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Significance of tumor budding in colorectal carcinoma – A tertiary care center study

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ABSTRACT

Background: Colorectal carcinoma is one of the leading causes of mortality worldwide. Although the current TNM staging helps in prognostic stratification, these cancers are known to show heterogeneous behavior within the same stage. A search for other prognostic histopathological factors has been in process. One such factor is tumor budding. **Aims and Objectives:** This study was aimed at enumerating tumor budding, stratifying it, and identifying correlation with other the clinicopathological factors. **Materials and Methods:** This was a retrospective study conducted in the Department of Pathology, Hassan Institute of Medical Sciences, Hassan. A total of 124 cases were studied. Archived blocks and slides were retrieved, reviewed, and assigned a tumor budding grade on H&E staining. Grade of tumor budding was correlated with various clinical and histopathological parameters for statistically significant association. **Results:** A total of 124 cases were studied which showed female preponderance (54.8%) in presentation and the most common age group being 61–70years (48%). Adenocarcinoma was the most common histological subtype (83.8%). Lymph node metastasis was observed in 52 cases (42%). The grade of tumor budding was low in 96 cases (77.5%), intermediate in 20 cases (16%), and high in 8 cases (6.5%). Statistically significant association was observed between grade of tumor budding and age, histological grade, lymphovascular invasion, and lymph node involvement. **Conclusion:** Tumor budding is an independent prognostic marker for adverse prognosis and predictor of lymph node metastasis. Enumerating tumor budding on routine H&E slide is an inexpensive method of providing additional prognostic factors for better patient management.

Key words: Colorectal carcinoma; Epithelial-mesenchymal transition; Prognostic factors; Tumor budding

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INTRODUCTION

Colorectal carcinoma is one of the leading causes of death in Western population as well as in India.[1,2] The gold standard for classification of colorectal cancer patients into different prognostic subgroups is by the TNM classification. However, colorectal carcinomas are known to vary in behavior and survival even within the same stages.[2,3] Additional prognostic biomarkers are needed for predicting the disease free intervals and rates of survival. Histopathological features such as lymphovascular invasion, tumor deposits in lymph nodes, and perineural invasion have shown promising results in predicting patient survival.[4]

In recent years, tumor budding has been in focus as a valuable prognostic marker and its importance has been highlighted by many gastrointestinal pathologists.[5] Tumor budding is defined as single cells or small groups of tumor cells up to four cell clusters within the tumor or at the invasive front.[3] It is believed that tumor budding is related to epithelial-mesenchymal transition in tumor pathogenesis. Tumor budding has been studied extensively in colon and rectal cancers. Researches are rapidly advancing in identifying its significance in carcinomas of the head-and-neck, upper gastrointestinal tract, breast, and lung.[5]

Tumor budding was not included in diagnostic pathology reports previously due to lack of standard assessment guidelines, existence of multiple systems for reporting with

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poor consensus between them, and poor reproducibility.[5-8] In 2016, International Tumor Budding Consensus Conference (ITBCC) classified tumor budding into three tier system, 0–4 buds as low budding (Bd 1), 5–9 buds as intermediate budding (Bd 2), and 10 or more buds as high budding (Bd 3). The ITBCC recommends the assessment of tumor budding especially in two scenarios pT1 and Stage II. It is an independent predictor of lymph node metastases in pT1 tumors and it is an independent predictor of survival in Stage II tumors.[3,5]

Numerous studies have shown that tumor budding can predict lymph node metastasis in early colorectal cancer and also an independent predictor of survival in Stage II colorectal cancer.[3,6,8] Studies like the SACURA trials have shown tumor budding to be an important decision-making factor for adjuvant chemotherapy in patients with Stage II colon cancer.[9]

Aims and objectives

This study was aimed at enumerating tumor budding, stratifying it, and identifying correlation with other the clinicopathological factors. The objectives included to identify and count tumor budding in resected colorectal carcinoma specimens and classify tumor budding into low, intermediate, and high grade; and to study the correlation of the grade of tumor budding with age, sex, tumor location, tumor size, histological grade, lymph node status, lymphovascular invasion, and perineural invasion.

MATERIALS AND METHODS

This study was a retrospective study of all resected colorectal carcinoma specimens received from the January 2016 to December 2020 in the Department of Pathology, Hassan Institute of Medical Sciences, Hassan. This study was approved by the Institutional Research Committee and Institutional Ethics Committee. Ethical permission was obtained for waiver of consent. Demography and clinical data were collected from the request forms. Cases with incomplete data were excluded from the study. Archived slides and blocks were retrieved. Blocks were recut and slides were made and stained with H&E stain. All tumor slides were examined under ×10 to identify hotspots. Tumor buds were counted under ×20 objective of Olympus CX41 microscope (Olympus Medical Systems India Pvt. Ltd., Gurgaon) with a field diameter of 22 mm. Thus, obtained bud count was divided by the normalization factor provided by ITBCC[3] to determine the tumor bud count per 0.785 mm^2. The budding grade was assigned as low (0–4 buds), intermediate (5–9 buds), and high (10 or more buds) based on the bud count which specified the absolute count per 0.785 mm^2. The tumor budding grade was analyzed to identify any correlation with the grade of tumor budding with age, sex, tumor location, tumor size, histological grade, lymph node status, lymphovascular invasion, and perineural invasion.

Statistical analysis was performed using SPSS software version 20. Fisher's exact test and Chi-square test were used to analyze the correlation of tumor budding with various clinicopathological parameters. $P<0.05$ was considered as statistically significant.

RESULTS

A total of 124 cases were included in this study, of which 56 (45.2%) cases were male and 68 (54.8%) cases were female. The age range was 29–80 years. The most common age group being 61–70 years which comprised of 48% of cases. The right colon and left colon were equally affected. The greatest tumor dimension ranged from 2 to 13 cm. The average greatest dimension of tumor was 5.8 cm. Adenocarcinoma was the most common histological type comprising of 104 (83.8%) cases followed by mucinous carcinoma involving 20 (16.2%) cases. The most of the adenocarcinomas were Grade 1 (51.6%) followed by Grade 2 (32.2%). Lymphovascular invasion was seen in 48 cases (38.7%) and perineural invasion was seen in 4 case (3.2%). The extent of tumor invasion was up to muscularis propria in 12.9% cases, invasion through the muscularis propria in 54.8% cases, and invasion of the visceral peritoneum was seen in 32.3% cases. Lymph node metastasis was observed in 52 cases (42%). The grade of tumor budding (Figure 1-4) was low in 96 cases (77.5%), intermediate in 20 cases (16%), and high in 8 cases (6.5%).

Correlation of various parameter was done with the grade of tumor budding which is depicted in Table 1. No statistical significance was observed between tumor budding grade and gender ($P=0.8572$), tumor site ($P=0.1767$), tumor size ($P=0.2354$), perineural invasion ($P=0.5473$), and extent of tumor invasion ($P=0.1818$). Statistically significant association was observed between grade of tumor budding and age ($P<0.0001$), histological grade ($P<0.0001$), lymphovascular invasion ($P<0.0001$), and lymph node involvement ($P<0.0001$).

DISCUSSION

This study was conducted to enumerate tumor budding, stratify it, and to identify correlation with various clinicopathological factors. We observed a female preponderance (54.8%) in tumor presentation. This was also observed by Munireddy et al., in their study, where they found statistically significant association of gender with tumor budding.[10] We observed equal presentation of tumors in both the right and left colon. Munireddy

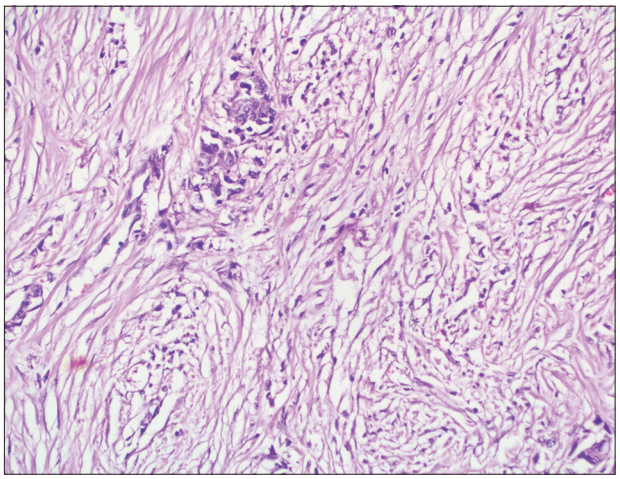

Figure 1: Tumor budding at the invasive edge – Low budding

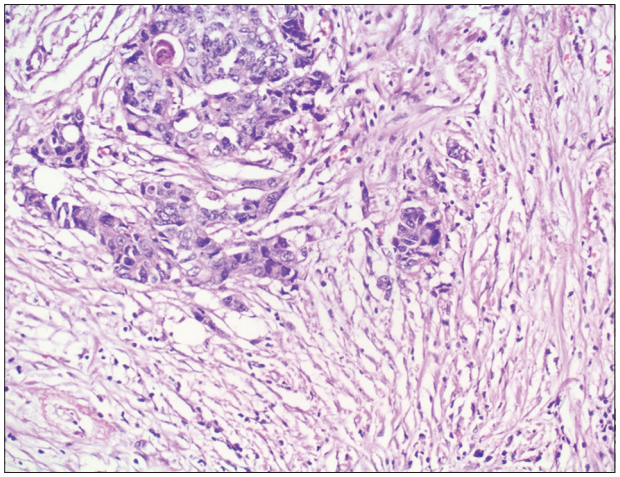

Figure 2: Tumor budding at the invasive edge – Intermediate budding

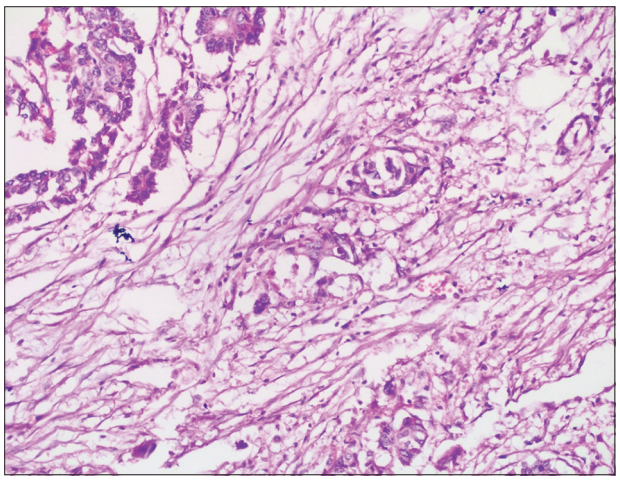

Figure 3: Tumor budding at the invasive edge – High budding

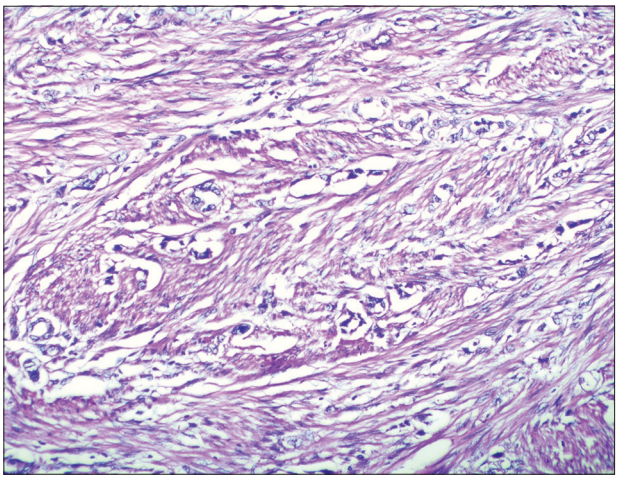

Figure 4: Tumor budding at the invasive edge – High budding

et al., showed right colon preponderance compared to Mehta et al., who showed the left colon predominance in their study.[10,11] In our study, this observations were not statistically significant. The most common age group of presentation was 61–70 years in our study which was similar to Munireddy et al., study.[10] We also observed that higher grade of tumor budding was associated with advancing age. This finding was statistically significant in our study.

Adenocarcinoma was the predominant histological type (83.8%) followed by mucinous carcinoma (16.2%). Similar observations were made by Munireddy et al.,[10] The study conducted by Mehta et al., also showed preponderance of adenocarcinoma followed by mucinous adenocarcinoma, signet ring cell carcinoma, and undifferentiated carcinoma.[11] The grade of tumor budding was low in 77.5%, intermediate in 16% cases, and high in 6.5% cases. The study by Lee and Chan showed low budding in 71% cases, intermediate in 9%, and high budding in 20% cases.[12] The majority of the tumors were of histological Grade 1 and showed significant correlation with tumor budding. Munireddy et al., and Sevda et al., studies also showed a positive correlation of histological grade with intensity of tumor budding, whereas Mondal et al., study showed no correlation of tumor budding with histological grade.[10,13,14]

Lymphovascular invasion showed positive correlation with tumor budding intensity whereas no statistically significant association was observed between perineural invasion and tumor budding. Similar observations were made by Roy et al.[8] On the contrary, Mondal et al., showed association of perineural invasion with tumor budding intensity.[13]

pT3 stage of tumors was the most frequent pT staging in our study and no statistically significant association was found with tumor budding. Roy et al., Mondal et al., Sevda et al., and Jagadale and Agarwal studies also showed pT3 stage of tumors as the majority and no statistically significant association with tumor budding.[8,13-15] However, Munireddy et al., showed statistically significant association

Table 1: Correlation of grade of tumor budding with various parameters

Correlation parameters	Number	Grade of tumor budding			P value
		Low	Intermediate	High	
Gender					
Male	56 (45.2%)	44	8	4	0.8572
Female	68 (54.8%)	52	12	4	
Age					
<60 years	44 (35.5%)	28	16	0	<0.0001
≥60 years	80 (64.5%)	68	4	8	
Tumor site					
Right	62 (50%)	44	12	6	0.1767
Left	62 (50%)	52	8	2	
Tumor size					
0–5 cm	64 (51.6%)	52	11	1	0.2354
6–10 cm	50 (40.3%)	36	8	6	
>10 cm	10 (8.1%)	8	1	1	
Histological grade					
1	66 (53.2%)	56	8	2	<0.0001
2	56 (45.2%)	40	12	4	
3	2 (1.6%)	0	0	2	
Lymphovascular invasion					
Present	48 (38.7%)	28	12	8	<0.0001
Absent	76 (61.3%)	68	8	0	
Perineural invasion					
Present	4 (3.2%)	4	0	0	0.5473
Absent	120 (96.7%)	92	20	8	
Extent of tumor invasion (pT)					
pT1	0	0	0	0	0.1818
pT2	16 (12.9%)	16	0	0	
pT3	68 (54.8%)	52	12	4	
pT4	40 (32.3%)	28	8	4	
Nodal status (N)					
0	72 (58%)	72	0	0	<0.0001
1	28 (22.6%)	24	4	0	
2	24 (19.4)	0	16	8	
3	0	0	0	0	
4	0	0	0	0	

of pT stage with tumor budding. We also observed increased frequency of tumor budding as well as higher grade of tumor budding with advanced pT stage; however, this observation was not statistically significant.

N0 was the most frequent nodal status in our study which was concordant with study done by Jagadale and Agarwal.[15] Association of tumor budding with nodal involvement was statistically significant with P<0.0001. Multiple previous studies have emphasized this association.[8-17] Rogers et al., described tumor budding as a predictor of lymphnode metastasis in node negative patients and can be a deciding factor for chemotherapy in such patients. Tumor budding can also help in predicting recurrence and long-term survival in colorectal carcinoma patients as it indicates an aggressive type of malignancy.[17]

Colorectal carcinomas have been known to have multistep carcinogenesis. Epithelial mesenchymal transition is one of the most important step leading to metastasis.[3] Tumor budding is observed at the invasive front of tumor and it symbolizes the morphological appearance of epithelial mesenchymal transition. It represents the effort of the tumor to detach from main tumor mass and metastasize. Several studies have established tumor budding as an independent adverse prognostic factor in colorectal carcinomas.[17] Hence, tumor budding can be used as a powerful predictor of aggressive nature of tumor and nodal metastasis.[3,11,16] Tumor budding is associated with other adverse prognostic factors such as lymph node metastasis, lymphovascular invasion, distant metastasis, higher tumor grade, and advanced TNM staging.[3] This can be considered as an indirect evidence for tumor budding to be a predictor of poor prognosis. Tumor budding can be especially helpful when there is no microscopically detectable lymph node metastasis. Inclusion of tumor budding in routine histopathology reports can be beneficial as the treating clinician can assess the prognosis of patients. It is a relatively simple exercise done on routine H&E slides with no extra staining or special procedures. Use of cytokeratin stains has been mentioned in the literature when there is obscuring of tumor stroma interface.[10] Many centers may not have the ×20 objective in the microscopes to enumerate tumor budding. In such situation, modifications on ×40 objective as suggested by Roy et al., can be considered.[8]

Limitations of the study

Small sample size and lack of follow-up data of the patients were the limitations of this study.

CONCLUSION

In our study, statistically significant association of grade of tumor budding with adverse prognostic factors such as advanced age, lymphovascular invasion, and lymph node status was noted. No statistically significant association was observed between tumor budding grade and gender, tumor site, tumor size, perineural invasion, and extent of tumor invasion. Enumerating tumor budding on routine H&E slide is an inexpensive method of providing additional prognostic factors in diagnostic pathology reports for better for patient management.

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Authors Contribution:
PN- Concept and design of the study, prepared first draft of manuscript, interpreted the results, statistical analysis, and interpretation; **HJ-** Concept and design of the study, statistical analysis and interpretation, reviewed the literature, and manuscript preparation; **TSS-** Concept, coordination, preparation of manuscript, and revision of the manuscript; and **KRN-** preparation of manuscript and revision of the manuscript.

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