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Original Article

Association of Tumour Budding With Histological Type and Grade, Pathological Stage and Lymph Node Metastasis in Colorectal Carcinoma

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ABSTRACT

Objective: To investigate the association between the tumour budding of colorectal carcinoma and its histological type, grade, lymph node metastasis and pathological stage.

Study Design: Cross-sectional study.

Place and Duration of Study: Histopathology Department, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Dec 2021 to Mar 2023.

Methodology: One hundred and twenty (120) colorectal carcinoma patients were examined for existence and severity using Hematoxylin and Eosin-stained sections. According to the number of tumour buds, cases were categorised as low grade (<10/200X), intermediate grade (10-19/200X), and high grade (>20/200X). These categories were related to lymph node involvement, histological type and grade, and pathological staging. In challenging cases, pan-cytokeratin immuno-histochemistry labelling was conducted to confirm tumour budding.

Results: The mean age of presentation was 55.78±12.47 years. The most common site of involvement was the ascending colon 66(55%), followed by the recto-sigmoid colon 29(24.2%). Most cases were conventional adenocarcinoma 80(67%), followed by mucinous carcinoma 31(26%). Most cases were moderately differentiated 62(52%) and were stage III 79(66%). Forty-two (35%) had low-grade, and thirty-four (28.3%) had intermediate-grade and high-grade tumour budding. Tumour budding significantly correlates with tumour size, histological grade, invasion extent, and lympho-vascular invasion (p-value <0.05).

Conclusion: Tumour budding is strongly associated with nodal metastasis and a high grade of colorectal carcinoma; thus, it must be considered an important independent adverse prognostic indicator for colorectal carcinoma.

Keywords: Colorectal carcinoma (CRC), Prognostic marker, Tumour budding.

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INTRODUCTION:

Colorectal carcinoma is one of the most commonly occurring epithelial tumours in the human body worldwide.[1,2] Pakistan was previously considered a low-risk zone for colorectal carcinoma; recent studies have reported an increase in the incidence of CRC. The incidence of colorectal carcinoma in Pakistan ranged from 4-6.5%.[3,4] The average age of diagnosis is 72 years for women and 68 years for men. Males are at higher risk of developing colorectal carcinoma. Certain prognostic factors play important roles in disease progression and selection of further treatment modalities like pathological staging, lymph node metastasis, tumour type and grade, lymphovascular invasion and nature of advancing edge of the tumour.[5]

The appearance of isolated single tumour cells or tiny clusters of up to 5 tumour cells in the stroma at the invasive front of the tumour is referred to as tumour budding. Clinically, it exhibits as an aggressive biological marker for colorectal cancer. International Tumor Budding Consensus Conference (ITBCC) 2016 proposed standardised tumour budding assess-ment and mandatory reporting in colorectal cancer patients to improve prognostic accuracy and advance treatment choices.[6] Besides clinical staging, lymphovascular invasion, and microsomal gene alterations, tumour budding is an independent poor prognostic indicator in colorectal cancer. Regrettably, a lack of interpretational uniformity with regard to the type, amount, and grade of tumour budding has prevented its widespread recognition as a must-declared factor.[7]

Our research aimed to determine potential links between tumour budding and histological grade, T-staging, lympho-vascular invasion, and nodal metastasis in colorectal tumours. Our focus would be presenting a practical aspect of assessment regarding tumour budding.

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METHODOLOGY

The cross-sectional study was conducted at the Histopathology Department, Armed Forces Institute of Pathology, Rawalpindi, from December 2021 to March 2023. Using the WHO sample size calculator, the sample size was determined taking anticipated frequency of colorectal adenocarcinoma at 7.7%.[7]

Inclusion Criteria: Patients aged 20-80 years, of either gender who underwent surgical resection of the large gut were included.

Exclusion Criteria: Patients who underwent prior chemotherapy, radiotherapy or both and metastatic carcinomas were excluded.

Using a non-probability consecutive sampling method, the study enrolled 120 cases of colorectal cancer on surgically excised specimens. In gross specimen analysis, 5-10 sections were taken from each tumour specimen. Sections were processed in a vacuum-assisted tissue processor, and Hematoxylin and eosin-stained sections were assessed simultaneously by two histopathologists for the presence and number of tumour buds in the invasive front, as shown in Figure-1 (a & b). UICC/ TNM classification system 8th edition was followed to stage the tumours.[8] Significant physical characteristics such as tumour diameter, histological type and grade, the extent of invasion, and lymph node involvement were noted. Nearly 10-12 spots in each specimen were considered at a magnification of 200X to determine the area with the highest tumour budding density. The pan-cytokeratin immunohistochemical stain revealed tumour budding in situations with high levels of inflammatory cells, haemorrhage, necrosis, and enhanced desmoplastic stromal reaction, as illustrated in Figure-1(c & d). Low-grade tumour budding (10/200X), intermediate-grade tumour budding (10-19/200X), and high-grade tumour budding (≥20/200X) were the three categories into which tumour budding density was further categorised.[9] Quantitative variables, including age, T-staging, lymph node involvement and tumour budding, were presented by calculating mean and standard deviation. In contrast, qualitative variables, including gender, lymphovascular invasion and tumour deposits, were presented by calculating frequency and percentages.

With the help of Statistical Packages for Social Sciences (SPSS) version 20 (IBM Corp., Armonk, NY), data were examined, and statistical calculations using Chi-square tests were used to check any association between the variables with average and highest tumour budding area. The *p*-value lower than or up to 0.05 was considered as significant.

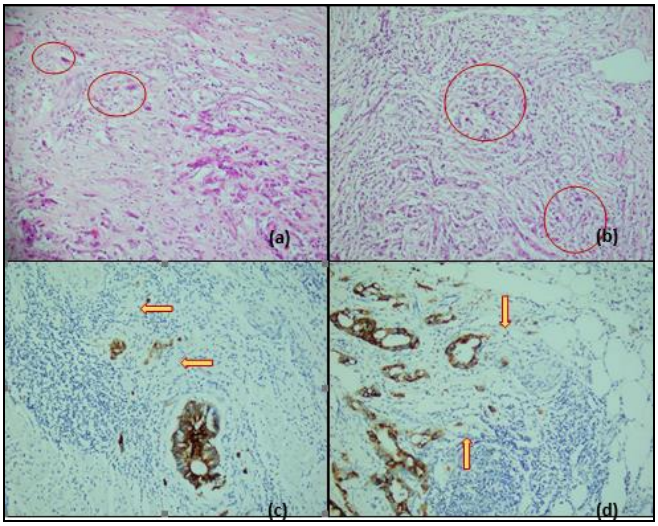

Figure: Tumor budding on hematoxylin and eosin (H&E) staining as marked by red circles in (a) and (b). Use of Pan-cytokeratin to highlight tumor budding in difficult cases because of excessive inflammation as marked by arrowheads in (c) and (d).

RESULTS

Among 120 cases of colorectal carcinoma recruited in the study, the average age was 55.78±12.47 years. 88(73%) cases were males, and 32(27%) were females.66(55%) The most common site of the primary tumour was ascending colon and cecum, accounting for the cases, followed by the recto-sigmoid area. In 66(60%) patients, moderately differentiated tumour grade was found, followed by 36(32.7%) cases with poorly differentiated tumours, and 8(7.3%) cases were well differentiated. 79(65.8%) patients had stage III tumours. As depicted in Table-I, nodal involvement was observed in 55(45.8%) patients. Figure illustrates hematoxylin and eosin staining of sections and immunohistochemical staining with pan-cytokeratin.

Of the 10-12 considered consecutive fields for counting tumour budding, there was no tumour budding in 10 (8.3%) cases, low-grade tumour budding (10/200X) was found in 42(35%) cases, intermediate-grade tumour budding (10-19/200X) was observed in 34(28.3%), and high-grade tumour budding (20/200X) was seen in 34(28.3%) cases. However, when the greatest tumour budding count per 200X field was assessed across the entire section, low-grade, intermediate-grade, and high-grade tumour budding were each observed in 29(24.2%), 38(31.7%) and 43(38.5%) of the instances, respectively, as shown in Table-II. The

association between different characteristics of tumour budding is summarised in Table-III.

Table-I: Characteristics of the Patients and Tumor Details (n=120)

Variables	Values
Age, years	55.8±12.5
Gender, n(%)	
Male	88(73.3%)
Female	32(26.7%)
Affected site, n (%)	
Right colon & cecum	66(55%)
Transverse colon	13(10.8%)
Left colon	12(10%)
Recto-sigmoid colon	29(24.2%)
Histological type, n (%)	
Adenocarcinoma	80(66.7%)
Mucinous adenocarcinoma	31(25.8%)
Signet ring adenocarcinoma	5(4.2%)
Neuroendocrine carcinoma	1(0.8%)
Gastrointestinal stromal tumor	3(2.5%)
Histological grade, n (%)	
Well-differentiated	10(8.3%)
Moderately-differentiated	62(51.66%)
Poorly-differentiated	48(40%)
Tumor size, cm	5.9±3.2
Lympho-Vascular Invasion, n (%)	
Seen	41(34.2%)
Not seen	79(65.8%)
Peri-Neural Invasion, n (%)	
Seen	27(22.5%)
Not seen	93(77.5%)
T-Stage, n (%)	
T1	2(1.7%)
T2	14(11.7%)
T3	79(65.8%)
T4	25(20.8%)
Lymph Node Involvement, n(%)	
Involved	55 (45.8%)
Uninvolved	65 (54.2%)

Table-I: Tumor Budding Depending on Average and Highest Count (n=120)

Tumor budding (Average count)	n (%)
No tumor budding	10(8.3%)
Low grade budding	42(35%)
Intermediate grade budding	34(28.3%)
High grade budding	34(28.3%)
Tumor Budding (Highest Count)	
No tumor budding	10(8.3%)
Low grade budding	29(24.2%)
Intermediate grade budding	38(31.7%)
High grade budding	43(35.8%)

DISCUSSION

Colorectal cancer is one of the most common malignancies in the world. Though it is thought that colorectal cancer is usually a disease of old age, in recent years, there has been a shift in the age of presentation. Now, more cases of this disease present early, as reported in many studies globally and locally. This age migration is more likely attributed to dietary factors and lifestyle modifications.[10] However, in Pakistan, no screening program is available for screening colorectal cancer.[11] In our study, the mean age of presentation was 55.78±12.47 years, as was seen in the study conducted by Pancione *et al*. in which the mean age of diagnosis of colorectal carcinoma is above 50 years.[12]

Colorectal cancer is more common in men, having a male-to-female ratio of 2.75. Worldwide, it is the second most common cancer in females with 9.2% and the third most common cancer in males with 10%, and this fact can be related to the increased smoking practice in males as compared to females.[13]

According to a study by Remo *et al*., 90–95% of colorectal carcinomas are classified as classic adenocarcinomas, while the second most common histological subtype is mucinous carcinoma.[14] According to a study conducted by El-Gendi *et al*., 90% of colorectal cancer is conventional adenocarcinoma.[15] Similar to what was shown in a study by Flaming *et al*., where 70% of the total colorectal carcinoma was moderately differentiated, the majority of the tumours in our study (66%) were moderately differentiated on histological differentiation.[16]

In our study, the ascending colon was the most common site of involvement in 66(55%) of cases, followed by the recto-sigmoid colon in 29(24.2%) cases. In contrast, in a study by Pancione *et al*., the sigmoid colon was the site of involvement in most cases. However, in older age groups, the ascending colon was the most common site of involvement in colorectal carcinoma.[17]

According to our results, in cases of colorectal carcinomas, high-grade tumour budding density is linked to higher histological grade, vascular invasion, and lymph node metastases. The study by Ueno *et al*. shows a direct correlation between colorectal cancer budding and spread. Our findings are consistent with previous studies who proposed a relationship between tumour budding and pathological factors in colorectal carcinomas, such as venous invasion and lymph node involvement.[18-20] These results support the hypothesis that tumour buds develop primarily from aggressive and more malignantly inclined cells. Despite a strong association between lymph node involvement and

Table-II: Association of Different Characteristics with Tumor Budding (n=120)

Characteristics	Tumor budding (Average count) n (%)		*p*-value	Tumor budding (Highest count) n (%)		*p*-value
	Low (10/200X) n=52	Moderate to Marked (>10/200X) n=68		Low (10/200X) n=39	Moderate to Marked (>10/200X) n=81	
Gender						
Male	37(30.8%)	51(42.5%)	0.104	29(24.2%)	59(49.2%)	0.374
Female	15(12.5)	17(14.2%)		10(8.3%)	22(18.3%)	
Tumor site						
Right colon	25(20.8%)	41(34.2%)	0.774	21(17.5%)	45(37.5%)	0.408
Transverse colon	6(5%)	7(5.8%)		3(2.5%)	10(8.3%)	
Left colon	21(17.5%)	20(16.7%)		15(12.5%)	26(21.2%)	
Tumor Size						
<5cm	31(25.8%)	23(19.2%)	0.009	23(19.2%)	31(25.8%)	0.019
5-10cm	18(15%)	38(31.7%)		14(11.7%)	42(35%)	
>10cm	3(2.5%)	7(5.8%)		2(1.7%)	8(6.7%)	
Histological Type						
Adenocarcinoma	34(28.3%)	46(38.3%)	0.723	26(21.7%)	54(45%)	0.936
Mucinous	15(12.5%)	16(13.3%)		12(10%)	19(15.8%)	
Signet ring	3(2.5%)	2(1.7%)		1(0.8%)	4(3.3%)	
Neuroendocrine	0(0%)	1(0.8%)		0(0%)	1(0.8%)	
GIST	0(0%)	3(2.5%)		0(0%)	3(2.5%)	
Histological Grade						
G-I	7(5.8%)	3(2.5%)	<0.001	5(4.2%)	5(4.2%)	<0.001
G-II	32(26.7%)	30(25%)		37(30.8%)	35(29.2%)	
G-III	13(10.8%)	35(29.2%)		7(5.8%)	41(34.2%)	
Lymph Node Involvement						
Seen	34(28.3%)	21(17.5%)	<0.001	29(24.2%)	26(21.7%)	<0.001
Not seen	18(15%)	47(39.2%)		10(8.3%)	55(45.8%)	
Extent of Invasion						
Submucosa	2(1.7%)	0(0%)	<0.001	2(1.7%)	0(0%)	<0.001
Muscularis propria	12(10%)	2(1.7%)		11(9.2%)	3(2.5%)	
Pericolorectal fat	37(30.8%)	42(35%)		25(20.8%)	54(45%)	
Metastasis	1(0.8%)	24(20%)		1(0.8%)	24(20%)	
Lympho-Vascular Invasion						
Seen	11(9.2%)	30(25%)	<0.001	7(5.8%)	34(28.3%)	0.002
Not seen	41(34.2%)	38(31.7%)		32(26.7%)	47(39.2%)	
PERI-neural invasion						
Seen	8(6.7%)	19(15.8%)	0.011	4(3.3%)	23(19.2%)	0.086
Not seen	44(36.7%)	49(40.8%)		35(29.2%)	58(48.3%)	

high-grade budding density, our findings showed no correlation between the number of affected lymph nodes and tumour budding density. In addition, our findings show that, in contrast to a previous study, the depth of tumour invasion is not associated with tumour budding density.[21] 47(78.3%) instances had high-grade budding at the area of the tumour segment with the highest bud count (hotspot). On the other hand, when the average number of buds per 200X field was taken into account, only 17(28.4%) of the 60 cases displayed high-grade budding (>10 buds), which is comparable to a study by Morodomi *et al*. (1989) that also used the average count option. In 27.5% of the tumours, high-grade budding was found.[19]

The results of our study reveal that high-grade tumour budding density is associated with aggressive phenotypical features in colorectal carcinoma, and it can be used as a practical and reliable parameter to identify higher malignancy potential. We suggest tumour budding as a risk factor for an adverse outcome in invasive colorectal carcinoma. Evaluating tumour budding can help improve the staging systems and treatment approach and can be an additional pathological parameter which helps determine tumour behaviour.

LIMITATIONS OF STUDY

Our study was limited by a small sample size and several observer pathologists. A large-scale multi-institutional study should be conducted to obtain more generalised results.

CONCLUSION

According to the findings of our study, high-grade tumour budding density can be relied on as a useful and accurate measure to detect higher malignancy potential because it is linked to aggressive phenotypical features in colorectal carcinoma. As a potential risk factor for a poor outcome in invasive colorectal cancer, we propose that tumour budding must be looked for in colorectal carcinoma. This extra pathological parameter can help improve staging systems and treatment strategies.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

WAK & BP: Conception, study design, drafting the manuscript, approval of the final version to be published.

MA & MUR: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

FR, HT & NK: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors 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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