



Original Article

# Tumor-infiltrating lymphocytes in colorectal carcinoma

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## Keywords:

Colorectal carcinoma;  
Tumor-infiltrating  
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Tumor stage;

## ABSTRACT

**Background:** Immunotherapy has become an important modality of treatment for CRC. The cells contributing to an effective immune response are CD8+ T cells of tumor-infiltrating lymphocytes. The present study aimed to assess the tumor-infiltrating lymphocytes on colorectal cancer and correlate them with other prognostic parameters.

**Materials and methods:** Cases of colectomy from April 2019 -Sept 2020 with a diagnosis of colorectal carcinoma were included in the study. Parameters i.e age, sex, size, location of the tumor, lymphovascular invasion, perineural invasion, T stage, N status, histological tumor type, and grade, resected margins were retrieved from the database of the department. The intensity of overall inflammatory cell reaction at the invasive tumor front was evaluated in H and E stained sections.

**Results:** 56 cases of colorectal cancers were reported during the study period with M: F ratio of 1.15:1. The age ranged from 23-79years with a mean age of 54.3years. The tumors were predominant in the colon (50 cases) than the rectum. Colonic carcinomas had high-grade tumor-infiltrating lymphocytes as compared to rectal carcinomas. The grade of tumor-infiltrating lymphocytes was statistically significantly associated with T, N status, and grade of tumors.

**Conclusions:** Inflammatory reactions at the invasive tumor front can be easily analyzed in resection specimens of colorectal cancers without additional costs. Low-grade tumor-infiltrating lymphocytes are indicative of high-stage tumors with a poor prognosis. Hence, the inclusion of tumor-infiltrating lymphocytes in pathological reports for risk stratification of the patient is mandatory.

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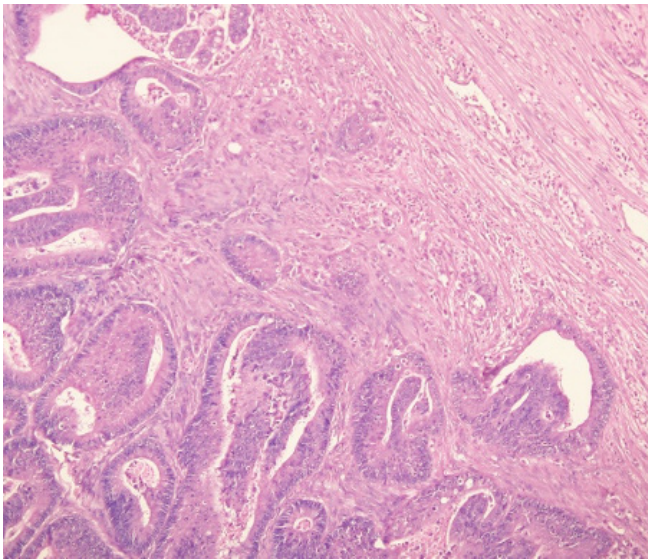
## INTRODUCTION

Globally colorectal cancer (CRC) accounts for one of the commonest malignant tumors of the gastrointestinal tract. It is estimated that in all new cases of malignant tumors, the incidence and mortality of CRC are 10.2% and 9.2%, respectively.<sup>1</sup> As one-third of the patients undergoing curative resection die within five years of surgery it is important to provide individualized therapy as per risk stratification to improve prognosis.<sup>2</sup> Immunotherapy has become an important modality of treatment for CRC.<sup>3-4</sup> It has been shown that in addition to tumor factors, the local tumor microenvironment (TME) i.e extracellular matrix,

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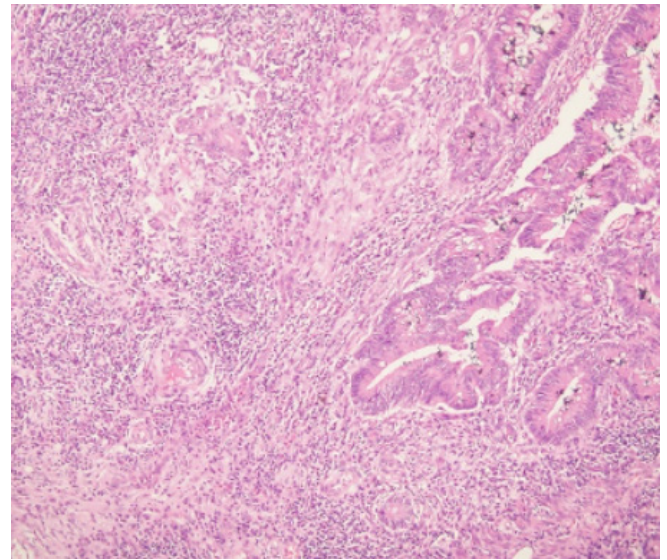
**Figure 1:** Low grade tumor infiltrating lymphocytes at the invasive tumor front of colonic adenocarcinoma (HE stain, X200)

immune cells, and cytokines play an important role in tumor proliferation, invasion, and metastasis. Thus, these are the prognostic indicators in colorectal carcinomas.<sup>5</sup>

The interactions among the different components of the tumor microenvironment mediate an immune response that is related to a favorable prognosis in colorectal cancer. The cells contributing to an effective immune response are CD8<sup>+</sup> T cells of tumor-infiltrating lymphocytes (TIL) that have cytotoxic effects, CD4<sup>+</sup> T helper cells (CD4<sup>+</sup>Th) that promote clonal expansion of antigen-specific CD8<sup>+</sup> T cells and help in the production of IFN- $\gamma$ , which thereby promote proliferation and functions of the effector molecules of CD8<sup>+</sup> T cells and NK cells.<sup>10-11</sup> Hence, it is reasonable to assess the activity of the immune system in tissue material, in the form of TILs present in the primary tumor. The present study aims to assess the TILs on H and E-stained sections in primary tumor front in patients with colorectal cancer and correlate it with other prognostic parameters.

## MATERIALS AND METHODS

We retrospectively reviewed the database of the Department of Pathology, Institute of Medicine, Maharajgunj, Nepal. Cases of colectomy with a diagnosis of colorectal carcinoma were included in the study. Patients who had undergone neoadjuvant chemo/chemoradiotherapy were excluded from the study. H and E stained sections of the tumor were retrieved from the archives and tumor-infiltrating lymphocytes were counted as proposed. The analyses of TILs were performed in the invasive front of the primary tumor mass. Overall inflammatory reaction was assessed by using a four-degree scale. Score 0 indicated an absence of reaction, 1 weak, 2 moderate, and 3 severe. Score 0 meant no increase of inflammatory cells, 1 indicated a mild and patchy increase of inflammatory cells at the invasive



**Figure 2:** High grade tumor infiltrating lymphocytes at the invasive tumor front (HE stain, X200)

margin, but with no destruction of invading cancer cell nests by the inflammatory cells. When inflammatory cells formed a band-like infiltrate at the invasive margin with a little destruction of cancer cell islets by inflammatory cells a score of 2 was given. A score of 3 denoted a very prominent inflammatory reaction, forming a cup-like zone at the invasive margin, and when the destruction of cancer cell nests was frequent and invariably present.<sup>8</sup> Also, to conduct statistical analysis, we divided the study group into Group 1 (low)-(1 level of stromal TILs) and group 2 (high)-(2 and 3 levels of stromal TILs).

Other parameters i.e age, sex, size, location of the tumor, lymphovascular invasion (LVI), perineural invasion (PNI), T stage, N status, histological tumor type, and grade, resected margins were retrieved from the database of the department. The parameters were analyzed using SPSS 20. Correlation with different parameters was determined using Pearson correlation.

## RESULTS

The study included 56 cases of colorectal carcinomas diagnosed on resection specimens received in the Department of Pathology from April 2019-September 2020. The study included 30 males and 26 females with an M:F ratio of 1.15:1. The age ranged from 23-79years with a mean age of 54.3years. The tumors were predominant in the colon (50cases) than the rectum. Out of the colonic carcinomas, 21 cases (42%) were located in the right side of the colon, 12 cases (24%) were in the transverse colon, 2 cases (4%) in the left-sided colon, and 15 cases (30%) in the sigmoid colon.

Of all colorectal carcinomas, 32.1% had a weak level of TIL (fig. 1), 53.6% moderate, and 14.3% strong levels

**Table 1A: Correlations between tumor-infiltrating lymphocytes and clinicopathological parameters**

Variables		Density of tumor-infiltrating lymphocyte		Total (%)	p-value
		Low	High		
Gender	Male	15	15	30 (53.6%)	0.5
	Female	15	11	26 (46.4%)	
Site	Colon	25	25	50(89.2%)	0.18
	Rectum	5	1	6(10.8%)	
Tumor Size	≤5cm	13	10	23(41.07%)	0.76
	>5cm	17	16	33(58.93%)	
Depth of Invasion	T1	0	1	1(1.8%)	0.004
	T2	5	3	8(14.3%)	
	T3	21	18	39(69.6%)	
	T4	4	4	8(14.3%)	
Lymphnode metastasis	N0	17	16	33(58.9%)	0.02
	N1	7	5	12(21.4%)	
	N2	6	5	11(19.7%)	

**Table 1B: Correlations between tumor-infiltrating lymphocytes and clinicopathological parameters**

Variables		Density of tumor-infiltrating lymphocyte		Total (%)	p-value
		Low	High		
Lymphovascular invasion	Positive	15	10	25(44.6%)	0.11
	Negative	15	16	31(55.4%)	
Perineural invasion	Positive	8	5	13(23.2%)	0.08
	Negative	22	21	43(76.8%)	
Histological grade	Grade 1	10	10	20(35.7%)	0.09
	Grade 2	13	12	25(44.6%)	
	Grade 3	7	4	11(19.7%)	
Histological type	Adenocarcinoma	24	23	47(83.9%)	0.004
	Mucinous adenocarcinoma	3	2	5(5.4%)	
	Signet ring cell carcinoma	3	0	3(8.9%)	
	MANEC	0	1	1(1%)	
Margin	Positive	1	2	3(8.9%)	0.09
	Negative	29	24	53(91.1%)	

of TILs (fig. 2). Colonic carcinomas had high-grade TIL as compared to rectal carcinomas. But it was statistically insignificant ( $P > 0.17$ ). The correlation of TIL with other parameters is displayed in table 1A and B.

## DISCUSSION

Carcinomas of the colon and rectum are one of the commonest cancers worldwide. According to gender, CRC is the third most common cancer in men (10%) and second in women (9.2%).<sup>8</sup>

Fifty-six cases of colorectal carcinomas were reported in resection specimens in one year. Thirty cases were seen in males and twenty-six cases were seen in females. Age ranged from 11-79 years with a mean age of 54.3 years. In

a similar study by Andreoni et al slightly higher number of cases was seen in males as compared to females. The age ranged from 24-88yrs, mean 63yrs.<sup>6</sup> Another study by Shah et showed 37cases of colorectal carcinomas in males and 36 cases in females. They reported a mean age of 52 years.<sup>7</sup>

The carcinomas were mostly localized to the colon (50cases). Out of the colonic carcinomas, 21 cases (42%) were located in the right side of the colon, 12 cases (24%) were in the transverse colon, 2 cases (4%) in the left-sided colon, and 15 cases (30%) in the sigmoid colon. Andreoni et al also found carcinomas predominantly localized to the colon rather than the rectum.<sup>6</sup> Another study reported adenocarcinomas located mostly in the sigmoid followed by rectum and caecum.<sup>9</sup>

Histologically, all colorectal carcinomas were adenocarcinomas in this study. In a study of histology of colorectal cancer, 96% were adenocarcinomas and other carcinomas included epidermoid carcinomas (squamous cell carcinomas) and carcinoids.<sup>9</sup> They found that mucinous and signet ring cell carcinomas were more common in the younger age group.<sup>9</sup> In this study, signet ring cell carcinomas were found in the younger age group. Another study by Nitsche reported mucinous adenocarcinoma more commonly in the right side of the colon.<sup>10</sup> All signet ring cell carcinomas and 50% of mucinous carcinomas were localized to the right side of the colon in our study. The number of mucinous adenocarcinomas and signet ring cell carcinomas was slightly higher in females as compared to males. With regards to mucin secreting tumors, a study found a higher proportion of these tumors in females.<sup>11</sup>

We found that moderately differentiated adenocarcinoma was the commonest histological grade, similar to a study by Stewart et al.<sup>9</sup> The TNM status showed most of the adenocarcinomas presenting in T3 and N0 status. This is similar to Nitsche et al's findings in their study of colorectal carcinomas.<sup>9</sup>

Circumferential resection margin (CRM) was the margin that was positive in all three margin positive cases. Histologically, they were adenocarcinomas with predominantly T3 status. A study by Balboa et al found CRM as the margin which was predominantly involved by tumor.<sup>12</sup>

It is stated in the literature that lymphocytic infiltration is a major immunological defense against tumor cells in solid tumors and is a potential predictor of colorectal cancer.<sup>13-15</sup> We found that TIL was weak in 32.1%, moderate in 53.6%, and strong in 14.3% of all colorectal cancers. In a similar study, weak infiltration was seen in 72 cases, moderate in 30 cases, and strong in 21 cases.<sup>16</sup> Colonic carcinomas (50%) presented with a high grade of TIL whereas only 16.6% of rectal cancers presented with high TIL. But this was not statistically significant (p-value 0.17). Other studies also reported the same.<sup>16</sup>

APC-lymphocytes stimulate the TIL, the degree and direction of activation and selectivity of stromal TILs depend on the type of antigen present on the tumor cell.<sup>17</sup> The first host defense against invasive tumor cells that further stimulate or inhibit the cellular response is provided by TILs located in the invasive tumor fronts.<sup>18</sup> We noted in this study that tumors with low TIL presented with a higher T and N status. In brief, we found a statistically significant correlation between the T and N status of cancers and the degree of TILs. A study by Jakubowska et al found a low incidence of TILs in the invasive tumor front associated with metastases to the local lymph nodes and extension of tumor beyond the nodule to the surrounding tissues.<sup>16</sup> These results are consistent with the observations of other studies

by Perez et al<sup>13</sup>, Huh et al<sup>19</sup>, and Pagès et al.<sup>20</sup> The decrease in intratumoral immune T-cell densities correlated with the growth of the primary tumor and the metastatic spread in a study by Mlecnik et al.<sup>21</sup>

Though a low grade of TIL infiltration was associated with LVI and PNI, it was not statistically significant in this study. However other studies have found a significant correlation between TIL and LVI and PNI.<sup>16</sup>

TILs were assessed in invasive tumor margins. Literature reports inflammatory reaction at the invasive margin as the predictor for both survival and recurrence-free survival. Macrophages and lymphocytes were the most valuable individual cell types as predictors of inflammatory response. In colorectal carcinomas, results indicate that CD8+ and FOXP3+ infiltrating cells, but not CD3+ T cells were the prognostic markers for disease-free survival and overall survival.<sup>5</sup>

## CONCLUSIONS

Inflammatory reactions at the invasive tumor front can be easily analyzed in resection specimens of colorectal cancers without additional costs. Low-grade TIL is indicative of high-stage tumors with a poor prognosis. Hence, the inclusion of TIL in pathological reports for risk stratification of the patient is mandatory.

**Conflict of interest: None**

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