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Original Article

Stromal And Intra-Epithelial Tumor

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ASSESSMENT OF STROMAL AND INTRA-EPITHELIAL TUMOR INFILTRATING LYMPHOCYTES IN COLORECTAL CARCINOMA AT ARMED FORCES INSTITUTE OF PATHOLOGY

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ABSTRACT

Objective: To conduct a morphological evaluation quantitatively of two types of tumor-infiltrating lymphocyte populations, including those located in the stroma and intraepithelial cancer structures, in patients with colorectal cancer.

Study Design: Cross sectional study.

Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi, from Jan to Jul 2019.

Methodology: Three levels of infiltration in the stroma by tumor infiltrating lymphocytes were determined. Level 1 was weak meaning (0-20% of stromal TILs), level 2 was moderate meaning (>20-50% of stromal TILs); and level 3 was strong meaning (50-90% of stromal TILs). TILs within tumor cells were divided into two groups. 0 meaning absent (no TILs present) and 1 meaning present (≥TILs in tumor cells)[1].

Results: Out of 30 cases 22 were males and 8 females. Ages ranged between 25-83 with a mean of 57 years and a standard deviation of ± 16.4 years. All of the cases were diagnosed cases of adenocarcinoma. The levels of stromal tumor infiltrating lymphocytes was weak in 1 case, moderate in 18 cases and strong in 11 cases whereas intraepithelial lymphocytosis was seen in 22 cases.

Conclusion: These results confirm that the infiltration of tumor infiltrating lymphocytes into the tumor in patients with colorectal carcinoma serves an important role in the invasion and progression of the disease, and should be considered in routine examinations.

Keywords: Colorectal carcinoma, Intraepithelial cancer, Tumor infiltrating lymphocytes.

INTRODUCTION

Colorectal carcinoma is one of the most common forms of cancer in the world today with an incidence of 20.1 and 14.6 per 100,000 per year for men and women respectively. Approximately 36% new cases have been noted in 2000 outside of industrialized countries showing that it is no longer an illness of developed countries. However, colorectal cancer is uncommon in Pakistan. A data based epidemiology study shows colorectal carcinoma to be seventh most common in males and ninth most common in females[2].

Immune response plays an important role in development of tumors which itself is a multistep process. The development and organization of tumor microenvironment is determined by local antitumor defense mechanisms. Presence of inflammatory cells in relative proportions in this area along with the composition of cell population affects the quality and characteristics of the inflammatory response[3]. Tumor infiltrating lymphocytes (TILs) are the lymphocytes directly isolated from tumor microenvironment. The TILs either release chemotactic and pro-inflammatory cytokines or recognize antigens and directly cause tumor lysis[4]. Evidence of cell membrane and cytoplasm destruction along with certain cases exhibited cell penetration and nucleus destruction within the tumor cells which were in direct contact with TILs as observed through electron microscopy[5].

Recently, immunotherapy has been used as means to inactivate or stimulate cell populations to activate immune response towards tumor cells[6]. This estimation to determine the degree and presence of infiltration by lymphocytes is used for the diagnosis of malignant melanoma. Positive prognostic markers for patients of malignant melanoma include infiltration between cancer cells and infiltration by lymphocytes at the edge of lesion[7]. Reduction in risk of lymph node metastasis and mortality caused by malignant melanoma is seen with increasing degree of infiltration by lymphocytes[8,9]. Patients with increased proportion of infiltration by TILs might respond to ipilimumab antibodies administration against cytotoxic T-lymphocyte associated protein 4, caused reduction of immune inhibition and promotion of immune response against tumor cells[10]. Moreover, another promising target for immunotherapy is programmed cell death protein 1 (PD1) protein and its ligand, which being expressed on

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active TILs of lymphoma and different malignancies including renal cell carcinoma.

METHODOLOGY

This cross sectional study was held at department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, from January to July 2019. Thirty pathologically proven patients with colorectal cancer were included in this study through convenience, non probability sampling. Sample size was calculated using WHO calculator, however sample size was limited due to number of resection samples received during these 6 months and inadequate fixation[11]. Tissue samples included in the study were resection specimens that had been processed and diagnosed at Histopathology department of Armed Forces Institute of Pathology, Rawalpindi. Colonoscopic biopsies, autolysed specimens or specimen showing extensive necrosis were excluded from this study. Study was approved by Institutional Review Board (FC-HSP17-24/READ-IRB/18/902) held at AFIP. Baseline clinico-pathologic data including patients' particulars, histological type and histological grade was noted. Formalin-fixed, paraffin-embedded blocks were sectioned at 3µm thickness. They were deparaffinized in xylene and rehydrated with decreasing concentration of ethanol.

The type of tumor growth, tumor size, histologic type, Dukes stages and grade of malignancy were determined by routine histopathologic evaluation of tumor sections. H&E slides were assessed to determine two morphologic types of TILs. One located within the stroma and other within intraepithelial structures of tumor by light microscopy at (200-400x magnification). The international TILs working group, 2014 guidelines were used to identify the TILs within the stroma. These were identified as a percentage of mononuclear inflammatory cells counted in 5 high power fields in the total stromal and intra-tumoral area, excluding crush artifacts, regressive hyalinization or necrosis. Three levels of infiltration in the stroma by tumor infiltrating lymphocytes were determined. Level 1 was weak meaning (0-20% of stromal TILs), level 2 was moderate meaning (>20-50% of stromal TILs); and level 3 was strong meaning (50-90% of stromal TILs). TILs within the tumor structures were counted in 5 HPF in the center of tumor excluding apoptotic bodies. For statistical analysis two groups were defined. 0 meaning absent (no TILs present) and 1 meaning present (≥ TILs in tumor cells).

Data was analyzed by SPSS version 24. Frequency and percentage was calculated for categorical variables such as gender, histological type, histological grade and TILs. Continuous variables were expressed as mean and standard deviation such as age. Percentage of categorical variables were compared using Pearson's chi-square test when was appropriate. The *p*-value ≤0.05 was considered significant.

RESULTS

Out of thirty cases, 22 were males 30 (73%) and 8 were female 30 (26%). Ages ranged between 25-83 with a mean of 57 years and a standard deviation of ± 16.4 years. All of the cases were diagnosed cases of adenocarcinoma 30 (100%). The levels of stromal TILs was weak in 1 case 30 (3.3%), moderate in 18 cases 30 (60%) and strong in 11 cases 30 (36%) whereas intraepithelial lymphocytosis was seen in 22 cases 30 (73%) (fig-1).

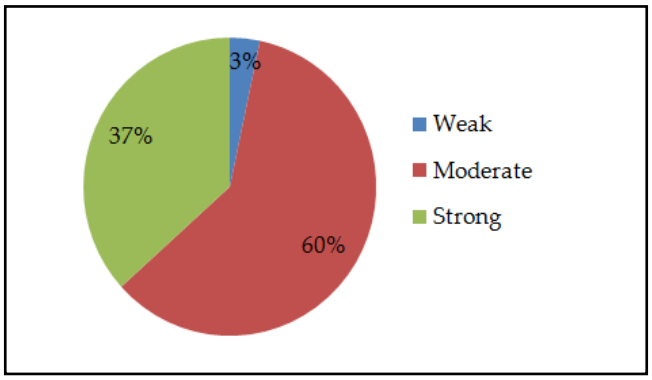

Figure-1: Frequency of stromal tumor infiltrating lymphocytes (n=30).

Eighteen cases had moderate stromal TILs 30 (60%). Within these cases showing moderate stromal TILs, majority of the cases were of grades 2 and 3; 10/18, 18 (55%) and 5/18, 18 (27%) respectively (*p*=0.34) (table-I). Similarly, of these 18/30 cases having moderate stromal TILs, 8 had primary tumor stage 3, 18 (44%) where as 5 cases had stage 2 disease 18 (27%) and stage 4 disease was seen in remaining 5 cases 18 (27%) (*p*-value= 0.293) (table-II). Moderate stromal TILs was associated with regional lymph node metastasis. 3 cases having moderate stromal TILs showed lymph node metastasis stage 1; 18 (16.6%) and 7 cases showed lymph node metastasis stage 2; 18 (38%) (*p*-value=0.015). Of the 18 cases having moderate stromal TILs, 4 showed tumor deposits 18 (22%) (*p*-value=0.102).

Presence of intraepithelial lymphocytosis was seen in 22/30 cases 30 (73%), these belonged predominantly grades 2 and 3; 12/22; 22 (54%) and 6/22; 22 (27%) respectively (*p*-value=0.687) (table-III). Of these 22 cases, 12 belonged to primary tumor stage-3; 22 (54%) (*p*-value=0.846) and 7 cases showed regional lymph node stage-2; 22 (31.8%) (*p*-value=0.723) (table-

IV). Only 4 cases showed presence of tumor deposits 22 (18%) (*p*-value=0.799).

Table-I: Correlation between tumor grade and stromal tumor infiltrating lymphocytes (n=30).

Grade	Stromal Tumor Infiltrating Lymphocytes			*p*-value
	Weak (%)	Moderate (%)	Strong (%)	
Grade 1	1 (3.3)	3 (10)	2 (6.6)	0.343
Grade 2	-	10 (33)	7 (23.3)	
Grade 3	-	5 (16.6)	2 (6.6)	

Table-II: Correlation between regional lymph node status and stromal tumor infiltrating lymphocytes (n=30).

Regional Lymph Nodes	Stromal Tumor Infiltrating Lymphocytes			*p*-value
	Weak (%)	Moderate (%)	Strong (%)	
Stage 0	-	8 (26.6)	10 (33.3)	0.015
Stage 1	1 (3.3)	3 (10)	1 (3.3)	
Stage 2	-	7 (23.3)	-	

Table-III: Correlation between tumor grade and intra-epithelial tumor infiltrating lymphocytes (n=30).

Grade	Intraepithelial Tumor Infiltrating Lymphocytes		*p*-value
	Absent (%)	Present (%)	
Grade 1	2 (6.66)	4 (13.3)	0.343
Grade 2	5 (16.6)	12 (40)	
Grade 3	1 (3.33)	6 (20)	

Table-IV: Correlation between lymph node status and intraepithelial tumor infiltrating lymphocytes (n=30).

Regional lymph nodes	Intraepithelial Tumor Infiltrating Lymphocytes		*p*-value
	Absent (%)	Present (%)	
Stage 0	4 (13.3)	14 (46.6)	0.723
Stage 1	2 (6.66)	3 (10)	
Stage 2	2 (6.66)	5 (16.6)	

DISCUSSION

Colorectal cancers occur independent of various pathogens, although, such type of cancers including malignant melanoma, kidney, lung and pancreatic cancers show increased occurrence in immunocompromised population[12]. A key role in anti-tumor defense is through the innate response of various inflammatory cells (NK cells, immature T lymphocytes, macrophages). Similarly cells involved in acquired response including antigen-presenting cells like CD8+ and CD45 Ro+ T cells are linked to colorectal cancer progression[13]. It has been observed that 50% of mice with T and B immunodeficiency developed tumors in the colon and lung simultaneously, with 80% of such tumors showed interferon resistance[14]. Jakubowska in 2017 indicated that tumor-infiltrating lymphocytes not only could serve a critical role in antitumor responses they can also provide a novel predictive and prognostic marker of colorectal carcinoma[15]. It also aimed to explain a detailed morphological assessment of two different types of TILs. One that is located within the stroma and another within the intra-epithelial structures. Similar evaluation has been conducted in the present study.

Jass *et al*[16] first attempted to classify the inflammatory lymphocytic infiltrate in colorectal carcinoma. This classification was based on a 4-poiny scale intensity assessment of infiltration by lymphocytes.

Later, Ogino *et al*[17] suggested that lymphocytic resposnse evaluation should also include four other parameters including Crohn's like reaction, peritumoral reaction, density of TILs ad intratumoral periglandular reaction. Similarly, Klinturp *et al*[18] also evaluated inflammatory reaction in the invasive margin and devised a classification system: 0 meant no inflammatory cell infiltrate increase, 1 was only mild or patchy increases: 2 was for significant inflammatory reaction with cell destruction and 3 was for florid 'cup-like' inflammatory infiltrate.

It has been seen that intraepithelial TILs once activated can bind to tumor parenchyma permanently once they are accompanied with tumor-associated antigen; single such tumor cell antigens can activated TIL to generate cytotoxic reactions, and the clone determines their degree of stimualtion[19]. Within TILs of stroma such stimulation is done through APC-lymphocyte sequence. Type of specific antigen on the tumor cell determines characteristics, selectivity and strength of stromal TILs clones. The degree of involvement of the immune response in CRC can be assessed by evaluation of presence of intraepithelial and stromal TILs.

It has also been observed that a decrease in TILs within the stroma is leads to involvement of lymphatic tubes, blood vessels, presence of lymph node metastasis and perineural space. This confirms that they are the major component of inflammatory cells involved in immune reposne. Huh *et al*[20] also observed that perineural invasion was identified to be correlated with low TIL grade. Additionally, development of the reponse and organization of TILs in the center of primary tumor stroma is affected by a weak inflammatory response in the invasive front. An increase in the severity of colorectal carcinoma as allocated by Dukes

and TNM staging systems is associate with a decrease in stromal TILs within the tumor. This decreased inflammation of TILS within the tumor can subsequently lead to cancer cell metastasis to distant organs and can also affect tumor size. As TILs affect stromal components' maturity and composition they can cause alteration of the potential of malignancy. Therefore, evaluation of TILs should be done in future histopathologic analysis.

CONCLUSION

The results of the present study demonstrate that the infiltration of tumor infiltrating lymphocytes into the tumor in patients with colorectal carcinoma can play a possible role in the prognosis and invasion of this disease, and should become a part of routine examinations.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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