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Tumor budding is associated with histological grade and Ki67 expression in a mastectomy cohort of invasive breast carcinoma

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Research Article

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Tumor budding is associated with histological grade and Ki67 expression in a mastectomy cohort of invasive breast carcinoma

Research

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Running title: Tumor budding in invasive breast carcinoma

Abstract

Tumor budding is a histomorphological manifestation of tumor-cell dissociation at the invasive front, but breast-specific assessment remains incompletely standardized. This secondary analysis included 55 invasive breast carcinoma tumors from 54 patients. Tumor budding was recorded as low grade (≤20 buds per 10 high-power fields) or high grade (>20 buds per 10 high-power fields). Two-sided Fisher exact or Fisher-Freeman-Halton exact tests were used, with Benjamini-Hochberg false-discovery-rate correction across 11 comparisons. High-grade budding was present in 32/55 tumors (58.2%). In the primary tumor-level analysis, high budding was associated with tumor-size category ($q=0.046$), pathological T category ($q=0.046$), histological grade ($q<0.001$), vascular invasion ($q=0.046$; conditional odds ratio 4.50, 95% confidence interval 1.21-19.51), and high Ki-67 ($q<0.001$; conditional odds ratio 63.46, 95% confidence interval 10.32-760.54). Lymph-node involvement was more frequent in high-budding tumors but was not significant after exact testing and multiplicity correction ($q=0.145$). In leave-one-record-out analyses for the bilateral tumors, histological grade and Ki-67 remained false-discovery-rate significant in both datasets, whereas size, T category, and vascular invasion were less stable. These findings support tumor budding as a low-cost invasive-front marker associated with adverse pathology, while highlighting the need for raw-count standardization, interobserver validation, and outcome-based external validation.

Keywords: tumor budding; invasive breast carcinoma; Ki-67; invasive front; vascular invasion; pathology biomarker

1 Introduction

Breast carcinoma is biologically heterogeneous, and pathological assessment integrates morphology, anatomical extent, histological grade, nodal status, vascular invasion, margin status, hormone-receptor expression, HER2 status, and selected proliferation markers [1-6]. These variables remain central to prognosis and treatment planning but do not fully describe cellular behaviour at the invasive tumor-host interface.

Tumor budding is defined as an isolated tumor cell or a cluster of up to four tumor cells at the invasive margin [7]. It is regarded as a histomorphological manifestation of reduced cell cohesion and invasive-front dissociation, with potential links to epithelial-mesenchymal plasticity, stromal interaction, vascular entry, and metastatic dissemination. Although standardized in colorectal carcinoma, breast carcinoma still lacks a universally accepted field area, scoring method, or reporting threshold [7].

Breast-cancer studies have related high tumor budding to larger tumor size, higher grade, vascular or lymphovascular invasion, nodal metastasis, recurrence, and adverse survival [8-18]. Recent studies have continued to compare breast-specific scoring approaches and evaluate relationships with clinicopathological and receptor variables, including data from South Asian cohorts [19-22]. Collectively, these reports support biological plausibility while also demonstrating substantial heterogeneity in counting fields, cut-offs, reader methods, and outcome definitions.

Ki-67 captures proliferative activity and is biologically distinct from invasive-front dissociation. Its interpretation is affected by pre-analytical variation, intratumoral heterogeneity, counting method, and cut-off selection [23-25]. Studying tumor budding alongside Ki-67 may therefore determine whether an invasive-front phenotype coexists with high proliferative activity.

The cohort was previously reported in an article focused on Ki-67 and clinicopathological variables [26]. The present article addresses a distinct primary endpoint: tumor budding. The specific objectives were to characterize the distribution of tumor budding, quantify its associations with recorded pathological variables using exact inference and multiplicity correction, and evaluate the robustness of the principal findings to the single patient who contributed two bilateral tumor records.

2 Materials and methods

2.1 Study design and setting

This hospital-based cross-sectional secondary histomorphological analysis was conducted in the Histopathology Section of a tertiary-care teaching hospital. The study included mastectomy specimens

diagnosed as primary invasive breast carcinoma during the documented study period from 1 September 2022 to 30 April 2024. The analysis comprised 55 tumors from 54 patients; one patient had bilateral tumors and therefore contributed two tumor-level observations. Adequately fixed mastectomy specimens with complete recorded variables were included, while improperly fixed specimens were excluded. All 55 tumor observations were retained in the primary analysis. Patient-level age and sex summaries counted the bilateral patient once. The study is reported with reference to STROBE recommendations for cross-sectional observational research [27].

Race and ethnicity were not recorded and were therefore unavailable for analysis. No race or ethnicity was inferred from names, geography, or other clinical information.

2.2 Histopathological and immunohistochemical assessment

Specimens were fixed in 10% neutral buffered formalin and processed routinely. Sections approximately 4 μm thick were prepared from representative paraffin blocks. Hematoxylin and eosin sections were used for histological typing, Scarff-Bloom-Richardson/Nottingham histological grading, pathological T-category assessment, and evaluation of tumor budding. ER and PR were interpreted according to ASCO/CAP recommendations, and HER2 was interpreted according to applicable ASCO/CAP guidance [5, 6]. HER2 results were classified as negative, equivocal, or positive; the single equivocal result was retained as such.

Vascular invasion was analyzed as recorded in the pathology data and is referred to as vascular invasion throughout this report. Pathological T category and lymph-node status were likewise analyzed as recorded. Complete pTNM stage groups were not reconstructed because a uniform complete-stage variable was unavailable. Histological type was grouped as invasive breast carcinoma of no special type (IBC-NST) or special histological type; the latter included invasive lobular carcinoma, carcinoma with apocrine differentiation, and mucinous carcinoma.

2.3 Tumor budding assessment

Tumor buds were searched at low magnification along the invasive front and evaluated at high power. A tumor bud was defined as an isolated carcinoma cell or a cluster of up to four carcinoma cells [7]. Mimickers, including stromal cells, macrophages, and inflammatory cells, were excluded by comparison with the cytological features of the invasive carcinoma. Buds were counted across 10 high-power fields corresponding to an approximate field area of 0.159 mm^2 per field in the study protocol. Tumor budding was recorded as low grade when ≤20 buds were present per 10 high-power fields and high grade when >20 buds were present.

2.4 Ki-67 assessment and analytic definitions

Ki-67 was assessed as nuclear staining in invasive tumor cells. At least 1,000 tumor cells were evaluated across 10 high-power fields in the study protocol, and the labelling index was calculated as the percentage of positively stained nuclei. Ki-67 was categorized as low (<20%) or high (≥20%); the recorded category was used without recalculation from the reported percentage ranges.

The largest recorded tumor dimension was used to derive three categories: ≤2 cm, >2-5 cm, and >5 cm. Pathological T category, lymph-node status, vascular invasion, margin status, ER, PR, HER2, Ki-67 category, tumor budding, and histological type were analyzed as recorded. Capitalization and leading or trailing spaces were standardized without changing clinical meaning. No value was imputed, and all 55 tumor observations were retained.

2.5 Statistical analysis

Categorical data are presented as counts and percentages. The primary analysis unit was the tumor record. Because several contingency tables contained sparse cells, two-sided Fisher exact tests were used for 2×2 comparisons and Fisher-Freeman-Halton exact tests were used for 2×k tables. Eleven comparisons were evaluated: tumor-size category, pathological T category, histological grade, lymph-node status, vascular invasion, margin status, ER, PR, HER2, histological group, and Ki-67 category. Benjamini-Hochberg false-discovery-rate correction was applied across these 11 tests, and adjusted values are reported as q values.

For 2×2 tables, conditional maximum-likelihood odds ratios with exact 95% confidence intervals were calculated. Cramér's V was used for multi-category associations and phi for binary associations. Because one patient contributed two bilateral tumor records, robustness was evaluated by repeating the full analysis after excluding each bilateral tumor record in turn. Statistical calculations were performed in Python 3.13 using SciPy 1.17 and a fixed-margin enumeration routine for 2×k exact tests. A two-sided $q<0.05$ was considered statistically significant after multiplicity correction.

2.6 Ethics approval and consent to participate

The study was reviewed and approved by the Institutional Ethics Committee of BLDE (Deemed to be University), Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura, India (approval number BLDE(DU)/IEC/681/2022-2023). All study procedures involving human participants, tissue samples, and data were performed in accordance with relevant guidelines and regulations and the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants in accordance with institutional requirements.

3 Results

3.1 Cohort characteristics

The study included 55 tumor observations from 54 patients. At the patient level, 53 patients were women and one was a man. Mean age was 52.2 years (standard deviation 12.3), median age was 52.5 years, and the range was 30-80 years. Tumor-level characteristics are summarized in Table 1.

IBC-NST accounted for 50/55 tumors (90.9%), and five tumors (9.1%) were special histological types. The cohort included 17 grade I, 26 grade II, and 12 grade III tumors. Lymph-node involvement was recorded in 37/55 tumors (67.3%), vascular invasion in 23/55 (41.8%), and involved margins in 4/55 (7.3%). ER was positive in 39/55 tumors (70.9%), PR in 31/55 (56.4%), and HER2 in 26/55 (47.3%); one tumor was HER2-equivocal. High Ki-67 category was present in 30/55 tumors (54.5%).

Table 1 Cohort characteristics

Variable	Overall distribution
Patients / tumor records	54 patients / 55 tumor records
Age (patient level)	Mean 52.2 ± 12.3 years; median 52.5; range 30-80
Sex (patient level)	Female 53 (98.1%); male 1 (1.9%)
Tumor size	≤2 cm: 5 (9.1%); >2-5 cm: 32 (58.2%); >5 cm: 18 (32.7%)
Pathological T category	T1: 5 (9.1%); T2: 31 (56.4%); T3: 11 (20.0%); T4: 8 (14.5%)
Histological grade	Grade I: 17 (30.9%); Grade II: 26 (47.3%); Grade III: 12 (21.8%)
Histological group	IBC-NST: 50 (90.9%); special type: 5 (9.1%)
Lymph-node status	Involved: 37 (67.3%); uninvolved: 18 (32.7%)
Vascular invasion	Present: 23 (41.8%); absent: 32 (58.2%)
Margin status	Involved: 4 (7.3%); uninvolved: 51 (92.7%)
ER status	Positive: 39 (70.9%); negative: 16 (29.1%)
PR status	Positive: 31 (56.4%); negative: 24 (43.6%)
HER2 status	Positive: 26 (47.3%); equivocal: 1 (1.8%); negative: 28 (50.9%)
Ki-67 category	High (≥20%): 30 (54.5%); low (<20%): 25 (45.5%)

Variable	Overall distribution
Tumor budding	High grade: 32 (58.2%); low grade: 23 (41.8%)

IBC-NST, invasive breast carcinoma of no special type. Patient-level age and sex were calculated after deduplicating the single bilateral patient.

3.2 Tumor budding distribution and pathological associations

High-grade tumor budding was present in 32/55 tumors (58.2%), while 23/55 (41.8%) had low-grade budding. The complete exact-test results, false-discovery-rate-adjusted q values, and effect estimates are shown in Table 2.

High-grade budding was associated with tumor-size category (exact p=0.021, q=0.046; Cramér's V=0.375), pathological T category (p=0.017, q=0.046; V=0.422), and histological grade (p<0.001, q<0.001; V=0.631). High-grade budding was absent among the five tumors measuring ≤2 cm and was concentrated in grade II and grade III tumors.

Vascular invasion was present in 18/32 high-budding tumors (56.3%) and 5/23 low-budding tumors (21.7%). High-grade budding was associated with higher conditional odds of vascular invasion (p=0.014, q=0.046; odds ratio 4.50, 95% confidence interval 1.21-19.51). Lymph-node involvement was more frequent in high-budding tumors (25/32, 78.1%) than in low-budding tumors (12/23, 52.2%), but this association did not meet the exact-test threshold after correction (p=0.079, q=0.145; odds ratio 3.20, 95% confidence interval 0.88-12.54). No significant association was detected with margin status, ER, PR, HER2, or histological group.

Table 2 Exact associations between tumor budding grade and pathological variables in the 55-tumor primary analysis

Variable	Low-grade budding (n=23)	High-grade budding (n=32)	Exact p	BH q	Effect estimate
Tumor size	≤2: 5; >2-5: 11; >5: 7	≤2: 0; >2-5: 21; >5: 11	0.021	0.046	Cramér's V=0.375
Pathological T category	T1: 5; T2: 10; T3: 6; T4: 2	T1: 0; T2: 21; T3: 5; T4: 6	0.017	0.046	Cramér's V=0.422
Histological grade	I: 15; II: 5; III: 3	I: 2; II: 21; III: 9	<0.001	<0.001	Cramér's V=0.631
Lymph-node status	Uninvolved: 11; involved: 12	Uninvolved: 7; involved: 25	0.079	0.145	OR=3.20 (95% CI 0.88-12.54)
Vascular invasion	Absent: 18; present: 5	Absent: 14; present: 18	0.014	0.046	OR=4.50 (95% CI 1.21-19.51)
Margin status	Uninvolved: 22; involved: 1	Uninvolved: 29; involved: 3	0.632	0.802	OR=2.25 (95% CI 0.17-124.82)
ER status	Negative: 6; positive: 17	Negative: 10; positive: 22	0.769	0.846	OR=0.78 (95% CI 0.19-2.95)
PR status	Negative: 9; positive: 14	Negative: 15; positive: 17	0.595	0.802	OR=0.73 (95% CI 0.21-2.46)
HER2 status	Negative: 11; equivocal: 1; positive: 11	Negative: 17; equivocal: 0; positive: 15	0.656	0.802	Cramér's V=0.163
Histological group	IBC-NST: 21; special: 2	IBC-NST: 29; special: 3	1.000	1.000	OR=1.08 (95% CI 0.11-14.06)
Ki-67 category	Low: 21; high: 2	Low: 4; high: 28	<0.001	<0.001	OR=63.46 (95% CI 10.32-760.54); φ=0.781

Exact p values are two-sided Fisher exact tests for 2×2 tables and Fisher-Freeman-Halton exact tests for 2×k tables. BH q values are Benjamini-Hochberg adjustments across 11 comparisons. OR, conditional odds ratio; CI, confidence interval; IBC-NST, invasive breast carcinoma of no special type.

3.3 Relationship between tumor budding and Ki-67

Tumor budding showed its strongest relationship with Ki-67 category. High Ki-67 was present in 28/32 high-budding tumors (87.5%) compared with 2/23 low-budding tumors (8.7%). The exact p value and adjusted q value were both <0.001. The conditional odds ratio for high Ki-67 in high- versus low-budding tumors was 63.46 (95% confidence interval 10.32-760.54), with phi=0.781. The wide confidence interval reflects small discordant-cell counts and should be interpreted cautiously.

Fig. 1 Tumor budding at the invasive front on hematoxylin and eosin stain. Panels A-C show detached single cells or small tumor-cell clusters within desmoplastic or adipose stroma at the invasive margin (original magnification ×40)

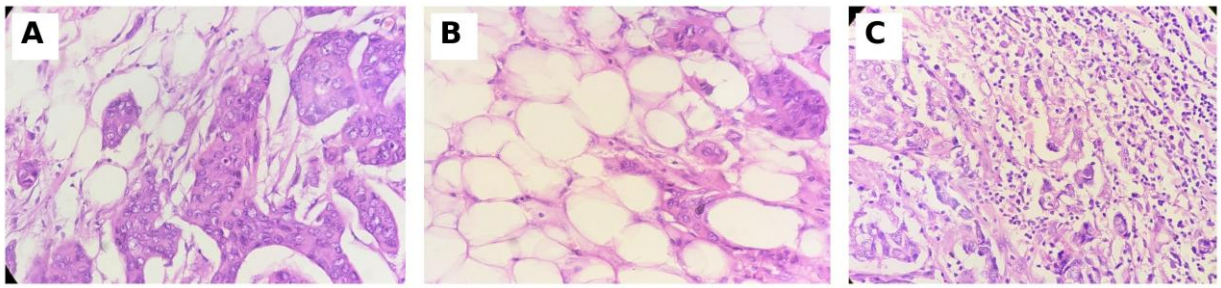
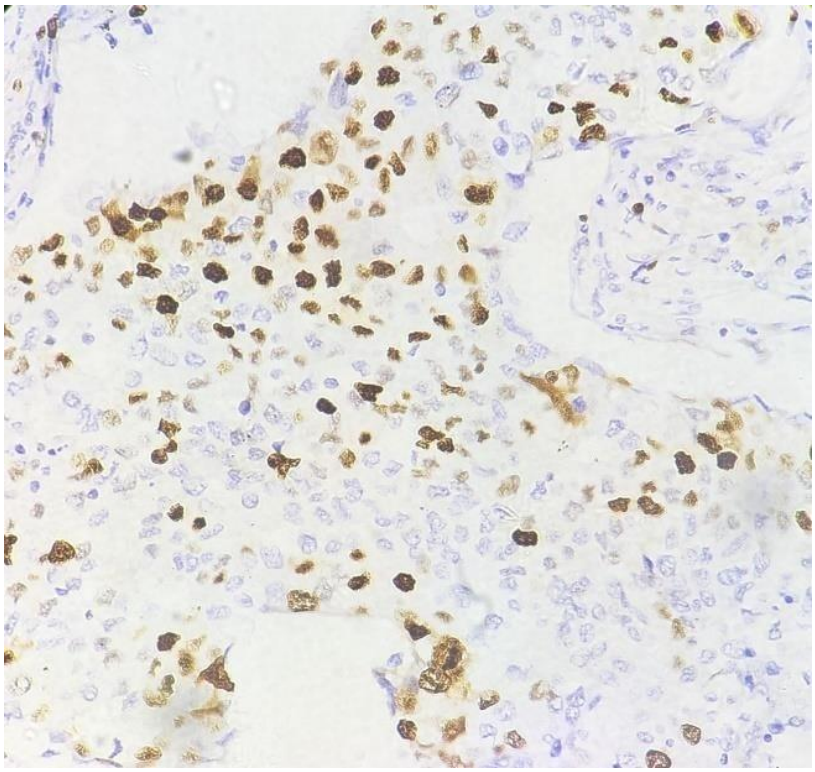


Fig. 2 Ki-67 immunohistochemistry showing nuclear labelling in invasive breast carcinoma cells (original magnification ×20)



3.4 Bilateral-record sensitivity analyses

Excluding either of the two bilateral tumor records produced two 54-tumor datasets (Table 3). Histological grade and Ki-67 remained false-discovery-rate significant in both analyses. Tumor size and pathological T category remained nominally significant, but their adjusted q values increased to 0.055 in both datasets. Vascular invasion remained significant after exclusion of the bilateral IBC-NST record (q=0.044) but not after exclusion of the bilateral lobular carcinoma record (q=0.055). Thus, grade and Ki-67 were the most stable correlates of high-grade budding in this dataset.

Table 3 Leave-one-record-out sensitivity analyses for the two bilateral tumor records

Association	Primary exact p / BH q	Exclude bilateral lobular record	Exclude bilateral IBC-NST record
Tumor-size category	0.021 / 0.046	0.025 / 0.055	0.025 / 0.055
Pathological T category	0.017 / 0.046	0.022 / 0.055	0.022 / 0.055
Histological grade	<0.001 / <0.001	<0.001 / <0.001	<0.001 / <0.001
Vascular invasion	0.014 / 0.046	0.024 / 0.055	0.012 / 0.044
Ki-67 category	<0.001 / <0.001	<0.001 / <0.001	<0.001 / <0.001

IBC-NST, invasive breast carcinoma of no special type.

3.5 Representative pathological findings

Representative tumor budding at the invasive front is shown in Fig. 1. Ki-67 immunohistochemistry showing nuclear labeling in invasive carcinoma cells is shown in Fig. 2.

4 Discussion

4.1 Principal findings

This analysis found high-grade tumor budding in 58.2% of invasive breast carcinoma tumor records. In the primary analysis, high budding clustered with larger tumor-size category, higher pathological T category, higher histological grade, recorded vascular invasion, and high Ki-67. After accounting for the bilateral patient through leave-one-record-out analyses, histological grade and Ki-67 were the most reproducible associations, whereas evidence for size, T category, and vascular invasion was more sensitive to inclusion of either bilateral record.

4.2 Biological and clinicopathological interpretation

The association between tumor budding and histological grade is biologically plausible. Histological grade integrates tubule formation, nuclear pleomorphism, and mitotic activity, while budding captures loss of cohesion and detachment at the invasive edge. Previous cohorts have similarly linked high budding with higher grade or other adverse morphological characteristics [8, 11, 12, 16, 19-22]. The present effect size for grade was large and remained stable in both sensitivity analyses.

The strong budding-Ki-67 relationship suggests that invasive-front dissociation can coexist with high proliferative activity. Similar relationships have been reported in other breast-cancer studies, although magnitude varies with counting method, selected field, and Ki-67 cut-off [16, 20-22]. Tumor budding and Ki-67 should not be treated as interchangeable: budding captures spatial invasive morphology, whereas Ki-67 estimates the proliferative fraction. Their combined assessment may describe complementary dimensions of tumor aggressiveness. Nevertheless, the large odds ratio and wide confidence interval in this modest dataset require external validation, and dichotomization of both variables may accentuate apparent separation.

Associations with tumor size, T category, and vascular invasion were directionally consistent with reports linking budding to local tumor extent, lymphovascular permeation, and metastatic potential [8-16, 19-22]. The bilateral-record sensitivity results, however, show that these findings were less stable than the grade and Ki-67 associations after multiplicity correction. Tumor size and pathological T category are also structurally related and should not be considered independent biological confirmations. The lymph-node result was directionally supportive but did not reach exact-test significance.

No meaningful association was identified with ER, PR, HER2, margin status, or grouped histological type. Published receptor findings have been inconsistent, and methodological heterogeneity in budding definitions probably contributes to between-study variation [13, 14, 16, 20-22]. The absence of association in this cohort suggests that tumor budding cannot be inferred from routine predictive immunohistochemistry alone.

4.3 Clinical relevance and contribution of the analysis

Tumor budding has practical appeal because it can be assessed on routine hematoxylin and eosin sections without additional laboratory cost. In settings where access to advanced molecular profiling is limited, a reproducible invasive-front marker could add contextual information to established pathological variables. However, this study does not establish independent prognostic value, treatment prediction, or a threshold ready for mandatory reporting.

The contribution of the present analysis is deliberately bounded. Tumor budding is an established research biomarker in breast carcinoma, and this cohort lacks outcome validation. The current work provides an implementation-focused analysis of an Indian mastectomy cohort using exact inference, multiplicity correction, effect-size reporting, and explicit sensitivity analysis for the only duplicated patient. The same cohort was previously reported in a Ki-67-centered analysis [26]; the present article addresses tumor budding as a distinct primary endpoint and uses Ki-67 only to evaluate its relationship with budding.

4.4 Future directions

Future validation should use calibrated field areas, raw bud counts, blinded dual-reader scoring, interobserver agreement, and prespecified handling of multifocal or bilateral disease. Prospective cohorts should evaluate whether budding improves recurrence or survival models beyond grade, size, nodal status, vascular invasion, receptor profile, and Ki-67. Digital and spatial quantification may also determine whether a continuous budding measure is more reproducible and clinically informative than a binary threshold.

5 Limitations

- Single-center cohort with 55 tumor records from 54 patients and no independent validation cohort.
- No recurrence, treatment-response, disease-free survival, or overall-survival endpoints were available; prognostic claims cannot be made from this analysis alone.
- Raw tumor-bud counts and reader-level scores were unavailable, preventing continuous-count analysis, threshold comparison, and interobserver-reproducibility assessment.
- Blinding of budding assessment to clinicopathological and immunohistochemical results was not documented, and cytokeratin-assisted adjudication was not recorded.
- Neoadjuvant-treatment status was unavailable.
- The pathology data did not distinguish lymphatic invasion from blood-vessel invasion.
- One patient contributed two bilateral tumor records; some associations were sensitive to exclusion of either record.
- The very strong Ki-67 association was based on dichotomized variables with small discordant cells, producing a wide confidence interval.

6 Conclusions

High-grade tumor budding was common in this cohort and was most robustly associated with higher histological grade and high Ki-67 category. Primary-analysis associations with tumor size, pathological T category, and vascular invasion were consistent with an adverse phenotype but were less stable in bilateral-record sensitivity analyses. Tumor budding is a promising low-cost histomorphological feature, but standardized counting, reproducibility testing, independent validation, and outcome-based studies are necessary before routine clinical reporting.

Data availability

The de-identified analytic dataset and statistical code supporting the findings are available from the corresponding author on reasonable request, subject to institutional ethics and privacy requirements. Accession-style identifiers will not be shared publicly.

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The authors declare that no external funding was received for this work.

Ethics approval and consent to participate

The study was reviewed and approved by the Institutional Ethics Committee of BLDE (Deemed to be University), Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura, India (approval number BLDE(DU)/IEC/681/2022-2023). All study procedures involving human participants, tissue samples, and data were performed in accordance with relevant guidelines and regulations and the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants in accordance with institutional requirements.

Clinical trial number: Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial or non-financial interests relevant to this work.

Author contributions

Mamatha K: Conceptualization, Methodology, Investigation, Resources, Supervision, Validation, Pathology review, Writing – review and editing. Upasana Sandilya: Investigation, Data curation,

Histopathological assessment, Methodology, Writing – original draft, Writing – review and editing. Sayandeep K. Das: Formal analysis, Data curation, Validation, Visualization, Statistical analysis, Writing – original draft, Writing – review and editing. All authors reviewed and approved the manuscript and agree to be accountable for the accuracy and integrity of the work.

Related publication and dual-publication disclosure

The cohort was previously described in a publication focused on Ki-67 and clinicopathological variables [26]. The present Research article addresses a distinct primary endpoint, tumor budding. Standalone Ki-67 associations from the earlier report are not reproduced; Ki-67 is included only to evaluate its relationship with tumor budding. All analyses reported here were performed specifically for the present tumor-budding study.

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