DOI: 10.7860/JCDR/2023/63006.18546

Original Article

Pathology Section

Assessment of Tumour Budding in Colorectal Carcinoma and its Correlation with Pathological Staging among Patients undergoing Resection at a Tertiary Care Hospital in Kerala, India

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ABSTRACT

Introduction: Colorectal Carcinoma (CRC) is one of the most commonly diagnosed carcinomas and a significant cause of cancer-related deaths worldwide. The prognosis and treatment decisions rely on the Tumour, Node, Metastasis (TNM) Staging system. However, some tumours are classified as low-risk based on TNM stage exhibit adverse outcomes. Therefore, the search for additional prognostic factors is necessary. Tumour budding is an established independent prognostic factor, with high-grade tumour budding consistently linked to lymph node metastasis, local recurrence, and distant metastasis.

Aim: To assess and grade tumour budding in CRC cases and examine its correlation with pathological staging.

Materials and Methods: A cross-sectional study was conducted on 95 patients between December 2019 and December 2021 at Sree Gokulam Medical College and Research Foundation, Venjaramood, Trivandrum, India. Resected specimens from CRC patients were processed, and Haematoxylin and Eosin (H&E) slides were examined for tumour budding assessment. Ten individual fields were scanned under a 10x objective to locate the hotspot area with the maximum number of tumour buds. Tumour buds were then counted under a 40x objective in the selected hotspot area. Tumour budding was categorised as low (0-1 bud), intermediate (2-4 buds), or high (5 or more buds). Immunohistochemistry (IHC) analysis with Pancytokeratin was carried out when assessment with H&E slides alone was difficult. The correlation between tumour budding and pathological staging was evaluated, along with its association with various histopathological parameters.

Results: The study included 95 patients, with a mean age of 68.22 years, comprising 58.95% males and 41.05% females. Low-grade tumour budding was observed in 42 (44.21%) cases, intermediate-grade budding in 34 (35.79%) cases, and high-grade tumour budding in 19 (20%) cases. There was a significant correlation between tumour budding and pathological staging (r-value=0.39) as well as the number of metastatic lymph nodes (r-value=0.34). The presence of lymph node metastasis and Lymphovascular Invasion (LVI) showed a statistically significant association (p-value <0.01).

Conclusion: Tumour budding grading is a valuable histopathological finding, as it increases with higher T stage and presence of nodal metastasis, aiding in the prediction of nodal metastasis and recurrence. It is positively correlated with pathological staging and the number of metastatic lymph nodes. Including tumour budding grade in the histopathology report can assist clinicians in assessing prognosis and making treatment decisions.

Keywords: Colorectal cancer, Pathological staging, Tumour bud grade

INTRODUCTION

CRC is one of the most commonly diagnosed carcinomas and a significant cause of cancer-related deaths in the Western world [1]. Despite being the most curable type of carcinoma of the gastrointestinal tract, it remains the second most common carcinoma in females after breast carcinoma and the third most common carcinoma in males after lung and prostatic carcinomas. The average age at diagnosis ranges from 60-70 years [2]. In 2020, it was estimated that over 1.9 million new cases of CRC would occur, making it the second leading cause of cancer-related mortality [3]. In India, the Annual Incidence Rates (AARs) for colon cancer and rectal cancer in men are 5.36 and 5.17 per 100,000, respectively. The AAR for colon cancer in women is 4.3 per 100,000. The incidence rate is higher in Thiruvananthapuram in the South region and lower in Chennai [4]. The average annual Crude Rate (CR) of CRC is nine, and it ranks among the top five common cancers (CR per 10^5) in men [5]. The crude incidence rate (CR per 105) in urban females is 10.6, while in rural regions, it is 7.7 [6].

Surgical resection is the primary treatment modality for most CRC patients. The prognosis and treatment decisions are based on the extent of the disease, as indicated by the American Joint Committee on Cancer (AJCC) TNM Staging system [7,8]. However, some tumours exhibit adverse outcomes despite being categorised as low-risk based on their TNM stage [8]. Thus, there is a need to search for additional prognostic factors in CRC assessment, which has become an important area of research. Some of the most useful histopathological factors studied include the nature of the advancing front, extramural venous invasion, tumour budding, and microsatellite instability.

In CRC neoplastic cells undergoing epithelial mesenchymal transition are histologically represented by the presence of tumour buds [9]. Tumour budding was first described by Imai as sprouting at the invasive front of the neoplasm, reflecting a more rapid tumour growth rate. Tumour budding is defined as the presence of single tumour cells or small clusters of upto four tumour cells at the invasive margin [10]. High-grade tumour budding is associated with increased expression of protein markers related to extracellular matrix degradation and increased proliferation. Markers of cell adhesion and migration, such as E-cadherin or syndecan-1, are decreased in the centre of tumours with high-grade tumour budding,

along with decreased phospho-AKT, which impacts cell survival by inhibiting apoptosis.

Recognised by the International Union against Cancer as an additional prognostic factor indicating adverse outcomes, tumour budding is now a well-established independent prognostic factor consistently linked to lymph node metastasis, local tumour recurrence, and distant metastasis. Tumour budding is considered a useful indicator of the presence of isolated neoplastic cells in lymph nodes of patients with node-negative colorectal cancers, warranting additional laparotomy in patients who have undergone local excision of T1 tumours [10]. Assessing tumour budding helps enhance prognostic accuracy and enables treatment decisions. Tumours with high-grade tumour budding have a significantly lower 5-year Disease-Free Survival (DFS) rate compared to those with low-grade tumour budding. High-grade tumour budding is considered a worse prognostic factor in Stage-II colon carcinoma and is a risk factor for recurrence, influencing the consideration of adjuvant treatment [11].

Various methods of grading tumour budding have been described by Hase K et al., Ueno T et al., Nakamura T et al., and Roy P et al., [12-15]. The standardised method for tumour budding assessment was formulated at the International Tumour Budding Consensus Conference (ITBCC) 2016, paving the way for reporting tumour budding in routine practice in the future [16]. Given the increased incidence of CRC cases in Thiruvananthapuram and the role of tumour budding in predicting local recurrence and metastasis, this study aims to assess tumour budding and determine the proportion of patients with low-grade, intermediate-grade, and high-grade tumour budding. Additionally, it will evaluate its correlation with pathological staging, the number of metastatic lymph nodes, and its association with other histopathological findings such as histological type, presence of lymph node metastasis, lymphovascular invasion, perineural invasion, and pathological staging.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology at Sree Gokulam Medical College and Research Foundation, Trivandrum, Kerala, India, from December 2019 to December 2021. Ethical committee clearance was obtained (SGMC-IEC no: 35/479/11/2019), and consent was obtained from patients before the commencement of the study.

Inclusion criteria: A total of 95 patients with Colorectal Cancer (CRC) who underwent surgical resection were included in the study. Out of the 95 cases, 58 were from the study period, and 37 were patients who underwent surgery between January 2015 and November 2019.

Exclusion criteria: Patients who received neoadjuvant chemotherapy or underwent surgery for recurrence were excluded from the study.

Study Procedure

Resected specimens of patients with CRC were fixed in 10% formalin. Relevant clinical information was collected using proformas. Grossing of specimens was performed, and adequate sections were taken and processed. Paraffin-embedded blocks were prepared, and sections were cut at a thickness of 4 µm. The sections were stained with H&E and examined. Tumour staging was done according to AJCC criteria [7,8]. Tumour budding assessment was performed according to the proposal by Roy P et al., [15]. The slide with the greatest degree of budding at the invasive front was selected first. Then, 10 individual fields were scanned under a 10x objective to find the hotspot area with the maximum tumour buds. Tumour buds (single tumour cells or clusters of upto four cells) were counted in the selected hotspot area under a 40x objective. Tumour budding grading was done as follows: low (0-1 bud), intermediate (2-4 buds), high (5 or more buds) [15].

Immunohistochemistry analysis with Pancytokeratin was performed for IHC in which there was a prominent inflammatory reaction, and assessment only with H&E slides was difficult. A mouse monoclonal antibody (IgG1) was used to identify cytokeratin expressed in the neoplastic cells. Ethylenediaminetetraacetic Acid (EDTA) buffer was used for antigen retrieval, and sections were kept under steam pressure for 15 minutes. They were then washed with Tris-buffered Saline (TBS) and covered with the primary antibody for one hour. Later, they were treated with an amplifier and polymer detector for 12 minutes each. Diaminobenzidine (DAB) chromogen was used and kept for 10 minutes. The sections were then washed and counterstained with haematoxylin.

The hospital medical records were searched for CRC cases who underwent resection at our hospital between January 2015 and November 2019. Formalin-fixed paraffin-embedded tissue blocks and H&E-stained slides of these cases were retrieved, and all tumour sections were reviewed. Fresh cuts from blocks were made, stained, and reviewed in cases where the original stain had faded. Tumour budding grading was performed as described above. The patients were interviewed with a questionnaire to obtain information about any local recurrence or distant metastasis after the surgical procedure.

STATISTICAL ANALYSIS

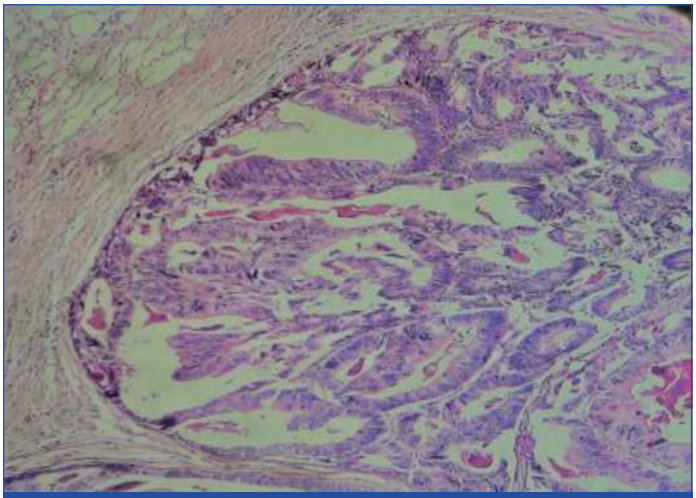
Microsoft Office Excel was used for data handling and preparation. The data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0 software. The results were expressed as percentages. Statistical tests such as Chi-square, Spearman rank correlation, and Mann-Whitney U test were used to determine the relationship between ordinal variables and compare ordinal parameters between groups. A p-value <0.05 was considered significant for all statistical interpretations.

RESULTS

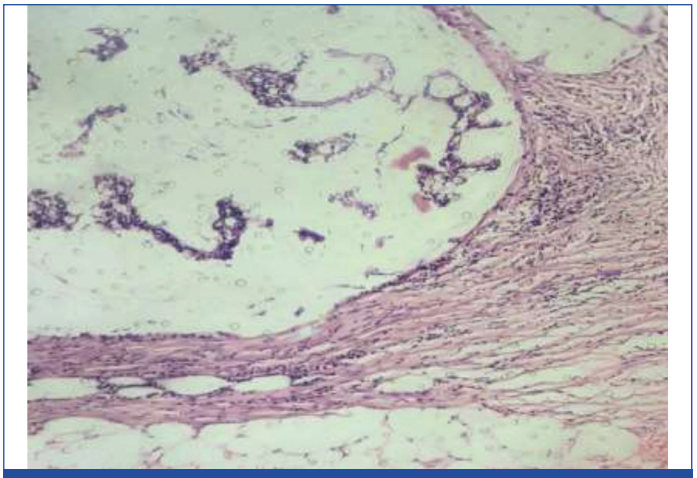
During the study period, a total of 95 cases were included in the analysis. The majority of the participants were in the age group of 71 to 80 years (n=32, 33.68%), while the least number of cases were in the younger age group of 31 to 40 years (n=2, 2.11%). The mean age of the participants was 68.22 years. Of the total cases, 58.95% were males (n=56) and 41.05% were females (n=39). The most common presenting complaint was bleeding per rectum (n=51, 53.68%), followed by constipation (n=15, 15.79%), weight loss and abdominal pain (n=11.58%). The most frequently received surgical specimen was low anterior resection (n=52, 54.73%), followed by sigmoidectomy (n=18, 18.95%). The most common site of tumour was the rectum (n=36, 37.89%), followed by the sigmoid colon (n=26, 27.37%) and rectosigmoid (n=13, 13.69%). Histologically, the majority of the cases were adenocarcinoma (n=84, 88.42%), while 11 cases were mucinous adenocarcinoma (11.58%). Most of the tumours were moderately differentiated (n=94, 8.94%). Lymphovascular invasion was observed in 32.63% of cases (n=31), and perineural invasion was seen in 14.74% of cases (n=14). Lymph node metastasis was present in 46.32% of cases (n=44). The majority of the tumours were classified as Stage III (n=37, 38.95%), while the least number of cases were Stage IV (n=8, 8.42%). The majority of the tumours were classified as T3 stage (n=72, 75.79%) [Table/Fig-1]. Tumour budding scoring was performed, with a minimum of zero buds and a maximum of 10 buds observed. Out of the 95 cases, 44.21% (n=42) showed low-grade tumour budding [Table/Fig-2a,b], 35.79% (n=34) showed intermediate-grade budding [Table/Fig-2c,d], and 20.00% (n=19) showed high-grade tumour budding [Table/Fig-2e,f]. IHC staining with tumour budding is shown in [Table/Fig-2g,h]. The association between histological type and tumour budding was not statistically significant (p-value=0.21) [Table/Fig-3]. Lymphovascular invasion was present in 16 cases with intermediate-grade tumour budding. The association between lymphovascular invasion and tumour budding was statistically significant (p-value <0.01) [Table/Fig-4]. Perineural invasion was most commonly seen in high-grade tumour

Characteristics		N (%)
Age (years)	31-40	2 (2.11)
	41-50	8 (8.42)
	51-60	20 (21.05)
	61-70	25 (26.32)
	71-80	32 (33.68)
	81-90	8 (8.42)
Gender	Male	56 (58.95)
	Female	39 (41.05)
Clinical presentation	Bleeding per rectum	51 (53.68)
	Constipation	15 (15.79)
	Loss of weight	11 (11.58)
	Abdominal pain	11 (11.58)
	Abdominal distension and vomiting	3 (3.16)
	Malena	4 (4.21)
Tumour site	Ascending colon	11 (11.58)
	Descending colon	9 (9.47)
	Sigmoid colon	26 (27.37)
	Rectosigmoid	13 (13.69)
	Rectum	36 (37.89)
Histological type	Adenocarcinoma	84 (88.42)
	Mucinous adenocarcinoma	11 (11.58)
Histological grade	Moderately differentiated	94 (98.94)
	Poorly differentiated	1 (1.06)
Lymphovascular invasion	Not identified	64 (67.37)
	Present	31 (32.63)
Perineural invasion	Not identified	81 (85.26)
	Present	14 (14.74)
Lymph node metastasis		
Present		44 (46.32)
Absent		51 (53.68)
pT stage	T1	6 (6.32)
	T2	14 (14.74)
	T3	72 (75.79)
	T4	3 (3.15)
pN stage	N0	51 (53.68)
	N1a	16 (16.84)
	N1b	9 (9.47)
	N2a	15 (15.80)
	N2b	4 (4.21)

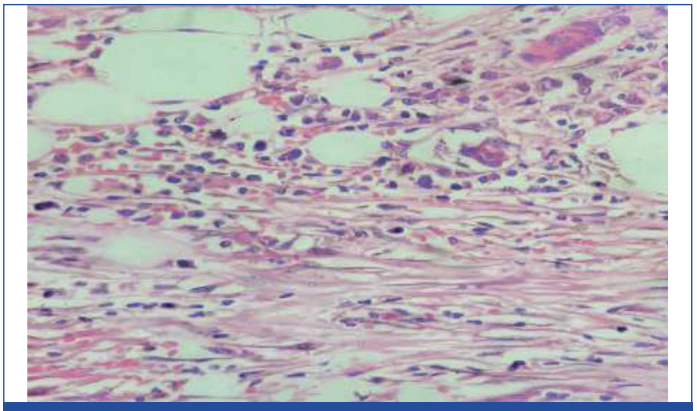
[Table/Fig-1]: Data on demographic and clinicopathological factors of 95 cases.



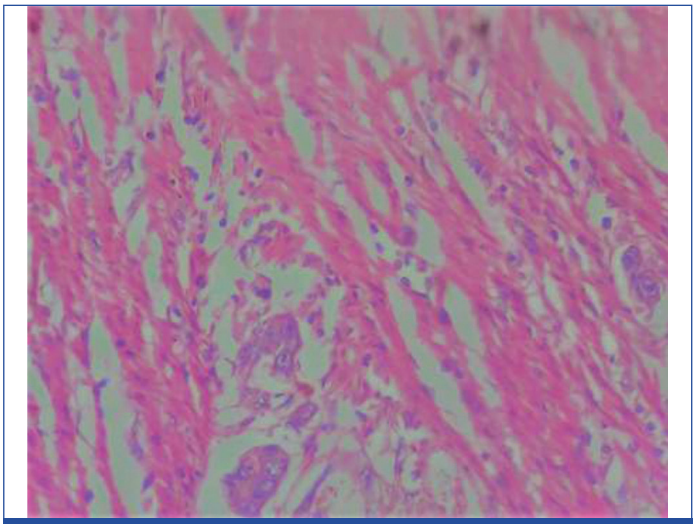
[Table/Fig-2a]: Low-grade tumour budding with zero buds (H&E, X400).



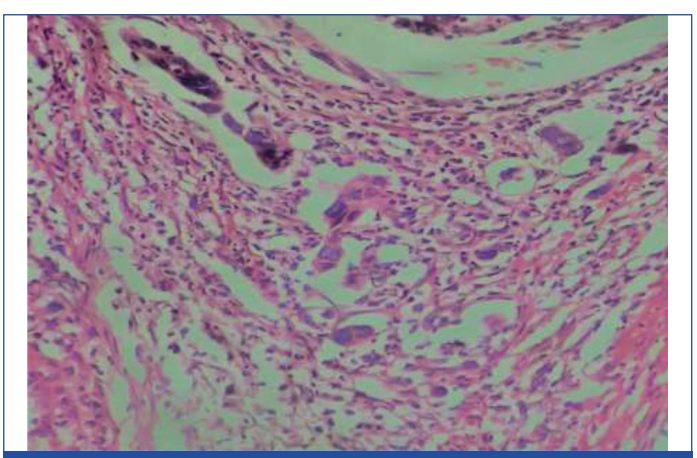
[Table/Fig-2b]: Low-grade tumour budding in mucinous adenocarcinoma with zero buds (H&E, X400).



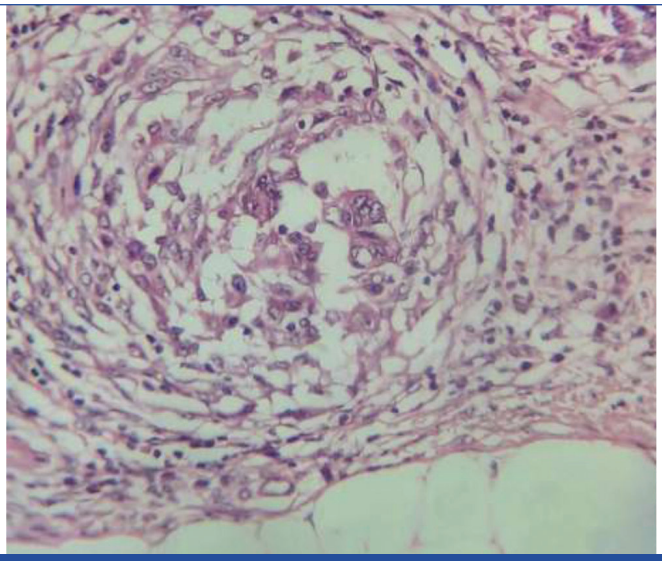
[Table/Fig-2c]: Intermediate-grade tumour budding with 2 tumour buds (H&E, X400).



[Table/Fig-2d]: Intermediate-grade tumour budding with 3 tumour buds (H&E, X400).

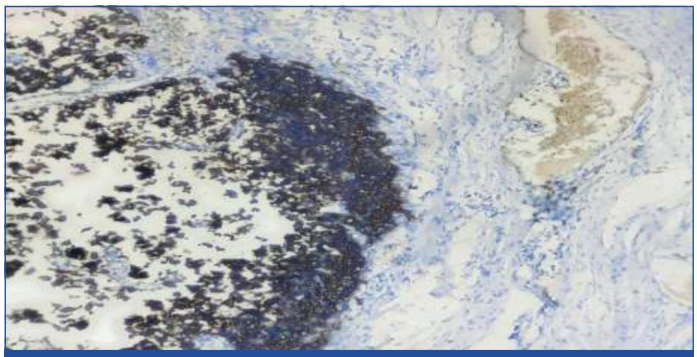


[Table/Fig-2e]: High-grade tumour budding with 7 tumour buds (H&E, X400).



[Table/Fig-2f]: High-grade tumour budding with 6 tumour buds (H&E, X400).

[Table/Fig-2g]: Intermediate tumour budding with 2 tumour buds (IHC, X400).



[Table/Fig-2h]: Low tumour budding with zero buds (IHC, X400).

budding cases (7 cases, 36.8%), and the association between perineural invasion and tumour budding was statistically significant (p-value: 0.002) [Table/Fig-5]. The majority of cases were in T3 stage (72 cases), and the correlation between T stage and tumour budding was statistically significant (r-value: 0.235, p-value: 0.02) [Table/Fig-6]. There was also a statistically significant correlation between the number of metastatic lymph nodes and tumour budding (r-value: 0.348, p-value: 0.001) [Table/Fig-7]. The association between lymph node metastasis and tumour budding was statistically significant (p-value: <0.01), with intermediate tumour bud grade being the most common in cases with lymph node metastasis (21 cases, 47.23%) [Table/Fig-8]. The most common AJCC stage was Stage III (37 cases), and the correlation between pathological staging and tumour budding was statistically significant (r-value: 0.39, p-value: 0.001) [Table/Fig-9]. Out of the 37 cases who underwent surgery between 2015 and 2019 and completed at least three years of follow-up, only one case (2.7%) showed local recurrence, four cases (10.8%) showed distant metastasis, and 18 cases reported death.

Histological type	Low-grade N (%)	Intermediate-grade N (%)	High-grade N (%)	χ^2	p-value
Adenocarcinoma	36 (85.7)	29 (85.3)	19 (100)	3.11	0.21
Mucinous adenocarcinoma	6 (14.3)	5 (14.7)	0		

[Table/Fig-3]: Association between histological type and tumour bud grade.

Lymphovascular invasion	Low-grade N (%)	Intermediate-grade N (%)	High-grade N (%)	χ^2	p-value
Not identified	37 (88.1)	18 (52.9)	9 (47. 4)	14. 88	p<0.01
Present	5 (11.9)	16 (47.1)	10 (52. 6)		

[Table/Fig-4]: Association between lymphovascular invasion and tumour budding grade.

Perineural invasion	Low-grade N (%)	Intermediate-grade N (%)	High-grade N (%)	χ^2	p-value
Not identified	41 (97.6)	28 (82.4)	12 (63.2)	12.72	0.002
Present	1 (2.4)	6 (17.6)	7 (36.8)		

[Table/Fig-5]: Association between perineural invasion and tumour budding grade.

T stage	Low-grade	Intermediate-grade	High-grade	Total
T1	4	1	1	6
T2	10	3	1	14
T3	27	30	15	72
T4	1	0	2	3
Total	42	34	19	95

[Table/Fig-6]: Correlation between T stage and tumour budding.
r=0.235, p-value=0.02 test applied-Spearman Rank Correlation and Chi-square

Lymph node status	Low-grade	Intermediate-grade	High-grade	Total
N0	33	12	6	51
N1a	2	9	5	16
N1b	5	3	1	9
N2a	1	9	5	15
N2b	1	1	2	4
Total	42	34	19	95

[Table/Fig-7]: Correlation between number of metastatic lymph nodes and tumour budding.
r=0.348, p=0.001-Spearman Rank Correlation and Chi-square

Tumour bud grade	Lymph node metastasis				Z#	p-value
	Present		Absent			
	Number of cases	Percentage (%)	Number of cases	Percentage (%)		
Low-grade	10	22.73	32	62. 75	3.79	p<0.01
Intermediate-grade	21	47.73	13	25. 49		
High-grade	13	29.54	6	11. 76		

[Table/Fig-8]: Association of tumour budding with lymph node metastasis.
#Mann-Whitney U Test

AJCC stage	Low-grade	Intermediate-grade	High-grade	Total
I	13	2	2	17
II	19	10	4	33
III	9	18	10	37
IV	1	3	4	8
Total	42	34	19	95

[Table/Fig-9]: Correlation between AJCC stage and tumour budding.
r=0.39, p-value: 0.001 #Spearman Rank Correlation and Chi-square

DISCUSSION

CRCs are the third most common carcinoma, accounting for 9.7% of all carcinomas. Now-a-days, the incidence of CRCs is rising in younger age groups due to modern dietary habits like increased consumption of red meat and lifestyle factors such as smoking, low physical exercise, and obesity. However, the mortality rate is low due to early detection, screening, and newer treatment modalities. Survival rates vary based on tumour behavior [17]. The most important prognostic factor in CRC is the pathological stage, but patients with the same stage can behave differently in terms of local recurrence and metastasis. Therefore, additional prognostic factors must be considered. Tumour budding is an emerging and promising prognostic factor as it is believed to be the first step in the metastatic process [18]. Present study assessed and graded tumour budding in CRC patients and found a statistically significant association between tumour budding and pathological staging.

Age is an important factor as the incidence of CRC is higher in older age groups and requires surveillance. The majority of CRC patients are above 50 years of age, with about 80% of colon cancer and 75% of rectal cancer patients being above 60 years of age at the time of detection [17]. In present study, the majority of cases were in the age group of 71 to 80 years (33.68%), which was comparable to previous studies [19]. The mean age in present study was 68.22 years. Among the participants, 56 were males and 39 were females. The majority were males (58.95%), consistent with previous research [20]. Most patients presented with bleeding per rectum 51 (53.68%), followed by constipation 15 (15.79%), loss of appetite 11 (11.58%) abdominal pain 11 (11.58%), similar to other studies [21]. The most common site of the tumour was the rectum 36 (37.89%), followed by the sigmoid 26 (27.37%), which aligns with previous findings [22].

The most frequent histological type was adenocarcinoma 84 (88.42%), followed by mucinous adenocarcinoma 11 (11.58%), and most tumours were moderately differentiated. These findings were similar to previous studies [23]. The association between histological type and grade with tumour budding was not statistically significant (p-value: 0.21), consistent with previous research [24]. Interestingly, seven cases arose from tubulovillous adenoma and three from villous adenoma, contrary to the expectation that villous adenomas have a higher chance of developing carcinomas [25].

Lymphovascular invasion was observed in 64 (67.37%) of cases, and perineural invasion in 14 (14.74%) of cases. Both lymphovascular invasion (p<0.01) and perineural invasion (p=0.002) were positively associated with tumour budding, similar to previous studies [22]. Another study showed that as tumour budding scores increased, lymphovascular invasion, perineural invasion, number of metastatic lymph nodes, and mortality rates also increased [23].

The majority of tumours (72 cases) were in T3 stage 72 (75.79%), indicating tumour extension through the muscularis propria into the pericolorectal fat, with no lymph node metastasis (pN0) in more than half of the cases (53.68%), consistent with previous research [26].

Tumour budding was low-grade in 42 cases (44.21%), intermediate-grade in 34 cases (35.79%), and high-grade in 19 cases (20.00%), similar to a previous study [26]. The 40x hotspot method was found to be more feasible in daily practice, as tumour buds were more easily appreciated on 40x magnification compared to 20x magnification, and counting was easier due to the smaller field area. Tumour budding assessed on routine H&E sections using both ×20 and ×40 worst field scores has high reproducibility and significant correlation with prognosis [3].

In Stage-I and Stage-II tumours, the majority of them show low-grade tumour budding. Thirteen out of 17 cases of Stage-I tumours show low-grade budding, and 19 out of 33 cases of Stage-II show low-grade budding. In Stage-III, 18 out of 37 cases show intermediate-grade tumour budding, and four out of eight cases of Stage-IV show high-grade budding. The correlation between AJCC stage and tumour budding was found to be statistically significant (r-value: 0.39, p<0.001), similar to the study by Mehta A et al., where an association between tumour budding and AJCC stage (p-value=0.021) was also found to be statistically significant [24].

The majority of cases in T1 and T2 stages show low-grade tumour budding. Thirty out of 72 cases in T3 stage showed intermediate-grade budding. The correlation between T stage and tumour budding was statistically significant (r-value: 0.235, p-value: 0.022). Petrelli F et al., in their meta-analysis, found that tumour budding was a significant prognostic marker for Stage-2 colorectal cancer, and high-grade tumour budding in these cases was associated with a 25% increase in the risk of death within five years [27]. Multivariate analysis revealed that tumour budding is an independent prognostic factor and is useful for identifying the subset of T3N0M0 patients who have a high risk of recurrence and would benefit from adjuvant treatment [28].

In a Delphi consensus study, the majority agreed that tumour budding score should be routinely assessed, and clinicians should take tumour budding into account when making decisions after local resection of pT1 CRC. When tumour budding is low-grade in pT1 CRC cases and there are no other risk factors, surgical resection is not necessary as the risk of nodal metastasis is considered very low. If intermediate or high-grade tumour budding is the only risk factor present, the need for additional surgical resection should be discussed, considering other clinical factors as well [29].

The majority of tumours with no lymph node metastasis (pN0) had low-grade tumour budding. The majority of tumours with lymph node metastasis had intermediate-grade (47.7%) and high-grade budding (29.5%). The association between lymph node metastasis and tumour budding was statistically significant (p<0.01), similar to the study by Mehta A et al., where an association between tumour budding and nodal involvement (p-value: 0.039) was also found to be statistically significant [24]. Most of the tumours with lymph node metastasis in four or more lymph nodes show high-grade tumour budding. The correlation between the number of metastatic lymph nodes and tumour budding was statistically significant (r-value: 0.348, p-value: 0.001), similar to the study done by Ozer SP et al., where the tumour budding score was positively correlated with the number of metastatic lymph nodes (p-value=0.011). The relationship between tumour budding score and lymph node stage is significant, and it is observed that as the degree of tumour budding increases, pN also increases [23].

Researchers found that the presence of tumour budding increased the risk of lymph node metastasis (OR-value of 6.44, 95% CI 5.26-7.87; p<0.0001) and concluded that it was an independent prognostic marker in pT1 CRC [29]. In the univariate analysis conducted by Kye BH et al., tumour budding was the only factor significantly correlated with lymph node metastasis [30]. Tumour budding was found to have high sensitivity (83.3%), acceptable specificity (60.5%), and a high negative predictive value (0.958) [27]. The association between tumour budding and tumour size, histological type, was not statistically significant (p-value=0.21), similar to the findings in the study by Mehta A et al., where no association was found between tumour budding and tumour size or histological type [24].

In a retrospective study of 37 participants, only one case had local recurrence, while four cases had metastasis. Approximately 51.4% of the patients followed-up were alive. The association between tumour budding and vital status was not statistically significant (p-value=0.18). However, a study by Ozer SP et al., found that patients with the presence of tumour buds had significantly lower cumulative survival rates compared to those without tumour buds. Additionally, patients with high tumour budding had significantly lower survival rates than those with low and moderate tumour budding grades (p-value <0.001) [23]. The incidence of recurrence was 6.4%, 12.1%, and 23.6% in the low, intermediate, and high

tumour budding groups, respectively (p-value <0.001). The grade of tumour budding was significantly associated with the incidence of recurrence in the liver, lung, lymph nodes, and peritoneum (p-value <0.001) [31].

Jäger T et al., investigated the prognostic significance of tumour budding for neoadjuvant treatment response in 128 rectal carcinoma patients and found that positive tumour budding was associated with poor 5-year relapse-free survival [32]. Changzheng Du et al., assessed the prognostic value of tumour budding in 96 rectal carcinoma patients after radiotherapy alone and consecutive curative resection and found that tumour budding in irradiated specimens was an independent prognostic factor for disease-free survival [33]. A multivariate proportional hazard model also found that the presence of budding was the only significant co-factor for postoperative survival [34].

In some cases, the assessment of tumour budding was difficult due to intense inflammatory infiltrate, and Pancytokeratin staining was done to identify the accurate tumour bud grade. Swathi M et al., found that there was an increased detection of high-grade tumour budding in IHC stained sections compared to H&E stained sections [21]. Satoh K et al., found that the high-grade of tumour budding assessed with cytokeratin stained sections detected more cases with venous invasion, lymphatic invasion, and lymph node metastasis compared to the budding grade assessed in H&E sections [22]. However, there was excellent concordance of tumour budding assessed on H&E slides and immunohistochemical stained slides of irradiated rectal carcinomas, indicating the feasibility of assessing tumour budding on H&E stained slides of irradiated specimens [27].

Tumour budding is an independent prognostic factor and high-grade tumour budding is associated with an increased chance of lymph node metastasis and recurrence [10]. It is now incorporated into the College of American Pathologists (CAP) protocol for reporting colorectal cancer and is to be reported according to ITBCC criteria [16,35]. Tumour budding can potentially be used to guide chemotherapy administration in patients. It has been found to be associated with worse survival in Stage-II CRC, especially in pT3N0M0 cases. Therefore, tumour budding can help in the decision about giving chemotherapy to high-risk patients with no lymph node metastasis [27]. Chemotherapy efficacy is comparable in high-grade and low-grade tumour budding groups, but the reduction in recurrence is greater in patients with a tumour bud count of 10 or more [36]. Tumour budding is also a reliable predictor for the chance of lymph node metastasis in T1 colorectal cancer, and in such cases, additional surgical resection may be warranted [37]. High-grade tumour budding is significantly associated with reduced cancer-specific survival, and it effectively stratifies patients' survival in primary operable colorectal cancer [38,39]. The reporting of tumour budding grade should be included in routine histopathology reports to help clinicians decide on further treatment and improve patient survival.

Limitation(s)

Tumour budding grading could not be done according to ITBCC criteria due to the unavailability of a x20 objective microscope. Additionally, not all patients could be followed-up, as some were referred elsewhere or lost to follow-up. Recurrence, metastasis, and vital status were only assessed in patients who underwent surgery between January 2015 and November 2019 and completed at least three years of postsurgical resection.

CONCLUSION(S)

Tumour budding is an important histopathological finding that is positively correlated with pathological staging and lymph node metastasis. It is preferential to mention the grade of tumour budding in histopathology reports, as it helps clinicians stratify patients into different risk categories and make decisions on further treatment modalities such as chemotherapy.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 25, 2023
- Manual Googling: Apr 13, 2023
- iThenticate Software: Jul 15, 2023 (20%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 21, 2023**
Date of Peer Review: **Mar 07, 2023**
Date of Acceptance: **Jul 19, 2023**
Date of Publishing: **Oct 01, 2023**