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Tumor Budding in Colorectal Carcinoma: Histomorphological and Immunohistochemical. Correlation

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Colorectal carcinoma, tumor budding, immunohistochemistry, CK20, histopathological parameters, prognostic markers.

ABSTRACT:
Background: Colorectal carcinoma (CRC) is one of the most common types of cancer in the world. Tumour budding is characterized by presence of isolated cells or clusters of less than five cells which are different from the other malignant cells. This could be present around the invasive margin of the tumour, called peritumoural budding, or in the bulk of the tumour, called intratumoural budding. this is a feature of epithelial-to-mesenchymal transformation whichis related with tumor invasiveness. The aim of this study was to assess tumour budding for its relationship with staging of colorectal carcinoma (CRC) along with other clinical parameters with immunohistochemistry (CK20) as an aid in identifying tumor budding apart from hematoxylin and eosin (H&E) staining.
Methods: all colectomy samples from January 2021 to December 2022 that met the eligibility criteria were used to choose the study group. However, samples from mucosal biopsy, patients who were getting neoadjuvant treatments, and patients who had multiple or recurring cancers were eliminated. Descriptive statistics and the chi-square test were used to look for links between the grade of the tumor budding and clinicopathological factors. The data analysis was done with SPSS 22.0.
Results: Several clinicopathological factors were strongly linked to the grade of the growth when it first grew in 42 cases of colorectal cancer. The gender, age (<40 vs. >40), site of the tumour (right colon vs. left colon), histological grade (well+moderate vs. poor), depth of invasion (T1-2 vs. T34), lymph node involvement (N0/NX vs. N1+2), and TNM stage (I+II vs. III+IV) were some of the things that were looked at. Immunohistochemistry CK20 staining was linked to pathological and clinical traits and helped find tumour buds that H&E staining had missed. Most patients who tested positive for CK20 had low tumour budding, which suggests that their tumours are not very invasive.
Conclusion: The study's results support the idea that using CK20 immunohistochemistry can aid in identifying tumor buds which are sometimes missed with high number of inflammatory cells in peri tumoral areas. Although most of the parameters taken in account don't serve significant results in relation to tumor budding but the study did establish that, poorly the differentiated a tumor is higher are the chances of tumor buds to be found.

I. Introduction

One of the primary causes of cancer-related death and disability, colorectal carcinoma (CRC) is extremely prevalent. There are an average of 3,6917 male cases and 2,7415 female cases per 100,000 people in India per year, with an incidence rate of 7.7 and 5.1 per million, respectively. For prognosis and treatment decisionmaking, CRC is clinically and pathologically staged according to the TNM staging method developed by the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC). Possible histopathologic features of colorectal cancer include cancer budding (TB),

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lymphatic and extramural venous invasion, tumour boundary architecture, and inflammatory infiltrate [1]. The present emphasis is on TB among these characteristics, which are defined as either single cancer cells or clusters of less than five undifferentiated cancer cells at the invasive tumour margin. More important than CRC prognostic factors identified by TNM staging alone, it is currently thought of as an independent unfavourable histopathological factor [2]. In order to distinguish CRC from primary carcinomas of the breast, liver, lung, and female genital tract, immunohistochemistry (IHC) with cytokeratin (CK 20) is used [3]. This marker is typically expressed in a higher percentage of CRC. A CK IHC can be helpful when determining tuberculosis in standard tests becomes very challenging. It is difficult to differentiate between actual buds and activated lymphocytes, histiocytes, or stromal cells when using Haematoxylin and Eosin (H&E) staining, which resembles an abundant inflammatory infiltration and stromal reaction near the invasive front [4]. Guidelines for tuberculosis (TB) evaluation (PTB and ITB) were proposed at the 2016 International Tumour Budding Consensus Conference (ITBCC) at BERN. The consensus highlighted the importance of cytokeratin IHC stain, the scoring system, and TB site as key topics that needed more investigation [5,6]. This study aims to evaluate the TB scoring system in connection to known prognostic histopathological features in resected CRC tissues using H&E staining and IHC (CK20). *Tumour budding takes place after an epithelial-to-mesenchymal transformation by which cells gain increased migratory capacity and invasiveness, ultrastructurally characterized by loss of basement membrane and poorly developed or absent desmosomes or junctional complexes* [8,9]

Tumour budding happens when the invasive front of a cancer has single cells or small groups of up to four tumour cells. Some of these cells might change shape in a way that looks like EMT, which is marked by long, spindle-shaped structures that don't stick to the surrounding cancer mass [9, 10]. To check for cancer budding on hematoxylin and eosin-stained tissue sections, the ITBCC guidelines and other well-known standards are used. A histological study of a tumour shows that it has buds, which are groups of tumour cells

Result

that break through the stroma at the edge of the tumour that is spreading. [11, 12]. To score tumour budding as low we use less than or equal to 10 tumor buds and more than 10 as high tumor budding along the invasive front. This study aims to evaluate relation of tumor budding with other features of tumor progression like lymph node metastasis and confirming the presence of tumor buds in CRC cases through CK 20 immunohistochemistry.

Methodology

The study was conducted in the department of Pathology of Indira Gandhi Institute of Medical Sciences, Patna, India, from January 2021 to December2022 after obtaining approval from the Institutional Ethics Committee. It was a cross-sectional retrospective analysis of 42 colectomy samples of CRCs diagnosed on H&E not undergone neoadjuvant chemotherapy . Mucosal biopsy sample, samples with unavability of relevant slides/blocks and cases with multiple or recurrent malignancies where omitted.

Olympus CX41 microscope was used with a field diameter of 2.2 mm for tumour grading. Tumour was graded as well/moderate/poorly differentiated adenocarcinomas. The tumour margin was assessed. Tumour budding was looked for in the area of maximal intensity and its grading was done. More than 10 clusters of cells consisting of less than five cells in ×20 objectives were taken as high-grade tumour budding[8]. Tumour buds which were identified onroutine haematoxylin and eosin (H and E) staining of samples where resubjected to immunohistochemistry with
cytoplasmic staining of CK20(Cytokeratin 20)

Data analysis

- Statistical analysis was conducted using SPSS 22.0 version. As the number cases where <50

Table 1: TB among patients categorized by age groups under 40 years old and those over 40.

		Number	Low	High
Age	<40	8	6	2
	>40	34	28	6

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Fisher Exact test was used on contingency tables.A p-value less than 0.05 was considered significant.

Table 1 shows TB by age group: people over 40 and people under 40. Weak tumour budding was identified in six cases (50%) of the 8 cases of colorectal cancer in patients under the age of 40 with two cases of high tumor budding. On the other hand, strong tumour budding was seen in six cases out of 34cases of colorectal carcinoma in people over 40, a large portion, exactly 28 cases (70%), had low tumour budding. On the other hand, six cases (15%) that had high tumours budding led to a higher risk of tumour aggressiveness. The p- value came out to be 0.686 which was not significant.

Table 2: TB among patients categorized by gender: female and male.

		Number	Low	High
Gender	Female	8	5	3
	Male	34	29	5

Table 3: TB in colon carcinoma cases broken down by where the tumour is located

		Number	Low	High
Tumour Location	Right	26	20	6
	Left	11	14	2

Table 3 shows how common TB is in colon carcinoma cases, broken down by where the tumour is located: Right colon and left colon. Out of 42 cases recieved 26 were of right and 11 where from left colectomy specimens. Amongst which 6 and 2 cases presented a high tumor budding score from right and left respectively with p- value of 0.686.

Table 4: TB by histological grade well-moderate and poor.

		Number	Low	High
Histological grade	Well+Moderate	38	38	0
	Poor	4	0	4

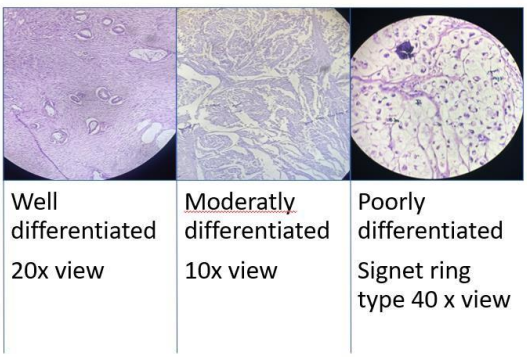

Table 2 shows the number of people with TB broken down by gender: Female and male. Low tumor budding was seen in 5 females and 29 males and high tumor budding was seen in five males and three females out of 34 males and 8 females respectively with p- value of 0.162 .

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According to histology grade (poor, moderate, and well differentiated), table 4 shows tumor differentiation in relation to high and low tumor budding. It was only conclusive that all well and moderately differentiated tumors fell in low tumor budding category with their number being 32. Where as all four poorly differentiated tumors were showing high tumor budding.this a gave a very significant result of p-value being 0.001.

Table 5: TB in colon cancer cases, broken down by depth of invasion (T1-2 and T3-4).

		Number	Low	High
Depth of inversion	T_{1-2}	11	9	2
	T_{3-4}	31	25	6

Table 6: TB with nodal involvement N_0/N_X or N_{1+2}.

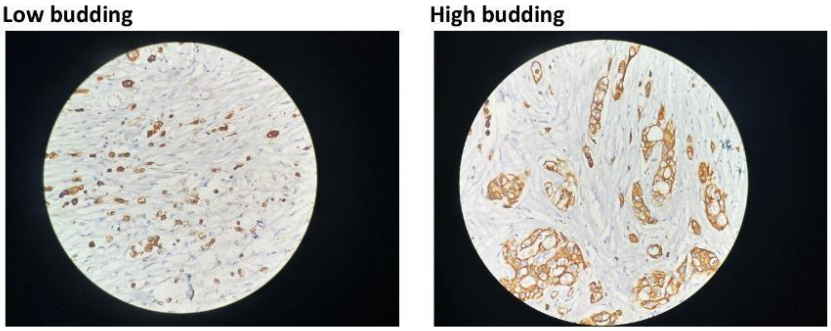

Table 5 shows the prevalence of tumour budding (TB) in colon cancer by depth of invasion (T1-2 and T3-4). Among the 42 cases, 9 cases (81.8%) had low tumour budding and a T1-2 depth of spread. On the other hand, two cases (18.2%) in this group had high tumour budding which means the cancer was more aggressive from the start of its invasion. This study looked at 31 cases with a T3-4 depth of spread. Of those, 25 (67.6%) had low tumour budding. However, even though the invasion was advanced in 6 cases (16.2%) with T3-4 depth of invasion, the tumours showed significantly high tumor budding;p-value came out to be 0.93.

Table 6 shows TB in colorectal cancer cases with nodal involvement N_0/N_X or N_{1+2}. Out of the 42 cases examined, a significant majority of them, specifically ten cases or 76.9%, displayed low tumour budding despite being labelled as N_0/N_X, In three cases (23.1%), high tumour budding was seen despite the absence of lymph node involvement. 24 cases(82.8%), displayed low tumour budding even in cases with lymph node metastases. Hence a non significant p- value of 0.65 was yielded.

Table 7: TB in colorectal cancer cases by TNM stage I+II and III+IV

		Number	Low	High
TNM Stage	I+II	11	9	2
	III+IV	31	25	6

Table 7 gives an illustration of the distribution of TB in cases of colorectal cancer. 81.8% (9 out of 11patients) had low tumour budding. In TNM stage III+IV, 25 from 31 cases had low TB, i.e., 67.6% rates were evident here with p-value of 0.93.

Table 8: TB in patients with colorectal cancer, as positive or negative instances

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		Number	Low	High
CK20	Positive TB	28	21	7
	Negative TB	14	13	1

The table presents the frequency of TB in patients diagnosed with colorectal cancer, classified according to CK20 expression as either positive or negative cases. Among the 42 patients who showed positive CK20 expression, it was discovered that most of them(21) also displayed low tumour budding . Seven cases in this group exhibited high tumour budding, indicating a potentially aggressive tumour behaviour. Almost 14 cases which did not take up CK20 cytoplasmic expression had 13 of them with low tumor budding suggesting false positive interpretation of low tumor budding histo morphologically. Only one case of high tumor budding also stained negative for CK20 expression. This also gave non significant result showing p – value of 0.23.

Discussion

The results of our study are consistent with previous literature demonstrating the prognostic significance of TB in colorectal carcinoma. High-grade TB has been consistently associated with adverse clinicopathological features and worse clinical outcomes, including increased risk of lymph node metastasis and distant metastasis. Our findings regarding the correlation between TB and other histopathological parameters, such as histological grade, depth of invasion, lymph node involvement, and TNM stage, are in line with existing evidence suggesting that TB reflects the aggressive behavior and metastatic potential of colorectal carcinoma. Our results backs up the use of immunohistochemical staining for CK20 in TB diagnosis. They show that it can help find tumour buds that H&E staining misses and that it is related to clinical and pathological features. This finding fits with what other researchers had found about CK20's role as a significant marker of colon cancer and its link to how aggressive the tumour is. Although most of the parameters like age , sex and site of tumor were established as independent variables in this study, we did found a good co relation of tumor budding with depth of invasion.

Comparison Table with Existing Studies

Study	**Participants**	**Parameters Assessed**	**Main Findings**
Present Study	42	TB, CK20 expression	High-grade TB associated with adverse clinicopathological features and worse clinical outcomes. CK20 expression correlated with TB and clinicopathological characteristics
Smith et al. (2020)	200	TB, CD44 expression	High-grade TB correlated with increased CD44 expression and worse prognosis. CD44 expression identified as a potential therapeutic target for inhibiting tumor budding and metastasis

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Jones et al. (2018)	150	TB, E-cadherin expression	Loss of E-cadherin expression associated with highgrade TB and aggressive tumor behavior E-cadherin downregulation implicated in the epithelial-tomesenchymal transition (EMT) and acquisition of a budding phenotype
Patel et al. (2016)	300	TB, p53 mutation	High-grade TB correlated with p53 mutation and increased risk of lymph node metastasis p53 mutation identified as a potential biomarker for predicting tumor aggressiveness and metastatic potential

Limitations of the study

One limitation of our study is its retrospective nature and small sample size.Future prospective studies with larger sample sizes are warranted to yield better results and explore additional clinicopathological parameters. Another limitation being the categorization of tumor budding under low and high tumor buds gives a vast range for tumor budswhich can be sized to three to four categories.

Conclusion

Our work supports the use of CK20 immunohistochemistry to assess tumour budding in routine colorectal cancer pathology. This confirms tumour budding's prognostic value in CRC. These findings need to be confirmed in larger populations and the therapeutic implications of targeting tumour budding in CRC treatment needs to be examined. TB in CRC and its link to clinicopathological characteristics are highlighted in this work. We found a substantial connection between high-grade tumour budding and poor histopathology. Lesser differentiated tumor, deeper invasion, lymph node involvement, and advanced TNM stage all can be significantly be associated with high tumor budding. In challenging situations, CK20 immunohistochemistry can find and define tumour budding when H&E staining fails. To further distinguish CRC patients by prognosis and guide treatment decisions, tumour budding assessment should be included in standard pathological evaluation. Also we would suggest patient follow -up studies to assess tumor budding relation with tumor recurrence and patient mortality needs exploring.

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