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Colorectal Carcinoma: Is There Any Correlation between Her2/neu Expression, Ki-67 Score, and Tumor Budding and Clinicopathological Parameters?—A Prospective Institution-Based Study

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Abstract


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Objectives Colorectal cancer is one of the most frequent cancers worldwide and is still a major cause of cancer mortality. Her2/neu, Ki67 score, and tumor budding are independent prognostic factors in colorectal carcinomas. The objectives of the study were to evaluate Her2/neu expression, Ki67 score, and tumor budding index at invasive margin in colorectal carcinoma and find out their possible correlations with different clinicopathological factors.

Materials and Methods An institution-based observational cross-sectional study was conducted for 18 months. Forty-one patients with histologically proven diagnosis of colorectal carcinoma were included. Histopathological and immunohistochemical analyses (Her2/neu and Ki-67) of each case were done.

Statistical Analysis Data analysis was done using the SPSS software.

Results A significant correlation was found between tumor budding status and pathological T stage, Dukes' and American Joint Committee on Cancer stages, and between tumor-infiltrating lymphocytes status and Ki-67 expression status ($p < 0.05$).

Conclusion The prognostic importance of tumor budding in colorectal carcinoma is very clear. Considering the small sample size of the present study, the prognostic values of Her2/neu and Ki-67 are required to be explored further in larger cohorts in the future.

Keywords

- colorectal carcinoma
- Her2/neu
- Ki-67
- tumor budding
- tumor-infiltrating lymphocytes

Introduction

Colorectal carcinoma is the third most common cancer diagnosed worldwide. It is also the second most common cause of cancer-related mortality.[1] The scenario remains rather bleak even though several novel screening and diagnostic modalities have come into practice. In India, colorectal carcinoma accounts for 3% of all cancers, but the incidence rates are rising alarmingly.[2] Novel prognostic markers are being explored every day to combat this deadly disease.

Tumor budding index has emerged as one of the most important prognostic parameters not only in colorectal carcinoma, but also in carcinomas of the head and neck,

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breast, esophagus, and lung.[3] Determination of tumor budding in colon cancer has played an important role in defining therapeutic strategies, especially regarding adjuvant and neoadjuvant therapies.[4]

Her2 (human epidermal growth factor receptor 2) is a proto-oncogene found on chromosome 17q21 and it encodes the protein, Her2, which is responsible for the proliferatioxn and differentiation of cells. Her2 is a therapeutic target in breast and gastric carcinoma patients, who show Her2 amplification or overexpression.[5] The role of Her2 in colorectal carcinoma is still being explored worldwide.[6]

Ki67 labeling index is an important prognostic parameter in different malignancies, including breast carcinoma and neuroendocrine tumors. However, the prognostic role of Ki67 labeling index in colorectal carcinoma has been noted with limited success in a few studies.[7]

The main aims of the present study were to find out the tumor budding index, Her2 status, and Ki67 labeling index in colorectal carcinoma patients and also to evaluate the correlation between these parameters and various clinicopathological aspects of the disease.

Materials and Methods

The study was performed after obtaining approval from the Institutional Ethical Committee (Ref no. – MC/KOL/IEC/NONSPON/873/01/2021, dated July 1, 2021).

A total of 41 histologically proven colorectal cancer patients attending the surgery and radiotherapy departments of the institution, over 18 months, for follow-up after surgery, were included in this study. They were subjected to a questionnaire for the collection of relevant clinical information.

The hematoxylin and eosin-stained histopathological slides were reviewed and relevant details including tumor budding index and tumor-infiltrating lymphocytes (TIL) score were recorded. A tumor bud is defined as single cells or small clusters of cells (up to 4 cells in a cluster) within the tumor or at the invasive front of the tumor.[8] In the present study, intratumoral tumor buds were not considered. All the sections taken from the tumor were first screened under low power objective ($\times$10) to determine the areas with the highest number of tumor buds at the invasive front. The tumor budding index was recorded as an average of 10 high-power fields (HPF) ($\times$40) and it was designated "low" if the score was less than 10 tumor buds/10 HPF and "high" when it was $\geq$ 10/10 HPF.[8] The cases in which there was heavy lymphocytic infiltration at the invasive front, obscuring the tumor buds, immunostain (PanCK) was used, to highlight them and facilitate scoring. In the present study, PanCK was used in 9 cases (21.9%).

TIL in the stroma of the tumor was also assessed at the invasive front of the tumor. The assessment was done according to the modified Klintrup–Makinen score and the stromal mononuclear cell infiltration at the invasive front was categorized into dour groups (excluding areas of crush artifact, necrosis, and hyalinization): 0–no increase in inflammatory cells; 1–patchy increase in inflammatory cells but no destruction of invading cancer cell islands; 2–band-like inflammatory cell infiltration with some destruction of cancer cell islands; and 3–very prominent inflammatory cell infiltration forming cup-like zone at the invasive margin with destruction of cancer cell islands.[9]

The formalin-fixed paraffin-embedded blocks were collected and relevant sections were stained with Her2/neu and Ki67 immunostains. Standardized immunoperoxidase techniques were used for staining using Her2-EP3 (Pathinsitu) and Ki-MIB1 (Pathinsitu) clones for Her2/neu and Ki67, respectively. Her2/neu scoring was done based on the system used to evaluate HercepTest: [6] 0–no immunoreactivity or immunoreactivity in $<$ 10% of tumor cells (negative); 1+ – faint weak immunoreactivity in $>$ 10% of tumor cells but only a portion of the membrane is positive (incomplete) (negative); 2+ – weak to moderate complete membrane immunoreactivity in $>$ 10% of tumor cells (weak positive); and 3+ – moderate to strong complete membrane immunoreactivity in $>$ 10% of tumor cells (positive).

Ki67 index was evaluated by calculating the percentage of tumor cells showing positive nuclear staining. When $<$ 25% positively stained tumor cells were found, it was considered as low Ki67-Li (labeling index) and a percentage of $\geq$ 25% as high Ki67-Li.[7]

All the relevant data were tabulated and analyzed to assess the correlation between tumor budding index, Her2/neu, Ki67 scores, and different clinicopathological features of colorectal carcinoma. The collected data was tabulated in a spreadsheet using Microsoft Excel 2019 and then statistical analysis was carried out using IBM SPSS statistics for Windows, version 26.0. Graphs were prepared using the GraphPad Prism for Windows, Version 9.0. Survival analysis was conducted to find the survival distributions of the patients according to the tumor budding index, HER2/neu, and KI-67 scores. Survival analysis was done with SPSS software by Kaplan–Meier analysis. A p-value of less than 0.05 was considered significant ($p < 0.05$).

Results

Among the 41 colorectal carcinoma patients included in the study, 34 (82.9%) were males and 7 (17.1%) were females. The age range was from 28 to 92 years, with a mean of 55.83 $\pm$ 16.37 years. Colon was involved in 30 (73%) patients and pure rectal carcinoma occurred in 11 (27%) patients. Well-differentiated carcinoma was reported in 7 patients (17.1%), moderately differentiated in 23 (56.1%), and poorly differentiated in 11 patients (26.8%). The tumors were staged as pT2 in 9 patients, pT3 in 25, and pT4 in 7 patients. Analysis of the tumor budding index showed high budding in 25 (61%) and low budding in 16 patients (39%). There was a significant correlation between tumor budding and the pT stage of the tumor (p-value: 0.001). There were 3 (7.3%) Her2/neu positive cases. There was no significant correlation found between Her2/neu expression and different clinicopathological parameters of colorectal carcinoma. High Ki67 expression was found in 23 patients (56.1%) and 18 (43.9%) patients showed low Ki67 score. A significant correlation was found between Ki-67 expression status and TIL score (p: 0.008) (►Table 1).

Table 1 Correlation between tumor budding score, Her2/neu status, Ki-67 score, and different clinicopathological parameters

Clinicopathological features.	Tumor budding score		HER2/neu status		Ki67 score	
	High tumor budding	Low tumor budding	HER2/neu negative	HER2/neu positive	High Ki67 score	Low Ki67 score
Gender						
Male	19 (55.9%)	15 (44.1%)	31 (91.2%)	3 (8.8%)	20 (58.8%)	14 (41.2%)
Female	6 (85.7%)	1 (14.3%)	7 (100%)	0 (0%)	3 (42.9%)	4 (57.1%)
Tumor site						
Ascending colon	1 (50%)	1 (50%)	2 (100%)	0 (0%)	1 (50%)	1 (50%)
Ascending colon and part of transverse colon	2 (100%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)
Caecum	6 (75%)	2 (25%)	6 (75%)	2 (25%)	5 (62.5%)	3 (37.5%)
Caecum and part of ascending colon	0 (0%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
Rectum	6 (54.5%)	5 (45.5%)	10 (90.9%)	1 (9.1%)	6 (54.5%)	5 (45.5%)
Sigmoid colon	8 (61.5%)	5 (38.5%)	13 (100%)	0 (0%)	7 (53.8%)	6 (46.2%)
Sigmoid colon and part of upper rectum	1 (50%)	1 (50%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Transverse colon	1 (50%)	1 (50%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Tumor grade						
G1	5 (71.4%)	2 (28.6%)	7 (100%)	0 (0%)	3 (42.9%)	4 (57.1%)
G2	14 (60.9%)	9 (39.1%)	22 (95.7%)	1 (4.3%)	13 (56.5%)	10 (43.5%)
G3	6 (54.5%)	5 (45.5%)	9 (81.8%)	2 (18.2%)	7 (63.6%)	4 (36.4%)
Tumor stage						
pT						
pT1	0 (0%)[a]	0 (0%)[a]	0 (0%)	0 (0%)	0 (0%)	0 (0%)
pT2	1 (11.11%)[a]	8 (88.89%)[a]	8 (88.89%)	1 (11.11%)	4 (44.44%)	5 (55.56%)
pT3	19 (76%)[a]	6 (24%)[a]	24 (96.00%)	1 (4.0%)	17 (68%)	8 (32%)
pT4	5 (71.43%)[a]	2 (28.57%)[a]	6 (85.71%)	1 (14.29%)	2 (28.57%)	5 (71.43%)
pN						
pN0	19 (67.86%)	9 (32.14%)	25 (89.29%)	3 (10.71%)	17 (60.71%)	11 (39.29%)
pN1	3 (42.86%)	4 (57.14%)	7 (100%)	0 (0%)	3 (42.86%)	4 (57.14%)
pN2	3 (50%)	3 (50%)	6 (100.0%)	0 (0%)	3 (50%)	3 (50%)
Dukes' staging						
A	1 (16.7%)	5 (83.3%)	5 (83.3%)	1 (16.7%)	4 (66.7%)	2 (33.3%)
B	17 (77.3%)	5 (22.7%)	20 (90.9%)	2 (9.1%)	13 (59.1%)	9 (40.9%)
C	6 (60%)	4 (40%)	10 (100%)	0 (0%)	5 (50%)	5 (50%)
D	1 (33.3%)	2 (66.7%)	3 (100%)	0 (0%)	1 (33.3%)	2 (66.7%)
AJCC staging						
I	1 (16.7%)	5 (83.3%)	5 (83.3%)	1 (16.7%)	4 (66.7%)	2 (33.3%)
II	17 (77.3%)	5 (22.7%)	20 (90.9%)	2 (9.1%)	13 (59.1%)	9 (40.9%)
III	6 (60%)	4 (40%)	10 (100%)	0 (0%)	5 (50%)	5 (50%)
IV	1 (33.3%)	2 (66.7%)	3 (100%)	0 (0%)	1 (33.3%)	2 (66.7%)
LVI						
Absent	17 (56.7%)	13 (43.3%)	27 (90%)	3 (10%)	18 (60%)	12 (40%)

(*Continued*)

Table 1 (*Continued*)

Clinicopathological features.	Tumor budding score		HER2/neu status		Ki67 score	
	High tumor budding	Low tumor budding	HER2/neu negative	HER2/neu positive	High Ki67 score	Low Ki67 score
Present	8 (72.7%)	3 (27.3%)	11 (100%)	0 (0%)	5 (45.5%)	6 (54.5%)
PNI						
Absent	20 (55.6%)	16 (44.4%)	33 (91.7%)	3 (8.3%)	21 (58.3%)	15 (41.7%)
Present	5 (100%)	0 (0%)	5 (100%)	0 (0%)	2 (40%)	3 (60%)
Tumor-infiltrating lymphocytes (TIL score)	High tumor budding	Low tumor budding	HER2 negative	HER2 positive	High Ki	Low Ki
Absent	5 (38.5%)	8 (61.5%)	13 (100%)	0 (0%)	6 (46.2%)[a]	7 (53.8%)[a]
Marked	7 (87.5%)	1 (12.5%)	7 (87.5%)	1 (12.5%)	7 (87.5%)[a]	1 (12.5%)[a]
Mild	8 (66.7%)	4 (33.3%)	11 (91.7%)	1 (8.3%)	3 (25.0%)[a]	9 (75%)[a]
Moderate	5 (62.5%)	3 (37.5%)	7 (87.5%)	1 (12.5%)	7 (87.5%)[a]	1 (12.5%)[a]
Vitality status						
Alive	17 (60.71%)	11 (39.29%)	2 (7.14%)	26 (92.32%)	18 (64.29%)	10 (35.71%)
Deceased	8 (61.54%)	5 (38.46%)	1 (7.69%)	12 (92.31%)	5 (38.46%)	8 (61.54%)

Abbreviations: AJCC, American Joint Committee on Cancer; LVI, lymphovascular invasion; PNI, perineural invasion.
[a]Denotes statistically significant parameters.

Discussion

The clinicopathological parameters of colon carcinoma found in the present study were comparable with those noted by Gianni et al in their work.[5]

Graham et al found a strong correlation between tumor budding and late stage of disease.[10] In the present study, a significant correlation between tumor budding and the pT stage of the tumor was noted. It has been suggested that tumor budding may be used as an indicator to decide therapeutic measures in cases of adenocarcinoma originating in a polyp. Further, the decision of adjuvant treatment in stage II carcinoma and that of neoadjuvant therapy when colorectal carcinoma is diagnosed in preoperative biopsies, rests, at least partially on tumor bud count.[8]

Melling et al concluded in their study that a high Ki67 index is a biomarker that may be used to determine the prognosis of patients with advanced stage of colorectal carcinoma.[7] Heidari et al and Farzand et al reported that Her2 expression may be used as prognostic marker of colorectal carcinoma.[11,12] No significant correlation was found between Her2/neu expression and different clinicopathological aspects of the patients in the present study, similar to that reported by Kilicarslan et al.[13]

A significant correlation was noted between Ki-67 expression status and TIL score in the present study. Jakubowska et al were of the opinion that TIL should be reported in all cases of colorectal carcinoma since they are an indicator of disease progression and prognosis.[14] Idos et al also noted that TIL and their subsets may be used as prognostic markers in colorectal carcinoma.[15]

Conclusion

Colorectal carcinoma is one of the most frequent cancers and still a major cause of cancer mortality, although the treatment regime has continuously improved. HER2/neu, Ki67, and tumor budding are very effective in treatment and prognostic purposes. There was a significant correlation of tumor budding with pathological T stage, Dukes' stage, and American Joint Committee on Cancer stage. A significant correlation was also obtained between Ki 67 score and TIL status.

It may be concluded that the prognostic importance of tumor budding is fairly well established. Increased tumor buds are significantly correlated with the advanced stage of colorectal carcinoma. Considering the small sample size of the present study, there is a need to explore the prognostic values of HER2/neu and Ki-67 further, in larger cohorts, in the future.[3,4,8]

Conflict of Interest

None declared.

References

1 Ahmad R, Singh JK, Wunnava A, Al-Obeed O, Abdulla M, Srivastava SK. Emerging trends in colorectal cancer: dysregulated signaling pathways (Review). Int J Mol Med 2021;47(03):14
2 Lavingia V, Gore AA. Time for colorectal cancer screening in India!. Indian J Cancer 2021;58(03):315–316
3 Boxberg M, Jesinghaus M, Dorfner C, et al. Tumour budding activity and cell nest size determine patient outcome in oral squamous cell carcinoma: proposal for an adjusted grading system. Histopathology 2017;70(07):1125–1137

4 Petrelli F, Pezzica E, Cabiddu M, et al. Tumour budding and survival in stage II colorectal cancer: a systematic review and pooled analysis. J Gastrointest Cancer 2015;46(03):212–218
5 Gianni L, Dafni U, Gelber RD, et al; Herceptin Adjuvant (HERA) Trial Study Team. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. Lancet Oncol 2011;12(03):236–244
6 Wang XY, Zheng ZX, Sun Y, et al. Significance of HER2 protein expression and *HER2* gene amplification in colorectal adenocarcinomas. World J Gastrointest Oncol 2019;11(04):335–347
7 Melling N, Kowitz CM, Simon R, et al. High Ki67 expression is an independent good prognostic marker in colorectal cancer. J Clin Pathol 2016;69(03):209–214
8 Roy P, Datta J, Roy M, Mallick I, Mohandas M. Reporting of tumor budding in colorectal adenocarcinomas using ×40 objective: a practical approach for resource constrained set-ups. Indian J Cancer 2017;54(04):640–645
9 Klintrup K, Mäkinen JM, Kauppila S, et al. Inflammation and prognosis in colorectal cancer. Eur J Cancer 2005;41(17): 2645–2654
10 Graham RP, Vierkant RA, Tillmans LS, et al. Tumor budding in colorectal carcinoma: confirmation of prognostic significance and histologic cutoff in a population-based cohort. Am J Surg Pathol 2015;39(10):1340–1346
11 Heidari Z, Mahmoudzadeh-Sagheb H, Jahantigh M, Charkhat Gorgich EA. Immunohistochemical expression of Ki67 and HER2 in colorectal cancer compared to adenomatous and normal samples. Int J Cancer Manag 2017;10:122–152
12 Farzand S, Siddique T, Saba K, Bukhari MH. Frequency of HER2/neu overexpression in adenocarcinoma of the gastrointestinal system. World J Gastroenterol 2014;20(19):5889–5896
13 Kilicarslan A, Dogan HT, Sungu N, Dogan M, Yalcin A, Dede DS. Association in colorectal carcinoma and clinicopathological features. Pol J Pathol 2018;69:143–149
14 Jakubowska K, Kisielewski W, Kańczuga-Koda L, Koda M, Famulski W. Stromal and intraepithelial tumor-infiltrating lymphocytes in colorectal carcinoma. Oncol Lett 2017;14(06):6421–6432
15 Idos GE, Kwok J, Bonthala N, Kysh L, Gruber SB, Qu C. The prognostic implications of tumor infiltrating lymphocytes in colorectal cancer: a systematic review and meta-analysis. Sci Rep 2020;10(01):3360

