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# Significance of Tumor Budding In Colorectal Carcinoma - Correlation with Histologic Prognostic Parameters

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## ABSTRACT

### Introduction

Tumor budding (TB) is defined as presence of single tumor cell or clusters of less than five cells at the invasive front of the tumor. TB in colorectal carcinoma has been shown to be associated with high tumor grade, lymphovascular invasion, local recurrence, and distant metastasis. Though it was not included in routine reporting earlier, the guidelines for reporting of TB were first given by ITBCC (International tumor budding consensus conference) in 2016 and it is now included in CAP (College of American Pathologists) protocol as a recommended element.

### Aims

The aims of the study are to score tumor budding in Hematoxylin and Eosin (H & E) slides of CRC, to compare the TB grade with histologic prognostic parameters (histologic grade, local invasion -pT, lymphnode status, lymphovascular invasion) and to evaluate inter- observer agreement of TB grading between two pathologists.

### Methods

This was a retrospective observational study conducted from June 2017 to June 2022, on 33 resected specimens of CRC. H & E slides and relevant clinicopathological data was collected. TB grading was assessed by two pathologists as low-Bd1, Intermediate-Bd2 and High-Bd3. The pathologist 1 graded tumor budding and evaluated the histologic

parameters- such as histologic grade, local invasion- pT, lymph node status (positive or negative) and presence/ absence of lymphovascular invasion. TB scoring was then assessed independently by Pathologist 2. Then, this was compared with the findings of Pathologist 1 to assess Inter- observer agreement.

**Results**

Out of 33 cases of CRC, 14 (42.4%) showed low TB- Bd1, 11 (33.3%) showed intermediate- Bd2 and 8 (24.2%) showed high tumor budding- Bd3. Lymphovascular invasion statistically correlated with TB with p- value- 0.037. No statistical correlation was found between TB and histological grade (P-value- 0.494), local invasion (p- value- 0.883), lymph node status (p- value- 0.186). Interobserver agreement between both the pathologists using kappa test showed substantial agreement (kappa value of 0.64) with p- value <0.001.

**Conclusion**

Tumor budding showed positive correlation with lymphovascular invasion and substantial agreement for Inter-observer reproducibility. Considering the importance of tumor budding as an evolving independent prognostic factor, it is recommended to make it a required element during reporting in all cases of CRC. This will be valuable in assessing the prognosis and commencing appropriate treatment protocols.

**Keywords**

Tumor budding, colorectal carcinoma, lymphovascularinvasion

**INTRODUCTION**

The concept of Budding was first described in 1920 by Broders and the term Tumor budding (TB) was given by Morodomi in 1989.[1] Tumor budding(TB) is defined as presence of single tumor cell or clusters of less than five cells at the invasive front of the tumor.[2] It is stratified into peritumoral budding and intratumoral budding.[3] It is one of the novel histopathological parameter which is being recognised as an important independent prognostic factor in colorectal carcinoma (CRC).[4] Tumor buds share identical features with malignant stem cells, reflecting the process of epithelial- mesenchymal transition. They show loss of E- cadherin which causes detachment of tumor cells from the main tumor mass and metastasize.[5] TB has been shown to be associated with high tumor grade, lymphovascular invasion, local recurrence and distant metastasis.[3] Even though TNM is accepted as gold standard assessment to predict clinical outcome it shows various outcomes within same tumor stage.[5] Studies are being done, so as to explore TB as an independent adverse prognostic and predictive factor in colorectal carcinoma.[4] The Union for International Cancer Control (UICC) accepts TB a reliable criteria.[6] Though it was not included in routine reporting earlier, the guidelines for reporting of TB were first given by ITBCC (International tumor budding consensus conference) in 2016 and it is now included in CAP (College of American Pathologists) protocol as a recommended element.[3] The aims of the study are to score tumor budding in Hematoxylin and Eosin (H & E) slides of CRC, to compare the TB grade with histologic prognostic parameters (histologic grade, local invasion -pT, lymphnode status, lymphovascular invasion) and to evaluate inter- observer agreement of TB grading between two pathologists.

**MATERIALS AND METHODS**

This was a retrospective observational study conducted from June 2017 to June 2022, on 33

resected specimens of CRC. H & E slides and relevant clinicopathological data was collected. TB grading was assessed by two pathologists. The pathologist 1 graded tumor budding and evaluated the histologic parameters (histologic grade, local invasion- pT, lymph node status, lymphovascular invasion). The correlation was investigated between TB and the histologic parameters. Then Pathologist 2 graded TB and the interobserver agreement between both the pathologists was assessed.

Histologic grading was carried out as per W.H.O guidelines. Tumors were graded as well differentiated (>95% glandular differentiation), moderately differentiated (50-95% glandular differentiation) and poorly differentiated (<50% glandular differentiation). Local invasion (pT) was categorized as per the TNM staging, AJCC 8th edition, i.epTX: Primary tumor cannot be assessed, pT0: No evidence of primary tumor, pTis: Carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae), pT1: Tumor invades the submucosa (through the muscularis mucosa but not into the muscularis propria), pT2: Tumor invades the muscularis propria,pT3: Tumor invades through the muscularis propria into peri-colorectal tissues, pT4a: Tumor invades the visceral peritoneum or invades or adheres to adjacent organ or structure, pT4b: Tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface ofthe visceral peritoneum). Then, the presence or absence of lymphnode metastasis, and lymphovascular invasion were recorded.

Scoring and grading tumor budding: This was done first by Pathologist 1 on H & E slides according to ITBCC 2016 guidelines. Thus, 10 fields were screened at 10x. Hotspot was identified at tumor invasive front. Using 20x tumor budding was counted in a single selected field (0.785mm$^2$). The tumor buds classified into three tier grading system as: low (Bd1)- 0 to 4, intermediate (Bd2)- 5 to 9, high (Bd3)- >10.

The TB grades were then correlated with prognostic parameters such as histologic grade, local invasion (pT), lymph node status (positive or negative) and presence/ absence of lymphovascular invasion.

TB scoring was then assessed independently by Pathologist 2. Then, this was compared with the findings of Pathologist 1 to assess Inter- observer agreement.

Statistical Analysis: Agreement for Interobserver variability was done using Cohen's kappa test and for other histopathological parameters by using Chi-square test with p- value of <0.05 was considered as significant. The Cohen's kappa test was interpreted as; ≤ 0 (No agreement), 0.01–0.20 (none to slight agreement), 0.21–0.40 (fair agreement), 0.41– 0.60 (Moderate agreement), 0.61–0.80 (Substantial agreement) and 0.81–1.00 indicates (almost perfect agreement)

**RESULTS**

A total of 33 cases were included in this study out of which 20 (60.6%) were males and 13 (39.3%) were females. The age range was between 49 to 67 yrs. Right colon (19, 57.5%) showed predominant involvement when compared with left colon 14 (14, 42.2%). Out of 33 cases, 14 (42.4%) showed low TB-Bd1 (Fig 1, 2), 11 (33.3%) showed intermediate- Bd2 (Fig 3) and 8 (24.2%) showed high tumor budding-Bd3 (Fig 4). TB was then compared with other histopathological parameters shown in Table. 1

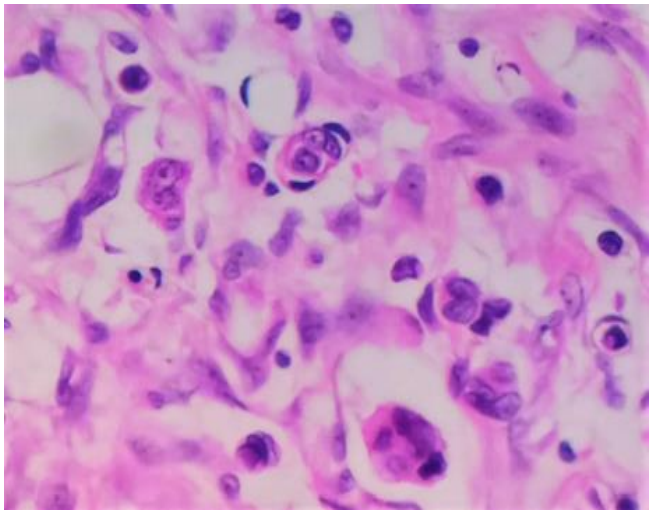

**Fig. 1: Tumor buds in clusters of 1-4( H & E, 400x)**

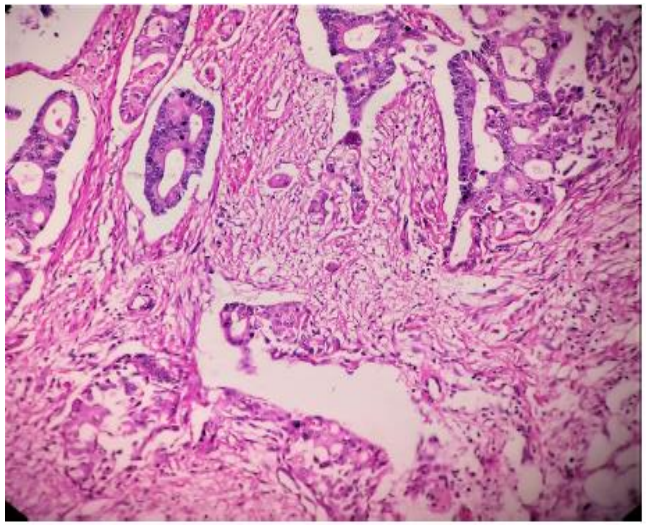

**Fig. 2: Low tumor budding (H & E, 200x)**

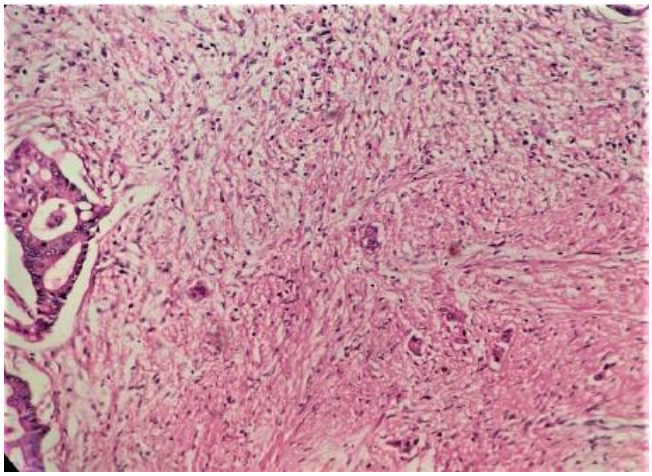

**Fig. 3: Intermediate tumor budding (H & E, 200x)**

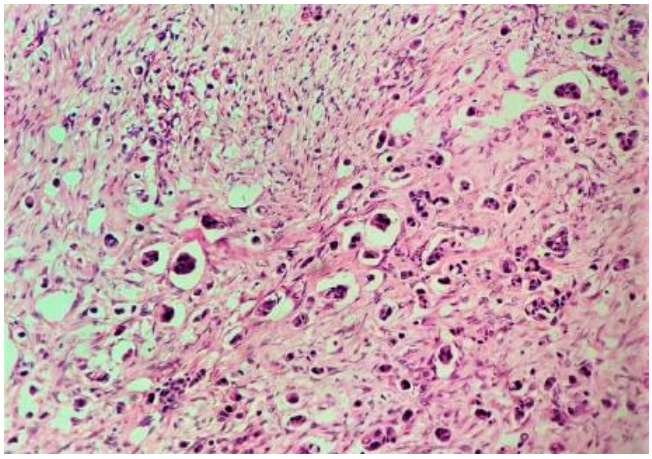

**Fig. 4: High tumor budding (H & E, 200x)**

TB was then compared with other histopathological parameters shown in Table. 1

**Table. 1. COMPARISION OF TUMOR BUDDING WITH HISTOLOGICAL PARAMETERS**

| HISTOLOGIC PARAMETERS | (n, %) | LOW (Bd1) n, (%) | INTERMEDIATE (Bd2) n, (%) | HIGH (Bd3) n, (%) | P- VALUE |
|---|---|---|---|---|---|
| Histologic grade | Well differentiated (8/33, 24.4%) | 4/8 (50%) | 3/8 (37.5%) | 1/8 (14.2%) | 0.494 |
| | Moderately differentiated (19/33, 57.5%) | 9/19 (47.3%) | 6/19 (31.5%) | 4/19 (21%) | |
| | Poorly differentiated (6/33, 18.1%) | 1/6 (16.6%) | 2/6 (33.3%) | 3/6 (50%) | |
| Local invasion | T2 (11/33, 33.3%) | 6/11 (54.5%) | 3/11 (27.2%) | 2/11 (18.1%) | 0.883 |
| | T3 (16/33, 48.8%) | 6/16 (37.5%) | 6/16 (37.5%) | 4/16 (25%) | |
| | T4 (6/33, 18.1%) | 2/6 (33.3%) | 2/6 (33.3%) | 2/6 (33.3%) | |
| Lymphnode status | Positive (10/33, 30.3%) | 2/10 (20%) | 4/10 (40%) | 4/10 (40%) | 0.186 |
| | Negative (23/33, 76.6%) | 12/23 (52.1%) | 7/23 (30.4%) | 4/23 (17.3%) | |
| Lymphovascular invasion | Present (13/33, 39.3%) | 2/13 (15.3%) | 6/13 (46.1%) | 5/13 (38.4%) | 0.037 |
| | Absent (20/33, 60.6%) | 12/20 (60%) | 5/20 (25%) | 3/20 (15%) | |

Lymphovascular invasion statistically correlated with TB with p- value- 0.037. This was seen in 13/33 cases with majority associated with Bd2 (6/13, 46.1%) and Bd3 (5/13, 38.4%).

No statistical correlation was found between TB and histological grade (P-value-0.494), local invasion (p-value- 0.883), lymph node status (p- value- 0.186). However, Bd3 was seen in 50% of poorly differentiated tumors, 21% of moderately differentiated and 14.2% of well differentiated tumors. Similarly, Bd3 was seen in 33.3% in pT4, 25% in pT3 and 18.1% in pT2. Also, 40% of tumors with positive lymphnode status showed Bd3 in contrast to only 17.3% with negative lymphnode status. Amongst cases showing lymphovascular invasion, Bd3 and Bd2 were seen in 38.4% and 46.1% cases respectively. (Table 1)

Interobserver agreement between both the pathologists using kappa test showed substantial agreement (kappa value of 0.64) with p- value <0.001, shown in Table 2.

TABLE. 2: INTER- OBSERVER AGREEMENT ANALYSIS FOR TUMOR BUDDING USING KAPPA TEST

| TUMOR BUDDING GRADE | PATHOLOGIST- 1 N (%) | PATHOLOGIST- 2 N (%) | KAPPA VALUE | P- VALUE |
|---|---|---|---|---|
| Low (Bd 1) | 14 (42.2%) | 11 (33.3%) | 0.64 | <0.001 |
| Intermediate (Bd 2) | 11 (33.3%) | 13 (39.3%) | | |
| High (Bd 3) | 8 (24.2%) | 9 (27.2%) | | |

## DISCUSSION

Tumor budding is a potential prognostic factor in colorectal carcinoma.[4] TB is stratified as intratumoral budding- ITB (presence of tumor cells within the tumor mass) and peritumoral budding- PTB (presence of tumor cells in invasive front of tumor).[3] The grade of tumor budding is strongly associated with presence of lymph node metastasis, distant metastasis, venous invasion.[4] It also indicates increased aggressiveness of tumor along with decreased survival rates.[3]

Due to lack of standardized assessment, ITBCC in 2016 recommended guidelines for reporting TB. Though it is not a required element in CAP protocol, it is recommended to report TB as an essential criterion especially in reporting carcinomas arising in polyps as well as for stage 1 and stage 2.[7] However, many studies have tried to assess the importance of PTB in relation to various histologic prognostic parameters in CRC.

The current study showed a male preponderance (20/33, 60.6%) of CRCs, which is similar to Demir A et al study.[8] The age group ranged from 49 to 67 years, whereas Naik P et al showed an age range of 61 to 70 years.[5] This study saw cases a decade earlier, possibly related to the increasing incidence of CRCs in a younger age group due to various lifestyle changes and genetic factors. Most of the cases showed right colon involvement (19/33 cases, 57.5%) similar

to Swathi M et al study (30/ 50 cases, 60%).[9]

A large majority of the tumors were moderately differentiated (19/33, 57.5%), followed by well differentiated (8/33, 24.4%) and poorly differentiated (6/33, 18.1%). Sadek S A et al study noted that majority of the tumors were moderately differentiated (51/92, 59.8%) followed by poorly differentiated (36/92, 39.2%) and well differentiated (5/92, 5.4%). [10] Most CRCs presented in pT3 (16, 48.8%), followed by pT2 (11, 33.3%), and pT4 (6, 18.1%). There were no cases in pT1 similar to Swathi M et al study, where majority of cases seen in pT3 (34/50, 68%) followed by pT2 (14/50, 28%) and pT4 (2/50,4%) with no cases in pT1.[9] A positive lymph node status was seen in 10/33, 30.3% cases where as Sadek S A et al noted the same in 36/92, 39.1% cases.[10] Lymphovascular invasion was seen in 11/33, 39.3% cases similar to Naik P et al (48/124, 38.7%).[5]

In the current study, most of the cases showed low and intermediate TB (42.2% and 33.3% cases respectively) with the least being high grade (24.2%). Naik P et al noted 77.5% and 16% as low and intermediate respectively, whereas only 6.5% cases were graded as high.[5] This difference could be attributed to subjectivity in grading and missing out small tumor buds.

A comparison of TB with histologic parameters in different studies has been represented in Table 3. On comparison of histological grade with TB grade, it was statistically insignificant like Mondal P et al study. However, Bd2 and Bd3 together were seen in 83.3% of poorly differentiated, 52.5% of moderately differentiated, and 51.7% of well differentiated carcinomas. There was a progressive increase in the number of tumor buds with histologic grade. Similarly, Mondal P et al noted Bd2 and Bd3 together in the single case (100%) of poorly differentiated, 82.5% of moderately differentiated, and 77.7% of well differentiated carcinomas. [11] Naik P et al noted a similar progressive increase.[5] [Table 3]

Comparison of local invasion with TB grade, was statistically insignificant like Naik P et al study.[5] However, Bd2 and Bd3 accounted for a large majority of cases in pT4 (66.6%) and pT3 (62.5%) when compared with pT2 (27.1%). There was a progressive increase in the number of tumor buds with increasing local invasion. Whereas, Naik P et al noted Bd2 and Bd3 in 23.5% (16/68) in pT3 and 30%(12/40) in pT4, no Bd2/Bd3 buds were found in pT1 and pT2, but all 16 cases in pT2 showed Bd1.[5] The ITBCC highly recommends counting of tumor budding in two scenarios mainly in pT1 and pT2.[4] TB in pT1 stage is associated with increased risk of lymph node metastasis, where patients may benefit from surgical resection alone. The current study did not have any cases in pT1. In pT2 CRC, TB is an indicator of short time disease free survival and patient with high TB can be considered for adjuvant therapy and it acts as a predictor of lymph node metastasis in node negative cases.[3] Numerous studies and meta-analysis have reported tumor budding to be an independent factor of poor survival and recurrence in stage 2 colorectal carcinoma, with outcome similar to those of patients with stage 3 colorectal carcinoma.

Tumor budding is also independent prognostic marker for adverse prognosis and predictor of lymph node metastasis.[5] Comparison of lymphnode status with TB grade, was statistically insignificant unlike Maqbool H et al and Naik P et al study.[12,5] This difference could be attributed to the smaller sample size in the current study when compared with

Maqbool H et al (n=50) and Naik P et al (n=124).However, of the 10 cases showing lymph node involvement, a large majority (80% cases) showed Bd2 and Bd3 (40% each).Whereas only 47.7% of lymph node negative cases showed Bd2 and Bd3. Maqbool H et al study noted Bd2 and Bd3 in 93.2% (26.6% and 66.6% respectively), in lymph node positive CRC (15/50, 30 %).[12] Comparison of lymphovascular invasion with TB grade, was statistically significant like Naik P et al study.[5] Of the 13 cases showing evidence of lymphovascular invasion, Bd2 and Bd3 together constituted a large majority of them (11, 84.5%). Among 48 CRCs which showed lymphovascular invasion, in Naik P et al study, Bd2 and Bd3 were seen 20 cases, 41.6% cases.[5]

**TABLE 3: TB AND HISTOLOGIC PARAMETERS - COMPARISON OF CURRENT STUDY WITH OTHER STUDIES**

| HISTOLOGIC PARAMETERS | | STUDIES | Bd1- n (%) | Bd2- n (%) | Bd3- n (%) |
|---|---|---|---|---|---|
| **Histologic grade** | Well differentiated | Current study | 4/8 (50%) | 3/8 (37.5%) | 1/8 (14.2%) |
| | | Mondal P et al | 8/36 (22.2%) | 4/36 (11.1%) | 24/36 (66.6%) |
| | | Naik P et al | 56/66 (84.4%) | 8/66 (12.1%) | 2/66 (3.03%) |
| | Moderately differentiated | Current study | 9/19(47.3%) | 6/19 (31.5%) | 4/19(21%) |
| | | Mondal P et al | 4/23 (17.3%) | 5/23 (21.7%) | 14/23 (60.8%) |
| | | Naik P et al | 40/56 (71.4%) | 12/56 (21.4%) | 4/56 (7.16%) |
| | Poorly differentiated | Current study | 1/6 (16.6%) | 2/6 (33.3%) | 3/6 (50%) |
| | | MondalP et al | 0 (0) | 1/1(100%) | 0 (0) |
| | | Naik P et al | 0/2 (0) | 0/2 (0) | 2/2 (100%) |
| **Local invasion** | T2 | Current study | 6/11 (54.5%) | 3/11 (27.2%) | 2/11 (18.1%) |
| | | Naik P et al | 16/16 (100%) | 0(0) | 0(0) |
| | T3 | Current study | 6/16 (37.5%) | 6/16 (37.5%) | 4/16 (25%) |
| | | Naik P et al | 52/68 (76.4%) | 12/68 (17.6%) | 4/68 (5.88%) |
| | T4 | Current study | 2/6 (33.3%) | 2/6 (33.3%) | 2/6 (33.3%) |
| | | Naik P et al | 28/40 (70%) | 8/40 (20%) | 4/40 (10%) |
| **Lymph node status** | Positive | Current study | 2/10 (20%) | 4/10 (40%) | 4/10 (40%) |
| | | Maqbool H et al | 1/15 (6.6%) | 4/15 (26.6%) | 10/15 (66.6%) |
| | | Naik P et al | 24/52 (46.1%) | 20/52 (38.4%) | 8/52 (15.3%) |
| | Negative | Current study | 12/23 (52.1%) | 7/23 (30.4%) | 4/23 (17.3%) |
| | | Maqbool H et al | 25/35 (71.4%) | 4/35 (11.4%) | 6/35 (17.1%) |
| | | Naik P et al | 72/72 (100%) | 0 (0) | 0 (0) |
| **Lymphovascular invasion** | Present | Current study | 2/13 (15.3%) | 6/13 (46.1%) | 5/13(38.4%) |
| | | Naik P et al | 28/48 (58.3%) | 12/48 (25%) | 8/48 (16.6%) |
| | Absent | Current study | 12/20 (60%) | 5/20 (25%) | 3/20 (15%) |
| | | Naik P et al | 68/76 (89.4%) | 8/76 (10.5%) | 0 (0) |

Interobserver agreement between two pathologists showed substantial agreement (0.64) with significant p- value, where as other studies like Koelzer VH et al showed moderate agreement (0.58) and Garfunkel R et al showed perfect agreement (0.86). [13,14]

A high tumor budding is associated with a high chance of relapse.[8] The concept of TB is based on epithelial-mesenchymal transition and thus TB may express different target molecules. The future investigations for these potential target molecules may be promising for an anti- budding therapy to specifically target the tumour cells responsible for local and distant metastases and consequently for tumour progression and decreased survival.[7]

The limitations of this study were the small sample size. Also, IHC was not performed to identify tumor buds, as many earlier studies have shown conflicting results.

**CONCLUSION**

Tumor budding showed positive correlation with lymphovascular invasion and substantial agreement for Inter-observer reproducibility. Considering the importance of tumor budding as an evolving independent prognostic factor, it is recommended to make it a required element during reporting in all cases of CRC. This will be valuable in assessing the prognosis and commencing appropriate treatment protocols.

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