ORIGINAL ARTICLE

Association of Tumour budding with P53 and Ki-67 Expression in Carcinoma Breast

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ABSTRACT

Background: Tumour budding is defined as a small cluster of cancer cells (less than 5 cells) and is the first step in invasion and metastasis. It has been best studied in colorectal carcinoma, where it is associated with a poor prognosis. Recently it has been studied in breast carcinoma, where it has been shown to be associated with adverse clinico-pathological parameters. ***Objective:*** To assess the tumour budding in cases of infiltrating ductal carcinoma of breast, and to correlate its relation to P53 and Ki67 expression. ***Methods:*** This retrospective study was conducted from September 2022 to September 2023. Tumor buds were assessed and graded into a three-tier grading system. P-53 and Ki-67 expression was graded based on percentage positivity of tumour cells. The correlation between tumour budding and p53 and Ki-67 expression was studied along with other clinic-pathological parameters. ***Results:*** Grade 1 tumour budding was observed in 30% cases, Grade 2 tumour budding was seen in 33.33 % and Grade 3 tumour budding were seen in majority of cases (36.67%,). There was a statistically significant correlation between tumour budding and increased Ki-67 expression (p=0.00065). P-53 expression and tumour budding showed no significant correlation (p=0.159). There was a significant association in relation to increasing histologic grade (p=0.0031) and presence of lympho-vascular invasion (p=0.044). ***Conclusion:*** Tumour budding is seen to associated with parameters favouring poor prognosis. Further studies with larger sample size are needed to ascertain its role as an added prognostic marker.

Keywords: Tumour budding, breast carcinoma, P-53, Ki -67, immunohistochemistry

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INTRODUCTION

The global leading cause of cancer incidence is breast cancer, crossing the incidence of lung cancer in 2020.[1,2] Prognostic factors such as hormone receptors, proliferative markers and genomic studies are under investigation, with few being used in our daily practice. However their availability is limited in routine / smaller laboratories.[3, 4] Tumour budding is a newer concept in the field of breast cancer. It consists of a small group of tumour cells (less than five) which are detached from the main tumour mass. They are usually observed at the invasive front of the tumour. Pathologists can identify tumour buds by haematoxylin and eosin (H&E) staining. In cases of severely necrotic tumours, or poorly differentiated tumours, immunoihistochemistry such as pan-cytokeratin (Pan-CK) can be used as well for tumour bud identification.[5]

They have been studied in malignancies of other organ systems such as gastric, colorectal, lung and oesophageal tumours. It is believed that cells froming a tumour bud have characterisitics of a cancer stem cell, due to their potenital for redifferentiation and migration.[6,7] Until now, tumour budding has been best studied in

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colorectal cancers and is accepted as an important prognostic factor as well. The recommendation for tumour budding in colorectal cancer have been given by the International Tumor Budding Consensus Conference (ITBCC) in 2017.[8]

P53 is a tumor suppressor gene located on chromosome 17 (17p31.1). Mutated p53 gene produces protein products that accumulate at a cellular level and can be immunohistochemically detected. P-53 expression has been associated with worse prognosis in breast cancer, but has not been recommended for routine use , as of yet.[9, 10] Ki-67 is a nonhistone nuclear protein that is tightly associated with the cell cycle and is expressed in all proliferating cell. It is a known marker of cell proliferative index. The utility of Ki-67 in various malignancies is being discovered.[9, 11]

The primary aim of this study was to assess the association of tumour budding in cases of infiltrating ductal carcinoma of breast with P53 and Ki67 expression in the same. The secondary aim was to correlate its relation to clinicopathological parameters (age, histologic grade, size of tumour, lymphovascular invasion, lymph node metastasis).

METHODS

This retrospective study was conducted in the department of pathology from September 2022 to September 2023. 30 cases of histopathologically confirmed invasive breast carcinoma (infiltrating ductal carcinoma) were included in this study. Only cases diagnosed as Infiltrating ductal carcinoma, No special type (NST) were included in the present study. Cases diagnosed as other variants of breast carcinoma, as well as cases who received neoadjuvant chemoradiotherapy to the breast were excluded from the study. Clinical data of all the cases was recorded. The routine hematoxylin and eosin (H & E) slides were studied for histologic grade, lympho vascular invasion and lymph node metastasis.

Evaluation of P-53 and Ki-67 staining: Immunohistochemical staining was performed for P-53 (Clone DO-7, DAKO) and Ki67(Clone Mib-1, DAKO), following the manufacturers instruction. Positive control tissue sections of tonsil for (P-53 and Ki67) were used. Grading of the immunohistochemical staining was done based on percentage of stain positivity and strength of staining intensity.

Grading of P53 staining:[9]

0%-10% stained = negative (-), grade 0.

10.1%-49% stained = positive, grade 1. (heterogenous and focal staining)

>50% stained = positive , grade 2. (homogenous, diffuse staining)

Grading of Ki-67 expression:[9]

Low proliferative: ≤ 20% Ki-67-positive cells

High proliferative: > 20% Ki-67-positive cells

Evaluation of tumour budding: Tumor buds were taken as a single or cluster of up to four tumor cells present at the invasive margin of the tumor. For tumour budding evaluation, the invasive front of the tumour was scanned in scanner power (×40 objective). Tumor buds were identified (100 x objective) and a hotspot area was selected. The individual cells morphology in the tumour buds were matched with those of the tumour proper, and only then, considered for counting. Counting of tumour buds was performed in ×200 objective per high power field (HPF) and was recorded. A three tier grading system was used. (Table 1).

Correlation of tumour budding with age, tumour size, histologic grade, lympho vascular invasion, lymph node metastasis, P-53 expression and Ki-67 expression was performed.

Table 1: Grading of Tumor budding according to ITBCC guidelines[7]

Grading of tumour budding	Number of tumour buds/HPF (×200)
Grade 1	0 4
Grade 2	4-9
Grade 3	≥10

Statistical analysis: Data was entered into excel sheet and analyzed using SPSS 23 software. The level of significance between tumour budding and various parameters were determined using Pearson's chi-square test. The test was considered statistically significant when p value was <0.05.

RESULTS

A total of 30 cases of infiltrating ductal carcinoma of breast were included; no special type were analyzed. The ages of the patients ranged from 45 years to 78 years (mean age-61.2 years). 56.67% cases had tumour on the right side (n=17) and

43.33% cases had tumour on the left side (n=13). The mean tumour size was 4cm. Majority of cases were Grade 2 (60%). Lympho-vascular invasion was noted in 40% cases and nodal metastasis was observed in 60% cases. Majority cases showed grade 2 pattern of P53 staining (46.67%) and grade 3 pattern of Ki-67 staining (50%).

Tumour budding was evaluated and was classified into three grades according to ITBCC. Tumour budding was seen in all cases. Grade 1 tumour budding was observed in 30% cases (n=9) (Fig. 1), Grade 2 tumour budding was seen in 33.33 % (n=10) (Fig. 2), and Grade 3 tumour budding were seen in 36.67% cases which comprised of the majority (n=11) (Fig. 3).

Correlation between tumour budding and various parameters revealed a significant association in relation to increasing histologic grade (p=0.0031) and presence of lympho-vascular invasion (p=0.044). However, there was no statistical association in relation to age (0.44). A majority of cases (76%) of grade 2 and grade 3 tumour budding were seen in cases displaying lymph nodal metastasis, however the relationship of tumour budding and nodal metastasis was not found to be statistically significant (p=0.279). With regards to immunohistochemical expression of P53 and tumour budding (Fig. 4 & 5), a trend of increasing tumour budding with increasing grade of P53 expression was noted. 45.45 % each (n=05), of cases displaying grade 3 tumour budding showed grade 2 and grade 3 pattern of P53 staining, respectively. However, the results were not statistically significant (p=0.159). Of the 11 cases displaying grade 3 tumour budding, 10 cases (90.9%) displayed high proliferative Ki-67 staining. There was a statistically significant correlation between tumour budding and increased Ki-67 expression (p=0.00065). (Fig. 6 &7).

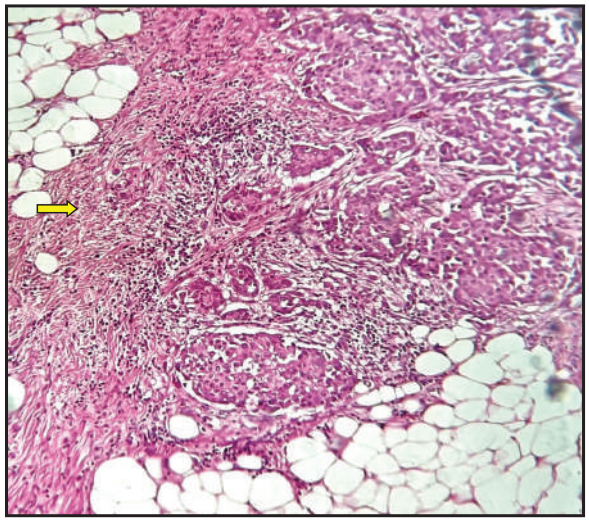

Figure 1: Grade 1 tumour budding (H&E, ×200)

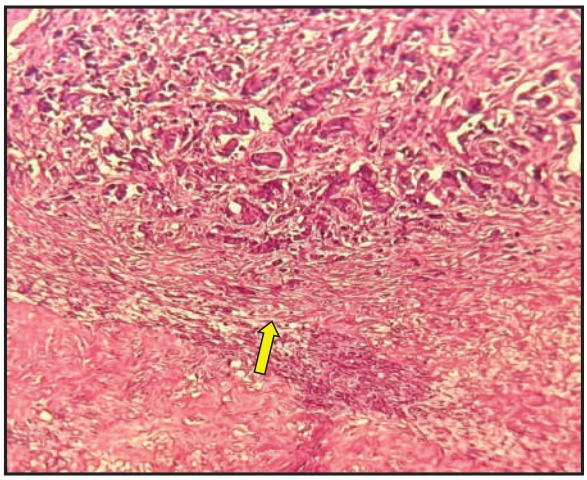

Figure 2: Grade 2 tumour budding (H&E, ×200)

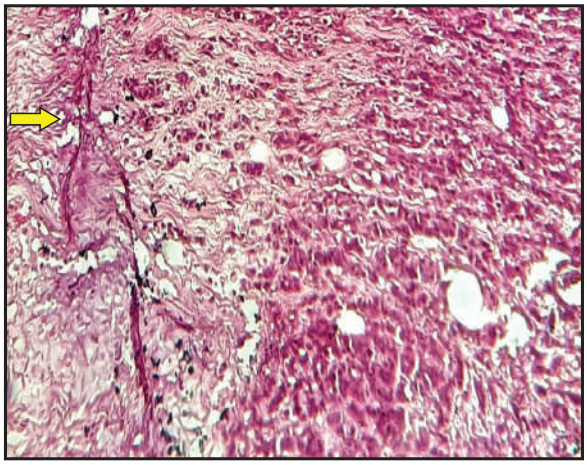

Figure 3: Grade 3 tumour budding (H&E, ×200)

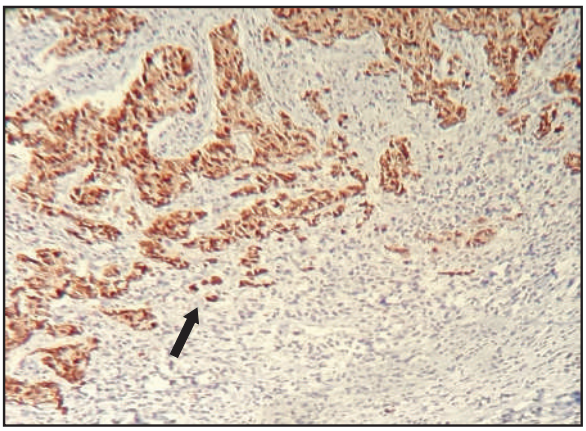

Figure 4: P- 53 grade 3 staining with grade 3 tumour budding (P-53, ×200)

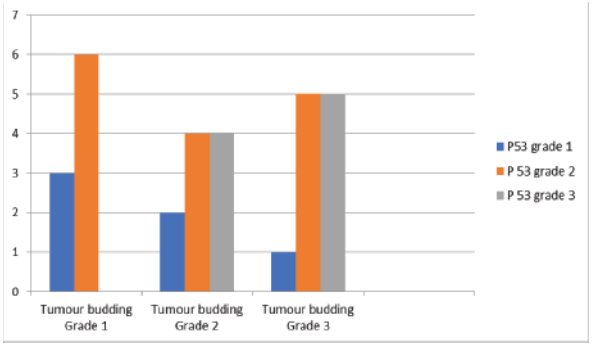

Figure 5: Bar diagram depicting p53 grade of staining with respect to grade of tumour budding.

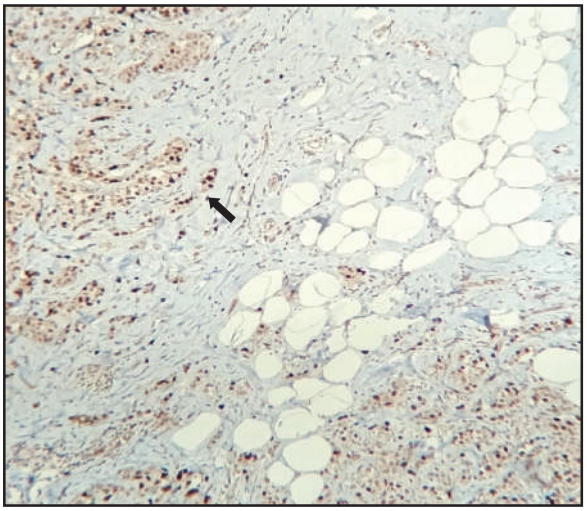

Figure 6: Ki-67 high proliferative index staining with grade 3 tumour budding (Ki-67, ×200)

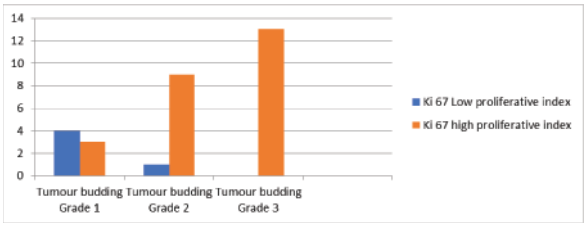

Figure 7: Bar diagram depicting Ki-67 grade of staining with respect to grade of tumour budding.

DISCUSSION

Invasion and metastasis are hallmarks of cancer, where in epithelial mesenchymal transition plays an integral role.[5] Tumour buds display epithelial mesenchymal transition as well as mesenchymal to epithelial transition, and are hence believed to play an important role in tumour invasion.[2,5] Tumour buds may also indicate the commencement of tumour cell detachment, following which individual cells may detach from the bud and further migrate, eventually leading to metastasis.[12,13] Previous studies have shown tumour budding to be a prognostic indicator of cancer in its early stages. With regard to breast carcinoma, studies have shown tumour budding to be associated with poor clinicopathological factors.[5] In association with conventional factors such as presence of lympho-vascular invasion and lymph node metastasis, tumour budding can be used as a measure of tumour aggressiveness.[14] In breast carcinoma, various methods for tumor bud evaluation have been employed in different studies. This led to a variation in observations, hence underlining the need for standardization of tumour bud evaluation criteria.[15] In view of this; a three tier method of counting tumour budding, recommended by ITBCC for colorectal carcinoma, has been used in this study.

In this study, we found a significant correlation between tumour budding and histologic grade. This was similar to findings reported by Agarwal et al.[16] There was no significant association found in relation to tumour size in this study. A majority of cases having grade 2 and grade 3 tumour budding were seen in cases displaying lymph nodal metastasis, despite absence statistically significant correlation. Salhia et al.[17] also reported lack of significant correlation with regard to tumour size and tumour budding; however thay found a staisitically significant association with regard to tumour budding and lymph node metastasis.

Lympho-vascular invasion and tumour budding showed a statistically significant correlation here. Similar findings were corroborated by Salhia et al.[17], Renuka et al.[18], Gabal et al.[19] and Gujam et al.[20] This could be due to the property of epithelial mesenchymal transition present in tumour buds, therby increasing their metastatic property with associated lympho-vascular invasion.[20]

In this study, a strong correlation between Ki-67 staining and tumour budding was observed. Miyuki et al.[21] and Silva et al.[22] observed higher grade of tumour budding associated with >20% of Ki-67 expression. A high proliferative index of Ki67 (>20%) is associated with increased tumour recurrence rate as well as organ metastasis.[21] Higher Ki-67 index correlation increased grade of tumour budding can point towards the role of tumour budding with worse prognosis in breast carcinoma. Approximately 30% of all breast cancer cases are associated with changes in the P53 gene expression.[23] Mutations contribute to a

negative action over wild type P53 leading to a "gain of capacity" feature ,and subsequent P5 3 overexpression.[24] The loss of normal function of p53 is associated with evolution and progression of breast cancer.[25] Li et al.[26] has reported significant correlation between higher levels of P53 expression and poor survival and increased mortality risk in breast carcinoma. In our study, we found increased grade of p53 expression in cases displaying increased tumour budding, which in turn is associated with poorer prognostic factors.

To our knowledge, there has been no previous study found to have correlated association between P-53 expression and tumour budding in breast carcinoma. A recent study performed in colorecal adenocarcinoma, found a positive correlation with regard to higher grade tumour budding with increased P-53 mutaions as well as presence of drug resistance.[27] Similar studies are warranted in the case of breast carcinomas as well to understand the exact association between P-53 expression and tumour budding.

CONCLUSION

Tumour budding was noted in all cases of our study and showed significant association with higher histologic grade, lympho-vascular invasion and Ki-67 expression. Further studies with a larger sample size and a standardized criteria for tumour bud evaluation in breast carcinoma are needed, to understand its role as a prognostic marker.

Conflicts of interest: None declared.

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Ethical approval: The study was approved by the Ethical Review Committee of Chamrajnagar Institute of Medical Sciences, Karnataka, India.

Authors' contribution: All authors were equally involved in conception, study design, data collection, statistical analysis, writing, editing and final approval of the manuscript.

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