Original Article

Pathological and Clinical Significance of Tumor Budding and Its Association with Epithelial-Mesenchymal Transition in Colorectal Carcinoma: A Retrospective Observational Study

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What's Known

- Tumor budding is linked to invasion, metastasis, and poor prognosis in colorectal carcinoma (CRC).
- Epithelial-mesenchymal transition (EMT) markers such as β-catenin, E-cadherin, Snail, and Zinc finger E-box-binding homeobox 1 (ZEB1) are frequently altered in CRC.
- Tumor budding has been associated with distant metastasis and other adverse clinicopathological features, but it is underutilized in standard pathology due to assessment challenges.

What's New

- This study highlights a significant correlation between tumor budding and EMT markers in CRC, providing a deeper understanding of invasion mechanisms.
- This finding contributes to our understanding of invasion mechanisms in CRC.

Abstract

Background: Metastases, not the primary tumor, account for most cancer-related deaths. Tumor budding, thought to represent epithelial-mesenchymal transition (EMT), has garnered attention due to its association with invasion and migration. This study aims to assess the pathological and clinical significance of tumor budding in colorectal carcinoma and its correlation with epithelial-mesenchymal transition.

Methods: In this retrospective observational study, tissue samples from 101 patients (no neoadjuvant treatment) were analyzed. Tumor budding was scored using International Tumor Budding Consensus Conference guidelines and classified into Budding 1 (BD1) (1-4 buds), Budding 2 (BD2) (5-9 buds), and Budding 3 (BD3) (10+ buds) per 0.785 mm². The tissue sample was subjected to immunohistochemistry to assess EMT markers: β-catenin, E-cadherin, Snail, and Zinc finger E-box-binding homeobox 1 (ZEB1).

Results: Tumor budding was significantly associated with advanced tumor stage (P=0.0001), deeper invasion (P=0.003), vascular invasion (P=0.001), perineural invasion (P=0.0001), and desmoplasia (P=0.010). Regional lymph node metastasis was seen in 93% of cases with tumor budding, and distant metastasis was found in eight cases (7.9%). Aberrant β-catenin expression was seen in 82 cases (81.2%), and aberrant E-cadherin in 65 cases (64.4%). Snail and ZEB1 positivity were observed in 55 (54.5%) and 32 (31.7%) cases, respectively. A significant correlation was found between aberrant β-catenin and ZEB1 (P=0.005). Although EMT markers coexisted frequently with tumor budding, no statistically significant association was observed.

Conclusion: The results of our study indicate that tumor budding is common in colorectal carcinoma and is significantly associated with advanced tumor stage, invasion, vascular and perineural invasion, and regional lymph node metastasis. Aberrant expression of EMT markers (β-catenin, E-cadherin, Snail, and ZEB1) was frequently observed, although no significant association with tumor budding was found.

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Keywords ● Epithelial-mesenchymal transition ● Colorectal neoplasms ● Prognosis ● Neoplasm invasiveness

Introduction

Colorectal carcinoma (CRC) ranks as the third most frequently diagnosed cancer and is the leading contributor to cancer-related mortality worldwide. It often develops from benign neoplastic lesions, such as adenomatous polyps and serrated polyps.[1]

As the molecular understanding of CRC advances, extensive research is being conducted to determine whether these histological or molecular traits can be used to predict treatment outcomes. Two potential classifications of CRCs have emerged from molecular and genetic investigations, including various gene expression analyses. The first classification is sporadic and unrelated to genetic susceptibility or family history, likely arising from environmental and nutritional factors.[2]

Nevertheless, a family history of CRC is present in 20-30% of patients with the disease, and 5% of these cancers arise in the context of conditions with Mendelian heredity. These include nonpolyposis disorders such as cancer familial syndrome (formerly Lynch II) and Hereditary Non-Polyposis colorectal cancer (HNPCC, formerly Lynch I), as well as conditions involving colonic polyps.[3, 4] The Fearon and Vogelstein model has long been accepted as the gold standard for understanding the genetic changes associated with CRC development.[5]

Given that molecular alterations are the primary cause of cancer, the ability of malignant tumors to invade nearby tissues and even metastasize to distant sites is a secondary factor. While cancer cells frequently exhibit anchorage-independent growth, normal tissue shows a strong correlation between cell adhesion and signaling, as evidenced by their reliance on anchoring for growth. Two of the most intriguing topics in tumor growth and metastasis are the epithelial-mesenchymal transition (EMT) of tumor cells and tumor budding.[5]

The shifts in adhesion and signaling in malignant cells, leading to metastasis, confirm many established theories in this field. Phenotypic alterations during EMT, including the invasion of the extracellular matrix and the departure of cancer cells from the primary tumor to form distant metastases, support the hypothesis that EMT is pathologically reactivated during malignant transformation.[6]

Numerous studies have linked tumor budding in CRC to unfavorable outcomes.[7, 8] The International Union Against Cancer has classified tumor budding as an "additional prognostic marker" alongside histological grade, perineural invasion, and tumor boundary.[9] However, several factors have made it challenging to incorporate tumor budding assessment into standard pathology reports.[10] The purpose of the present study is to identify tumor budding in patients with CRC and correlate the results with EMT.

Materials and Methods

Ethical Approval and Case Selection

This study was approved by the Ethics Committee of the University of Duhok, Duhok city, Iraq, and the Duhok Directorate General of Health in Duhok city, Iraq (Approval number: 13062021-7-17). Written informed consent was obtained from all participants.

Evaluation of Tumor Budding and Its Relationship with Clinical Features

In this retrospective observational study, 101 paraffin-embedded, formalin-fixed CRC tissue blocks were collected between January 2017 and May 2023 in Duhok city, Iraq. All relevant resection specimens and hematoxylin and eosin-stained slides were reassessed by other skilled pathologists, blinded to the clinical outcome. The histopathological details of each tumor were obtained from diagnostic records provided by various attending pathologists. CRC cases diagnosed with endoscopic biopsy or treated with neoadjuvant therapy were excluded. A cohort was used to establish a histopathological cutoff for "high" tumor budding and to validate its prognostic significance using an established scoring system.[8] According to the tumor budding scoring guidelines from the International Tumor Budding Consensus Conference,[11] cases positive for tumor budding were categorized into three groups: BD1 (1–4 buds/0.785 mm^2), BD2 (5–9 buds/0.785 mm^2), and BD3 (10 or more buds/0.785 mm^2).

Immunohistochemistry

The following EMT markers were tested: Snail, Zinc finger E-box-binding homeobox 1 (ZEB1), E-cadherin, and β-catenin. The tissue sections were incubated with the following primary antibodies at room temperature and the indicated dilutions: anti-β-catenin (1:200, Dako, Denmark), anti-E-cadherin (1:100, Dako, Denmark), anti-ZEB1 (1:150, Abcam, UK), anti-Snail (1:500, GeneTex, USA), and anti-vimentin (1:100, Dako, Denmark). Immunohistochemical staining was performed using the DAKO Kit system (DAKO, Denmark) along with a peroxidase/DAB Kit (DAKO).

The reactivity was assessed based on the percentage of positive cells and staining intensity. Staining intensity was classified into four levels: negative (0), weak (1), moderate

(2), and high (3). Five groups were established based on the percentage of positively stained cells: 0-5% (0), 6-25% (1), 26-50% (2), 51-75% (3), and 76-100% (4). A staining index score between 0 and 12 was calculated by multiplying the staining intensity score by the percentage of positive cells. A staining index score between 6-12 indicated positive protein expression, while a score between 0–6 indicated negative protein expression. The subcellular localization of the staining (nucleus, cytoplasm, and membrane) was independently evaluated for β-catenin and E-cadherin. Aberrant expression of E-cadherin and β-catenin was indicated by ectopic staining in the cytoplasm or nucleus and the absence of membrane staining.[12, 13]

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics software, version 26.0 (IBM Corp., Armonk, NY, USA). Categorical data were presented as numbers and percentages (n, %). The Chi square test and Fisher's exact test were used to assess associations between categorical variables. A P value <0.05 was considered statistically significant.

Results

Clinicopathological Characteristics of CRC Patients

A total of 101 patients (48 women and 53 men) diagnosed with CRC enrolled in the study. The patients' ages ranged from 18 to 83 years, with a mean age of 53.90 years. Tumor grade data showed that moderately differentiated tumors were the most prevalent, accounting for 76.2% of cases, while well-differentiated and poorly differentiated tumors accounted for 15.8% and 7.9%, respectively. Conventional adenocarcinoma was the most common histological type, found in 88 cases (87.1%), while mucinous carcinoma and signet ring cell carcinoma were found in 12 cases (11.9%) and

Table 1: Clinicopathological characteristics of colorectal carcinoma patients

Variants		n (%)
Age	<50	31 (30.7)
	≥50	70 (69.3)
Sex	Male	53 (52.5)
	Female	48 (47.5)
Location of Tumor	Right	43 (42.6)
	Left	58 (57.4)
Histological Types	Conventional adenocarcinoma	88 (87.1)
	Mucinous adenocarcinoma	12 (11.9)
	Signet ring cell adenocarcinoma	1 (1.0)
Grade	Well differentiated	8 (7.9)
	Moderate differentiated	77 (76.2)
	Poorly differentiated	16 (15.8)
Stage	I	22 (21.8)
	II	34 (33.7)
	III	37 (36.6)
	IV	8 (7.9)
Tumor Invasion	T1	5 (5.0)
	T2	26 (25.7)
	T3	56 (55.4)
	T4	14 (13.9)
Regional lymph node metastasis	N0	58 (57.4)
	N1	26 (25.7)
	N2	17 (16.8)
Distant metastasis	M0	93 (92.1)
	M1	8 (7.9)
Vascular Invasion	Positive	73 (72.3)
	Negative	28 (27.7)
Perineural Invasion	Positive	51 (50.5)
	Negative	50 (49.5)
Desmoplasia	Positive	66 (65.3)
	Negative	35 (34.7)
Lymphocytic Infiltration	Positive	69 (68.3)
	Negative	32 (31.7)
Total		101 (100)

one case (1.0%), respectively. Stage III was the most frequent, present in 37 cases (36.6%), while stage IV was the least common, present in eight cases (7.9%). More than half of the tumors (57.4%) were located in the left colon (58 cases). Data are shown in table 1.

Tumor Budding among CRC Patients

Out of 101 patients, 64 cases (63.4%) exhibited tumor budding. Table 2 summarizes all the relevant data. The most prevalent category was low (BD1), while the least common category was high (BD3). Figures 1 and 2 illustrate the tumor budding categories. Tumor budding and advanced tumor stage, tumor invasion, vascular invasion, perineural invasion, and desmoplasia were significantly associated. The link between regional lymph node metastases and tumor budding was highly significant (P=0001). Table 3 shows that N1 revealed 23/26 (88.5%) cases, and N2 revealed 17/17 (100%) cases affected by tumor budding.

Immunohistochemical Stains

Aberrant Epithelial Markers (β-catenin and E-cadherin) in CRC Patients

As shown in table 4 and figures 3 and 4, out of the 101 samples examined, 82 (81.2%) showed aberrant β-catenin localization, whereas 65 (64.4%) showed aberrant E-cadherin.

Table 2: Categorization of tumor budding of colorectal carcinoma patients

Categorization of Tumor Budding	Positive Tumor Budding n (%)	Negative Tumor Budding n (%)	Total n (%)
Low (BD1)	27 (26.7)		
Intermediate (BD2)	21 (20.8)		
High (BD3)	16 (15.8)		
Total	64 (63.4)	37 (36.6)	101 (100%)

BD1: Budding 1; BD2: Budding 2; BD3: Budding 3

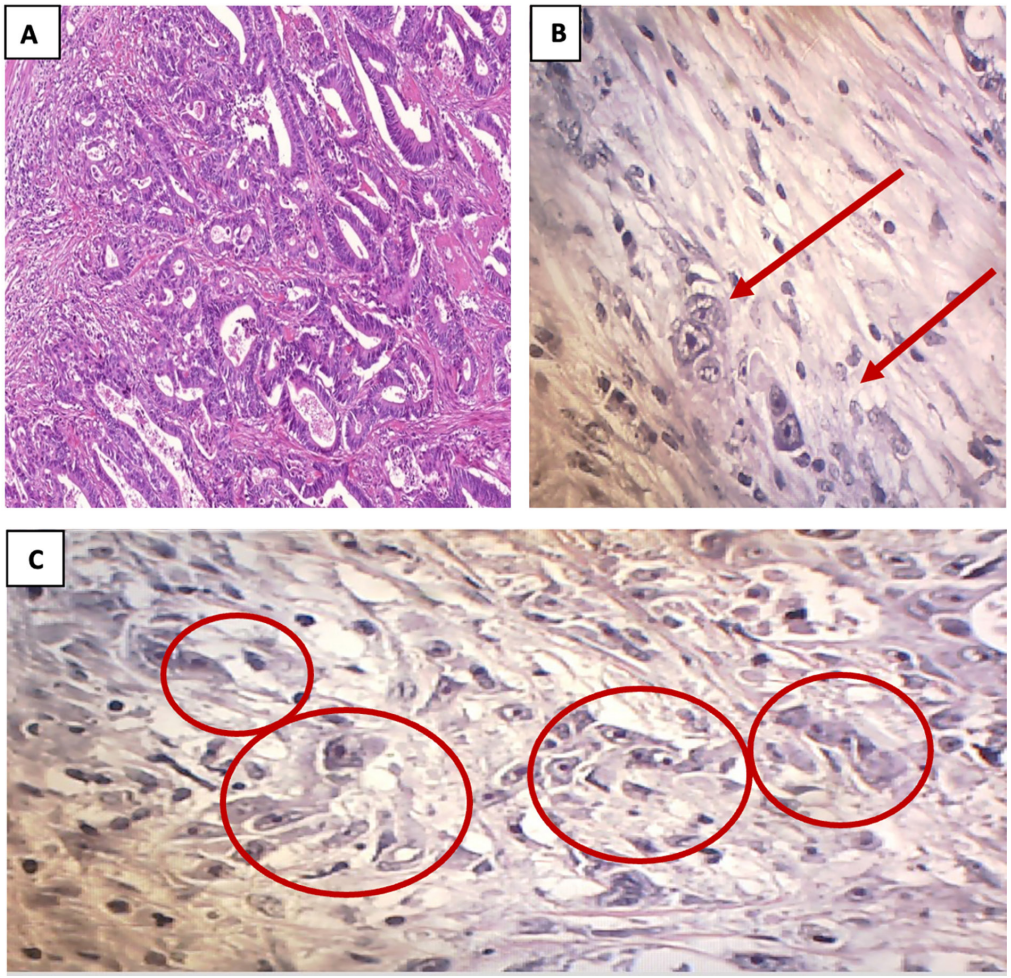

Figure 1: Histological images show tumor budding in colorectal carcinoma: (A) typical architecture of moderately differentiated adenocarcinoma (20×); (B) BD2 with 5–9 buds (red arrows) (40×); and (C) BD3 with ≥10 buds (red circles) (40×). BD2: Budding 2; BD3: Budding 3

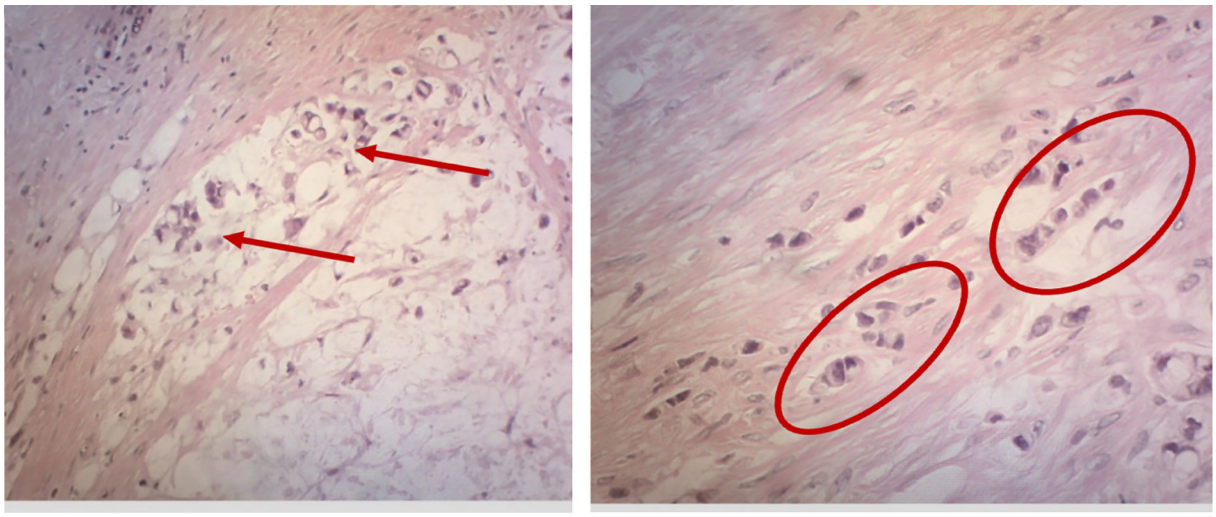

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Figure 2: Histological images show mucinous carcinoma with malignant epithelial cells in mucin pools (red arrows) and high-grade tumor budding (BD3) (red circles), indicating poor prognosis (40×).

Table 3: Association between tumor budding with clinicopathological characteristics of colorectal carcinoma patients

Variants		Tumor Budding			P value
		Positive n (%)	Negative n (%)	Total n (%)	
Age	<50	24 (77.4)	7 (22.6)	31	0.073*
	≥50	40 (57.1)	30 (42.9)	70	
Sex	Male	35 (66.0)	18 (34.0)	53	0.680*
	Female	29 (60.4)	19 (39.6)	48	
Location of Tumor	Right	27 (62.8)	16 (37.2)	43	0.999*
	Left	37 (63.8)	21 (36.2)	58	
Histological Type	Conventional Adenocarcinoma	56 (63.6)	32 (36.4)	88	0.846**
	Mucinous Adenocarcinoma	7 (58.3)	5 (41.7)	12	
	Signet Ring Cell Adenocarcinoma	1 (100.0)	0	1	
Grade	Well Differentiated	3 (37.5)	5 (62.5)	8	0.122**
	Moderate Differentiated	48 (62.3)	29 (37.7)	77	
	Poorly Differentiated	13 (81.3)	3 (18.8)	16	
Stage	I	4 (18.2)	18 (81.8)	22	0.0001**
	II	19 (55.9)	15 (44.1)	34	
	III	34 (91.9)	3 (8.1)	37	
	IV	7 (87.5)	1 (12.5)	8	
Tumor Invasion	T1	1 (20.0)	4 (80.0)	5	0.003**
	T2	11 (42.3)	15 (57.7)	26	
	T3	40 (71.4)	16 (28.6)	56	
	T4	12 (85.7)	2 (14.3)	14	
Regional lymph node metastasis	N0	24 (41.4)	34 (58.6)	58	0.0001*
	N1+N2	40 (93)	3 (7)	26	
Distant metastasis	M0	57 (61.3)	36 (38.7)	93	0.252**
	M1	7 (87.5)	1 (12.5)	8	
Vascular Invasion	Positive	54 (74.0)	19 (26.0)	73	0.001*
	Negative	10 (35.7)	18 (64.3)	28	
Perineural Invasion	Positive	41 (80.4)	10 (19.6)	51	0.0001*
	Negative	23 (46.0)	27 (54.0)	50	
Desmoplasia	Positive	48 (72.7)	18 (27.3)	66	0.010*
	Negative	16 (45.7)	19 (54.3)	35	
Lymphocytic Infiltration	Positive	43 (62.3)	26 (37.7)	69	0.826*
	Negative	21 (65.6)	11 (34.4)	32	
Total		64 (63.36)	37 (36.63)	101	

*Chi square; **Fisher exact test; Significant P value≤0.05

Table 4: Subcellular localization of β-catenin and E-cadherin in colorectal carcinoma tissue samples

Marker	Localization		
	Normal expression (membranous) n (%)	Aberrant expression (cytoplasm and nucleus) n (%)	Total n (%)
β-catenin	19 (18.8)	82 (81.2)	101 (100%)
E-cadherin	36 (35.6)	65 (64.4)	101 (100%)

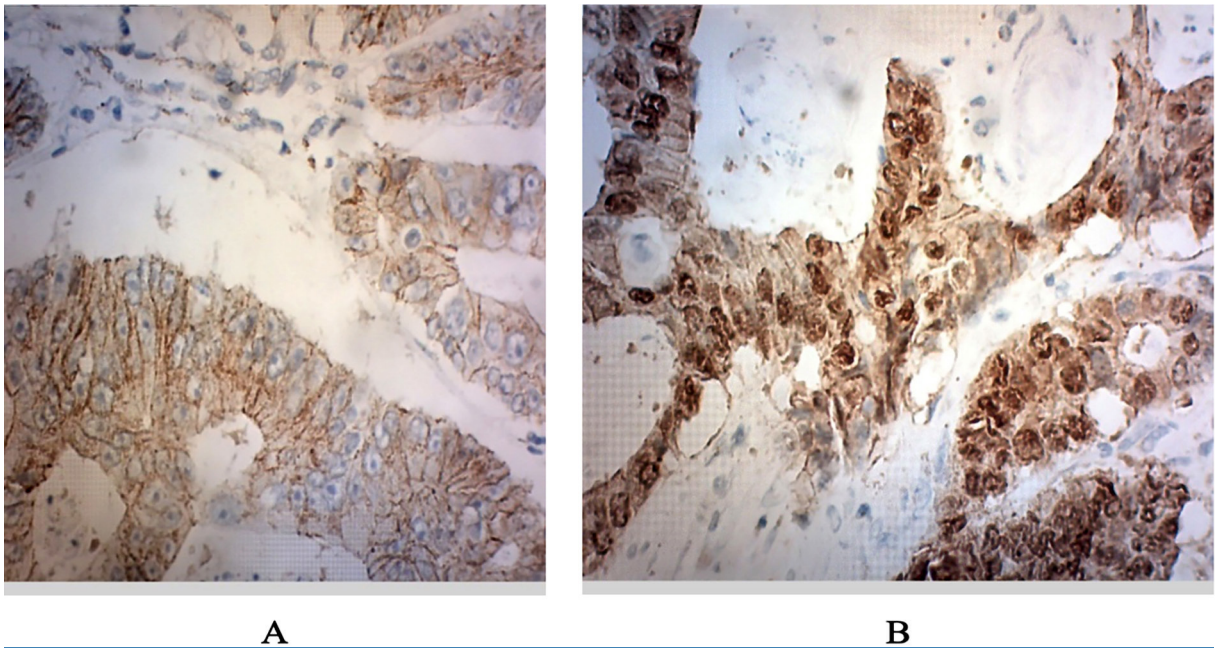

Figure 3: Immunohistochemical staining shows normal membranous β-catenin in tumor cells (A) and aberrant cytoplasmic/nuclear β-catenin (B), reflecting Wnt/β-catenin pathway activation and EMT promotion (40×).

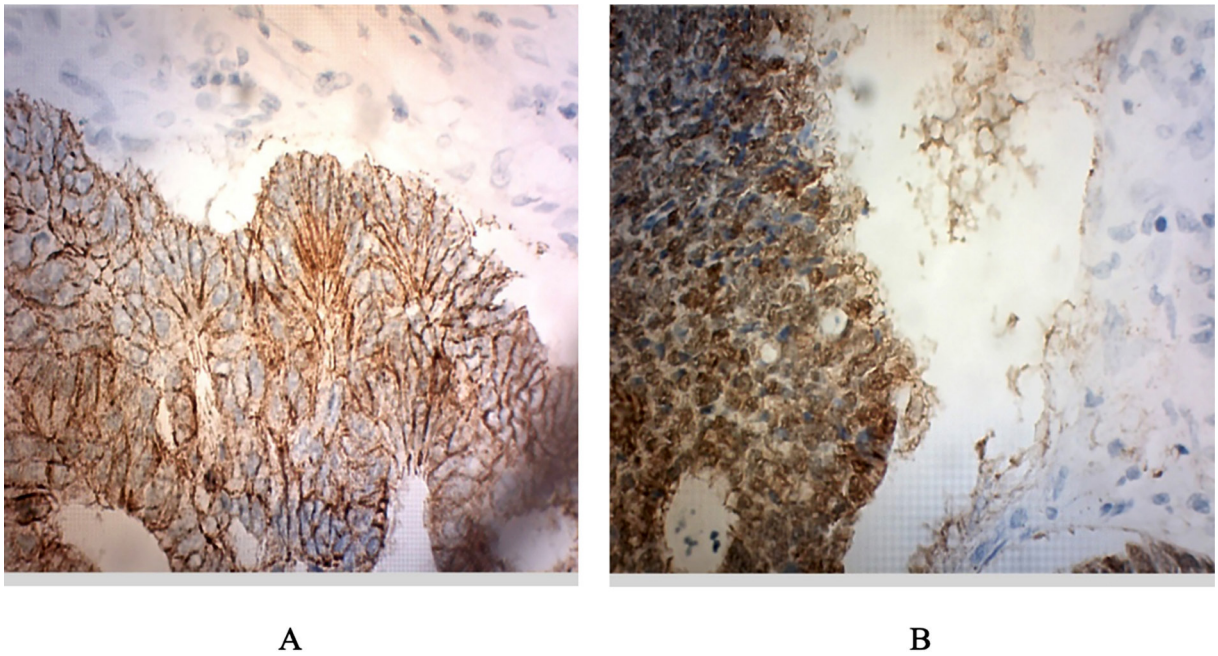

Figure 4: Immunohistochemistry shows normal membranous E-cadherin (A) and aberrant cytoplasmic/nuclear E-cadherin (B), indicating reduced adhesion and EMT-associated mesenchymal shift (40×).

In addition, table 5 demonstrates the relationship between the aberrant subcellular localization of E-cadherin and β-catenin and the clinicopathological characteristics of CRC patients. The study revealed a positive correlation between aberrant β-catenin expression and histological types (P=0.049), tumor invasion (P=0.004), and perineural infiltration (P=0.023). The correlation between clinicopathological characteristics and E-cadherin was not statistically significant.

Table 5: Association between subcellular localization of β-catenin and E-cadherin with clinicopathological characteristics of colorectal carcinoma patients

Variants		β-catenin				E-cadherin			
		Normal expression n (%)	Aberrant expression n (%)	Total n	P value	Normal expression n (%)	Aberrant expression n (%)	Total n	P value
Age	<50	8 (25.8)	23 (74.2)	31	0.273*	12 (38.7)	19 (61.3)	31	0.822*
	≥50	11 (15.7)	59 (84.3)	70		24 (34.3)	46 (65.7)	70	
Sex	Male	11 (20.8)	42 (79.2)	53	0.622*	16 (30.2)	37 (69.8)	53	0.299*
	Female	8 (16.7)	40 (83.3)	48		20 (41.7)	28 (58.3)	48	
Location of Tumor	Right	6 (14.0)	37 (86.0)	43	0.315*	14 (32.6)	29 (67.4)	43	0.676*
	Left	13 (22.4)	45 (77.6)	58		22 (37.9)	36 (62.1)	58	
Histological Type	Conventional adenocarcinoma	14 (15.9)	74 (84.1)	88	0.049**	30 (34.1)	58 (65.9)	88	0.487*
	Mucinous adenocarcinoma	4 (33.3)	8 (66.7)	12		6 (50.0)	6 (50.0)	12	
	Signet Ring Cell Adenocarcinoma	1 (100.0)	0	1		0	1 (100.0)	1	
Grade	Well Differentiated	3 (37.5)	5 (62.5)	8	0.299**	2 (25.0)	6 (75.0)	8	0.056**
	Moderate Differentiated	13 (16.9)	64 (83.1)	77		24 (31.2)	53 (68.8)	77	
	Poorly Differentiated	3 (18.8)	13 (81.3)	16		10 (62.5)	6 (37.5)	16	
Stage	I	7 (31.8)	15 (68.2)	22	0.365**	7 (31.8)	15 (68.2)	22	0.641**
	II	6 (17.6)	28 (82.4)	34		10 (29.4)	24 (70.6)	34	
	III	5 (13.5)	32 (86.5)	37		16 (43.2)	21 (56.8)	37	
	IV	1 (12.5)	7 (87.5)	8		3 (37.5)	5 (62.5)	8	
Tumor Invasion	T1	4 (80.0)	1 (20.0)	5	0.004**	2 (40.0)	3 (60.0)	5	0.901**
	T2	5 (19.2)	21 (80.8)	26		9 (34.6)	17 (65.4)	26	
	T3	10 (17.9)	46 (82.1)	56		19 (33.9)	37 (66.1)	56	
	T4	0 (0)	14 (100)	14		6 (42.9)	8 (57.1)	14	
Regional lymph node metastasis	N0	13 (22.4)	45 (77.6)	58	0.639**	17 (29.3)	41 (70.7)	58	0.197*
	N1	4 (15.4)	22 (84.6)	26		10 (38.5)	16 (61.5)	26	
	N2	2 (11.8)	15 (88.2)	17		9 (52.9)	8 (47.1)	17	
Distant metastasis	M0	18 (19.4)	75 (80.6)	93	1.000**	33 (35.5)	60 (64.5)	93	0.999**
	M1	1 (12.5)	7 (87.5)	8		3 (37.5)	5 (62.5)	8	
Vascular Invasion	Positive	11 (15.1)	62 (84.9)	73	0.156*	25 (34.2)	48 (65.8)	73	0.649*
	Negative	8 (28.6)	20 (71.4)	28		11 (39.3)	17 (60.7)	28	
Perineural Invasion	Positive	5 (9.8)	46 (90.2)	51	0.023*	18 (35.3)	33 (64.7)	51	0.999*
	Negative	14 (28.0)	36 (72.0)	50		18 (36.0)	32 (64.0)	50	
Desmoplasia	Positive	12 (18.2)	54 (81.8)	66	1.000*	24 (36.4)	42 (63.6)	66	0.999*
	Negative	7 (20.0)	28 (80.0)	35		12 (34.3)	23 (65.7)	35	
Lymphocytic Infiltration	Positive	12 (17.4)	57 (82.6)	69	0.785*	22 (31.9)	47 (68.1)	69	0.271*
	Negative	7 (21.9)	25 (78.1)	32		14 (43.8)	18 (56.3)	32	
Total		19	82	101		65 (64.4)	36 (35.6)	101	

*Chi square; **Fisher exact test; Significant P value ≤0.05

Table 6: Expression of mesenchymal transitional markers (Snail and ZEB1) in Colorectal cancer

Marker	Expression		
	Positive n (%)	Negative n. (%)	Total n (%)
Snail	55 (54.5)	46 (45.5)	101 (100%)
ZEB1	32 (31.7)	69 (68.3)	101 (100%)

ZEB1: Zinc finger E-box binding homeobox 1

Expression of Mesenchymal Markers (Snail and ZEB1) in CRC Patients

Snail and ZEB1 are typically expressed abnormally and are localized within the cytoplasm of tumor cells, as presented in figures 5 and 6. Out of the 101 cases, Snail reactivity was found in 55 cases (54.5%) and ZEB1 reactivity in 32 cases (31.7%). Tables 6 and 7 illustrate the correlation between Snail and ZEB1 expressions and the clinicopathological characteristics of CRC patients; was not statistically significant.

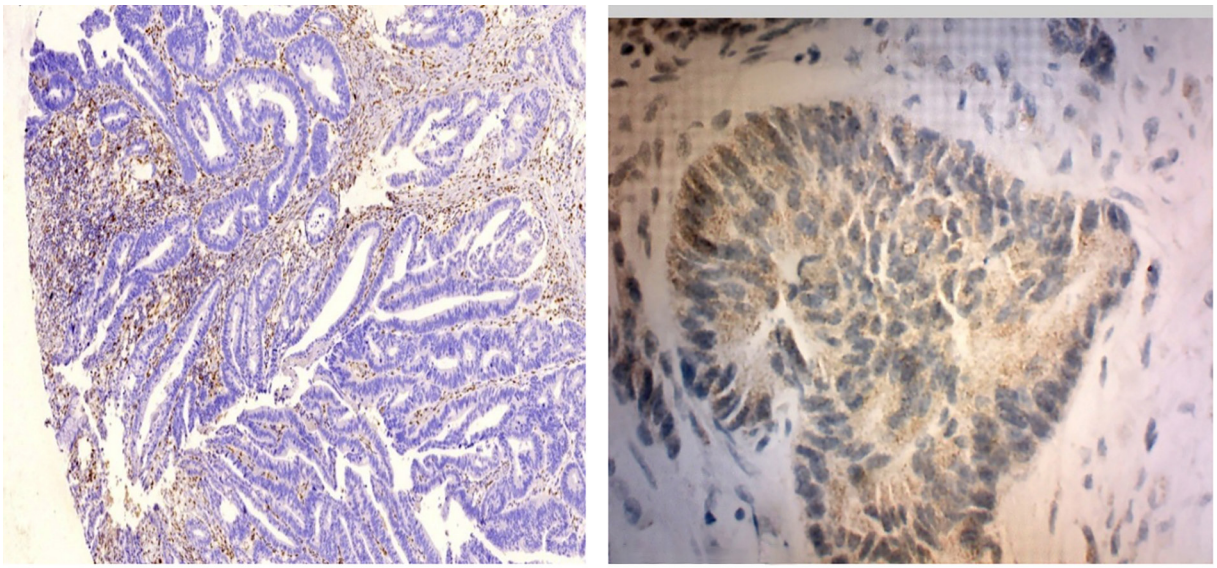

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Figure 5: Immunohistochemistry reveals Snail absence in tumor cells with stromal cell presence (A) (20×) and positive Snail expression in tumor cells (B), suggesting EMT-associated transcriptional changes (40×).

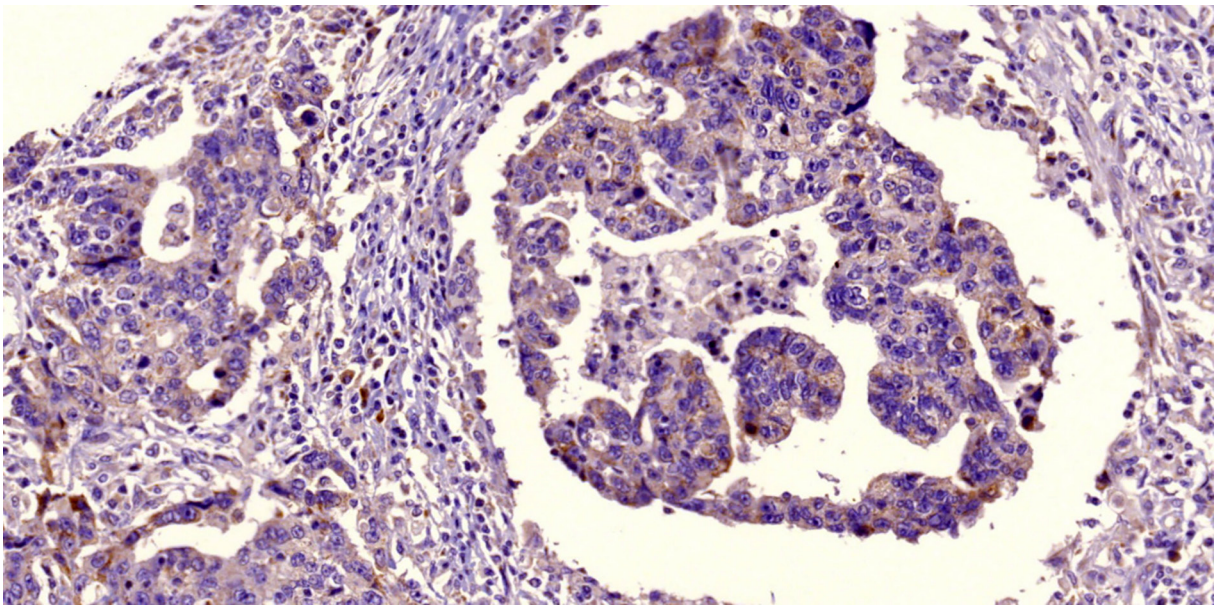

Figure 6: Immunohistochemistry shows cytoplasmic ZEB1 expression in tumor cells, indicating EMT activation and increased tumor invasiveness.

Association between Epithelial and Mesenchymal Markers (EMT)

The association between aberrant β-catenin and E-cadherin with mesenchymal marker expression (Snail and ZEB1) in CRC patients is shown in table 8. Aberrant β-catenin displays a significant association with ZEB1 (P=0.005).

Relationship between Tumor Budding and EMT Expression

The relationship between tumor budding and the expression of EMT markers, β-catenin, E-cadherin, Snail, and ZEB1 is presented in tables 9 and 10. The data showed that there is a significant correlation between tumor budding and Snail (P=0.002), while other EMT markers were not statistically significant.

Discussion

In this study, we evaluated the pathological and clinical significance of tumor budding TB and its association with EMT markers in CRC. Tumor budding was observed in 63.4% of cases and significantly correlated with advanced stage, tumor invasion, distant metastasis, vascular invasion, perineural invasion, and desmoplasia.

Table 7: Correlation between mesenchymal transitional marker expression (Snail and ZEB1) with clinicopathological characteristics of colorectal carcinoma patients

Variants		Snail				ZEB1			
		Positive n (%)	Negative n (%)	Total (%)	P value	Positive n (%)	Negative n (%)	Total (%)	P value
Age	<50	20 (64.5)	11 (35.5)	31	0.200*	12 (38.7)	19 (61.3)	31	0.357*
	≥50	35 (50.0)	35 (50.0)	70		20 (28.6)	50 (71.4)	70	
Sex	Male	25 (47.2)	28 (52.8)	53	0.162*	16 (30.2)	37 (69.8)	53	0.831*
	Female	30 (62.5)	18 (37.5)	48		16 (33.3)	32 (66.7)	48	
Location of Tumor	Right	27 (62.8)	16 (37.2)	43	0.163*	18 (41.9)	25 (58.1)	43	0.083*
	Left	28 (48.3)	30 (51.7)	58		14 (24.1)	44 (75.9)	58	
Histological Type	Conventional Adenocarcinoma	50 (56.8)	38 (43.2)	88	0.284**	29 (33.0)	59 (67.0)	88	0.827**
	Mucinous Adenocarcinoma	5 (41.7)	7 (58.3)	12		3 (25.0)	9 (75.0)	12	
	Signet Ring Cell Adenocarcinoma	0 (0)	1(100.0)	1		0 (0.0)	1 (100.0)	1	
Grade	Well Differentiated	5 (62.5)	3 (37.5)	8	0.686**	2 (25.0)	6 (75.0)	8	0.999**
	Moderate Differentiated	43 (55.8)	34 (44.2)	77		25 (32.5)	52 (67.5)	77	
	Poorly Differentiated	7 (43.8)	9 (56.3)	16		5 (31.3)	11 (68.8)	16	
Stage	I	12 (54.5)	10 (45.5)	22	0.338**	5 (22.7)	17 (77.3)	22	0.703**
	II	21 (61.8)	13 (38.2)	34		11 (32.4)	23 (67.6)	34	
	III	20 (54.1)	17 (45.9)	37		14 (37.8)	24 (62.2)	37	
	IV	2 (25.0)	6 (75.0)	8		2 (25.0)	6 (75.0)	8	
Tumor Invasion	T1	2 (40.0)	3 (60.0)	5	0.394**	1 (20.0)	4 (80.0)	5	0.600**
	T2	15 (57.7)	11 (42.3)	26		6 (23.1)	20 (76.9)	26	
	T3	33 (58.9)	23 (41.1)	56		19 (33.9)	37 (66.1)	56	
	T4	5 (35.7)	9 (64.3)	14		6 (42.9)	8 (57.1)	14	
Regional lymph node metastasis	N0	34 (58.6)	24 (41.4)	58	0.247*	17 (29.3)	41 (70.0)	58	0.092*
	N1	15 (57.7)	11 (42.3)	26		6 (23.1)	20 (76.9)	26	
	N2	6 (35.3)	11 (64.7)	17		9 (52.9)	8 (47.1)	17	
Distant metastasis	M0	53 (57.0)	40 (43.0)	93	0.137**	30 (32.3)	63 (67.7)	93	0.999**
	M1	2 (25.0)	6 (75.0)	8		2 (25.0)	6 (77.0)	8	
Vascular Invasion	Positive	41 (56.2)	32 (43.8)	73	0.658*	24 (32.9)	49 (67.1)	73	0.812*
	Negative	14 (50.0)	14 (50.0)	28		8 (28.6)	20 (71.4)	28	
Perineural Invasion	Positive	27 (52.9)	24 (47.1)	51	0.842*	19 (37.3)	32 (62.7)	51	0.286*
	Negative	28 (56.0)	22 (44.0)	50		13 (26.0)	37 (74.0)	50	
Desmoplasia	Positive	33 (50.0)	33 (50.0)	66	0.294*	22 (33.3)	44 (66.7)	66	0.660*
	Negative	22 (62.9)	13 (37.1)	35		10 (28.6)	25 (71.4)	35	
Lymphocytic Infiltration	Positive	37 (53.6)	32 (46.4)	69	0.833*	23 (33.3)	46 (66.7)	69	0.652*
	Negative	18 (56.3)	14 (43.8)	32		9 (28.1)	23 (71.9)	32	
Total		55 (54.5)	46 (45.5)	101		32 (31.7)	69 (68.3)	101	

*Chi square; **Fisher exact test; Significant P value≤0.05; ZEB1: Zinc finger E-box binding homeobox 1

Table 8: Association between subcellular localization of β-catenin and E-cadherin with mesenchymal transitional marker expression (Snail and ZEB1) in colorectal carcinoma patients

Marker	Expression	β-catenin				E-cadherin			
		Normal Expression n (%)	Aberrant Expression n (%)	Total	P value	Normal Expression n (%)	Aberrant Expression n (%)	Total	P value
Snail	Positive	10 (18.2)	45 (81.8)	55	1.000*	16 (29.1)	39 (70.9)	55	0.149*
	Negative	9 (19.6)	37 (80.4)	46		20 (43.5)	26 (56.5)	46	
ZEB1	Positive	1 (3.1)	31 (96.9)	32	0.005*	9 (28.1)	23 (71.9)	32	0.373*
	Negative	18 (26.1)	51 (73.9)	69		27 (39.1)	42 (60.9)	69	
Total %		19 (18.8)	82 (81.2)	101		36 (35.6)	65 (64.4)	101	

*Chi square; Significant P value ≤0.05; ZEB1: Zinc finger E-box binding homeobox 1

Table 9: Relationship between tumor budding and epithelial-mesenchymal transition markers in colorectal carcinoma

Criteria		Tumor Budding			P value
		Positive n (%)	Negative n (%)	Total n	
β-catenin	Positive	53 (64.6)	29 (35.4)	82	0.605*
	Negative	11 (57.9)	8 (42.1)	19	
E-cadherin	Positive	39 (60.0)	26 (40.0)	65	0.394*
	Negative	25 (69.4)	11 (30.6)	36	
Snail	Positive	30 (54.5)	25 (45.5)	55	0.062*
	Negative	34 (73.9)	12 (26.1)	46	
ZEB1	Positive	21 (65.6)	11 (34.4)	32	0.826*
	Negative	43 (62.3)	26 (37.7)	69	
Total n (%)		64 (63.37)	37 (36.63)	101	

*Chi square, Significant P value ≤0.05; ZEB1=Zinc finger E-box binding homeobox 1

Table 10: Relationship between tumor budding categorization and epithelial-mesenchymal transition markers

Tumor Budding Categorization	β-catenin		E-cadherin		Snail		ZEB1		Total n (%)
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	
Negative (BD0)	29 (78.4)	8 (21.6)	26 (70.3)	11 (29.7)	25 (67.6)	12 (32.4)	11 (29.7)	26 (70.3)	37 (100)
Low (BD1)	22 (81.5)	5 (18.5)	16 (59.3)	11 (40.7)	10 (37.0)	17 (63.0)	10 (37.0)	17 (63.0)	27 (100)
Intermediate (BD2)	19 (90.0)	2 (10.0)	13 (60.0)	8 (40.0)	10 (50.0)	10 (50.0)	5 (20.0)	16 (80.0)	20 (100)
High (BD3)	12 (75.0)	4 (25.0)	10 (62.5)	6 (37.5)	10 (62.5)	6 (37.5)	6 (37.5)	10 (62.5)	16 (100)
P value	0.678*		0.797*		0.579*		0.092*		

*Chi square; Significant P value ≤0.05; BD0: Budding 0; BD1: Budding 1; BD2: Budding 2; BD3: Budding 3

Immunohistochemistry revealed frequent aberrant expression of β-catenin and E-cadherin and positivity for Snail and ZEB1. Although EMT markers were commonly expressed alongside tumor budding, no statistically significant association was found, suggesting that tumor budding may independently serve as a stronger indicator of tumor aggressiveness in CRC.

Despite intensive efforts to elucidate the mechanisms behind tumor development and migration, they remain elusive and contentious. Since Stephen Paget's groundbreaking 19th-century work introduced the concept of seed and soil, a significant amount has been written and accomplished in the field of cancer metastasis.[14]

There is a good possibility of forecasting the tumor's course and the tumor management approach by considering the dynamics of tumor progression and metastasis. Tumor budding is a histological phenomenon that has been observed in several tumors characterized by the presence of individual or tiny clusters of malignant cells in the tumor stroma. Tumor budding is a real tumor growth pattern that can include zero buds to many buds. It is classified based on the number of buds present. However, it is debatable if the cutting artifact is the cause of a small number of tumor buds per defined area.[15]

This study confirmed that CRC patients had a significant tumor budding rate. Tumor budding was discovered in 64 cases (63.4%) out of 101 patients. It revealed that 21 and 27 patients (26.7%, 20.8%) had low and intermediate tumor budding, respectively, and that 16 patients (15.8%) had significant tumor budding. These results were consistent with those of Pyo JS and others,[16] who found that 135 (50.8%) and 32 (12.0%) of the patients had low and high tumor budding, respectively.

There was a significant association between tumor budding and aggressive tumor behavior, including stage, tumor invasion, regional lymph node metastasis, vascular invasion, perineural invasion, and desmoplasia. Tumor budding was also shown to be an independent prognostic factor linked to overall survival in CRC.[17-19]

Tumor budding was found to be an independent prognostic factor linked to lymph node metastases in numerous published studies.[19-21] Out of 43 patients in the current study identified with lymph node metastases, 40 (93%) demonstrated a correlation with tumor budding.

The subcellular distribution and loss of membranous β-catenin and E-cadherin in CRC tumor samples revealed a high percentage of aberrant β-catenin and E-cadherin expression,

81.2% and 64.4%, respectively, in an immunohistochemical analysis conducted to examine markers related to cell adhesion. In contrast, the mesenchymal markers ZEB1 and Snail exhibited high frequency, 32 (31.7%) and 55 (54.5%), respectively. These results were consistent with previous research.[22, 23]

Aberrant β-catenin and E-cadherin expression patterns and clinicopathological parameters showed a significant association, particularly with histological type, tumor invasion, and perineural invasion. These results agreed with Gao and others,[24] Hussein and colleagues,[25] and Bruun and others,[26] respectively.

Another study stated that there was no significant link between the prognosis and accumulation of β-catenin and E-cadherin in the cytoplasm and/or nucleus.[27]

Furthermore, the current study confirmed a feature of EMT: many cases showed mesenchymal transitional characteristics while maintaining epithelial characteristics. For instance, 16 cases (29.1%) had positive E-cadherin and Snail while β-catenin was normal, and 10 cases (18.2%) had positive Snail. ZEB1 and E-cadherin demonstrated a similar correlation, with nine cases (28.1%) showing positive ZEB1 and normal E-cadherin. ZEB1 expression was the lone exception, showing aberrant β-catenin in 31 cases (96.9) and normal β-catenin in one case (3.1). The sequences of tumor transition from epithelial to mesenchymal features and subsequent tumor invasion highlighted the same correlation.[28]

Tumor budding and EMT markers have a high frequency association. In 53/82 (64.6%), 39/65 (60.0%), 30/55 (54.5%), and 21/32 (65.6%) cases, tumor budding was concurrently present with abnormal β-catenin, E-cadherin, ZEB1, and Snail. Although this discovery did not have statistical significance, it did indicate a high rate of connection and suggested that the presence of EMT markers may not be a key concurrent factor in determining tumor budding status. The current study included 66 individuals (65.3%) with a high proportion of desmoplasia.

This study had certain limitations. The results were dependent on a particular sample set while leaving out important variables. Because of its relatively small sample size, the study methodology fell short of forecasting long-term outcomes, particularly the prognosis and survival rate, underlining the necessity of conducting thorough research to explore the clinical implications of tumor budding in CRC.

Conclusion

In repetitive histological sections of CRC, tumor budding is prevalent and can be measured independently. It significantly correlates with the metastasis of lymph nodes in staging parameters. In CRC, A significant correlation was found between aberrant β-catenin, E-cadherin, and expression of ZEB1 and Snail, which reflects that EMT markers are frequently altered and linked to tumor invasion and perineural infiltration. EMT has a high correlation with tumor sprouting. When compared to individuals with any level of tumor budding, EMT is less indicative.

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Authors' Contribution

The authors conceived the study, performed the research, analyzed the data, and wrote the manuscript. The authors read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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