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Original Article

Pathology Section

Association of Tumour Budding with Histomorphological as Well as Hormonal Receptors Status in Breast Carcinoma Cases: A Cross-sectional Study

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ABSTRACT

Introduction: The most common cancer in India among females is breast carcinoma. To improve the diagnostics and prognostics of breast cancers various prognostic factors are being evaluated worldwide where Tumour Budding (TB) is one of the important prognostic indicator. TB is considered as the first step process of tumour metastasis and invasion, related to the Epithelial-Mesenchymal Transition (EMT).

Aim: To study TB in breast carcinoma and its association with key prognostic markers in breast cancer.

Materials and Methods: The present study was a cross-sectional study conducted in the Central Laboratory, Histopathology Section in Rajarajeswari Medical College and Hospital, Bengaluru, Karnataka, India between the period of January 2022 to January 2023. A total of 30 Modified Radical Mastectomy (MRM) breast carcinoma cases were included in the study. Grossing of the adequately fixed specimen by buffered formalin (10%) was done and representative bits taken and processed. Haematoxylin and Eosin (H&E) stained sections were evaluated for TB. Counting of TB was done. Association between the TB and pathological variables like hormone receptor status such as Oestrogen Receptor (ER), Progesterone Receptor (PR) and HER2/neu receptor status were studied. Association between the TB and other histological parameters such as lymphovascular invasion, metastasis and necrosis which are key prognostic markers were studied. The results were interpreted and analysed by using few statistical tests including Z-test and p-value with significance.

Results: In the present study, total number of 30 cases of breast carcinoma were included where Invasive ductal breast carcinoma, Not Otherwise Specified (NOS) was the most common histological type of carcinoma constituting 27 cases (90%). In all the cases TB were studied and further categorised into high TB and low TB. A 7 (23.33%) cases showed high TB and 23 (76.66%) cases showed low TB. In high TB 6 cases showed ER positivity, three cases PR positivity and five cases showed HER2/neu positivity. In high-grade TB 4 (13.33%) cases shows lymphovascular invasion, 6 (20.00%) cases shows lymph node metastasis and 3 (10.00%) cases shows tumour necrosis. With the above findings and with the help of standard deviation (Z-test) and p-value, significant association was observed in high TB with ER positivity with p-value of 0.03 and high TB shows significant association with lymph node metastasis with p-value of 0.03.

Conclusion: TB has been proved to be an important individual prognostic factor in colon and oesophageal carcinomas, and the findings of the current study showed that high TB was significantly associated with ER positive status and lymph node metastasis which also demonstrates the high utility of TB in prognostication of breast carcinoma. Hence, it was conclude that TB is an easy to identify and unique significant prognostic factor in evaluating invasive breast carcinoma cases.

Keywords: Epithelial-Mesenchymal transition, Lymphovascular invasion, Metastasis, Prognostic factor, Tumour necrosis

INTRODUCTION

Carcinoma of breast is the most common type of cancer in India and the second most common cause of mortality worldwide [1]. The burden of incidence of breast cancer and its mortality is continuously growing and most cases are diagnosed at an advanced invasive stage. To improve this it requires early identification and evaluation of various prognostic markers including biomarkers status, lymphovascular invasion, perineural invasion, lymph node metastasis, histological grade and type along with TB [2,3].

TB is one of the important prognostic indicator which is a small cluster of one to five tumour cells at the invasive front, which has detached from the tumour bulk. Peripheral tumour buds are defined as tumour buds in 10 consecutive fields counted from the peripheral area of the tumour. Intertumoural buds are defined as tumour buds in 10 consecutive high-power fields from within the area of tumour itself [2,3].

TB has been identified as the initial step in the process of metastasis where the tumour buds are detached tumour cells from the tumour bulk, which acquires EMT, where the detached cancer cells lose their epithelial characteristics and acquire charecteristics linked to mesenchyme to become motile thus metastasise [3,4].

So TB is considered as the first step process of tumour metastasis and invasion, related to the EMT [5]. Abundant research work on TB has been done on tumours of the head and neck, lungs, stomach, oesophagus and colon. However, there is limited information about the role of TB in breast cancer. Hence, the present study aimed to study the association of TB with key prognostic markers such as (Oestrogen) ER status, Progesterone (PR) status, Human epidermal growth factor receptor 2 (HER2/neu) status, lymphovascular invasion and metastasis and tumour necrosis. To study TB in breast carcinoma and its association with key prognostic markers in breast cancer.

MATERIALS AND METHODS

This cross-sectional study - conducted in the Central Laboratory, Histopathology section in Rajarajeswari Medical College and

Hospital, Bengaluru, Karnataka, India, from January 2022 to January 2023. Approval of research by the Institutional Ethics Committee was taken with IEC NO RRMCH-IEC/217/2023.

Sample size: Total 30 MRM breast carcinoma cases were included in the study. The sample size was calculated using the formula $n=Z^2pq/d^2$. Taking p=50%, Z=1.96 and allowable error of 18%, the minimum sample size was calculated to be 30. Hence 30 cases of MRM specimens were included in this study based on feasibility.

Inclusion criteria: All new cases of MRM specimens received in histopathology section were included in the study in between January 2022 to January 2023.

Exclusion criteria: Recurrent breast carcinomas were excluded from the study.

Study Procedure

All the MRM specimens received were adequately fixed in buffered formalin (10%) for approximately 12-24 hours. Grossing of the specimen was done and representative bits taken and processed. H&E stained sections were evaluated first to look for TB. Counting of TB was done. Atleast three sections were evaluated for TB. Association between the TB and pathological variables like ER, PR and HER2/neu receptor status and histological parameters such as lymphovascular invasion, metastasis and necrosis were studied and analysed.

Evaluation of the tumour buds was done as follows:

- The invasive front of breast carcinoma was identified in scanner. (4X).
- Tumour buds were searched in low power (10X).
- Tumour buds were examined under high power (40X).
- The mimickers of tumour buds like inflammatory cells, multinucleated giant cells, fibroblasts, endothelial cells, smooth muscle cells and artifacts were excluded by examining under high power (40X).
- Nuclear and cytoplasmic characteristics of tumour bud cells were compared with those of the invasive tumour cells by examining under high power (40X objective).
- Number of tumour buds counted in 10 High Power Fields (HPFs) were noted.
- To evaluate TB 10 HPF were used and they were classified as high grade (≥10 per10HPF)/ low grade (< 10 per 10 HPFs).

For ER and PR immunohistochemistry interpretation as per Allred Scoring System [6]:

- The Total Scores (TS) for ER and PR are given as TS = PS + IS. (PS-Proportion Score, IS- intensity of staining).
- TS of 0 and 2 are negative scores.
- TS of 3, 4, 5, 6, 7, and 8 are positive scores.
- The proportion score (PS) (0-5) and the % positive tumour cells are respectively, 0 (0%), 1 (<1%), 2 (1-10%), 3 (11-33%), 4 (34-66%), 5 (67-100%).
- The Intensity of Staining (IS) for the nuclear positivity of the cells graded as 0, 1, 2, and 3 was as none, mild, moderate, and strong, respectively.

For HER2 immunohistochemistry interpretation [7]:

- **Positive**

  Score 3+: tumour displays complete, intense circumferential membranous staining in >10% of tumour cells (readily appreciated using a low power objective and observed within a homogenous and contiguous invasive cell population).

- **Equivocal**

  Score 2+: weak to moderate complete membrane staining observed in >10% of invasive tumour cells.

  Other less common patterns also considered equivocal.

  Circumferential membrane staining that is intense but within ≤ 10% of tumour cells.

  IHC staining that is moderate to intense but incomplete (basolateral or lateral) often seen in micropapillary breast cancer.

- **Negative**

  Score 1+: incomplete faint membrane staining and within >10% of invasive tumour cells.

  Score 0: no staining observed or incomplete faint/barely perceptible membrane staining within ≤10% of invasive tumour cells.

STATISTICAL ANALYSIS

Data were then entered in Microsoft Excel Sheet and analysed using Statistical Package for the Social Sciences (SPSS) v 20.0 (trial version). Descriptive statistics including percentages were analysed. Inferential statistics including Z-test and p-value with significance were analysed and interpreted.

RESULTS

A total number of 30 cases of breast carcinoma were included in the study. Maximum cases were of age group of 40-60 years 16 (53.33%) followed by 8 (26.67%) cases of age group 61 and above and 6 (20%) cases of age group 20-40 years as shown in [Table/Fig-1]. The most common side involved was right side more than left-side breast as shown below in [Table/Fig-2]. Most common site involvement was upper outer quadrant with reference to [Table/Fig-3]. A 19 cases (63%) shows tumour size ≤ 5 cm and 11 (37%) cases shows tumour size >5 cm. Invasive ductal breast carcinoma, Not Otherwise Specified (NOS) was the most common histological type carcinoma constituting 27 cases (90%) followed by invasive lobular carcinoma, mixed infiltrative ductal carcinoma and metaplastic carcinoma one case each (3%), which is depicted in [Table/Fig-4].

The TB was evaluated in all 30 cases. TB was further classified into high TB (tumour buds >10/10 HPF) and low TB. (Tumour Buds <10/10 HPF). In all the 30 cases tumour buds were studied in areas with the highest concentration of buds ("hotspots") depicted in [Table/Fig-5,6] shows a high power field of high grade TB of more than 10 tumour buds.

Age group (years)	n (%)
20-40	6 (20.00)
41-60	16 (53.33)
Above 61	8 (26.67)
Total	30 (100)

[Table/Fig-1]: Distribution of cases according to age.

Laterality	High n (%)	Low n (%)	Total (N)
Right	5 (27.78)	13 (72.22)	18
Left	2 (16.67)	10 (83.33)	12
Total	**7**	**23**	**30**

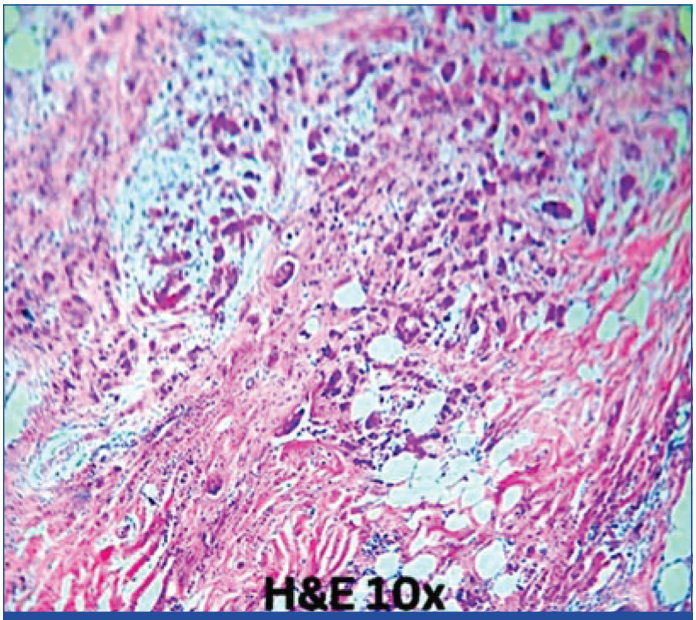
[Table/Fig-2]: Distribution of cases-based on laterality.

Quadrant	n (%)
Upper outer quadrant	25 (83)
Upper inner quadrant	2 (7)
Lower inner quadrant	1 (3)
Lower outer quadrant	2 (7)
Total	**30 (100)**

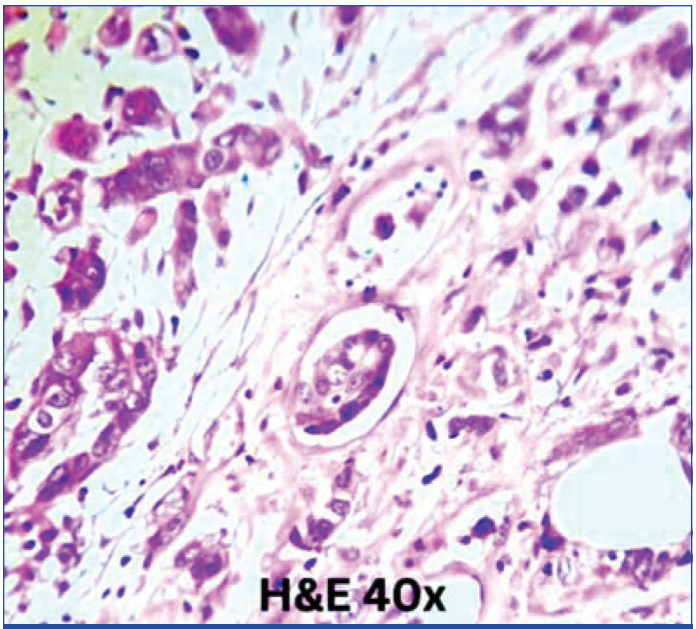
[Table/Fig-3]: Distribution of cases-based on quadrant.

Diagnosis	Cases	High tumour budding n (%)	Low tumour budding n (%)
Invasive ductal carcinoma	11	2 (18.18)	9 (81.82)
Invasive lobular carcinoma	1	0	1 (100.00)
Inavsive ductal carcinoma	16	4 (25.00)	12 (75.00)
Mixed invasive ductal carcinoma	1	1 (100.00)	0
Metaplastic carcinoma	1	0	1 (100.00)
Total	**30**	**7**	**23**

[Table/Fig-4]: Distribution of cases based on diagnosis.



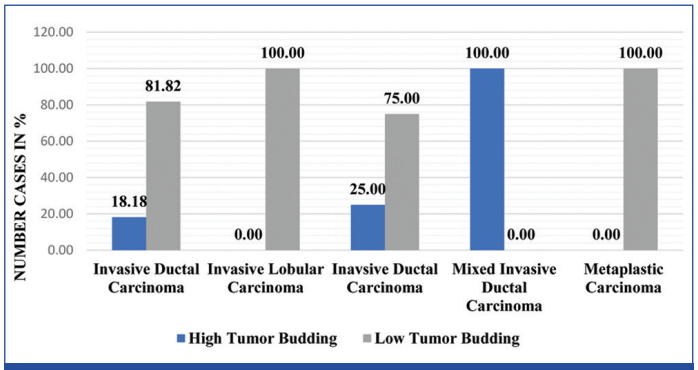
[Table/Fig-5]: Highest concentration of buds -hotspots (H&E 10X)



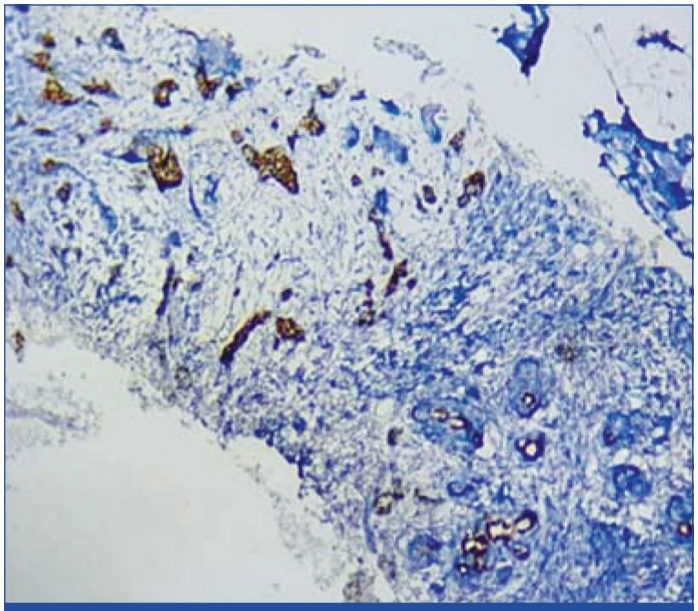
[Table/Fig-6]: High grade tumour budding (H&E 40x).

Study of TB with ER, PR and HER2/neu

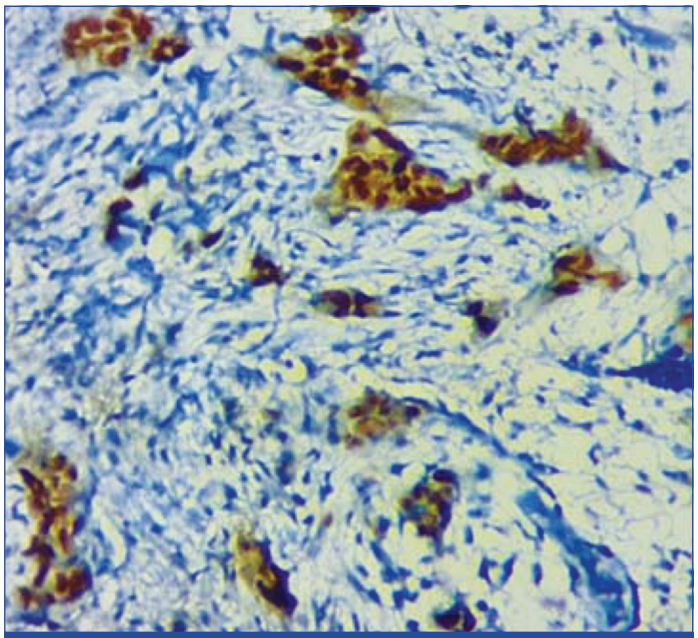
In the present study, TB was studied in immunohistochemistry studies done in all the cases. Markers ER, PR and HER2NEU was done in all the 30 cases. High and low TB in all 30 cases were compared with diagnosis and depicted in which high TB was seen in mixed invasive ductal carcinoma than in Invasive ductal carcinoma NOS [Table/Fig-7]. Out of 30 cases of breast carcinoma, 7 (23.33%) cases shows high TB and 23 (76.66%) cases shows low TB. In high TB 6 (85.71%) cases shows ER positivity {one case depicted in [Table/Fig-8] showing ER positivity in high TB area}, 3 (42.85%) cases PR positivity {[Table/Fig-9] showing PR positivity in high TB area} and 5 (71.42%) cases shows HER2/neu positivity {[Table/Fig-10] showing HER2/neu positivity in high tumour bud}. In low TB 9 (39.13%) cases shows ER positivity and 11 (47.82%) cases shows positivity for PR and Her2 neu. With the help of standard deviation (Z-test) and p-value, significant association was <0.05 observed in high TB with ER positivity which is shown in the [Table/Fig-11].



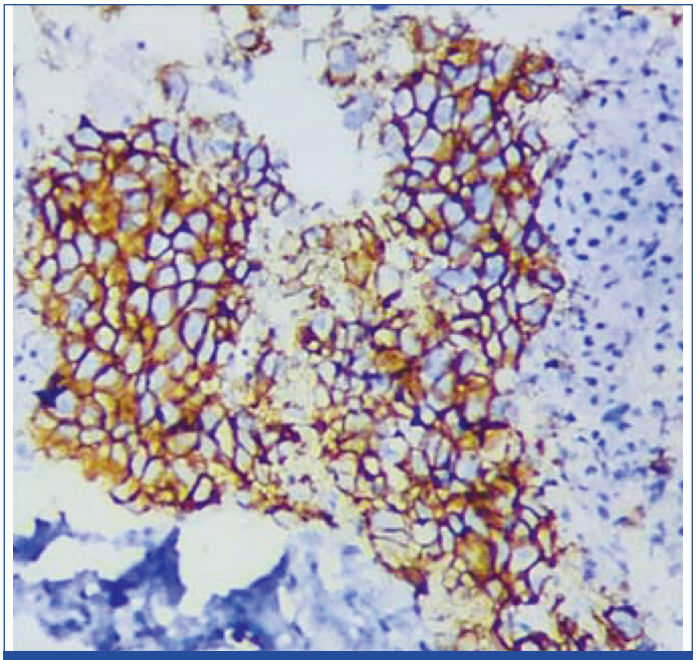
[Table/Fig-7]: Comparison of high and low tumour budding (TB) across histological diagnosis.



[Table/Fig-8]: ER positivity in high TB area (10x).



[Table/Fig-9]: PR positivity in high TB area (40X).



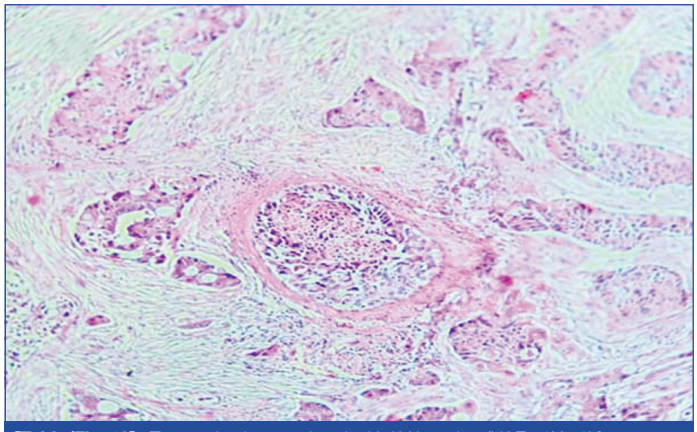
[Table/Fig-10]: HER2/neu positivity in TB (40x).

Positive	TB		Z-Test	p-value
	High	Low		
ER	6/7	9/23	2.158	0.030
PR	3/7	11/23	-0.230	0.818
HER2/neu	5/7	11/23	1.096	0.271

[Table/Fig-11]: Study of Tumour Budding (TB) with ER, PR & HER2/neu (Significant Association of TB with ER Positivity).

Tumour Budding (TB) Association with Histopathological Parameters

The TB association with histopathological parameters such as lymphovascular invasion, lymph node metastasis and tumour necrosis was studied in all 30 cases. In high grade TB 4 (13.33%) cases shows lymphovascular invasion as seen in [Table/Fig-12], 6 (20.00%) cases shows lymph node metastasis and 3 (10.00%) cases shows tumour necrosis. Whereas in low TB 19 (63.33%) cases shows lymphovascular invasion, 9 (30.00%) cases shows lymph node metastasis and 8 (26.67%) cases shows tumour necrosis with the help of z-value, High TB shows significant association with lymph node metastasis which was shown in [Table/Fig-13].



[Table/Fig-12]: Tumour buds associated with LV invasion (H&E 10X,40X).

Parameters		TB		Z-value	p-value
		High grade n (%)	Low grade n (%)		
LV invasion	Present	4 (13.33)	19 (63.33)	-1.394	0.164
	Absent	3 (10.00)	4 (13.33)		
Lymph node metastasis	Present	6 (20.00)	9 (30.00)	2.158	0.03
	Absent	1 (3.33)	14 (46.67)		
Tumour necrosis	Present	3 (10.00)	8 (26.67)	0.696	0.69
	Absent	4 (13.33)	15 (50.00)		

[Table/Fig-13]: TB association with histopathological parameters.

DISCUSSION

The TB is one of the mechanisms of cancer invasion and metastasis. TB was first recognised and described by Imai in stomach cancer [5]. It is recognised as a strong adverse prognostic factor and has been studied in detail in colorectal cancer and now has been included in the specific guidelines for the management of colorectal cancer [8].

Budded cells in breast cancer show EMT like molecular phenotype. Since EMT cells with migratory and invasive properties, TB at the invasive margin was hypothesise as the first step of invasion and metastasis, implied that TB would be a more sensitive indicator of aggressiveness when compared with traditional histopathological variables, such as node metastases and Lymphovascular Invasion (LVI) [9].

In all the 30 cases tumour buds were studied in areas with the highest concentration of buds ("hotspots") were selected for scoring in accordance with Rathod GB et al., and Kumarguru BN et al., [1,10].

In the present study, the age range was a majority in between 40-60 years which was in comparable to Rathod GB et al., and Buch AC et al., [1,3]. The majority of the studies considered only invasive breast cancer NOS. In contrast, the current study included various histological types of breast cancer in concordance with Rathod GB et al., as TB from a ruptured tumour capsule is a common pathway shared by all types of breast carcinoma [1].

The researchers in the Rathod GB et al., and Huang T et al., and this study counted tumour buds in 10 fields using an X40 (HPF) objective lens [1,4]. In contrast, Kumar N used an X20 (HPF) objective lens and counted tumour buds in five fields [6]. It is advisable to count tumour buds in 10 fields for good accuracy.

There was a good and clear correlation found between high TB and the size of the tumour, the number of metastasised lymph nodes, and the ER PR status of the tumour in most of the studies.

In the current study, high-grade TB showed a significant correlation with ER positivity (85.7%) which was similar to the study done by Masilamani DS and Kanmani P (85.2%), Agarwal R (68.75%) and Salhia B et al., (89.2%) in contrast [2,9,11] to study done by Malla S et al., which showed no association between TB and hormone receptor status [5].

In present study, there was significant association of high TB with lymph node metastasis, these finding were concurrent with the study done by Rathod GB et al., Masilamani DS and Kanmani P, Buch AC et al., Gupta V and Patel M and Kumarguru BN et al., [1,2,3,6,10].

The present study confirmed high-grade TB was significantly associated with oestrogen positivity receptor status and lymph node metastasis.

In the study conducted by Huang T et al., compared as many of 10 various studies on TB to know its novel role in breast carcinoma along with clinical applications and future prospects of tumour markers concluded that TB can predict lymphatic invasion and lymph node involvement and play an increasingly prominent role in recurrence, metastasis and chemotherapy resistance [4]. But, more research is needed to explore the role of TB in breast carcinoma as there is no consensus on the precise definition of TB in breast carcinoma and the evaluation method. Therefore, it is necessary to establish consistent pathological criteria to identify and quantify TB to improve TB counting's accuracy and repeatability in clinicopathological practice [4].

Very few literature are there to compare TB with therapy effects where Gujam FJ et al., showed strong association (p=0.001) was observed between treatment status and high-grade TB but much large scale research is required to confirm these findings [12].

Thus, TB has been proved to be an important individual prognostic factor in colon and oesophageal carcinomas, and the findings of the current study also demonstrates the high utility of TB in prognostication of breast carcinoma.

Limitation(s)

The important limitation of the current study is small sample size. Hence, larger studies are needed to establish TB as an important adverse independent prognostic marker in breast cancers.

CONCLUSION(S)

High TB was significantly associated with ER positive status and lymph node metastasis. TB would be of great help to the clinicians to assess the prognosis of the patient, especially its association with lymphovascular invasion in the absence of lymph node metastases. Hence, it was concluded that TB is an easy to identify and unique significant prognostic factor in evaluating invasive breast carcinoma cases.

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