Original Article

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Histological Study of Tumour Budding in Colorectal Adenocarcinoma and Its Association with Tumor Stage and Nodal Status

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ABSTRACT

Introduction: CRC is commonest human carcinoma and leading causes of cancer-related death worldwide. "Tumor budding" is 4 or more tumor cells in invasive front of colorectal adenocarcinoma glands invading up to adjacent stroma, can act as independent prognostic marker for tumor staging which changes prognosis and further treatment plans

Objective: Association of tumor budding with stage of tumour and lymph node status.

Methods: This is a retrospective study from Jan 2019 – Jan 2022. H&E sections prepared from resected total/hemi/partial colectomy specimens to assess tumor bud count following International tumour budding consensus conference (ITBCC) 2016 criteria "Tumour budding assessed in 1 hotspot (in a field measuring 0.785 mm2) at the invasive front with average 10 consecutive fields with highest bud count in one field (hotspot) under 200x magnifications (field area=0.785 mm2) using Magnus Decahead microscope. The budding was graded as low (0-4 buds), intermediate (5-9 buds), high (10 or more buds).

Results: Analysis done according to ITBCC tumor bud scoring shows increased tumor budding is associated with positive higher tumor grade (p value: 0.026) and stage (p value - 0.024) but not with nodal status (p value: 0.096).

Conclusion: Current study shows tumor bud score is associated with positive lymph node status, higher tumor grade and stage. However, the study needs to be validated by a larger sample size and follow up studies.

Keywords: *Tumor Budding, IBCC, AJCC Stage, Lymph Node Metastasis, Colorectal Adenocarcinoma*

Introduction

"Tumor budding", defined as 4 or more tumor cells in invasive front of colorectal adenocarcinoma containing neoplastic cells from the neoplastic glands invading up to the adjacent stroma, can act as independent prognostic marker of outcome for staging and invasive nature of the tumor [1]

Colorectal carcinoma (CRC) is commonest human carcinoma and leading causes of cancer-related death worldwide. Prognosis and further treatment plans will be based on the stage of disease correlating with TNM staging system [2]

Endoscopically biopsy specimen of pT1 colorectal cancer, tumor budding is associated with high risk of lymph node metastases. Henceforth, patients with budding can undergo early surgical resection [3]

Some stage II colorectal cancer patients come with worse survival than stage III colorectal cancer

patients (who receive adjuvant chemotherapy Stage II patient doesn't get chemotherapy unless perforation, lymphovascular invasion) hence, tumor budding can be an "independent predictor of recurrence and survival in stage II colorectal" [3]

Intratumoral budding (ITB) is identified in biopsies prior to surgery could help to assess the patients who would qualify for neo-adjuvant therapy can detect the future outcome of tumor regression [3]

The goal of this study is to evaluate Tumor budding in histopathologically confirmed cases of adenocarcinoma of the colon and rectum as important predictor and its association with tumor staging and lymph nodal metastasis as a indicator for predicting the aggressiveness of Colorectal carcinoma in hospital based setting in Mangalore

Materials and Methods

Source of data

The study will be conducted in the central diagnostic laboratory of a tertiary care teaching hospital in Dakshina Kannada district. Anonymized tissue blocks from surgically resected specimens from the colon – Caecum, ascending, transverse, descending, sigmoid colon and rectum will be included in the study.

Method of collection of data (including sampling procedure, if any) Sample size

On the basis of the study conducted by Anurag Mehta et al (1) .It was observed that 71.6% cases(Tumour budding

count association with low grade tumour) with 95% confidence interval and 10% absolute allowable error. The sample size estimated for the study is 81.3% approximate equal to 81

Using the formula, , n=(Z(1-∝/2) P^((1-p)))/L

Sampling technique:

Purposive sampling technique will be adopted to select the subjects who meet the inclusion criteria.

Study design: It is a retrospective based study

Duration of the study: Retrospective study from Jan 2019 to Jan 2021 had been taken for the study.

Inclusion criteria: Histopathologically proved, newly diagnosed cases of adenocarcinoma of the caecum, ascending, transverse, descending, sigmoid colon and rectum, samples received from patients of age more than 18 years, both genders will be considered in the study.

Exclusion criteria: Adenocarcinoma of the gastric, small intestine, appendix, Mucinous cell carcinoma, Post- chemotherapy and post- radiotherapy cases, Benign neoplasms.

Methodology

Cases which are diagnosed as adenocarcinoma of colon and rectum and also fulfill the inclusion criteria are considered. The patient's case history and clinical examination will be obtained from the medical records. Relevant laboratory and radiological investigation reports will be noted.

For histopathological examination, sections of 3 to 5µm thickness are made with blocks from surgically resected specimens. These sections are initially stained with hematoxylin and eosin and will be assessed to note the grade and differentiation. H & E sections prepared from resected total/hemi/partial colectomy specimens were assessed to note tumor bud count. Tumor bud count was done following (ITBCC) 2016 criteria "Tumour budding was assessed in 1 hotspot (in a field measuring 0.785 mm^2) at the invasive front.[1]" The average bud count in 10 consecutive fields and the highest bud count in one field (hotspot) were assessed under 200x magnification (field area=0.785 mm^2) using Magnus Decahead Microscope.

The budding was graded as Low (0-4 buds), Intermediate (5-9 buds), High (10 or more buds).

Statistical analysis

Statistical analysis of the data was done using SPSS 23 software, descriptive statistics were calculated and summarized. These data included the average (mean and standard deviation), frequency and percentage. Descriptive and inferential statistical analysis has been carried out in the present study. The association between the attributes is found using chi square test. Level of significance was set at $p<0.05$.

Results

Of the **81** cases included in the study, average age 58.271±11.190 ranging from (24years —90). A total of 29 patients (35.8%) were female patients and 52 patients (64.2%) are male patients. Tumors classified according to American journal of cancer committee (AJCC) Stage I was reported in 52 cases (64.2%) patients, stage II in 9 cases (11.1%) patients, stage III in 19 case (23.5%) patients and stage IV in 1 case (1.2%) patients. 4 (4.9%) cases of Poorly differentiated, 49 cases (60.5%) and 28 cases (34.6%) of well differentiated patients. There are 61 (75.3%) cases with no nodal positivity, 13 (16%) cases with N1 status, 6 cases (7.4%) and 1 case (1.2%) with N2M1.

Stage I had 5 cases (9.61%) with High Count (HC), 16 cases (30.76%) with Intermediate count (IC) and 31 cases (59.61%) with Low count (LC). Stage II had 1 case (11%)) with HC, 6 cases (66.66%) with IC and 2 cases (22.22%) with LC. Stage III had 3 cases (15%) with HC, 13 cases (68%) with IC and 3 (15.78%) cases with LC. Stage IV had 1 case with IC. As the tumor stage increase more cases are seen with HC of tumor budding. There is significant association between AJCC stage and tumour budding**.** The Statistical analysis of the data were done using SPSS 23 software and association between the attributes is found using chi square test= 32.62. P value = 0.024. Level of significance was set at $p<0.05$.

Discussion

The present study done following Lugli A et al recommendations for reporting ITB in CRC based on ITBCC – 2016 consensus conference [1]. The study shows increased tumor budding score is associated with higher tumor stage (p value - 0.024). This result is similar to that of Mehta et al. [2] who found statistically significant correlations with AJCC stage (p-value 0.021). In the present study, there is no *significant* association between tumor budding score and nodal status (p value=0.096) in pT1 stage (n=61). However, 28 cases of pT1 CRC with N0 nodal status have high to intermediate tumor bud score. Stage II CRC with high tumor bud score has worse survival as compared to stage III. These patients can be started with adjuvant therapy to improve the survival outcome.

A study done by Anurag Mehta et al , showed that most of the cases were moderately differentiated adenocarcinoma (75%)with morphology of tumor invasion into the pericolic/subserosal fat (66.6%) and were stage III (38.3%). Nodal involvement was present in 50% cases. Correlations

Table 1: Crosstabulation of Patients gender, tumor budding score and AJCC stage.

Characteristic		Frequency	Percentage %
Gender	Female	29	35.8%
	Male	52	64.2%
Tumour budding score	Low	36	44.4%
	Intermediate	36	44.4%
	High	9	11.1%
AJCC stage	I	52	64.2%
	II	9	11.1%
	III	19	23.5%
	IV	1	1.2%

Table 2: Patient with Tumor Grade and lymph node status.

Grade Of Tumour	Poor	4	4.9%
	Moderate	49	60.5%
	Well	28	34.6%
Nodal Status	N0	61	75.3%
	N1	13	16%
	N2	6	7.4%
	N2M1	1	1.2%

Table 3: Association between AJCC stage and tumor budding.

AJCC STAGE	Tumour Budding			Total	P value
	High count	Intermediate count	Low count		
I	5 (9.61%)	16 (30.76%)	31(59.61%)	52	**0.024***
II	1(11.11%)	6(66.66%)	2(22.22%)	9	
III	3(15.78%)	13(68.42)	3(15.78%)	19	
IV	0	1(100%)	0	1	
Total	**9(11.11%)**	**36(44.44%)**	**36(44.44%)**	**81**	

Table 4: Association Between Tumor Budding Score And Nodal Status.

Tumour budding score	Nodal status				P value
	N0	N1	N2	N2M1	
High	6 (9.83%)	2 (15.38%)	1 (10%)	0	0.096
Intermediate	22 (36.06%)	8 (61.53%)	5(90%)	1(100%)	
Low	33 (54.09%)	3 (23.07%)	0	0	
Total: 81	**61**	**13**	**6**	**1**	

Table 5: Table Shows Association Between Grade Of Tumour And Nodal Status.

Grade of tumour	Nodal status				P value
	N0	N1	N2	N2M1	
Poor	3(4.9%)	0	1 (16.66%)	0	0.026*
Moderate	32 (52.45%)	11 (84.61%)	4 (66.66%)	1 (100%)	
Intermediate	0	0	1 (16.66%)	0	
Well	26 (42.62)	2 (15%)	0	0	
Total	**61**	**13**	**6**	**1**	

There exist significant association between grade of tumour and nodal status. (P value = 0.026 <0.05).

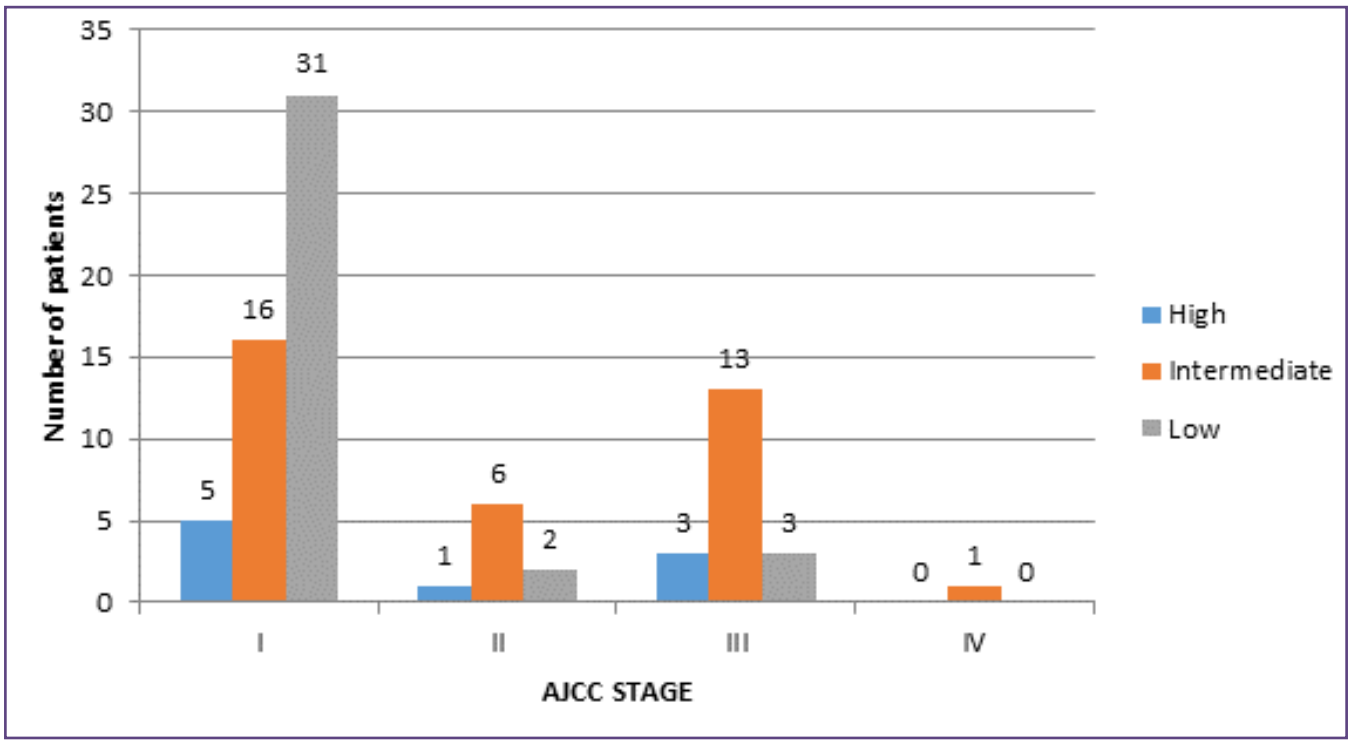

Fig. 1: The figure shows their association between AJCC stage and tumor budding score There exist significant association between AJCC stage and tumor budding. (P value = 0.024<0.05).

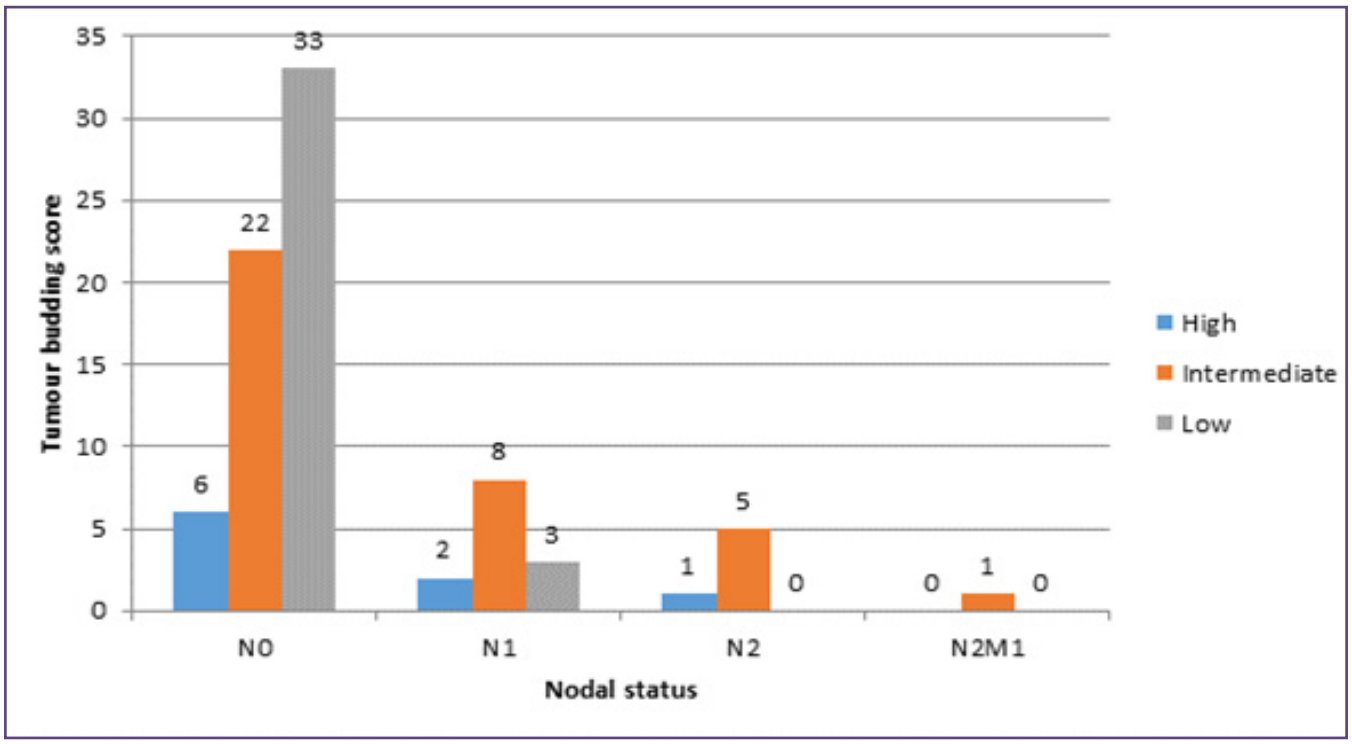

Fig. 2: Association between tumour budding score and nodal status . P value = 0.096 shows with no significant association.

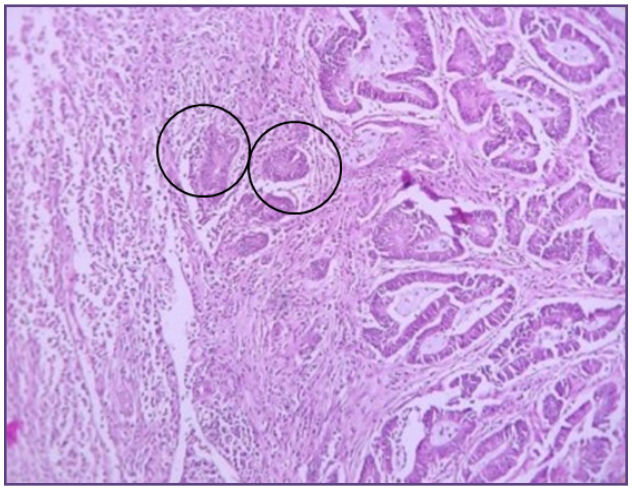

Fig. 3: H&E in 20X, circle shows tumour invasive front with tumour buds cluster "having 4 or more cells in a nest".

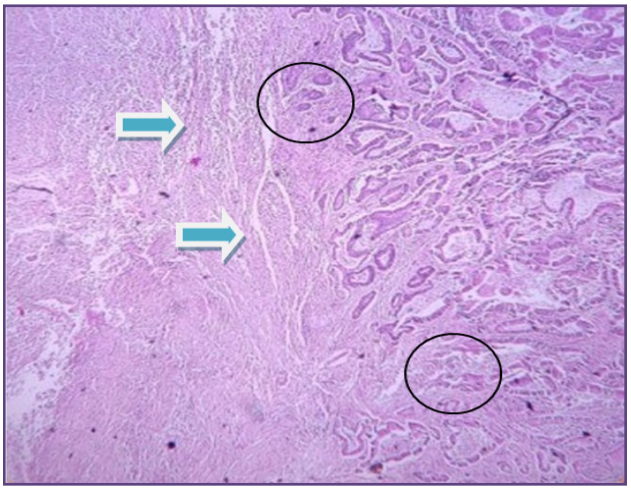

Fig. 4: H & E in 20X, Tumor buds "having 4 or more cells in a nest Tumor buds at invasive front, Arrow shows desmoplastic stroma with fibrosis.

between tumor budding and nodal involvement (*p*-value 0.039) and AJCC stage (*p*-value 0.021) were found to be statistically significant. Thus Tumor budding is trustworthy indicator of lymph nodal metastasis & higher stage of tumor predicting the aggressive outcome of CRC[2]

A similar study by Roy P et al in-resource constraints hospitals with dual study done in 40X magnification due to unavailability of microscope with 20X magnification in normal day to day use in India, Then the results were cross examined with 20X had similar high reproducibility and correlates significantly with prognosis. [4]

Higher count of tumor is more associated with higher grade which shows the link with the fragmentations and spread of the disease which proves that some Stage II patients had worst outcome compared to stage III. [5]

The Mechanism of epithelial–mesenchymal transition causes loss of cell adhesion molecules, cytoskeletal changes, increased production of extracellular matrix proteins, apoptosis survival with degrade basement membrane to evade resulting in increased migratory ability and invasiveness [3].

The stroma plays a crucial role in both the process and maintenance of Tumor budding, as well as the cell's spread. The relevance of CD10 expression in benign stromal cells need to be investigated. Because CD10 and MMP have structural similarities, it is postulated that CD10 could produce a microenvironment that aids cancer cell invasion and metastasis. Furthermore, there is a strong link between MMP-9 expression in budding cells and a more aggressive tumour phenotype [6].

A major risk factor which is seen associated with CRC ADC was obesity related which causes complex interactions with malignant cell transformation causing insulin resistance, growth factor , steroid hormone dysregulation, and chronic inflammation tumor microenvironment to stimulate carcinogenesis with cells undergoing epithelial mesenchymal pathway through a leptin-associated pathway had a similar link to β-catenin dysregulation[7] .

In a study conducted by Zolbec I et al shows that cases with 0 tumor budding with T2 N0 M0 also shows worst prognosis which is seen due to different histological type with mucinous, colloid type where stroma plays an important role of budding phenotype and spread due to stromal microenvironment and spread of it. [8]

T cell lymphocyte densities around inflammation shows intraepithelial CD3+, CD8+ , CD45+ lymphocytes cells were inversely associated with tumour budding at invasive front. suggest that anti-tumour immunity based on cytotoxic T cells causes suppression of microinvasion. High-grade tumour budding was linked to shorter colorectal cancer-specific survival

PDC grade and stage. [9]

Peritumoral budding PTB counted on surgically resected specimens and Intratumoral budding ITB counted on biopsy specimens which are often missed due to mucosal biopsies with cases with higher Intratumoral budding have more of invasive front and aggressiveness in later stage, hence ITB needed to employed on regular reporting of biopsies. [10]

"Pseudo budding" (maximum 4 cells) is a term used for artifacts and fragmentations mimicking ITB . so, use of Pancytokeratin as an tumor marker can be used to see stromal rection surrounding ITB. [11]

AJCC stage II CRC (pT3/4, pN0, M0) consists of patients with various overall survival years ranging from 66.5% (stage IIA) to 37.3% (stage IIC) compared to 5-year survival of 73.1% (stage IIIA) and 46.3% (stage IIIC) for node-positive patients. Thus, stage II at later stage is seen to have bad prognosis due to early relapse of CRC after surgery because of micro metastasis. [12]. In addition, high tumor budding is associated with aggressive lymphovascular space invasion but the use of tumor budding as a parameter in making treatment decisions have been advocated in stage II disease [13]

Patients with stage III CRC are usually offered adjuvant chemotherapy, while with stage II are not offered unless associated high-risk features such as tumour perforation, lymphovascular invasion, poor tumour differentiation that high tumor budding was associated with poor disease-free survival and act as independent prognostic factor. [14, 15]

This is an retrospective study done on institution where surgically resected specimen's PTB were not evaluated with Pan cytokeratin marker to differentiate from tumor fragmentation. Another drawback is of low sample size and lack of patient's follow-up details which are not evaluated with the overall survival of the patients and lack of family history investigations. Thus, further this studies needed with history of presentation, Pan CK evaluation, patients follow-up and evaluation for overall survival outcome needed to be recorded and evaluated.

Conclusion

The present study concludes that higher tumor bud score is associated with higher tumor grade and stage. There was no association between tumor bud score and nodal status. Biopsy specimens are not been the study needs to be validated by a larger sample size and follow up studies

Abbreviations

CRC: Colorectal, ADC: Adenocarcinoma, AJCC: American journal of cancer committee, MP : Matrix metalloproteinase, CD: Clusters of differentiation, ITB : Intratumoral budding : Peritumoral budding, PanCK: Pancytokeratin, HC: High count, IC: Intermediate count, LC: Low count, T: Tumor, N: Node , M: Metastasis

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Ethical consent approval

Granted as per guidelines of the Institutional Ethics committee. Global consent for research was taken at the time of surgery.

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Competing interests

No Conflict of interest

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