ Hybridisation (FISH) testing was performed for four cases (5.6%) of borderline (HER2 2+) expression cases.

The authors found no significant association between BRAF V600E expression and HER2 expression (p-value=0.62). Concurrent positivity for BRAF V600E and HER2 was not observed in any of the cases.

DISCUSSION

The present study evaluated the immunohistochemical expression of BRAF V600E and HER2 in CRC and their association with various clinicopathological parameters. The main findings of the current study were that BRAF V600E expression was significantly associated with advanced stage, higher tumour grade, lymph node metastasis, lympho-vascular invasion and mucinous histology, whereas HER2 expression showed limited association, with significance only for tumour grade.

BRAF V600E expression: In the present study, among 72 known cases of CRC, 16 (22.22%) cases were positive, 7 (9.72%) cases were equivocal and 49 (68.06%) were negative for BRAFV600E expression. In comparison, previous studies have reported varying positivity rates Vakiani E et al., observed 39.3%, Zhang X et al., reported 3.9%, and Seppala TT et al., found 12.3% positivity for BRAF V600E expression [12-14]. The variation in prevalence across studies may be due to differences in sample size, methodology and detection techniques.

In the present study, BRAF V600E expression showed significant association with poor differentiation, advanced tumour stage, lymph node metastasis, lympho-vascular invasion and mucinous histology, suggesting a more aggressive tumour phenotype. Similar associations have been reported by Chen D et al., which reported significant association between BRAF V600E and advanced TNM staging and mucinous histology, Li WQ et al., which reported significant association between BRAF V600E mutation and poor differentiation [15,16], Zhang X et al., which reported CRC tumours with poor differentiation had a higher incidence rate of BRAFV600E mutation than those with moderate/well differentiation and O'Riordan E et al., which reported high incidence of poorly differentiated tumours were in the BRAFMUT cohort compared with the BRAFWT group [13,17].

But the studies done by Zhang X et al., reported no significant association between BRAF V600E and bowel invasion, lymph node involvement and Lympho-Vascular Space Invasion (LVSI) and Kadiyska TK et al., reported moderate differentiation is one of the common characteristic features seen in BRAF Mutated (MUT) tumours [13,18].

It is important to note that the IHC600 antibody used in the study detect only the BRAF V600E mutant protein and does not identify non-V600Emutations such as L597V, D594N, G466V. Therefore, immunohistochemistry serves as a screening tool and does not replace molecular testing. Equivocal BRAF V600E IHC cases ideally require molecular confirmation, which could not be performed in the present study due to financial and resource constraints.

HER2 expression: In the present study, the authors found that 5.56% (4 cases) and 4.17% (3 cases) were borderline and strongly positive for HER2 expression and 90.27% (65 cases) showed negative expression. This result is in concordance with study done by Schuell B et al., [19] which showed 1% moderate and 3% strongly positivity and Zhang X et al., showed 3.96% positivity whereas this result is in discordance with study done by Shabbir A et al., which showed 78.9% positivity, possibly due to differences in scoring criteria, antibody clones and interpretation [13,20].

All HER2 borderline cases were advised for FISH testing, however, due to financial and resource constraints, molecular confirmation could not be performed.

In the present study, HER2 expression showed significant association only with well to moderately differentiated tumours. Our result is in discordance with study done by Shabbir A et al., which reported pattern of membranous HER2 expression more in high-grade CRC [20] and Zhang X et al., which reported no significant association with tumour differentiation [13].

However, the authors did not find any significant association between HER2 expression and Age, Gender, tumour site, tumour size, depth of invasion, lymph node involvement, tumour stage, histopathological type and LVSI. This result is consistent with the study done by Schuell B et al., which reported that there was no significant association of HER2 expression with gender and age [19], and Shabbir A et al., which reported no significant association was found between HER2 staining with age of the patient, tumour site, histologic type, LVSI [20], tumour invasion, lymph node metastasis and stage of the tumour but discordance with study done by Zhang X et al., which reported significant association with tumour stage and depth of invasion [13].

No concurrent positivity for BRAF V600E and HER2 was observed in this study, suggesting that these alterations represent distinct molecular subsets in CRC.

The present study has several limitations. The small sample size limits the generalisability of the findings and unavailability of follow-up data precludes the assessment of survival outcomes and direct evaluation of prognostic significance. The molecular confirmation of BRAF mutation and HER2 amplification was not performed and IHC alone may not detect non-V600E BRAF mutations.

Future multicentric studies incorporating larger sample sizes, long term follow-up and comprehensive molecular profiling are required to validate these findings. Despite its limitations, this study provides regional data from Eastern India, where limited published literature exists on combined BRAF V600E and HER2 expression in CRC.

Limitation(s)

The present study was limited by a short duration, small sample size, single centre (72 cases from one region), and lack of survival and follow-up data. Absence of confirmatory molecular testing and inability to detect non-V600E BRAF mutations. Multivariate studies could not be performed due to the relatively small sample size and limited number of outcome events. Broader, multicentric research with molecular analysis is needed for more representative findings.

CONCLUSION(S)

In summary, BRAFV600E expression in CRC is significantly associated with high-grade, invasive and advanced-stage tumours, highlighting its role as a marker of aggressive disease. HER2 expression, on the other hand, shows limited association with clinicopathological features, except for a significant association with well to moderately differentiated histology, indicating a distinct

biological behaviour. The combined use of immunohistochemistry and molecular testing remains crucial for accurate characterisation of these biomarkers. Integration of such molecular insights into routine diagnostic and therapeutic pathways will contribute to more individualised treatment strategies and improved patient outcomes in CRC.

Acknowledgement

The authors would like to acknowledge the important contributions of all the technicians, staff, and faculty members of the Department of Pathology and Department of Surgery, R. G. Kar Medical College, Kolkata, in our research.

REFERENCES

[1] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-63. Doi: 10.3322/caac.21834.

[2] Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988;319(9):525-32. Doi: 10.1056/NEJM198809013190901.

[3] Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21(11):1350-56. Doi: 10.1038/nm.3967.

[4] Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK, et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline summary from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. J Clin Oncol. 2017;13(5):333-37. Doi: 10.1200/JOP.2017.022152.

[5] Fanelli GN, Dal Pozzo CA, Depetris I, Schirripa M, Brignola S, Biason P, et al. The heterogeneous clinical and pathological landscapes of metastatic Braf-mutated colorectal cancer. Cancer Cell Int. 2020;20:30. Doi: 10.1186/s12935-020-1117-2.

[6] Caputo F, Santini C, Bardasi C, Cerma K, Casadei-Gardini A, Spallanzani A, et al. BRAF-mutated colorectal cancer: clinical and molecular insights. Int J Mol Sci. 2019;20(21):5369. Doi: 10.3390/ijms20215369.

[7] Śmiech M, Leszczyński P, Kono H, Wardell C, Taniguchi H. Emerging BRAF mutations in cancer progression and their possible effects on transcriptional networks. Genes (Basel). 2020;11(11):1342. Doi: 10.3390/genes11111342.

[8] Madani SH, Sadeghi E, Rezaee A, Sadeghi M, Khazaei S, Amirifard N, et al. Survey of HER2-neu expression in colonic adenocarcinoma in the west of Iran. Asian Pac J Cancer Prev. 2015;16(17):7671-74.

[9] Djaballah AS, Daniel F, Milani A, Ricagno G, Lonardi S. HER2 in colorectal cancer: the long and winding road from negative predictive factor to positive actionable target. Am Soc Clin Oncol Educ Book. 2022;42:1-14. Doi: 10.1200/EDBK_351354.

[10] Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, et al. Global Cancer Observatory: Cancer Today [Internet]. Lyon (France): International Agency for Research on Cancer; 2024 [cited 2024 Dec 9]. Available from: https://gco.iarc.who.int/today.

[11] Weiser MR. AJCC 8th edition: colorectal cancer. Ann Surg Oncol. 2018;25(6):1454-55. Doi: 10.1245/s10434-018-6462-1.

[12] Vakiani E, Yaeger R, Brooke S, Zhou Y, Klimstra DS, Shia J. Immunohistochemical detection of the BRAF V600E mutant protein in colorectal neoplasms. Appl Immunohistochem Mol Morphol. 2015;23(6):438-43.

[13] Zhang X, Wu J, Wang L, Zhao H, Li H, Duan Y, et al. HER2 and BRAF mutation in colorectal cancer patients: a retrospective study in Eastern China. Peer J. 2020;8:e8602. Doi: 10.7717/peerj.8602.

[14] Seppälä TT, Böhm JP, Friman M, Lahtinen L, Väyrynen VM, Liipo TK, et al. Combination of microsatellite instability and BRAF mutation status for subtyping colorectal cancer. Br J Cancer. 2015;112(12):1966-75.

[15] Chen D, Huang JF, Liu K, Zhang LQ, Yang Z, Chuai ZR, et al. BRAFV600E mutation and its association with clinicopathological features of colorectal cancer: a systematic review and meta-analysis. PLoS One. 2014;9(3):e90607. Doi: 10.1371/journal.pone.0090607.

[16] Li WQ, Kawakami K, Ruszkiewicz A, Bennett G, Moore J, Iacopetta B. BRAF mutations are associated with distinctive clinical, pathological and molecular features of colorectal cancer independently of microsatellite instability status. Mol Cancer. 2006;5:2.

[17] O'Riordan E, Bennett MW, Daly L, Power DG. The implication of BRAF mutation in advanced colorectal cancer. Ir J Med Sci. 2022;191(6):2467-74. Doi: 10.1007/s11845-021-02689-x.

[18] Kadiyska TK, Konstantinova DV, Atanasov VR, Kremensky IM, Mitev VI. Frequency and application of the hot spot BRAF gene mutation (p.V600E) in the diagnostic strategy for hereditary nonpolyposis colorectal cancer. Cancer Detect Prev. 2007;31(3):254-56.

[19] Schuell B, Gruenberger T, Scheithauer W, Zielinski CH, Wrba F. HER2/neu protein expression in colorectal cancer. BMC Cancer. 2006;6:123.

[20] Shabbir A, Mirza T, Khalid AB, Qureshi MA, Asim SA. Frequency of HER2/neu expression in colorectal adenocarcinoma: A study from developing South Asian Country. BMC Cancer. 2016;16(1):855. Doi: 10.1186/s12885-016-2912-y.

PARTICULARS OF CONTRIBUTORS:

1. Junior Resident, Department of Pathology, R G Kar Medical College, Kolkata, West Bengal, India.
2. Associate Professor, Department of Pathology, R G Kar Medical College, Kolkata, West Bengal, India.
3. Associate Professor, Department of Pathology, R G Kar Medical College, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Rina Barman,
Department of Pathology, Kolkata, West Bengal, India.
E-mail: dr.rinabarman@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Dec 15, 2025
- Manual Googling: Mar 05, 2026
- iThenticate Software: Mar 11, 2026 (1%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Nov 15, 2025**
Date of Peer Review: **Jan 07, 2026**
Date of Acceptance: **Mar 12, 2026**
Date of Publishing: **Jul 01, 2026**