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Evaluation of tumor stroma ratio in stage I to IV colorectal carcinoma at a tertiary care hospital in the Morang district, Nepal

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ABSTRACT

Background

The tumor stroma ratio has previously been shown to be a strong prognostic factor in colorectal carcinoma. It correlates with high-risk parameters like tumor stage, lymph node metastasis, and lymphovascular invasion. The present study aimed to analyze the tumor stroma ratio (TSR) in Stage I to Stage IV colorectal cancer and correlate it with tumor stage, lymphovascular invasion, and lymph node metastasis.

Material and methods

A total of 50 patients whose resection specimens had been received in the Department of Pathology, Birat Medical College Teaching Hospital, Nepal, during the study period. 8 cases who had neo-adjuvant chemotherapy were excluded. The invasive front of the tumor was evaluated using a 10x objective for the stroma percentage and was categorized into the high stroma (>50%) and low stroma (≤50%) TSR.

Results

Of 42 cases, 19 (45.2%) tumors have a low tumor stroma ratio, and 23(54.8%) show a high tumor stroma ratio. The low TSR is strongly associated with the high pathological stage of the tumor ($p < 0.001$) and the high lymph node stage ($p < 0.001$). Low TSR tumors were more common on the left side ($p < 0.001$). Lymphovascular invasion is associated with high stroma compared to low stroma ($p < 0.001$). The increasing tumor stage from I to IV strongly correlated with the TSR ($p < 0.001$).

Conclusion

The current study confirms the significant association between TSR and the corresponding tumor stage, metastasis in the lymph node, and lymphatic and vascular invasion.

Keywords
Carcinoma, Colorectum, Lymph node metastasis, Tumor Stage, Tumor stroma ratio.

Background

Colorectal (CRC) carcinoma is the most common malignant digestive tract tumor globally. According to GLOBOCAN 2020 data, CRC is the third most diagnosed cancer in the world, corresponding to 10% of all cancer diagnoses [1]. In Nepal, the incidence of colorectal carcinoma is 5.5%, the sixth most common cancer after lung, cervix, breast, and gallbladder, and it accounts for 6.1% of mortality [2]. The tumor, node, and metastasis (TNM) staging system is the most used method to determine the prognosis and guide treatment [3]. The TNM classification is based mainly on the depth and extent of tumor invasion. Still, there is always a need for additional predictive and prognostic markers that can guide the patient's course of disease [3, 4]. Various biomarkers specific to genetic pathways, gene mutations, the origin of cells, genetic expression, and tumor immune response have been proposed to identify the prognosis and recurrence of colorectal carcinoma [5, 6]. The drawback is their high cost and sophisticated lab technique. So, a biomarker based on simple histopathological examination is desirable and, in turn, cost-effective and sustainable [3, 6]. With that, TSR, also known as tumor-stroma percentage, evaluated on the most invasive front of tumor in haematoxylin and eosin-stained sections, is a promising biomarker and correlates with worse prognosis. Moreover, TSR has also been shown to predict the response to adjuvant therapy, with high TSR showing resistance to chemotherapy [6-10].

The present study aimed to analyze TSR in Stage I to Stage IV colorectal cancer and to correlate tumor stroma ratio about tumor stage, lymphovascular invasion, and lymph node metastasis.

Material and methods

Study design, participants and the experimental procedure

A total number of 50 patients were included, and they were advised to visit the hospital laboratory. A verbal and written consent was taken. Resection specimens of all colorectal cancer patients during the study period were fixed in 10% neutral buffered formalin, appropriate margins were inked, grossed, and processed in a tissue processor, paraffin-embedded tissue blocks were sectioned in 5-micrometer thickness, and slides were stained with Hematoxylin and Eosin stain. Slides were evaluated under a light microscope, and the most invasive part of the tumor was evaluated using the 10X field, and the fields were scored in 10% increments. Tumor cells should be present in all four edges of the selected field. The stroma percentage was estimated and was scored in four groups: 1: TSR >75%, 2: 50% <TSR< or equal to 75, 3: 25%<TSR < or equal to 50%, 4: TSR < or equal to 25%. The four groups' values were categorized into two groups: low stroma (≤50%) (Figure 1A) and high stroma (>50%) (Figure 1B). Respective stage I to IV of the tumor, metastasis in lymph nodes with a total number of positive lymph nodes and presence of lymphatic and/or vascular invasion, distant metastasis in each low and high TSR was evaluated.

Inclusion criteria

An individual whose right or left hemicolectomy, abdominoperineal resection (APR), and low anterior resection (LAR) have been performed for colorectal carcinoma and whose samples were received in the Department of Pathology for histopathology assessment from June to September 2024 were considered in this study.

Exclusion criteria

Patients who have received Neoadjuvant chemotherapy/ Radiotherapy before surgery were excluded.

Ethical committee approval and informed consent

The Birat Medical College Teaching Hospital's Ethical Committee (IRC-PA-389/2024) approved the study. The participants gave consent before the study, which was conducted according to the Declaration of Helsinki.

Data interpretation and statistical analysis

After obtaining the laboratory results, an analysis was done using the Statistical Package for the Social Sciences (SPSS) version 16. Descriptive statistics were analyzed in terms of percentage and frequency distribution. The chi-square test was used to evaluate the relationship between TSR and clinicopathological parameters, and P values <0.05 were considered significant statistically.

Results

The clinicopathological features of patients are elaborated on in Table 1. Out of a total of 42 patients, male patients (n=24, 57%) outnumbered female patients (n=18, 43%) with colorectal carcinoma. We found no statistical significance between TSR and patients' gender and age. The maximum number of patients were diagnosed histologically as adenocarcinoma, NOS (n = 36, 86%), followed by mucinous adenocarcinoma (n = 4, 10%) and signet ring cell carcinoma (n = 2, 5%).

Table 1: Clinicopathological features of patients and their respective association with Tumor stroma ratio

Clinicopathological characteristics				Tumor stroma ratio		P value
		n	(%)	High	Low	
Age in years at diagnosis	<73	39	93	22	17	0.372×
	≥73	3	7	1	2	
Gender	Male	24	57	15	9	0.245
	Female	18	43	10	8	
pT stage	pT1	8	19	8	0	< 0.001*
	pT2	15	36	15	0	
	pT3	6	14	0	6	
	pT4	13	31	0	13	
pN stage	pN0	14	33	13	1	< 0.001*
	pN1	11	26	10	1	
	pN2	17	40	0	17	
Histology	Adenocarcinoma, NOS	36	86	18	18	0.161v

	Mucinous adenocarcinoma	4	10	4	0	
	Signet-ring cell carcinoma	2	5	1	1	
Localization	Right side	25	60	22	3	< 0.001*
	Left side	17	40	1	16	
Lymph nodes	<12 nodes	28	67	23	5	< 0.001*
	≥ 12 nodes	14	33	0	14	
Lymphovascular invasion	Present	21	50	2	19	< 0.001
	Absent	21	50	21	0	
Distant metastasis	Present	3	7	0	3	0.048
	Absent	39	93	23	16	

×p>0.05, statistically not significant, *p<0.05, statistically significant

Of 42 cases, 19 (45.2%) tumors have a low tumor stroma ratio, and 23(54.8%) tumors show a high tumor stroma ratio. Low TSR was significantly associated with the high pathological stage of the tumor (p <0.001) and the high lymph node stage (p <0.001). All the pT1 (n= 8, 19%) and pT2 (n=15, 36%) tumors have low stroma, whereas no tumor with low stroma was found to be in the pT3 and pT4 stage. All the tumors in the pT3 (n=6, 14%) and pT4 (n=13, 31%) groups had high stroma and low TSR. For the Nodal status, the stroma low percentage in the N0 group (n = 13) is 92.8% and in the N1 group (n = 10) 90.9%. All the N2 (n= 17, 40%) group tumor was found to have high stroma.

Low TSR tumors were more common on the left side (p <0.001), and high TSR tumors were more common on the right side (p <0.001), showing a site-specific aggressiveness of the tumors. In the present study, high TSR shows less than 12 lymph node extractions and less lymph node involvement, while low TSR shows an increased number of lymph node extractions and increased lymph node involvement with strong statistical significance (p <0.001).

Similarly, lymphovascular invasion is associated with high stroma in the tumor-invasive front, whereas the low stroma-containing tumor shows the absence of lymphovascular invasion (p <0.001). In the present study, only three patients with low TSR show distant metastasis.

Relation with tumor stage I to IV (Table 2)

Table 2: Relation of tumor Stage I to IV and Tumor stroma ratio

Tumor stage	Number (n=42)		Tumor stroma ratio		P value
	n	(%)	High	Low	
Stage I	13	31	13	0	
Stage II	1	2	0	1	< 0.001
Stage III	25	60	10	15	
Stage IV	3	7	0	3	

The maximum number of patients were in stage III (n=25, 60%), followed by stage I (n=13, 31%) and stage IV (n=3, 7%), as shown in Table 2. Only one patient was in stage II (n=1, 2%). In our study, the increasing stage of the tumor from I to IV strongly correlated with the TSR (p <0.001). All the patients with tumor stage I have high TSR (n=13, 31%). Most patients with tumor stage III have low TSR (n=15, 60%). All the patients who presented in stage IV (n=3, 7%) had low TSR colorectal carcinoma showing metastasis to distant organs.

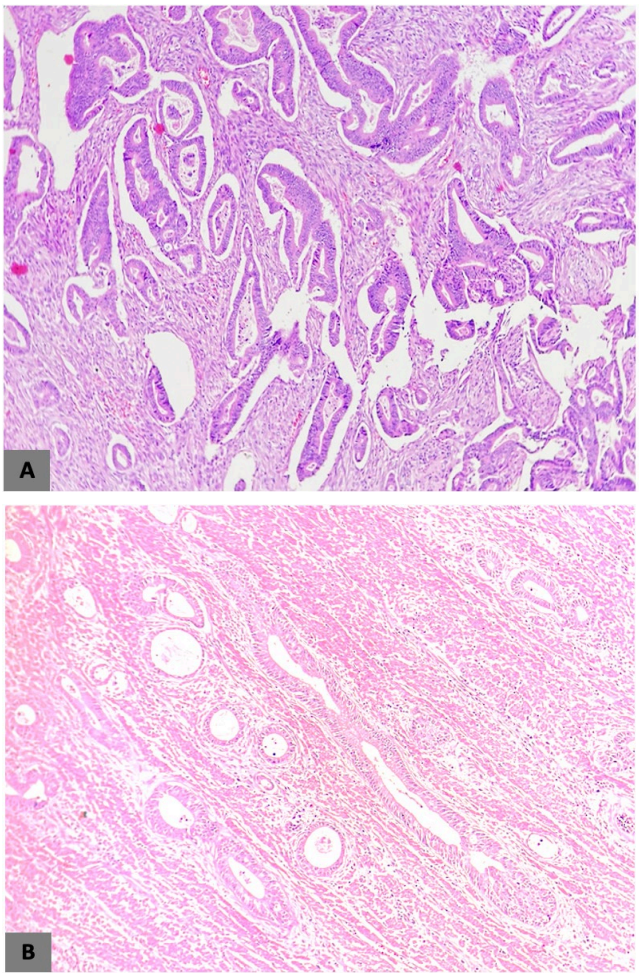

Figure 1: A: Tumor showing high Tumor stroma ratio (low stroma content <50%), Hematoxylin and Eosin stain, 400x. B: Tumor with low Tumor stroma ratio (high stroma content >50%), Hematoxylin and Eosin stain, 400x.

Discussion

The tumor and its stroma ratio is a prognostic parameter in patients with colon and rectum cancer [11-13]. The classification of TSR as low and high can be added in routine pathology reporting in combination with the TNM classification to categorize the patients at increased risk of recurrence [12, 3]. In addition, TSR also provides information on the addition of adjuvant chemotherapy and offers individualised treatment for a selected group of patients [11].

The evaluation of TSR was first highlighted by Mesker in colorectal cancer [12]. Histopathological assessment of TSR to generate prognostic information is simple yet rapid and convincing compared to molecular and machine learning-based markers, which take time, require sophisticated technologies and are expensive [12, 14]. In a low-resource

country like Nepal, developing simple histology-based biomarkers can be effective and cost-minimizing in the long run. Hence, with the current study, we aim to analyse the same in H and E-stained sections and identify the association of TSR with clinicopathological characteristics in Nepalese colorectal cancer patients in the Eastern region of Nepal. To the best of our search, this is the first study conducted in Nepal that evaluates the significance of tumor stroma ratio and high-risk parameters.

The present study reveals a significant correlation between low TSR and advanced disease stage, highlighting the prognostic potential of low and high TSR in colorectal carcinoma, supported by other studies [10, 12, 15]. In the present study, 100% of stage I cancer showed a high TSR, which is in contrast to other studies that showed a weak association of TSR in early-stage colorectal cancer [14, 16]. This result in the current study can be used as a baseline further to evaluate the association between Stage I cancer and TSR. The significance of TSR in stage II cancer in the present study is too early to comment on, as there was only one patient in stage II with low TSR. In the current research, Stage III tumors have low TSR, further suggesting that stroma-rich environments become more prevalent as tumors progress and are associated with poor disease outcomes. Other studies support this finding [15]. However, in contrast, Mesker et al. mention the low sensitivity of stroma and carcinoma percentage for stage III tumors, which can lead to undertreatment of patients [12].

In the current study, we excluded cases who had received Neo-adjuvant chemotherapy/ Radiotherapy as the prior therapy can induce a significant stromal reaction, giving a false positive impression of tumor stromal reaction. We also excluded the small biopsy specimens, as it would be difficult to identify the most invasive front of the tumor for TSR evaluation.

We also observed a significant association between TSR and pathological T stage ($p < 0.001$), pathological N stage ($p < 0.001$) and lymphovascular invasion ($p < 0.001$), which is comparable to other similar studies [15, 17]. These findings suggest that tumors with low TSR are more likely to have a higher stage, which indicates a deeper level of tumor invasion and an increase in metastasis to lymph nodes [9, 11, 15, 17]. Furthermore, the increased number of colorectal carcinomas with lymphovascular invasion in tumors with low TSR emphasize the aggressive behaviour of tumors rich in stroma.

Though in the current study, right-sided tumors outnumbered the left-sided ones, the stroma-rich tumors were more common on the left side than the right side, showing the site-specific aggressiveness of the tumor ($p < 0.001$). A similar finding was reported by Smith et al. and Dang et al. [6, 7]. However, some other studies show no statistical significance between the tumor site and TSR [11, 17].

Only three patients presented with distant metastasis in this study. All these cases had low TSR ($p=0.048$), indicating that TSR plays a role in the metastatic behaviour of colorectal carcinoma. However, larger cohorts are required to confirm these findings.

In a study by Eriksen AC et al. [11], a statistically significant association was found between the histopathological type of adenocarcinoma NOS with low TSR. Still, we did not find any association between TSR and tumor histologic type.

Conclusion

The present study highlights the role of TSR as an indicator of high-risk features in colorectal cancer. A low TSR is associated with a high tumor Stage, lymphovascular metastasis, and increased positive lymph nodes. Incorporating TSR into routine reporting practice, in addition to TNM staging, can add value in determining the course of disease and help provide individualized treatment to a selected group of patients.

Limitations and future scope of the study

The present study's limitation is its small sample size, especially of Stage II colorectal cancer patients, which limits the generalizability of the findings in the Nepalese population. Despite that, the current study comprehensively assesses the correlation between TSR and high-risk prognostic parameters. We haven't followed up with patients in the current study, but in the next phase of this study, we plan to follow all these patients and correlate the TSR with overall survival, disease-free survival, and their response to therapy.

Relevance of the study

The present study shows how TSR estimation in a resource setting can contribute to the prognosis and assessment of the tumor's lymphovascular invasion and nodal stage.

Abbreviations

Abdominoperineal resection (APR), Colorectal cancer (CRC), Hematoxylin and Eosin (H&E), Low anterior resection (LAR), Tumor, node and metastasis (TNM), Tumor stroma Ratio (TSR)

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Authors' contribution

a. Study planning: DK
b. Data collection: DK
c. Data analysis/ interpretation: DK
d. Manuscript writing: DK
e. Manuscript revision: DK, PY, SBP
f. Final approval: DK, PY, SBP
g. Agreement to be accountable for all aspects of the work: DK, PY, SBP

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