Original article

Clinicopathological Significance of Tumor Budding in Breast Carcinoma

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ABSTRACT

Background: Tumour budding Tuberculosis is a new prognostic marker whose role in breast cancer is still under evaluation. Our aim was to study Tuberculosis in breast carcinoma and correlate it with other prognostic markers.

Methods: A descriptive cross-sectional study was conducted over 2 years on 75 invasive breast carcinoma specimens and biopsies. Hematoxylin and Eosin sections were examined for tumour grade, stage, molecular subtype, necrosis, lymphovascular invasion inflammation and counting of Tuberculosis. Lymph node metastasis was studied only in mastectomies. TB was defined as a cluster of 1-5 tumour cells and counted in 10 consecutive 400X fields. The cut-off for high grade TB was taken as ?10 per 10 HPFs. Immunohistochemical staining was done for molecular subtyping and differentiating Tuberculosis from mimickers. Statistical analysis was done using chi square test and Fischer's exact test.

Results: Tuberculosis was present in 66/75 cases; 53% (n=35) were high grade. Among these, majority were of T2 (74%, n= 26), grade 2 (52%, n= 18), luminal A (34%, n= 12), had 3+ inflammation (46%, n= 16) and peripheral tumour buds (54%). Necrosis and lymphovascular invasion were absent in 77% and 71%, respectively. Lymph node metastasis was seen in 63% (n= 25/28) cases. Statistically significant association (p= 0.016) was observed between degree of inflammation and Tuberculosis grade. However, no significant association was observed between TB and other prognostic markers of breast carcinoma.

Conclusions: In our study, association of Tuberculosis with different prognostic markers was appreciated but was not statistically significant. However, it highlights need for standardization of Tuberculosis reporting.

Keywords: Breast Neoplasms; epithelial-mesenchymal transition; prognosis; tumor budding.

INTRODUCTION

Cancer statistics 2020 report by national cancer registry programme states breast cancer as the most common cancer in females.[1] Most cases are diagnosed at an advanced stage which necessitates early identification and evaluation.[2]

Tumor budding is a new morphological marker for the invasion and spread of tumor.[3] The 2017 international tumor budding consensus (ITBCC) standardized tumor budding classification and the reporting guidelines are now included in College of American Pathologists (CAP) protocol.[3,4] The ITBCC defines tumor bud (TB) as a cluster of 1-4 tumor cells whereas most existing studies define TB as a cluster of 1-5 tumor cells.[4-13] Tumor buds can be uniquely targeted for therapies and used for predicting the response to treatment.[8] It was first described in colorectal carcinomas but now is recognised in other tumors such as lung, breast, oral, oesophagus, gastric, larynx, etc.[3,14] According to literature, increased tumor budding is associated with a short survival time in breast cancer patients.[8]

METHODS

A descriptive cross-sectional study was conducted at the Department of Pathology in Pimpri (Pune) within a period of September 2020-August 2022. Institutional

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ethical sub-committee clearance (IESC) was obtained (Research Protocol number: IESC/PGS/2020/186). Informed consent was obtained from all individual participants included in the study.

Total 75 breast carcinoma specimens and biopsies of women having infiltrating ductal carcinoma (IDC) were included in the study. Cases with any other type of breast cancer, only in situ component, previous history of radio and chemotherapy, male breast and tissues with inadequate material were excluded.

Study protocol: Details of the patient were obtained from the records and files in the department. Post modified radical mastectomy (MRM) specimens or biopsy was fixed in buffered formalin (pH: 7.0-7.4) for approximately 12-24hrs. Grossing of the specimen was done and sections were taken following the protocols used in the department.[15] In mastectomy cases, a slide with the most representative area i.e. tumor tissue along with surrounding normal breast tissue was selected for examination. In case of breast biopsies, a single block and section prepared from the same was examined. The Hematoxylin and Eosin (H&E) stained slides were studied under the microscope for the histological type and grade, pathological stage, necrosis, lymphovascular invasion (LVI), inflammation and counting of tumor buds. The histological type was determined according to the WHO (2019) Classification of breast tumors.[16, 17] Elston and Ellis modified Scarff-Bloom-Richardson (SBR) grading system was used for histopathological grading of the tumors.[18] Staging classification of the mastectomies was done by the pathological TNM staging (pTNM) and biopsies were classified as per the clinical staging (cTNM) according to AJCC, 8th Edition.[19] We scored inflammation as 1+, 2+ or 3+ on the basis of mild, moderate or severe degree of inflammation. (Figure. 1A-B)

Counting and grading of tumor buds: A tumor bud was defined as a single tumor cell or a group of 1-5 tumor cells. Tumor buds were counted in 10 consecutive high power fields (HPFs) i.e. 400X. The area of each field was 0.283 mm^2. The areas with the most invasive front of tumor were selected for counting since these areas have high density of tumor buds. The morphological features of tumor cells were compared with the invasive cells of main tumor to avoid the possibility of counting mimickers of TB like macrophages, histiocytes, endothelial cells or artifacts. Areas of necrosis were avoided from the fields of counting.[5, 13] When all the tumor buds in 10 consecutive fields were counted from the peripheral area of the tumor only, these tumor buds were termed as “peripheral tumor buds” (PTBs). (Figure. 2A) In cases with no peripheral area of tumor seen, the counting of tumor buds was done in 10 consecutive high power fields from within the area of tumor itself. These tumor buds were termed as “intratumoral buds” (ITBs).[10] (Figure. 2B) In few cases with less than 10 peripheral high power fields of tumor, the counting was done from the available number of peripheral fields along with few fields from within the areas of tumor to complete 10 consecutive counting high power fields. In such cases, both peripheral tumor buds (PTBs) and intratumoral buds (ITBs) were counted and the summation of both (PTBs + ITBs) was taken as the final tumor budding count. To ensure reliability, the counting for each case was done by two different pathologists and an average of both the counts was taken as the final tumor budding count. To identify the cut off values for tumor budding, the receiver operator curve (ROC) was done with molecular subtype. The cut-off for high and low grade TB was >9 (sensitivity- 57.58 and specificity- 61.70). The AUC was 0.570 (p= 0.304) which shows that tumor budding is not a good indicator to distinguish luminal and non- luminal. (Figure. 3) Hence, we considered a count of ≥10 per 10 HPFs as high grade TB (Figure. 4A) and a count of < 10 per 10 HPFs as low grade of TB (Figure. 4B). Various other studies decided the cut off for grading TB on the basis of ROC curve analysis with LN metastasis or cancer specific survival or overall survival.[5, 9, 12, 20, 21]

Immunohistochemical (IHC) staining: In our study the IHC was done: (1) On all the cases for molecular sub-typing using the primary antibodies ER, PR and HER2. (2) On those cases where the tumor buds were difficult to differentiate from its mimickers like macrophages, endothelial cells, fibroblasts and histiocytes. (Figure. 5A-B) The primary antibodies used were CD68, CD34 and CK7. Sections to be studied were cut at 3-4 µm on charged slide or Poly-L-Lysine coated slide followed by deparaffinization and rehydration. DAKO antigen retrieval buffer (high pH) was used for antigen retrieval. The slides were then washed in a diluted DAKO wash buffer for 5 minutes. For staining- Incubation and all the staining materials and reagents were at room temperature. Endogenous peroxidase blocking was done for 10 minutes followed by washing with a buffer for 2-3 minutes. Finally, primary antibodies ER (SP1 clone), PR (SP2 clone), HER2 (SP3 clone), CD68, CD34 and CK7 (OV- TL 12/30 clone) were used for an incubation time of 60 minutes each and then washed with buffer. For the reporting of ER and PR receptors, Allred system of scoring was used as proposed by College of American Pathologists (CAP) and American Society of Clinical Oncology (ASCO). According to this system of scoring, two parameters are used for the quantification of ER and

PR receptors i.e. the proportion score (proportion or the number of stained cells) and intensity score (intensity of staining i.e. pale or dark). The final score is calculated by adding the Proportion Score and Intensity Score with score range of 0-2: negative and 3-8: positive.[22] Reporting of HER2 was done according to the latest published guidelines.[23]

Statistical analysis: The data was entered in Microsoft excel. Epi Info by Centers for Disease Control and Prevention (CDC): version 7 and WinPepi by J. H. Abramson: version 11.65 were used for analysis. The qualitative data was presented in the form of numbers and percentages. To find out the association between categorical variables, chi square and fisher's exact test were applied.

RESULTS

The general distribution of cases on the basis of tumor budding is shown in Table. 1. LN metastasis was evaluated only in the mastectomies (n= 28/75). In the present study, equal number of cases had presence and absence of LN metastasis. No statistically significant association was found between TB and other prognostic factors. The distribution of cases on the basis of tumor budding grade is shown in Table. 2. High grade TB was seen in 35/ 66 cases. No significant association was observed between TB grade and other prognostic markers of breast except degree of inflammation. The grade of TB and inflammation were directly correlated with p-value of 0.016.

Table 1. Tumor budding with other important clinicopathological parameters.

Prognostic factor	TB	Observation				Total	p-value
Age *		20-40	41-60	61 and above			
	Present	11 (17%)	38 (57%)	17 (26%)		66	0.693
	Absent	2 (22%)	6 (67%)	1 (11%)		9	
	Total	13 (17%)	44 (59%)	18 (24%)		75	
Laterality *		Left	Right				
	Present	38 (58%)	28 (42%)			66	0.073
	Absent	2 (22%)	7 (78%)			9	
	Total	40 (53%)	35 (47%)			75	
Tumor size *		T1	T2	T3	T4		
	Present	1 (2%)	29 (74%)	10 (15%)	6 (9%)	66	0.2596
	Absent	1 (11%)	6 (67%)	2 (22%)	0 (0%)	9	
	Total	2 (3%)	55 (73%)	12 (16%)	6 (8%)	75	
Tumor grade *		Grade 1	Grade 2	Grade 3			
	Present	7 (10%)	40 (61%)	19 (29%)		66	0.761
	Absent	0 (0%)	6 (67%)	3 (33%)		9	
	Total	7 (9%)	46 (62%)	22 (29%)		75	
Molecular subtype *		Luminal A	Luminal B	Her2	Triple negative		
	Present	25 (38%)	12 (18%)	13 (20%)	16 (24%)	66	0.729
	Absent	3 (33%)	2 (23%)	3 (33%)	1 (11%)	9	
	Total	28 (37%)	14 (19%)	16 (21%)	17 (23%)	75	
Necrosis *		Present	Absent				
	Present	14 (21%)	52 (79%)			66	1.000
	Absent	2 (22%)	7 (78%)			9	
	Total	16 (21%)	59 (79%)			75	
Lymphovascular invasion *		Present	Absent				
	Present	16 (24%)	50 (76%)			66	0.674
	Absent	1 (11%)	8 (89%)			9	
	Total	17 (23%)	58 (77%)			75	
LN metastasis *,†		Present	Absent				
	Present	14 (56%)	11 (44%)			25	0.222
	Absent	0 (0%)	3 (100%)			3	

Table 1. Tumor budding with other important clinicopathological parameters.						
Prognostic factor	TB	Observation			Total	p-value
	Total	14 (50%)	14 (50%)		28	
Inflammation ‡		1+	2+	3+		
	Absent	-	-	-	-	
	Present	23 (35%)	21 (32%)	22 (33%)	66	-
	Total	23 (35%)	21 (32%)	22 (33%)	66	
Location of TB ‡		ITB	PTB	Mixed		
	Present	24 (36%)	37 (56%)	5 (8%)	66	-
	Absent	-	-	-	-	
	Total	24 (36%)	37 (56%)	5 (8%)	66	

Table 1. footnotes:

*Fischer's exact test

†In MRM cases

‡In cases with TB

Table 2. Grading of tumor buds and their association with known prognostic markers.							
Prognostic factor	TB	Observation				Total	p-value
Age *		20-40	41-60	61 and above			
	High	5 (14%)	21 (60%)	9 (26%)		35	0.84
	Low	6 (19%)	17 (55%)	8 (26%)		31	
	Total	11 (17%)	38 (58%)	17 (25%)		66	
Laterality *		Left	Right				
	High	22 (63%)	13 (37%)			35	0.501
	Low	16 (52%)	15 (48%)			31	
	Total	38 (58%)	28 (42%)			66	
Tumor size †		T1	T2	T3	T4		
	High	0 (0%)	26 (74%)	6 (17%)	3 (9%)	35	0.873
	Low	1 (3%)	23 (74%)	4 (13%)	3 (10%)	31	
	Total	1 (2%)	49 (74%)	10 (15%)	6 (9%)	66	
Tumor grade †		Grade 1	Grade 2	Grade 3			
	High	5 (14%)	18 (52%)	12 (34%)		35	0.269
	Low	2 (6%)	22 (71%)	7 (23%)		31	
	Total	7 (10%)	40 (61%)	19 (29%)		66	
Molecular subtype †		Luminal A	Luminal B	Her2	Triple negative		
	High	12 (34%)	4 (12%)	8 (23%)	11 (31%)	35	0.262
	Low	13 (42%)	8 (26%)	5 (16%)	5 (16%)	31	
	Total	25 (38%)	12 (18%)	13 (20%)	16 (24%)	66	
Necrosis *		Present	Absent				
	High	8 (23%)	27 (77%)			35	0.967
	Low	6 (19%)	25 (81%)			31	
	Total	14 (21%)	52 (79%)			66	

Table 2. Grading of tumor buds and their association with known prognostic markers.						
Prognostic factor	TB	Observation			Total	p-value
Lymphovascular invasion *		Present	Absent			
	High	10 (29%)	25 (71%)		35	0.559
	Low	6 (19%)	25 (81%)		31	
	Total	16 (24%)	50 (76%)		66	
LN metastasis †, ‡		Present	Absent			
	High	10 (63%)	6 (37%)	16	35	0.434
	Low	4 (44%)	5 (56%)	9	31	
	Total	14 (56%)	11 (44%)	25	66	
Inflammation †, §		1+	2+	3+		
	High	7 (20%)	12 (34%)	16 (46%)	35	0.016
	Low	16 (52%)	9 (29%)	6 (19%)	31	
	Total	23 (35%)	21 (32%)	22 (33%)	66	
Location of TB †,§		ITB	PTB	Mixed		
	High	13 (37%)	19 (54%)	3 (9%)	35	1.000
	Low	11 (36%)	18 (58%)	2 (6%)	31	
	Total	24 (36%)	37 (56%)	5 (8%)	66	

Table 2. footnotes:

*Chi square test

†Fischer's exact test

‡In MRM cases

§In cases with TB

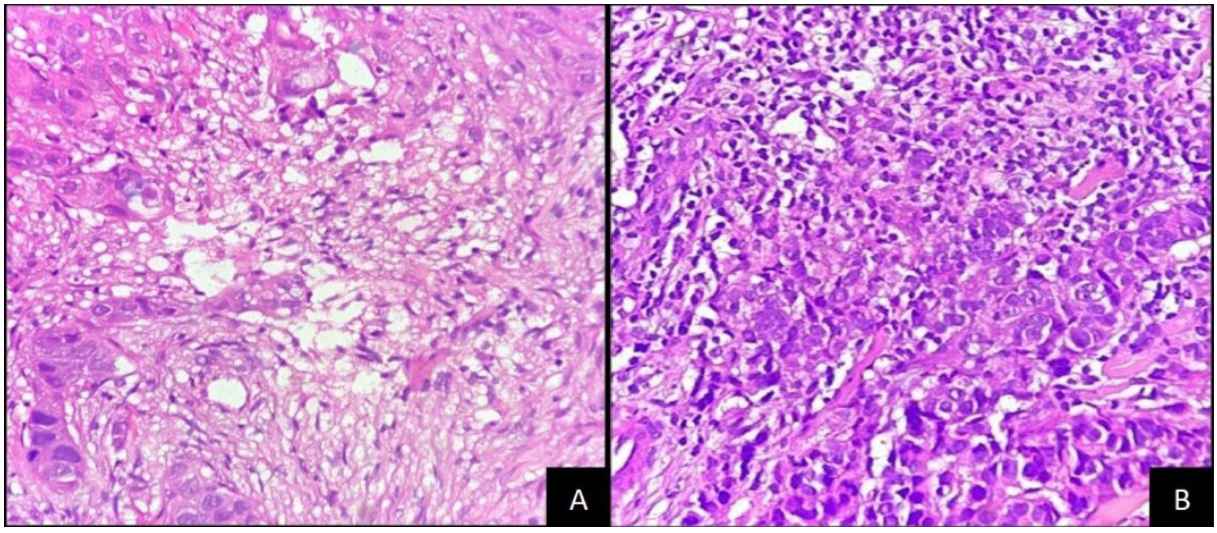

Figure 1. A-B. H&E stained sections in a case of invasive ductal carcinoma breast with mild/1+ inflammation (A) and severe/3+ inflammation (B).

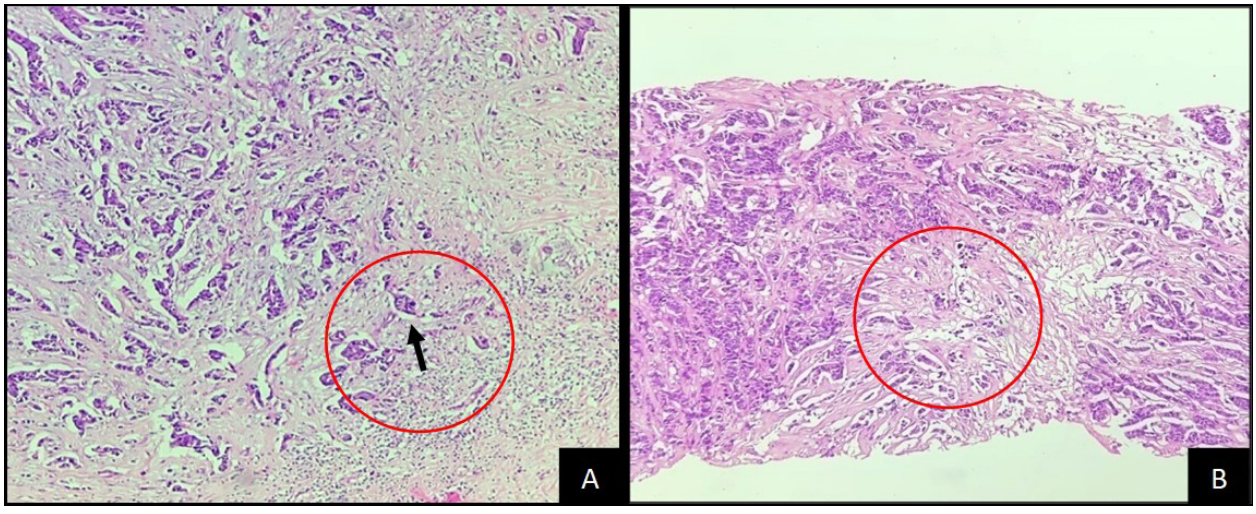

Figure 2. A. Peripheral tumor buds (PTBs) in the tumor infiltrating area. (H&E, 100X)
Figure 2B. Intratumoral buds (ITBs) in a relatively hypocellular area within the tumor itself. (H&E, 100X)

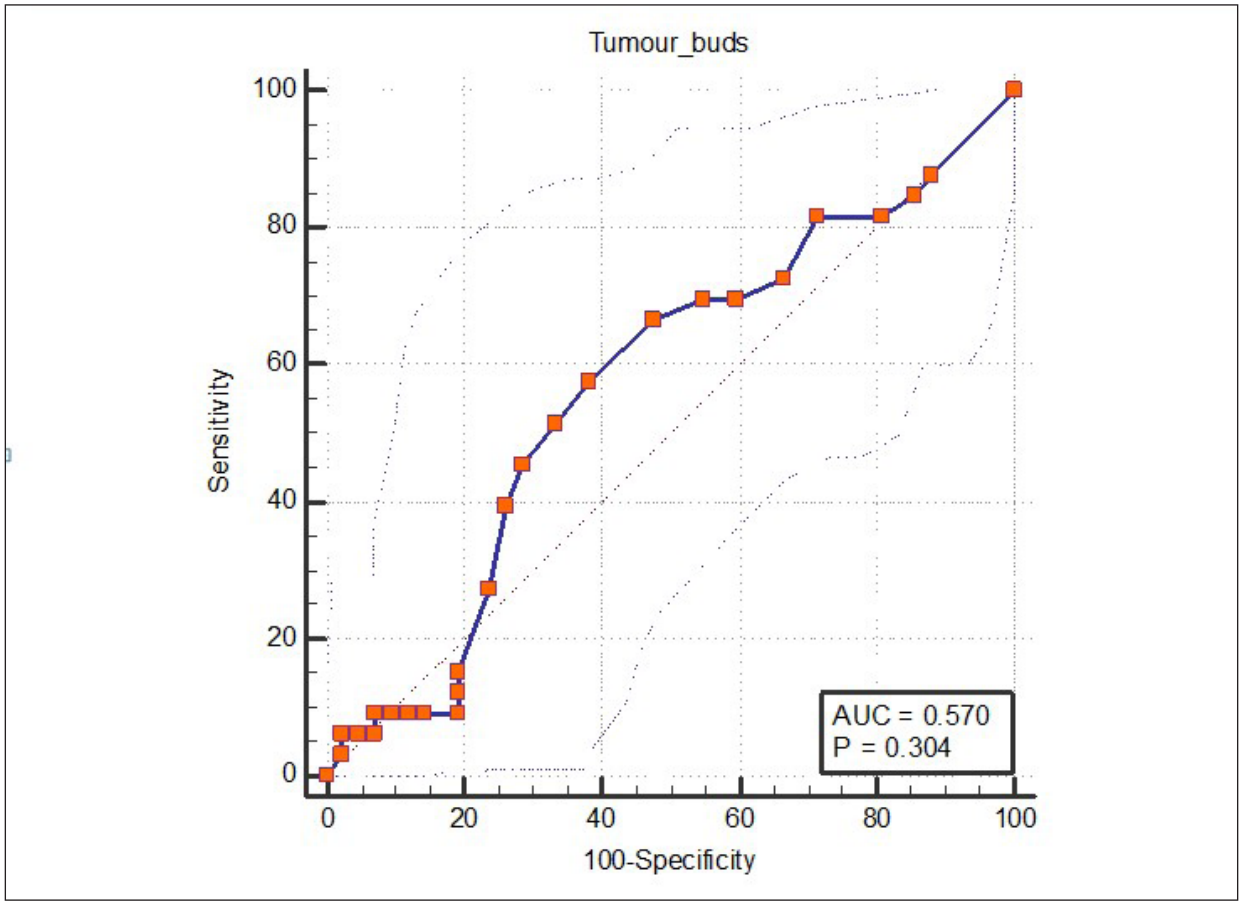

Figure 3. Cut-off value of high grade TB by ROC curve.

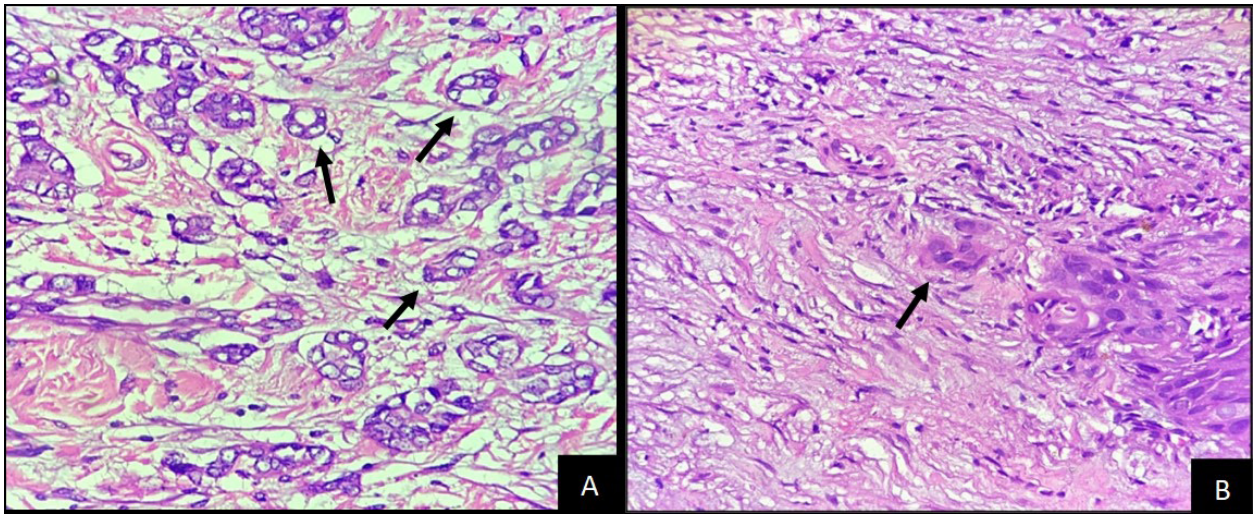

Figure 4. A Photomicrograph showing high grade TB (arrows). (H&E, 400X) Figure 4B. Photomicrograph showing low grade TB with a single cluster of 5 tumor cells (arrow). (H&E, 400X).

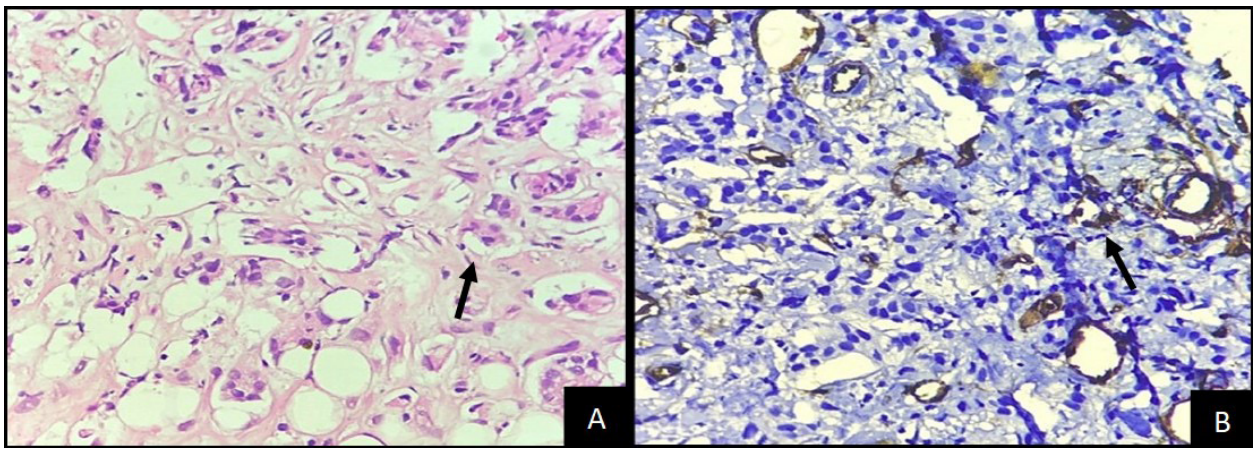

Figure 5. A. Image showing cells resembling TB (arrow). (H&E, 400X) Figure 5. B. Image showing cytoplasmic positivity of those cells confirming endothelial cells (arrow). (IHC: CD34, 400X).

DISCUSSION

Tumor budding was first described by Imai in stomach cancer.[14, 24-26] The emerging utility of this concept is to identify its prognostic significance.

On the basis of various population-based studies conducted nation-wide, the average age group of patients with breast cancer was found to be 50-53 years.[2] In the present study, 81% patients were above 40 years of age. Similar observations were made by Gujam et al, Gabal et al and Kumarguru et al.[5,7,9] In the present study, the ratio of involvement of left breast to right breast was 1.13. This was concordant with the results obtained by the studies conducted by Amer et al, Buch A et al and Kumarguru et al.[5, 27, 28] However, no significant impact of laterality was found on the tumor features, symptoms and 5-year survival of patients.[27] Similar to the studies conducted by Gabal et al and Agrawal R et al, tumors in stage T2 were most common.[7, 11] Gujam et al showed that 60% of the cases in their study had tumor size of </= 20mm.[9]

In the present study, majority of the cases had tumor of grade 2 (62%). The results were similar in the studies conducted by Masilamani et al and Gabal et al.[6, 7] On observing the molecular subtyping, majority of the cases were of Luminal A type (37%) followed by Triple negative cancers (23%) in this study. Laedrach C et al, Gujam et al and Masilamani et al. confirmed that the most common type of tumor was Luminal A type.[6, 8, 9] In the study, necrosis was mostly absent in the cases. Similar results were obtained by Gabal et al in 2018.[7] Gujam et al in their study found out that 52% of the cases showed high tumor necrosis.[9] In terms of LVI, our results were in agreement with the studies done by Laedrach C et al and Masilamani et al where most of the cases had absence of lymphovascular invasion.[6, 8] Gabal et al in their study observed that most of the cases had presence of lymphovascular embolization.[7] Not in concordance with the studies conducted by Gujam et al and Agrawal R et al, LN metastasis was seen to be equally present and absent in our study.[9,11] Gabal et al observed that most of the cases were positive for LN metastasis.[7]

Tumor budding in breast carcinoma:

Tumor buds are a part of tumor microenvironment which result from EMT and loss of cell adhesion molecules like E-cadherin. TB was counted in 10 HPFs of 400X. Agrawal R et al counted TB on 20X and a cut off of ≥10 was considered high grade.[11] Salhia B et al counted TB in both surgically resected specimens and core biopsies at 400X magnification.[10] The most appropriate area for counting TB is considered to be the invasive border of tumor.[12, 20, 21, 29] Due to the difficulty in evaluation of morphology in invasive lobular carcinoma and much better appreciation of TB in invasive ductal carcinoma, only IDC cases were included in the study. Okcu O et al explained that transcription factors like ZEB 1 and 2, SNAIL and TWIST which have role in EMT are positive in tumor buds but are negative in lobular carcinoma.[21]

In our study, association of TB with other prognostic markers of breast cancer was found to be statistically insignificant. The sample size in our study was small which might have underpowered the findings. TB was present commonly in tumors of T2, grade 2 and Luminal A subtype. Necrosis and LVI were absent in majority of the cases with TB. Similar findings were observed by Gabal et al but necrosis was absent in 65.9% of their cases.[7] On observing the pattern of inflammation in the cases with TB we found that maximum cases had mild inflammation and were given a score of 1+ (35%). Gabal et al and Gujam et al used Klintrup criteria to score inflammatory infiltrates. Absence of inflammation was given a score of 0, score 1 for mild inflammation, score 2 for band like inflammation and score 3 for extensive or florid cup-like inflammation. Their study along with the present study showed that most of the cases with TB showed weak inflammation.[7, 9] We divided the cases with TB on the basis of its location into peritumoral (PTBs), intratumoral (ITBs) and mixed (PTB + ITB). ITBs were counted only in cases where tumor peripheral area for counting PTBs was not enough and we had to enter the tumor area for further counting. Salhia et al, in their study also discussed that identifying PTBs and ITBs in biopsies is very challenging as the biopsies are taken from the tumor mass itself and due to random sampling there is very less area of tumor infiltration.[10] Renuka IV et al showed that high PTB was significantly associated with LN metastasis and LVI but not with tumor size, age of the patient, tumor grade and ER status. They also noted that high ITB was associated significantly with LVI, nodal status and tumor grade.[30]

In the present study, 37/66 cases with TB had PTBs in abundance. The association of PTBs and ITBs individually with other prognostic markers could not be carried out due to lack of homogeneity and standardization.

LN metastasis was frequently positive in cases with TB similar to the study of Gabal et al. This can be explained by the fact that more the number of tumor cells separate from the tumor, more should be the potential for metastasis.[7]

Association between TB grade and other prognostic markers of breast carcinoma:

In the present study and the studies conducted by Gujam et al, Liang F et al and Kumarguru et al, no significant association was observed between high grade TB and age or laterality.[5, 9, 20] In contrast, a study done by Gonzalez L O et al showed that the age of patient correlated significantly with the TB grade.[13]

A pattern of high grade TB was seen in stage T2 and grade 2 but no significant association of the tumor grade or tumor size was noted with high grade TB. The results were in correlation with the studies by Gonzalez L O et al and Gujam et al.[9, 13] Okcu O et al, Liang F et al and Agrawal R et al demonstrated that not tumor grade but tumor size was significantly related to high grade TB.[11, 20, 21] The studies conducted by Gonzalez et al, Gabal et al, Agrawal R et al and the present study showed no significant association between the hormone receptor status and high TB grade.[7, 11, 13] In a study by Masilamani et al, TB grade was consistently correlated with the HER2 status.[6] Gujam et al observed that high grade TB is associated with positive status of ER receptor.[9] Okcu O et al noted significant correlation of high grade TB with negative PR receptor status.[21] Although both lack of hormonal receptor positivity (HER2 enriched and triple negative subtypes) and increased number of TBs are expected to be common in aggressive form of tumor, the association between the two is still a topic of debate as no standardized system for TB estimation in breast carcinoma has been introduced till now.

The results of the present study and a study by Gujam et al showed no significant association between high grade TB and necrosis. According to Gujam et al, TB grade and degree of inflammation were inversely correlated.[9] This was in opposition to the results of our study as we observed that majority of the cases with high grade TB were given a score of 3+ for inflammation. There was a statistically significant association between the higher grade of TB and increased inflammation (p= 0.016). Our observation can be explained by a theory of Jiang B et al which states that tumor cells form stable pairs with lymphocytes known as “tumor lymphocyte chimeras” (TLCs) that facilitate its migration to distant organs.[31]

It was observed that high grade TB cases showed presence of LN metastasis but absence of lymphovascular invasion. However, similar to the studies by Gonzalez et al and Agrawal R et al, the correlation was not statistically significant.[11, 13] Gabal et al showed that high grade TB is related to LN metastasis but not related to lymphovascular invasion.[7] Contrasting results were observed by the studies conducted by Okcu O et al, Kumarguru et al, Liang F et al and Gujam et al where the higher grade of TB was significantly associated with LN metastasis and LVI.[5, 9, 20, 21] Despite the discrepancy in the results of our study along with various studies, we believe that there is a strong specificity of high grade TB for LN metastasis. In context to the location of TBs, PTBs/ITBs were not found to be significantly related to high grade of TB in our study.

Limitations: One important limitation of this study was a small sample size. The number of biopsies included in the present study fairly exceeded the number of mastectomies. Due to this, the association between TBs and LN metastasis could not be carried out satisfactorily. IHC staining to distinguish TB from its mimickers was done only in selected cases. We counted ITBs along with PTBs in both mastectomies and biopsies, wherever required. However, the association of ITBs and PTBs individually with other prognostic markers could not be carried out due to lack of homogeneity and standardization.

Futurescope: A systematic method of scoring TBs in breast carcinoma is yet to be introduced. Tissue microarray can be used to scan and select the area of interest. A clear understanding of the role of PTBs and ITBs is needed. Mammaprint and Oncotype Dx are also good prognostication methods but are very expensive as compared to TB analysis. We recommend the standardization of definition of TB, selection of area for counting, the power of objective, the number of fields to be examined, cut-off for high and low grade TB, specific IHC staining and a reporting format for TB.

CONCLUSIONS

In our study we tried to observe the association of TB with other known prognostic markers of breast cancer. Although only inflammation was found to be statistically related to TB grade, we still suggest that TB can be a promising marker of tumor aggressiveness. Further studies with bigger sample size can be done for better understanding of the role of TB as a prognostic marker. The introduction of a standardized method of scoring and reporting TB is still awaited.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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