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Original Research Paper

Oncopathology

CORRELATION OF TUMOR INFILTRATING LYMPHOCYTES AND TUMOR STAGING IN RECTAL CARCINOMA: A STUDY IN A TERTIARY CANCER CARE CENTRE IN NORTH EAST INDIA

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ABSTRACT Colorectal cancer is responsible for 10% of cancer cases and 9.4% of cancer-related deaths and it's one of the commonest cancers in north-east India. Recognition of the prognostic effect of tumor-infiltrating lymphocytes and tumor-related immune responses has recently taken interest of the researcher and many of them are trying to find a prognostic implication of the same. Many scorings system have been developed for this purpose. Here we are using the scores by International TILs Working Group (ITWG) and will try to find the relation between tumor infiltrating lymphocytes and tumor staging considering gender, age, size of tumor, histopathological findings, grading, lymphovascular invasion, perineural invasion, depth of invasion, nodal status and metastastic potential. All findings will be assessed for significance using p value and final correlation will be done using Pearson correlation index.

KEYWORDS :

INTRODUCTION:

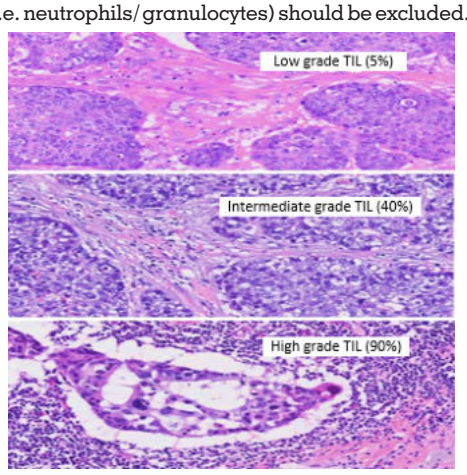
Colorectal cancer is a clinically common malignant tumor of the end part of large intestine. Global Cancer Statistics reveals that 0.94 million deaths occurred out of 1.9 million newly diagnosed rectal carcinoma patients in 2020, which is responsible for 10% of cancer cases and 9.4% of cancer-related deaths [1]. It has been shown that local tumor microenvironment comprising of immune cells, cytokines and associated matrix play an important role as a prognostic marker. The prognostic effect of tumor-infiltrating lymphocytes and tumor-related immune responses has recently become increasingly recognized and incorporation of targeted therapy based on the findings are helpful in increasing patient survival period [2]. The cells contributing to an effective immune response are CD8+ T cells that have cytotoxic effect, CD4 T helper cells that promote clonal expansion of antigen specific CD8 T cells along with B cells and NK cells [3]. So here, we will try to find whether these tumor infiltrating lymphocytes play any role in tumor staging and their importance in maintaining tumor microenvironment. Also, as the new treatment protocols mostly targets the local factors rather than the systemic factors, so we will also try to have and overview of scope and immunotherapy by targeting these lymphocytes.

MATERIAL AND METHOD:

The study was carried out retrospectively in Oncopathology department, Dr. Bhubaneswar Borooah Cancer Institute, from 1st July, 2022 to 30th June 2023. Cases of colectomy with a diagnosis of rectal adenocarcinoma were included in the study (well, moderate, poorly differentiated, mucinous adenocarcinoma and signet ring cell carcinoma). Patients who did not have detailed information and had undergone neoadjuvant chemo/chemoradiotherapy were excluded from the study. Also, metastatic carcinoma to rectum were excluded. Total sample size was 87 (Fisher's formula). Clinical details of the patients were retrieved including age, sex, and symptoms, duration and family history. As soon as we received the specimen, we fixed the specimen in 10% formalin, processed in an automated tissue processor, paraffin embedded and was cut in 4–5-micron thickness at rotatory microtome and H & E staining was done for histopathological examination. The TIL grading score was recommended from the International TILs Working Group (ITWG) and categorized into 3 groups: low (0% to 10%), intermediate (15% to 50%), and high (55% to 100%).

Summary of the ITWG adapted for Assessing TILs in CRC [4]

TILs in CRCs should be assessed in the stromal compartment only and reported as a percentage of the stromal area. Stromal TILs evaluation should be confined to the borders of the invasive tumor. TILs in zones of necrosis, fibrosis, and abscess formation should be excluded. TILs should be reported as a continuous variable, that is an average of the stromal TIL density over the entire section, rounded to the nearest 5%. All mononuclear inflammatory cells (lymphocytes and plasma cells) should be scored, but other inflammatory cells (i.e. neutrophils/granulocytes) should be excluded.

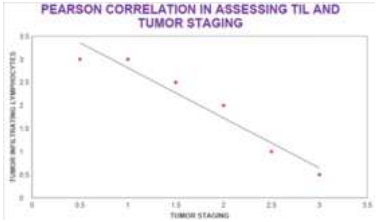


RESULT AND OBSERVATION:

The summary of the results obtained are mentioned in the tabulated form:

Criteria	Category	Low TIL	Intermediate TIL	High TIL	Percentage	P value
Gender	Male	27 (31.03%)	13 (14.94%)	7 (8.05%)	47 (54.02%)	1.2

	Female	21 (24.14%)	10 (11.5%)	9 (10.34%)	40 (45.98%)	
Age	<60 years	13 (14.94%)	12 (13.8%)	11 (12.64%)	36 (41.38%)	0.9
	>60 years	35 (40.23%)	11 (12.64%)	5 (5.75%)	51 (58.62%)	
Size	<5 cm	5 (5.74%)	14 (16.09%)	10 (11.5%)	29 (33.33%)	0.01
	>5 cm	43 (49.42%)	9 (10.35%)	6 (6.9%)	58 (66.67%)	
HPE	Adenocarcinoma	42 (48.27%)	21 (24.14%)	16 (18.39%)	79 (90.80%)	0.009
	Mucinous AC	4 (4.6%)	2 (2.3%)	0	6 (6.90%)	
	Signet ring AC	2 (2.3%)	0	0	2 (2.30%)	
Grade	WDAC	23 (26.42%)	12 (13.8%)	10 (11.5%)	45 (51.72%)	0.04
	MDAC	18 (20.69%)	8 (9.19%)	5 (5.75%)	31 (35.63%)	
	PDAC	7 (8.05%)	3 (3.44%)	1 (1.15%)	11 (12.64%)	
LVI	Positive	29 (33.3%)	5 (5.74%)	2 (2.4%)	36 (41.38%)	0.001
	Negative	19 (21.84%)	18 (20.69%)	14 (16.09%)	51 (58.62%)	
PNI	Positive	21 (24.14%)	9 (10.34%)	4 (4.6%)	34 (39.08%)	0.9
	Negative	27 (31.03%)	14 (16.08%)	12 (13.8%)	53 (60.91%)	
DOI	T1	9 (10.35%)	8 (9.19%)	6 (6.9%)	23 (26.44%)	0.042
	T2	12 (13.8%)	6 (6.9%)	5 (5.74%)	23 (26.44%)	
	T3	13 (14.94%)	6 (6.9%)	5 (5.74%)	24 (27.58%)	
	T4	14 (16.08%)	3 (3.46%)	0	17 (19.54%)	
Nodes	N0	10 (11.5%)	7 (8.05%)	11 (12.63%)	28 (32.18%)	0.007
	N1	12 (13.8%)	11 (12.64%)	4 (4.6%)	27 (31.04%)	
	N2	26 (29.89%)	5 (5.74%)	1 (1.15%)	32 (36.78%)	
Metastasis	M0	9 (10.35%)	19 (21.84%)	11 (12.63%)	39 (44.82%)	1.42
	M1	16 (18.39%)	3 (3.45%)	4 (4.6%)	23 (26.44%)	
	M2	23 (26.44%)	1 (1.15%)	1 (1.15%)	25 (28.74%)	



Pearson correlation showing correlation of tumor infiltrating lymphocytes and tumor staging

DISCUSSION:

The statistically significant parameters showing reverse correlation with TIL, i.e. as the TIL increases, the decreasing parameters are: **(1)** Size of tumor **(2)** Histopathology of tumor **(3)** Grade of adenocarcinoma **(4)** Lymphovascular invasion **(5)** Depth of invasion (T) **(6)** Lymph node status (N).

Shovana Karki et al. in the year 2021 also found similar correlation, however, the study also showed significant relationship with PNI but not with tumor size and LVI [5]. A study by Jakubowska et al (2019) 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 [6]. Consistent results are found with the observations of other studies by Huh et al (2012) [7], Mlecnik et al (2011) and Pagès et al(2008) [8]. The decrease in intratumoral immune T-cell densities correlated with the growth of the primary tumor and the metastatic spread.

CONCLUSION:

Increased focus on the tumor microenvironment has identified inflammatory infiltrate as a critical predictor of disease activity impacting patient prognosis. As one-third of the patients undergoing curative resection die within five years of surgery due to recurrence or metastasis, it is important to provide individualized therapy as per risk stratification to improve prognosis. In rectal carcinoma, lymphocytic infiltration into the tumor has been associated with good outcomes, and prevention of its exhaustion and apoptosis in tumors is the goal of immunotherapy, especially immune checkpoint inhibitors. In 2017, Pembrolizumab was approved by the FDA for the treatment of all dMMR-MSI-H metastatic solid tumors, becoming the first biomarker-based cancer treatment regimen. Now a days, the PD-1 inhibitors Pembrolizumab and Nivolumab, led a to durable response in patients with metastatic microsatellite instability-high colorectal carcinomas. Another inhibitor, Ipilimumab, a fully-humanized monoclonal antibody that blocks CTLA-4 and increases immune response, has also been approved by the FDA for patients who have previously received chemotherapy. Epacadostat, an Indomethacin 2,3-double oxygenase 1 (IDO1) inhibitor, that causes activation of mostly T lymphocytes was planned to combine with pimuzumab and azacytidine in the MSS CRC [9]. Inhibition of MEK, a downstream effector of the RAS-MAPK pathway, was found to induce PD-L1 upregulation, leading to the development of combination of MEK and PD-L1 inhibitors [10]. Monalizumab, a clinically used antibody targeting NKG2A, which causes suppression of NK cells, has been developed to promote NK cell function and has shown the potential to enhance the efficacy of anti-PD-1 therapy [11].

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