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EXPRESSION AT TUMOUR BUDDING SITES IN COLORECTAL ADENOCARCINOMAS: TRANSLATING MORPHOLOGIC SCORE INTO CLINICALLY USEFUL RESULTS

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ABSTRACT

Background: Tumour budding is recognized as an independent prognostic factor in colorectal adenocarcinoma. Increased nuclear expression of β-catenin at tumour budding sites has been implicated in the epithelial-mesenchymal transition. This study evaluated the pathogenetic role of tumour budding through nuclear β-catenin expression and examined its correlation with lymphovascular invasion in colorectal adenocarcinoma.

Material and Methods: This prospective and retrospective study included biopsy-proven colorectal carcinoma cases diagnosed over a two-year period at the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai. Of 199 colorectal adenocarcinomas (NOS), 60 randomly selected cases were included for detailed clinicopathological evaluation. Tumour budding was graded at the invasive front using conventional hematoxylin and eosin staining according to the ITBCC 2016 guidelines and by β-catenin immunohistochemistry. β-Catenin expression was analyzed and correlated with histopathological parameters.

Results: The highest incidence of colorectal carcinoma occurred in patients aged 51–60 years. Females constituted 55% of cases, and proximal tumours accounted for 51.7%. Tumours measuring <5 cm comprised 53.3% of cases, while 53.3% were Stage II. Lymphovascular invasion was absent in 71.7% of cases. By conventional hematoxylin and eosin assessment, 55% of cases demonstrated intermediate tumour budding (B2, 5–9 buds). High tumour budding (B3, >10 buds) was observed in patients aged 51–60 years, and all 14 B3 cases (100%) were moderately differentiated adenocarcinomas. Using β-catenin immunohistochemistry, 43.3% of cases were categorized as intermediate tumour budding (B2). Membranous β-catenin expression was observed in 61.7% of cases, whereas nuclear β-catenin expression was observed in 11.7%. No significant improvement in tumour budding detection was achieved using β-catenin immunohistochemistry compared with conventional hematoxylin and eosin evaluation.

Conclusions: Tumour budding can be assessed using both conventional hematoxylin and eosin staining and β-catenin immunohistochemistry. High tumour budding may provide additional prognostic information; however, no statistically significant association with lymphovascular invasion was demonstrated in this study. However, β-catenin immunohistochemistry did not significantly improve the detection of tumour budding over conventional histopathological assessment.

KEYWORDS: Colorectal adenocarcinoma; Tumour budding; β-Catenin; Immunohistochemistry; Lymphovascular invasion; Epithelial-mesenchymal transition.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and one of the leading causes of cancer-related mortality. Globally, CRC accounts for approximately 8.9% of all cancers. In India, the average annual incidence is 7.7 and 5.1 per million population among men and women, respectively. Although CRC predominantly affects individuals older than 50 years, recent studies have demonstrated an increasing incidence among younger patients with more aggressive disease behaviour. Furthermore, several Asian countries have experienced a two- to four-fold increase in CRC incidence during the past few decades, largely attributed to changes in dietary habits and lifestyle (1–9). The development of colorectal adenocarcinoma is influenced by both environmental and genetic factors. Dietary factors, including high consumption of animal fat and red meat with low fibre intake, together with obesity and physical inactivity, contribute significantly to colorectal carcinogenesis. Molecularly, colorectal carcinoma comprises heterogeneous tumour subtypes arising through distinct pathways of carcinogenesis, resulting in differences in biological behaviour, histomorphology and protein expression (10–18). Among the molecular pathways implicated in colorectal carcinogenesis, the APC/Wnt signalling pathway is one of the best characterized. APC gene mutations result in activation of Wnt signalling, leading to stabilization and nuclear translocation of β-catenin. Under physiological conditions, β-catenin forms a complex with E-

cadherin at the cell membrane, maintaining epithelial cell adhesion and polarity. Nuclear accumulation of β-catenin functions as an oncogenic transcription factor and represents a hallmark of epithelial–mesenchymal transition (EMT). Nearly 90% of colorectal carcinomas demonstrate dysregulation of the Wnt signalling pathway, with APC mutations occurring in approximately 60% of cases (17–20). Tumour budding, defined as isolated single tumour cells or clusters of fewer than five tumour cells at the invasive front, is considered the morphological counterpart of epithelial–mesenchymal transition. Increased nuclear β-catenin expression and reduced E-cadherin expression have consistently been demonstrated in tumour buds compared with the main tumour body. Besides conventional pathological staging parameters such as tumour depth and lymph node status, tumour budding has emerged as an independent adverse prognostic factor associated with tumour aggressiveness and poor clinical outcome (21–28). The present study evaluated the pathogenetic significance of tumour budding in colorectal adenocarcinoma by assessing nuclear β-catenin expression using immunohistochemistry and correlating tumour budding with lymphovascular invasion.

MATERIALS AND METHODS

Study Design and Study Population

This prospective and retrospective study included adult patients with histopathologically confirmed colorectal adenocarcinoma diagnosed at the Institute of Pathology, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai. During the study period, 212 colectomy specimens were received for histopathological examination. Among these, seven cases were diagnosed as benign and non-neoplastic lesions, six as other malignant colorectal neoplasms, and 199 as colorectal adenocarcinoma, not otherwise specified (NOS). Of the 199 colorectal adenocarcinomas, 32 were well differentiated, 91 were moderately differentiated, and 76 were poorly differentiated. Sixty cases were selected randomly for detailed histopathological and immunohistochemical evaluation.

Inclusion and Exclusion Criteria

Adult patients aged 26–80 years with histopathologically confirmed primary colonic or rectal adenocarcinoma who underwent hemicolectomy, abdominoperineal resection, or low anterior resection were included.
Patients younger than 26 years and resection specimens diagnosed as mucinous carcinoma, signet-ring cell carcinoma, or neuroendocrine carcinoma were excluded.

Clinicopathological Evaluation

Clinical and pathological data, including age, sex, tumour site, tumour size, operative procedure, histological grade, tumour stage, depth of invasion, lymphovascular invasion, and perineural invasion, were retrieved from surgical pathology records.
Representative sections from all selected specimens were processed routinely and stained with hematoxylin and eosin. Among the 60 selected cases, four (6.7%) were well differentiated, 55 (91.7%) were moderately differentiated, and one (1.6%) was poorly differentiated. These cases were evaluated for tumour budding on hematoxylin and eosin sections and by β-catenin immunohistochemistry.

Assessment of Tumour Budding on Hematoxylin and Eosin Sections

Tumour budding was assessed according to the recommendations of the International Tumor Budding Consensus Conference (ITBCC), 2016. Four-micrometre-thick hematoxylin and eosin-stained sections were examined to identify the invasive tumour front. Ten fields were initially screened under ×10 magnification to identify the tumour budding hotspot. Tumour buds within the hotspot were counted under ×20 magnification. Bud counts were normalized using a correction factor of 0.810, corresponding to the field diameter (FN 18) of the Olympus 20i microscope.
Tumour budding was categorized according to the ITBCC three-tier grading system:

- Bd1 (low budding): 0–4 buds
- Bd2 (intermediate budding): 5–9 buds
- Bd3 (high budding): ≥10 buds

Immunohistochemistry

Immunohistochemical analysis of β-catenin was performed on formalin-fixed, paraffin-embedded tissue sections using a super-sensitive polymer horseradish peroxidase detection system based on non-biotin polymer technology. Four-micrometre tissue sections were mounted on positively charged slides. Heat-induced antigen retrieval was performed before incubation with rabbit monoclonal anti-β-catenin antibody. Immunoreactivity was detected using a horseradish peroxidase-conjugated polymer secondary antibody with diaminobenzidine as the chromogen.

Antigen	Vendor	Clone/Species	Dilution	Positive Control
β-Catenin	PathnSitu	Rabbit monoclonal	Ready to use	Non-neoplastic colonic mucosa

Evaluation of β-Catenin Expression

β-Catenin immunoreactivity was evaluated separately at the tumour centre and invasive front according to the scoring system previously described.[19]
Staining intensity was graded as:

- **0:** No staining

- **1:** Weak staining
- **2:** Moderate staining
- **3:** Strong staining

The extent of staining was scored as:

- **0:** ≤5%
- **1:** 6–25%
- **2:** 26–50%
- **3:** 51–75%
- **4:** 76–100%

Membranous β-catenin expression was considered preserved when more than 80% of tumour cell membranes demonstrated staining; otherwise, expression was considered reduced. High cytoplasmic and nuclear β-catenin expression was defined as staining in more than 50% of tumour cells.

Tumour budding at the invasive front was graded according to the ITBCC 2016 three-tier classification (Bd1, Bd2, and Bd3).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 23.0. Tumour budding assessed on hematoxylin and eosin sections and by β-catenin immunohistochemistry was correlated with clinicopathological variables, including age, sex, tumour size, tumour site, histological grade, stage, and lymphovascular invasion. Associations were analysed using the Chi-square test. Correlations between β-catenin immunohistochemical findings, tumour budding assessed on hematoxylin and eosin sections, tumour stage, and lymphovascular invasion were also evaluated. A P value <0.05 was considered statistically significant.

Ethics approval: The study was approved by the Institutional Ethics Committee, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai. The study was conducted in accordance with institutional ethical guidelines.

RESULTS

A total of **199 colorectal adenocarcinoma (NOS)** cases were diagnosed during the study period, of which **60 randomly selected cases** were included for detailed histopathological and immunohistochemical evaluation. All study cases were conventional colorectal adenocarcinoma (NOS).

Clinicopathological Characteristics

The clinicopathological characteristics of the study population are summarized in **Table 1**. Gross evaluation of selected hemicolectomy specimens demonstrated exophytic and classic ulcerated tumor masses traversing the mucosal tissue wall (**Figure 1**). Most tumors were **moderately differentiated (91.7%)**, whereas **6.7%** were well differentiated and **1.6%** were poorly differentiated. The highest incidence was observed in patients aged **51–60 years (26.7%)**, followed by the **41–50 years** and **61–70 years** age groups (21.7% each). Females constituted **55.0%** of the study population, while males accounted for **45.0%**. Proximal colonic tumors were the most common (**51.7%**), followed by distal colonic (**28.3%**) and rectal tumors (**20.0%**). Tumors measuring **<5 cm** constituted **53.3%** of cases, while **46.7%** measured **5–10 cm**. Stage II disease was the predominant pathological stage (**53.3%**), followed by Stage III (**30.0%**), Stage I (**13.3%**), and Stage IV (**3.3%**). Lymphovascular invasion was present in **28.3%** of cases and absent in **71.7%**. On conventional light microscopy, these moderately differentiated variants presented classical patterns of complex infiltrating malignant gland profiles driving into an adjacent desmoplastic stromal response (**Figure 2**)

Table 1. Clinicopathological characteristics of the study population (N = 60)

Characteristic	Category	n	%
Age (years)	≤40	9	15.0
	41–50	13	21.7
	51–60	16	26.7
	61–70	13	21.7
	71–80	6	10.0
	>80	3	5.0
Sex	Female	33	55.0
	Male	27	45.0
Tumor site	Proximal colon	31	51.7
	Distal colon	17	28.3
	Rectum	12	20.0
Tumor size (cm)	<5	32	53.3
	5–10	28	46.7
Histological grade	Well differentiated	4	6.7
	Moderately differentiated	55	91.7

	Poorly differentiated	1	1.6
Pathological stage	I	8	13.3
	II	32	53.3
	III	18	30.0
	IV	2	3.3
Lymphovascular invasion	Present	17	28.3
	Absent	43	71.7

Association of Clinicopathological Variables with Tumor Stage

No statistically significant association was observed between pathological stage and age (**P = 0.493**), gender (**P = 0.739**), tumor site (**P = 0.678**), or tumor size (**P = 0.521**). In contrast, lymphovascular invasion showed a highly significant association with pathological stage (**P = 0.0005**).

Table 2. Association between clinicopathological variables and pathological stage

Variable	Pearson χ^2	P value	Significance
Age	14.429	0.493	Not significant
Gender	1.260	0.739	Not significant
Tumor site	3.988	0.678	Not significant
Tumor size	2.257	0.521	Not significant
Lymphovascular invasion	26.365	0.0005	Significant

Tumor Budding on Hematoxylin and Eosin Sections

Tumor budding assessed according to the ITBCC 2016 criteria demonstrated Bd2 (intermediate budding) as the predominant category (55.0%), followed by Bd3 (23.3%) and Bd1 (21.7%) (Table 3). Evaluation within verified hotspot areas under ×20 magnification highlighted clear micro-clusters or detached single tumor cells scattered through hypercellular bands of stroma (**Figure 3**). High-density detachment zones showed an extreme loss of cohesive structural architecture, confirming advanced local cellular migration (**Figure 4**), which was monitored alongside standard cell nest configurations under higher magnifications.(Figure 8)

Table 3. Tumor budding assessed on hematoxylin and eosin sections

Tumor budding grade	Bud count	n	%
Bd1	0–4	13	21.7
Bd2	5–9	33	55.0
Bd3	≥10	14	23.3

A significant association was observed between tumor budding grade and patient age (**Pearson χ^2 = 19.571, P = 0.034**). However, tumor budding did not show significant associations with pathological stage (**P = 0.731**), histological grade (**P = 0.695**), or lymphovascular invasion (**P = 0.972**) (Table 4).

Table 4. Association between tumor budding (H&E) and clinicopathological variables

Variable	Pearson χ^2	P value	Significance
Age	19.571	0.034	Significant
Pathological stage	3.595	0.731	Not significant
Histological grade	2.221	0.695	Not significant
Lymphovascular invasion	0.057	0.972	Not significant

β-Catenin Immunohistochemical Expression

Tumor budding assessed by β-catenin immunohistochemistry demonstrated **Bd2** in **43.3%**, **Bd1** in **26.7%**, **Bd3** in **18.3%**, while **11.7%** of cases showed negative staining (Table 5).

Predominant membranous β-catenin expression was observed in 61.7% of cases. Intact cohesive tumor nests tracked with strong linear membranous boundary patterns overall (**Figure 5**). Detailed cellular mapping across peripheral tumor zones showed a distinct stabilization of the protein complex at intact borders, with cytoplasmic migration shifts starting to express as cellular networks fragmented (**Figure 6**). Crucially, immunohistochemistry precisely highlighted small detached micro-clusters and single isolated tumor cells tracking through the matrix wall, clearly detailing individual buds via localized expression shifts (**Figure 7**)

Table 5. Tumor budding assessed using β-catenin immunohistochemistry

Tumor budding grade	n	%
Bd1	16	26.7
Bd2	26	43.3
Bd3	11	18.3

Negative	7	11.7

Table 6. β-Catenin expression at the invasive front

Expression pattern	n	%
Membranous	37	61.7
Cytoplasmic	9	15.0
Nuclear	7	11.7
Negative	7	11.7

Association of β-Catenin-based Tumor Budding with Clinicopathological Variables

No statistically significant association was identified between β-catenin-based tumor budding and histological grade (**P = 0.131**) or pathological stage (**P = 0.965**) (Table 7).

Table 7. Association between β-catenin-based tumor budding and clinicopathological variables

Variable	Pearson χ^2	P value	Significance
Histological grade	9.848	0.131	Not significant
Pathological stage	2.984	0.965	Not significant

Correlation Between Hematoxylin and Eosin Tumor Budding and β-Catenin Immunohistochemistry

A highly significant correlation was observed between tumor budding assessed on hematoxylin and eosin sections and β-catenin immunohistochemistry (**Pearson χ^2 = 74.861, P = 0.0005**) (Table 8).

Table 8. Correlation between tumor budding assessed on hematoxylin and eosin sections and β-catenin immunohistochemistry

Variable	Pearson χ^2	P value	Interpretation
Tumor budding (H&E) vs β-catenin	74.861	0.0005	Significant positive correlation

Tumor Budding and Lymphovascular Invasion in Stage II Tumors

Among the **32 Stage II tumors**, **50.0%** demonstrated intermediate tumor budding (Bd2) on hematoxylin and eosin sections, while **46.9%** showed Bd2 using β-catenin immunohistochemistry. Lymphovascular invasion was absent in **90.6%** of Stage II tumors (Table 9).

Table 9. Tumor budding and lymphovascular invasion in Stage II tumors (N = 32)

Parameter	Category	n	%
Tumor budding (H&E)	Bd1	8	25.0
	Bd2	16	50.0
	Bd3	8	25.0
Tumor budding (β-catenin)	Bd1	8	25.0
	Bd2	15	46.9
	Bd3	6	18.8
	Negative	3	9.4
Lymphovascular invasion	Absent	29	90.6
	Present	3	9.4

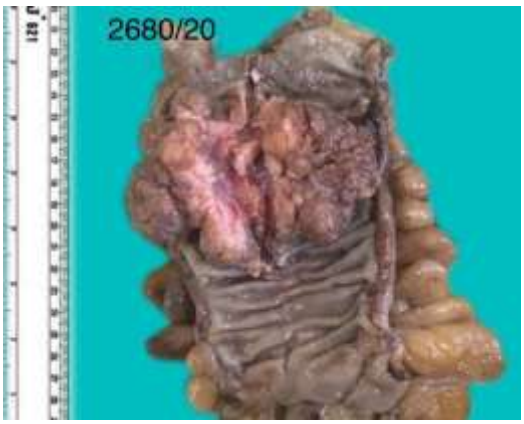

Figure 1. Gross specimen of a colorectal adenocarcinoma resection. The image shows an exophytic, fungating, and ulcerated mass involving the colonic mucosa, along with an adjacent reference ruler indicating tumor sizing.

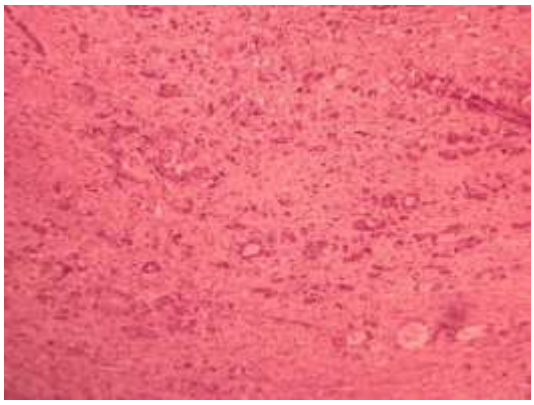

Figure 2. Colorectal adenocarcinoma (Hematoxylin and Eosin, ×100). Representative low-power view of a moderately differentiated colorectal adenocarcinoma showing infiltrating malignant glands embedded within a desmoplastic stroma at the invasive front.

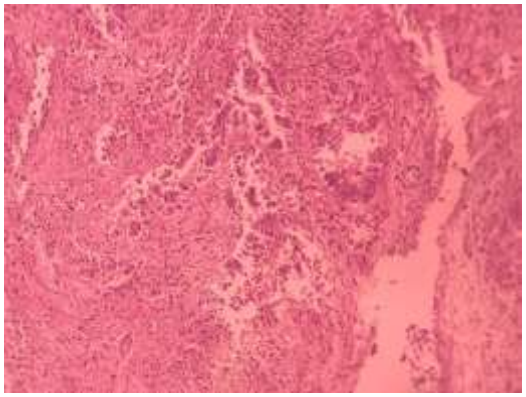

Figure 3. Tumor budding hotspot (Hematoxylin and Eosin, ×200). High-power microscopic view highlighting clusters of isolated tumor single cells or small aggregates of fewer than 5 tumor cells at the invasive tumor front, indicative of intermediate-to-high tumor budding.

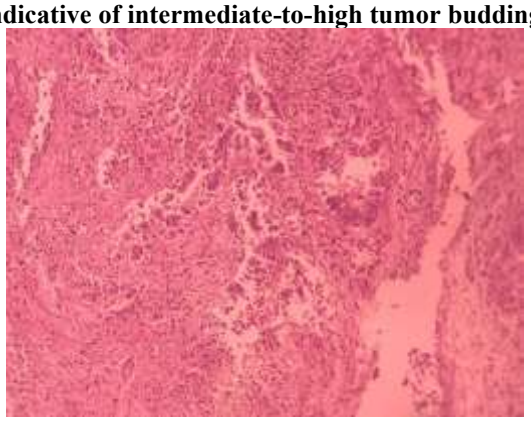

Figure 4. High-density tumor budding front (Hematoxylin and Eosin, ×200). Marked architectural stromal infiltration showing a distinct loss of glandular cohesion, showcasing morphologic evidence of active epithelial-mesenchymal transition (EMT) at the invasive front.

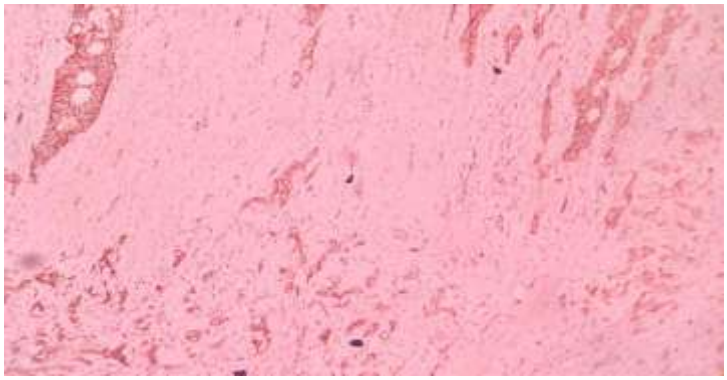

Figure 5. β-Catenin immunohistochemical staining (×100). Low-power overview demonstrating strong, preserved membranous β-catenin localization within the larger cohesive neoplastic glandular margins.

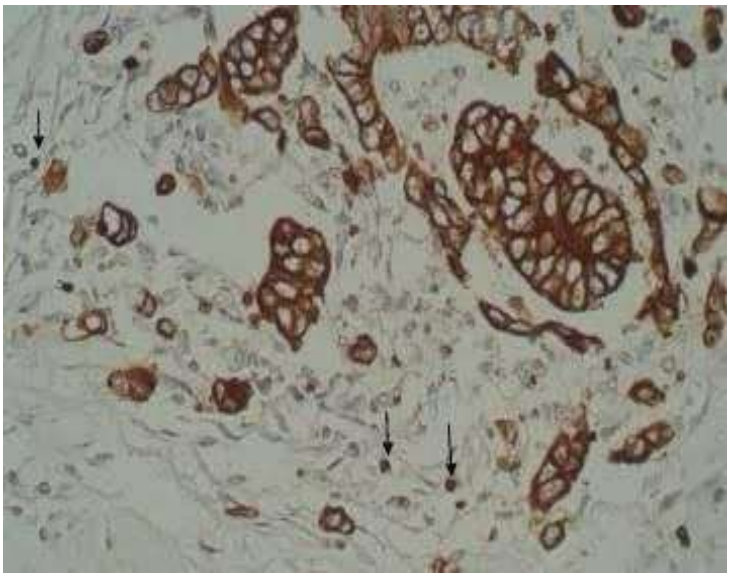

Figure 6: β-Catenin expression patterns at tumor cell membranes (×200). Microscopic view confirming distinct linear membranous stabilization within established margins, with early cytoplasmic accumulation appearing in migrating sub-glandular configurations.

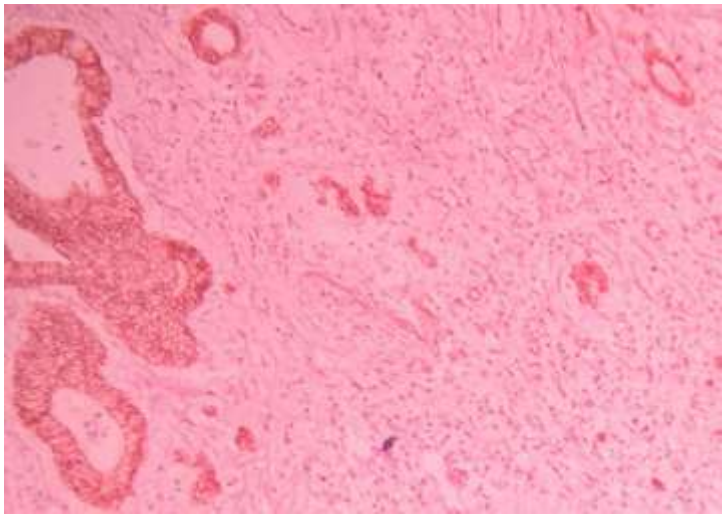

Figure 7. High-power evaluation of tumor buds by β-Catenin immunohistochemistry (×400). Immunohistochemical delineation clearly identifying single isolated tumor cells and micro-clusters (marked by arrows) tracking through the extracellular matrix, highlighting areas of nuclear/cytoplasmic localization shifts.

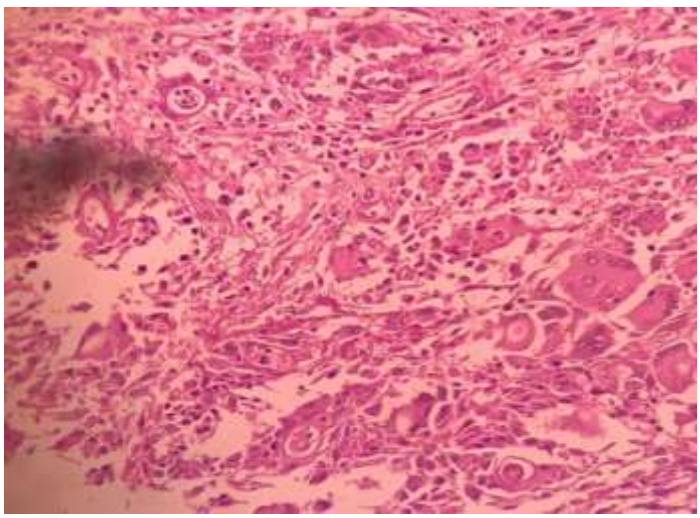

Figure 8. High-power focus of invasive cellular nests (Hematoxylin and Eosin, ×400). Detailed view showing clear separation of dysplastic single cells and cellular cords pushing against stromal defenses, showcasing the morphological parameters monitored during routine ITBCC risk stratification

DISCUSSION

Tumour budding is increasingly recognized as an adverse histopathological feature in colorectal carcinoma and has been incorporated into routine pathological reporting following the recommendations of the International Tumor Budding Consensus Conference (ITBCC 2016). It represents tumour cell dedifferentiation at the invasive front and is regarded as the morphological manifestation of epithelial–mesenchymal transition (21,22,24–28).

In the present study, moderately differentiated adenocarcinoma constituted the predominant histological subtype, with Stage II disease representing the largest proportion of cases. The highest incidence was observed in the 51–60-year age group with a slight female predominance, reflecting the clinicopathological profile of the study population.

Assessment of tumour budding using hematoxylin and eosin sections according to the ITBCC 2016 recommendations demonstrated intermediate tumour budding (Bd2) as the predominant category. A statistically significant association was observed between tumour budding grade and patient age, whereas no significant association was identified with pathological stage, histological grade or lymphovascular invasion. These findings indicate that tumour budding can be reproducibly assessed using routine hematoxylin and eosin sections following standardized ITBCC recommendations (21–23).

β-Catenin immunohistochemistry demonstrated predominantly membranous expression at the invasive front, whereas nuclear expression was observed in only 11.7% of cases. Nuclear localization of β-catenin has been associated with activation of the Wnt signalling pathway, epithelial–mesenchymal transition and tumour progression in colorectal carcinoma (20,25–27). However, membranous expression predominated in the present study.

A major finding of the present study was the highly significant correlation between tumour budding assessed on hematoxylin and eosin sections and tumour budding evaluated using β-catenin immunohistochemistry ($P = 0.0005$). Although immunohistochemistry may facilitate identification of tumour buds in selected cases, particularly in the presence of inflammatory infiltrates, conventional hematoxylin and eosin assessment demonstrated comparable performance. These findings support routine histopathological evaluation of tumour budding using ITBCC recommendations (22,28).

Lymphovascular invasion demonstrated a significant association with pathological stage but not with tumour budding. Among Stage II colorectal carcinomas, intermediate tumour budding was the predominant category using both conventional histology and β-catenin immunohistochemistry, whereas lymphovascular invasion was absent in most cases. Previous studies have suggested that tumour budding provides additional prognostic information and may aid risk stratification in Stage II colorectal carcinoma (14–19,28).

The principal strength of the present study is the comparative evaluation of tumour budding using both conventional hematoxylin and eosin staining and β-catenin immunohistochemistry according to standardized ITBCC recommendations. However, the relatively small sample size and single-centre design may limit the generalizability of the findings.

CONCLUSION

Tumour budding can be reliably assessed using routine hematoxylin and eosin staining, while β-catenin immunohistochemistry demonstrates strong concordance with conventional assessment. However, β-catenin immunohistochemistry did not significantly improve tumour budding detection over conventional histopathological evaluation. Lymphovascular invasion showed a significant association with pathological stage, whereas no significant association was observed between tumour budding and lymphovascular invasion. Routine assessment of tumour budding may therefore provide valuable prognostic information and complement conventional pathological staging in colorectal carcinoma.

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