



A Retrospective Study of Association of Tumor Budding, Tumor Microenvironment, and Clinicopathological Characteristics of Invasive Breast Carcinoma

Tanwi Singh¹ Kalpana Chandra¹ Nidhish Kumar¹ Anuja Mishra¹ Sanjeet Singh¹ Anju Singh¹
Umesh Kumar¹ Praveen Kumar²

¹ Pathology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India

² General Medicine, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India

Address for correspondence Kalpana Chandra, MD, Pathology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India (e-mail: kalpana.chandra.14@gmail.com).

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Abstract

Background Tumor budding (TB) has been identified in many solid cancers and thought to be involved in invasion and is the initial step in the metastatic process. Limited information is available documenting the role of tumor budding in breast carcinoma. With this aim, the present study evaluates the association of tumor budding, tumor microenvironment, and its correlation with clinicopathologic parameters.

Materials and Methods A total of 102 cases were archived and evaluated for peripheral and intra tumoral budding along with tumor microenvironment on hematoxylin and eosin (H&E) slides.

Statistical Analysis Correlation between tumor budding, tumor microenvironment, and other classical clinicopathological parameters was studied by Chi-square test. A *p*-value less than 0.05 was considered significant.

Results Females constituted 99 cases out of 102 and 3 were males. We found 55.9% and 44.1% of patients in the age group less than or equal to 50 and greater than 50, respectively. Also, 65.6% of cases presented with small tumor size less than or equal to 5 cm, 80.39% with lymph node metastasis, and 76.4% with lympho-vascular emboli. High peripheral tumoral budding (PTB) was seen in 45.10%, low peripheral tumoral budding in 54.9%, high ITB in 53.9%, and low ITB in 46.1%. Necrosis was found only in 39.21%. Significant statistical association of PTB was found with lymph node metastasis, lymphovascular emboli, and tumor necrosis, whereas ITB with tumor grade, lymph node metastasis, lympho-vascular emboli, and necrosis. Both PTB and ITB showed no statistically significant correlation with age and size of the tumor.

Conclusion Tumor budding is an independent adverse prognostic factor in invasive breast carcinoma. However, further work is needed to establish a standard method for the quantification of this parameter, which will help in effective stratification of patients in terms of disease-free survival and likely outcome.

Keywords

- ▶ intratumoral budding
- ▶ invasive breast carcinoma
- ▶ peripheral tumor budding
- ▶ prognosis
- ▶ tumor budding

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Introduction

Breast cancer ranks as the most frequently diagnosed cancer in females accounting for 24% of all female cancers reported globally. It contributes for 11.6% of cancer in both sexes.¹ Breast cancer is well known for its heterogeneity with different histological and molecular subtypes showing differences in behavior and propensity to metastasize.² In recent years, tumor budding (TB) has been identified in many solid cancers as an important contributing factor in terms of prognosis and cancer-free survival. To date, colorectal cancer characterizes tumor budding at its best. TB is defined as single tumor cells or small clusters of tumor cells (less than five), which detaches from the main tumor mass.³ From a pathophysiologic point of view, TB represents cancer cells that are caught in the process of invasion and is the initial step in the process of metastasis.⁴ This unique phenomenon was first pointed out by Imai in 1954. He described it as a morphological feature that was observed at the invasive front of the tumor and later referred to as tumor sprouting.⁵ TB can be assessed at two sites, namely at the invasive front (peripheral TB) or inside the tumor (intra-TB). In context of breast carcinoma, limited information is available that documents the role of TB in determining the behavior and clinical outcome.

It has been documented by many researchers such as Salhia⁶ and Gujam⁷ et al that tumor buds are associated with lymph node metastasis and lympho-vascular invasion, which play a crucial role in cancer-specific survival. In our country, as the major population belongs to poor socio-economic status, determination of TB at the time of routine hematoxylin and eosin (H&E) reporting will be time and cost-effective as 30% of patients die due to widespread metastasis.⁸

In future, TB may play an important role in the prognostication of modified radical mastectomy (MRM) patients. After the establishment of standard criteria for its quantification, it may come up as an additional independent adverse prognostic factor. With this aim, the present study evaluates the association of TB, tumor microenvironment, and its correlation with clinicopathologic parameters.

Materials and Methods

The study was conducted at a tertiary care referral hospital in the department of pathology. It was a retrospective study done over a period of 2 years from January 2018 to December 2019. We included all MRM specimens received at the histopathology laboratory reported as invasive breast carcinoma not otherwise specified (NOS). Institutional ethical committee clearance was taken. Patient data were collected from departmental records.

All the H&E slides were retrieved and the histomorphological data were reevaluated for the presence of TB and its score, histological grade (modified Bloom–Richardson), lymphovascular invasion, and lymph node metastasis. Lumpectomy and core biopsy specimens along with slides received for review from outside were excluded from the present study. Patients

with prior history of chemotherapy were also excluded. Further evaluation of tumor microenvironment, specifically in terms of necrosis, tumor stroma percentage (TSP), and tumor inflammatory cell infiltrates (TILs) was performed. Tumor stroma percentage was evaluated on H&E slides. The invasive front of the tumor was assessed on scanner view ($\times 4$) and then representative single-field was examined under $\times 10$ magnification in such a way that tumor cells were present at all the four sides of the image. The percentage of stroma was then calculated. Cases with TSP less than 50% and more than 50% were categorized into low grade and high grade, respectively. For evaluation of TILs, we used the Klintrup–Makinen method to grade the TILs.⁹

Assessment and Scoring of Tumor Bud

1. The invasive front of the tumor was identified on scanner view for evaluation of peripheral tumor budding (PTB). Similarly, tumor bud within the growth/center referred as intra tumoral budding (ITB) was also identified.
2. A bud was identified as an isolated single cancer cell or a group of up to five cancer cells. The highest bud count per field was used as the number of buds.
3. Tumor buds were searched in low power ($\times 10$) at both the sites and then counted on high power ($\times 40$) for 10 consecutive fields.
4. Cases with positive TB were further categorized into low grade (≤ 10) and high grade (> 10) at both sites.

Data were then entered into an MS Excel sheet and further compiled, listed, interpreted, and analyzed using the Statistical Package for the Social Science (SPSS version 20) software. We tried to assess the correlation, if any, between TB, tumor microenvironment, and other classical clinicopathological parameters.

Results

A total of 102 cases were archived and included in the study. In our study, the majority were females constituting 99 cases out of 102, and 3 were males. We found 55.9% cases and 44.1% cases in less than or equal to 50 and greater than 50 age groups, respectively. The majority of the cases presented with small tumor size less than or equal to 5 cm (67 [65.6%]). We evaluated both PTB and ITB in all 102 cases.

Association of PTB with clinicopathological parameters (**► Table 1**).

Significant statistical association of PTB was found with lymph node metastasis ($p = 0.00$), lymphovascular emboli ($p = 0.001$), and tumor necrosis ($p = 0.015$). However, age of the patient, tumor size, and tumor grade showed no significant correlation with PTB.

Association of intra-tumoral budding with clinicopathological parameters: A significant positive correlation of ITB with tumor grade, lymph node metastasis,

Table 1 Association of peripheral tumor budding (PTB) with histopathological parameters

Parameters	Tumor budding: high grade	Tumor budding: low grade	p-Value
Age (y)			0.906
≤ 50	26	31	
> 50	20	25	
Tumor size (cm)			0.742
≤ 5	31	36	
> 5	15	20	
Necrosis			0.015
Present	24	16	
Absent	22	40	
Lymph node metastasis			0.00
Present	44	38	
Absent	02	18	
LVI			0.001
Present	42	36	
Absent	04	20	
Tumor grade			0.332
G1	03	08	
G2	31	38	
G3	12	10	

Table 2 Association of intra tumoral budding with histopathological parameters

Parameters	Tumor budding: high grade	Tumor budding: low grade	p-Value
Age (y)			0.613
≤ 50	32	25	
> 50	23	22	
Tumor size (cm)			0.957
≤ 5	36	31	
> 5	19	16	
Necrosis			0.012
Present	26	11	
Absent	29	36	
Lymph node metastasis			0.017
Present	49	33	
Absent	06	14	
LVI			0.005
Present	48	30	
Absent	07	17	
Tumor grade			0.042
G1	03	08	
G2	36	33	
G3	16	06	

lymphovascular emboli, and necrosis was found. However, ITB showed no statistically significant correlation with age of the patient and size of the tumor (→ **Table 2**).

We assessed the tumor microenvironment including necrosis, TSP, and TILs. We found 66 (64.7%) patients with low TILs and 36 (35.3%) with high TILs. In the present study, 70 (68.62%) out of 102 cases had high TSP, while 32 (31.37%) patients had low TSP.

Discussion

Tumor budding is a histological process seen in many cancers. It is believed that TB displays an epithelial-to-mesenchymal transition-like process that gains migratory potential and resistance to apoptotic signals.¹⁰ These characteristics would advocate TB as a more sensitive predictor of aggressiveness and adverse prognostic factor as compared to traditional clinicopathologic variables. Invasive breast carcinoma, NOS being the most common histological subtype, was included in our study.¹¹

Up till now, a uniformed method of assessment and reporting of TB in colorectal adenocarcinoma has been established by the International Tumor Bud Consensus Conference (ITBCC) and published in 2017.¹² This has also been advocated by the College of American Pathologists (CAP).^{13,14} However, no

uniformity and reporting of TB in breast carcinomas has been achieved till date. A few authors have used ×20 objective, while others including the present study had used ×40 objectives to count TB. It is difficult to differentiate TB from its mimickers, especially on an H&E section on a low-power objective. Secondly, in resource-poor countries such as ours, the availability of ×20 objective is a practical issue even at many institutes. So, we suggest it would be better to count the TB at ×40 objectives on a H&E section. A few authors have used the hotspot field method, while a few, including the present study, have used consecutive field method.

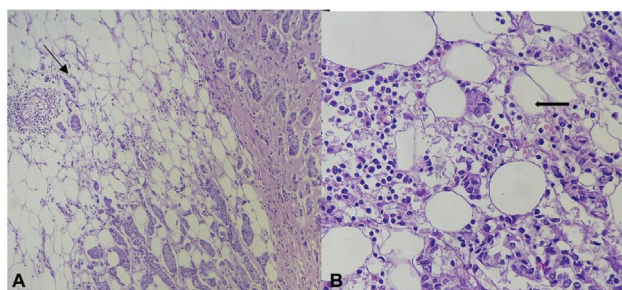
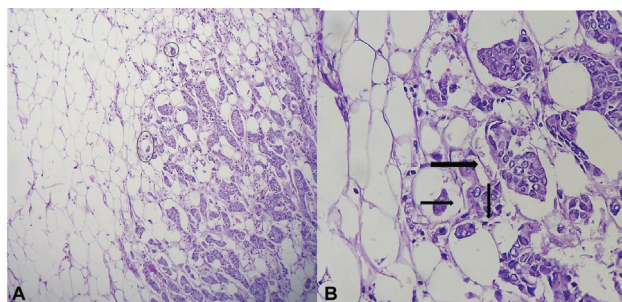
A few researchers have used immunohistochemistry (IHC)-stained slides for the assessment of TB; however, a study done by Van Wyk et al¹⁵ concluded that IHC did not improve the detection TB over H&E. We also used H&E sections for counting TB in our studies (→ **Table 3**).

The total number of cases in our study was 102, in which 3 patients were male. This was in contrast to other studies, where only female patients were included. The maximum clustering of cases was observed in the fifth decade with second peak in the seventh decade. Similar age range was found by Gujam et al.⁷

In our study, the majority of the patients had low-grade PTB (→ **Fig. 1**). This finding was similar to that of Liang et al² who found a higher number of patients with low PTB. The

Table 3 Comparison of sociodemographic variables with tumor budding parameters

Parameters	Present study	Kumarguru et al ¹⁶	Sriwidyani et al ¹⁷	Gujam et al ⁷	Salhia et al ⁶
Total number of cases	102	50	70	474	148
Age	< 50	Sixth decade (53.14 y)	Fifth decade (48.6 y)	> 50 y	61 y (median)
Lesion	Invasive Breast Carcinoma	Invasive breast carcinoma	Invasive breast carcinoma	Invasive breast carcinoma	Invasive breast carcinoma
Power of objective	×40 [HPF]	×40 [HPF]	×40 [HPF]	×20 [HPF]	×40 [HPF]
Number of fields	10	10	5	5	10
Tumor budding cut off	> 10	> 20	> 10	> 20	> 4
Tumor budding range	0–23/10 HPF	5–32/10 HPF	2–40/5 HPF	–	–
Staining	H&E	H&E	IHC (cytokeratin)	H&E	IHC (Pan cytokeratin)

**Fig. 1.** (A and B) Low PTB. Tissue section of invasive breast carcinoma of no special type displaying one peripheral tumor bud (black arrow) at the invasive front of the tumor (H&E, ×100 and ×400).**Fig. 2.** (A and B) High PTB. Tissue section of invasive breast carcinoma of no special type displaying multiple peripheral tumor buds (black arrows and circles) at the invasive front of the tumor (H&E, ×100 and ×400).

above findings differed from those of Salhia et al⁶ who found 79.7% of cases with high-grade PTB (►Fig. 2) and 20.3% of cases with low-grade PTB. The range of PTB in our study was 02-23/10 high power field (hpf), which is in close approximation with that reported by Liang et al.²

In this study, we found a statistically significant correlation of PTB with lymph node metastasis and lymphovascular invasion. We also found a significant association of PTB with tumor necrosis, which was similar to the study done by Kumarguru et al.¹⁶ However, Gujam et al,⁷ in their study, found no association of PTB with tumor necrosis. In the present study, PTB did not show any significant association with age of patients, tumor size, and the overall histological grade. This finding was similar to KumarGuru et al,¹⁶ Gujam et al,⁷ and Salhia et al.⁶ However, Sriwidyani et al¹⁷ observed significant association of PTB with histological grade, while Liang et al² found a significant association of tumor size with PTB (►Table 4).

ITB: While PTB is becoming a topic of interest amongst researchers, limited data about ITB are available in the literature. Zlobec et al¹⁸ evaluated ITB in colorectal adenocarcinomas and found that ITB, if evaluated in preoperative biopsies, predicts lymph node and distant metastasis. In a similar way, there is need for extensive research to evaluate ITB in BCs. In future, this may establish ITB as an independent prognostic factor.

There was a significant correlation of ITB (►Figs. 3 and 4) with tumor grade as well as LVI and lymph node (LN)

Table 4 Comparison of association (*p*-value) of clinicopathological parameters with peripheral tumor budding in different studies

Parameters	Present study	Kumarguru et al ¹⁷	Sriwidyani et al ¹²	Gujam et al ⁷	Salhia et al ⁶	Liang et al ²
Lymph node metastasis	0.00	< 0.001	< 0.003	< 0.009	–	< 0.05
Lymphovascular invasion	0.001	0.001	0.001	0.001	0.015	0.001
Necrosis	0.015	0.004	–	0.107	–	–
Grade	0.332	0.884	0.03	0.099	–	0.163
Tumor size	0.742	–	–	0.469	–	0.014
Age	0.906	0.729	–	0.08	–	0.513

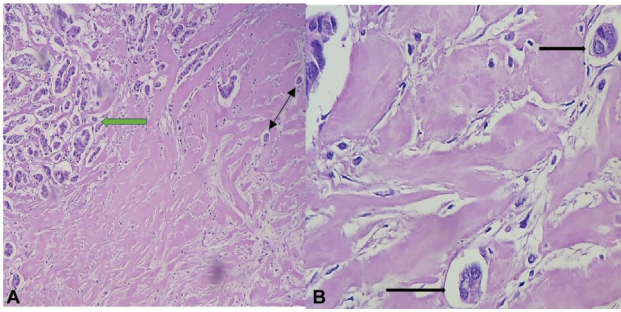


Fig. 3. (A and B) Low ITB. Tissue section of invasive breast carcinoma of no special type displaying two intratumoral buds (black arrow) within the tumor mass (green arrow) (H&E, $\times 100$ and $\times 400$).

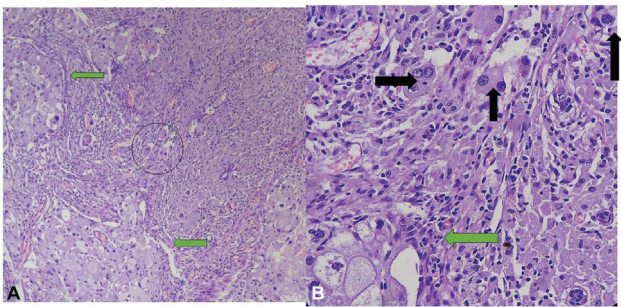


Fig. 4. (A and B) High ITB. Tissue section of invasive breast carcinoma of no special type (tumor proper; green arrow) displaying three tumor buds (black arrows) within the tumor mass (H&E, $\times 100$ and $\times 400$).

Table 5 Comparison of association (*P*-value) of clinicopathological parameters with Intra tumoral budding in different studies

Parameters	Present study	Renuka et al ¹⁹	Salhia et al ⁶
Lymph node metastasis	0.017	0.000	–
Lympho-vascular Invasion	0.005	0.002	–
Necrosis	0.012	–	–
Grade	0.042	0.048	0.036
Tumor size	0.957	–	–
Age	0.613	–	–

metastasis, found similar to the study by Renuka et al.¹⁹ There was no significant correlation of ITB with age, gender of patients, and tumor size in this study (\blacktriangleright Table 5).

Assessment of tumor microenvironment (TME): The microenvironment surrounding the stroma plays an important role in the survival, growth, proliferation, and progression of breast cancer. The TME includes necrosis, TSP, and TILs.⁷ Tumor stroma is one of the components of TME that represents the proportion of the tumor associated with stroma. Many researchers have observed potential prognostic value of TSP in their studies in various solid cancers. In this study,

we have considered the broader aspect of TME. In the present study, 70 out of 102 cases had high TSP ($> 50\%$), while 32 patients had low TSP. The other evaluated component of TME in this study was TILs. We found 66 patients with low TILs and 36 with high TILs. The lack of use of IHC in our study limited the assessment of both TSP and TILs. Hence, we chose not to evaluate the association of TME with other clinicopathological parameters.

Limitations

Because this was a retrospective study with smaller sample size, we could not relate the TB grade with recurrence and patient's mortality. The lack of use of IHC limited the assessment of TME with other clinicopathological parameters.

Conclusion

We observed a significant association of TB with lymph node metastasis, lymphovascular invasion, tumor grade, and necrosis. This suggest TB as a reproducible and an independent adverse prognostic factor in invasive breast carcinoma, NOS. However, further work is needed to establish a standard method for the quantification of this parameter, which in future can complement traditional prognostic markers. This will help in effective stratification of patients in terms of disease-free survival and likely outcome.

Conflict of Interest

None declared.

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