SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: 10.36346/sarjpm.2022.v03i03.002

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

The Relation between Tumor Infiltrating Lymphocyte and Classical Tumor Staging in Colorectal Carcinoma (Semi-Quantitative Study by Immun histochemistry in a Group of Iraqi Patients)

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Article History: | Received: 28.05.2022 | Accepted: 24.06.2022 | Published: 30.06.2022 |

Abstract: ***Background:*** Inflammatory response had a role in cancer progression, presence of noticeable inflammation within the tumor and its margin may play an important prognostic role in colorectal carcinoma. ***Objective***: The goal of the study is to estimate the classical H&E lymphocytes infiltration pattern, CD3, CD8 cells percentage, and their relation with surgical staging TNM. ***Materials and Methods:*** Thirty cases of colorectal carcinoma were collected from a privet lab in Baghdad from May 2021 to August 2021. The clinicopathological data were taken from patient's reports. The pathological samples were taken from tumor invasive margin as paraffin blocks, for each block one H&E slide and two slides for immune staining for CD3, CD8. ***Results:*** The tumor size ranged from≤ 3-5cm, twenty-three cases (79%) were moderately differentiated adenocarcinoma, nineteen cases (65%) diagnosed as stage III. Tumor infiltrating lymphocytes (K-M) grade shows grade 1 in seventeen cases (59%), grade 2 in ten cases (34%), and grade 3 in three (7%) cases. Sixteen cases (55%) show high intraepithelial CD3, five cases (17%) show high intraepithelial CD8, four cases (14%) with low lymphocytes/stromal ratio, eighteen cases (61%) show moderate lymphocyte/stromal infiltration, and seven cases (24%) with high lymphocytes/stromal infiltration. there were significant statistical relations between intraepithelial CD3 and stromal CD3/CD8 ratio (p=0.010); intraepithelial CD3 cells and stromal/tumor ratio (p=0.024); intraepithelial CD8 cells and patient age (p=0.019); and between tumor size and stromal /lymphocytes ratio(p=0.025); no significant relation between K-M grade, intraepithelial cells, stromal/tumor ratio and TNM staging nor tumor grade. ***Conclusions:*** The relation between classical TNM staging and tumor infiltrating lymphocytes are independent; each one of them measures different aspect of cancer cells behavior, and both of them are important for patient prognosis. There is variation in tumor infiltrating lymphocytes, intraepithelial lymphocyte (CD3, CD8) which clarified by immune histochemical method. Automated calculation method (immunoscore) is superior**.**

Keywords: Colorectal carcinoma, CD3, CD8, H&E, immunohistochemistry**.**

INTRODUCTION

White blood cells (WBC), or lymphocytes are very important component in the formation and the constituents of the immune system, lymphocytes usually helps our body in fight off and defense against infections by killing the cells that cause the disease and eliminate them via B or T cells that identify the undesirable cells like cancer, when cancer cells start to grow up, they are recognized by lymphocytesand go through the tumor and penetrate it, this step called Tumor infiltrating lymphocytes (TILs) and start to kill and destroy the cancer cells (Badalamenti *et al.,* 2019), (Paijens *et al.,* 2021). However, this process may be

braked or prevented if the tumor transmit some signals that may weakened the response of the immune system, and thus recent studies focus on how to develop and improve the immune cell / or TILs in order not to be affected by these tumor signals and can be used as cells therapy (Bagchi *et al.*, 2021).

Colorectal cancer (colon and rectal cancer CRC), is a cancer that affect the large intestine and reach the colon and its lower part (rectum), usually this type of cancer affected with race, age personal history of the patient, however colon cancer takes long period of time to developed the polyps to cancer tumors (Dominic *et al.*, 2020), (Recio-Boiles *et al.*, 2022). Pathological evaluation is very important to assess the depth of the disease to decide prognosis of the cancer and to choose when to start the therapy or the surgery to the tumor (Keller *et al.*, 2020). Many factors such as early diagnosis, the detection of immunohistochemical markers, gene mutation (molecular study), and TNM staging, all these factors may determine the prognosis of the disease.

Recently, in assessing colorectal cancer, the inflammatory response of the local host is a very important and significant to determine the progression of the disease and to verify the oncological patient outcome and the presence of obvious inflammation in the infiltrate cells that may play an important in the survival. The recent studies confirmed the correlation between cancer cell and the immune system particularly, the stomal lymphocyte and infiltrating lymphocytes (Palomero *et al.*, 2020), (Tobin *et al.*, 2021).

The current study was carried out to estimate the classical H&E lymphocytes infiltration pattern, CD3, CD8 cells percentage, and their relation with surgical staging TNM.

MATERIALS AND METHODS

Thirty cases of colorectal carcinoma where collected from Dr. Raji Al-Hadithi private histopathology labs and Al-Qiema hospital from May 2021-August 2021, clinical data as age, sex, tumor type, location, size, grade and TNM staging where taken from patients reports, pathological samples were taken from tumor invasive margin as paraffin blocks, for each block one H&E slide and two slides for immune staining for CD3,CD8.Slides were prepared for immunohistochemical staining, by dewaxing, antigen heat induced retrieval with pH (Dako DM828) solution for 20 mints in water bath at 95 °C (Boenisch 2013).

Each case were stained with CD3 (DakoIS503), CD8 (DakoIR62361-2), the immune histochemical staining protocol was followed as in the reference method (Boenisch 2013), the immune staining by HRPT/ chromogranin detection kit (DakoK8023).

Both H&E and immunohistochemical stained slides were examined by pathologists for tumor type, grade, invasive margin,semi-quantitative, histopathological assessment of the density of the generalized inflammatory cell infiltrate by applying the Klintrup-Mäkinen (KM) on hematoxylin and eosin-stained (H&E) section of the deepest point of tumor invasion using a 4-point scale (0- no increase, 1- mild or patchy increase in inflammatory cells, 2- prominent inflammatory reaction forming a band at the invasive margin, and 3- florid cup-like infiltrate at the invasive edge with destruction of cancer cell islands).

The tumor stroma ratio (10), lymphocytes to stroma ratio **(TILs 2015)** (low when lymphocytes less or equals 10% of the stroma connective tissue, intermediate 10-50% and high when lymphocytes more than 50%of the stroma) (Hendry *et al.*, 2017) were also estimated, and the number of Cd3, CD8 positive cells from immunohistochemical stained slides was calculated as number of cells/HPF. Staging data depending on tumor size, depth of invasion, number of involved lymph nodes, and tumor grade also interpreted. The staging appendix below shows the staging and was applied in the current study **(AJCC Cancer Staging Manual 2017).**

Staging Appendix

Symbol	Description (TNM staging)	Symbol	Description	Symbol	Stage grouping
PT	Primary tumor	PN	Regional lymph nodes	Stage 0	Tis N0 M0
TX	primary tumor cannot be assessed	NX	Regional lymph nodes cannot be assessed	Stage I	T1 - T2 N0 M0
T0	no evidence of primary tumor	N0	no regional lymph node metastasis	Stage IIA	T3 N0 M0
Tis	carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)	N1	Metastasisin 1-3 regional lymph nodes	Stage IIB	T4a N0 M0
TI	tumor invades submucosa (through the muscularis mucosa but not into the muscularispropria),	N1 a	Metastasisin 1 regional lymph node	Stage IIC	T4b N0 M0
T2	tumor invades muscularispropria, T3: tumor invades through the	N1b	Metastasis in 2 - 3 regional lymph nodes	Stage IIIA	T1 - T2 N1 / N1c M0

	muscularispropria into the pericolorectal tissues				T1 N2a M0
T4	T4a: tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum), T4b: tumor directly invades or adheres to other adjacent organs or structures	N1C	No regional lymph nodes are positive but there are tumor deposits in the subserosa, mesentery or nonperitonealized pericolic or perirectal / mesorectal tissues	Stage IIIB	T3 - T4a N1 / N1c M0 T2 - T3 N2a M0 T1 - T2 N2b M0
		N2	Metastasis in 4 or more regional lymph nodes	Stage IIIC	T4a N2a M0 T3 - T4a N2b M0 T4b N1 - N2 M0
		N2a	Metastasis in 4-6 regional lymph nodes	Stage IVA	Any T any N M1a
		N2b	Metastasis in 7 or more regional lymph nodes	Stage IVB	Any T any N M1b
				Stage IVC	Any T any N M1c

RESULTS

In the current study the age group ranged from 50-70 years with most cases between ≤60-70 years, there was slight Male predominance (55%). The tumor size ranged from ≤ 3 to 5 cm, twenty-three cases (79%) were moderately differentiated adenocarcinoma, nineteen cases (65%) diagnosed as stage III. Tumor infiltrating lymphocytes estimated by Klintrup–Mäkinen (K-M) grading method shows grade1 in seventeen cases (59%), grade 2 in ten cases (34%), and grade3 –distractive in three (7%) of the case. Sixteen cases (55%) show high intraepithelial CD3, and five cases (17%) show high intraepithelial CD8, four cases (14%) with low lymphocytes/stromal ratio, eighteen cases (61%) show moderate lymphocyte/stromal infiltration, and seven cases (24%) with high lymphocytes/stromal infiltration. The percent of CD3, CD8 are listed in table 2, while tables 3 represent the statistical relations between CD3/CD8 % with patient's clinic-pathological data. there were significant statistical relations between intraepithelial CD3 and stromal CD3/CD8 ratio (p=0.010) and between intraepithelial CD3 cells and stromal/tumor ratio (p=0.024)table(6), also there was significant relation between intraepithelial CD8 cells and patient age (p=0.019); significant relation between tumor size and stromal /lymphocytes ratio(p=0.025)(tables 4,5); no significant relation between K-M grade, intraepithelial cells, stromal/tumor ratio and TNM staging nor tumor grade.

Table 1: Patients clinic-pathological data

		No	%
Age (years)	<50years	4	13.8
	50---59	7	24.1
	60---69	14	48.3
	=>70years	4	13.8
	Mean±SD (Range)	59.5±10.0 (40-85)	
Gender	Male	16	55.2
	Female	13	44.8
Tumor size	<3x3cm	9	31.0
	3x3---	6	20.7
	4x4---	8	27.6
	=>5x5cm	6	20.7
Tumor infiltrating lymphocytes K-M	Patchy/focal	17	58.6
	Band like	10	34.5
	Distraction	2	6.9

T Stage	T1	2	6.9
	T2	4	13.8
	T3	19	65.5
	T4	4	13.8
N Stage	N0	17	58.6
	N1	6	20.7
	N2	6	20.7
Grade	Well differentiated	4	13.8
	Moderately diff.	23	79.3
	Poorly diff.	2	6.9
Intraepithelial CD3(cells/HPF)	High	16	55.2
	Low	13	44.8
	Mean±SD (Range)	4.7±2.1 (2-8)	
Intraepithelial CD8 (cells/HPF)	High	5	17.2
	Low	24	82.8
	Mean±SD (Range)	3.0±1.6 (0-6)	
Stromal CD3%P (cells/HPF)		38.9±18.2 (8-70)	
Stromal CD8%P (cells/HPF)		16.5±8.8 (2-35)	
Stromal CD8/CD3 ratio	Low (<50%)	17	58.6
	High (=>50%)	12	41.4
Stromal CD8/CD3 ratio		44.9±19.8 (7.14-80)	
Tumor/stromal (%)		69.7±13.8 (40-90	
Lympho/stromal (%)	Low	4	13.8
	Moderate	18	62.1
	High	7	24.1

Table 2: The number and percent of stromal / intraepithelial CD3 and CD8

		No	%
Intraepithelial CD3 (cells/HPF)	High	16	55.2
	Low	13	44.8
	Mean±SD (Range)	4.7±2.1 (2-8)	
Intraepithelial CD8 (cells/HPF)	High	5	17.2
	Low	24	82.8
	Mean±SD (Range)	3.0±1.6 (0-6)	
Stromal CD3%P (cells/HPF)		38.9±18.2 (8-70)	
Stromal CD8%P (cells/HPF)		16.5±8.8 (2-35)	
Stromal CD8/CD3 ratio	Low (<50%)	17	58.6
	High (=>50%)	12	41.4
Stromal CD8/CD3 ratio		44.9±19.8 (7.14-80)	
Tumor/stromal (%)		69.7±13.8 (40-90)	
Lympho/stromal (%)	Low	4	13.8
	Moderate	18	62.1
	High	7	24.1

Table 3: Statistical relations between CD8/CD3 ratio and patient's clinic-pathological data

		Stromal CD8/CD3 ratio				P value
		Low (<50%)		High (=>50%)		
		No	%	No	%	
Age (years)	<50years	3	75.0	1	25.0	0.249
	50---59	2	28.6	5	71.4	
	60---69	10	71.4	4	28.6	
	=>70years	2	50.0	2	50.0	
Gender	Male	9	56.3	7	43.8	0.774
	Female	8	61.5	5	38.5	
Tumor size	<3x3cm	2	22.2	7	77.8	0.025*
	3x3---	5	83.3	1	16.7	
	4x4---	7	87.5	1	12.5	
	=>5x5cm	3	50.0	3	50.0	

Tumor infiltrating lymphocytes K-M	Patchy/focal	12	70.6	5	29.4	0.055
	Band like	3	30.0	7	70.0	
	Distraction	2	100	-	-	
T Stage	T1	1	50.0	1	50.0	0.423
	T2	2	50.0	2	50.0	
	T3	13	68.4	6	31.6	
	T4	1	25.0	3	75.0	
N Stage	N0	10	58.8	7	41.2	0.842
	N1	4	66.7	2	33.3	
	N2	3	50.0	3	50.0	
Grade	Well differentiated	2	50.0	2	50.0	0.186
	Moderately diff.	15	65.2	8	34.8	
	Poorly diff.	-	-	2	100	
Intra epithelial CD3	High	6	37.5	10	62.5	0.010*
	Low	11	84.6	2	15.4	
Intra epithelial CD8	High	1	20.0	4	80.0	0.054
	Low	16	66.7	8	33.3	
Lympho/stroma (%)	Low	3	75.0	1	25.0	0.132
	Moderate	8	44.4	10	55.6	
	High	6	85.7	1	14.3	
*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 levels.						

Table 4: Statistical relations between intraepithelial CD3 lymphocytes and patients clinic-pathological data

		Intra epithelial CD3				P value
		High		Low		
		No	%	No	%	
Age (years)	<50years	3	75.0	1	25.0	0.604
	50---59	3	42.9	4	57.1	
	60---69	7	50.0	7	50.0	
	=>70years	3	75.0	1	25.0	
Gender	Male	8	50.0	8	50.0	0.534
	Female	8	61.5	5	38.5	
Tumor size	<3x3cm	6	66.7	3	33.3	0.563
	3x3---	2	33.3	4	66.7	
	4x4---	4	50.0	4	50.0	
	=>5x5cm	4	66.7	2	33.3	
Tumor infiltrating lymphocytes K-M	Patchy/focal	7	41.2	10	58.8	0.145
	Band like	8	80.0	2	20.0	
	Distraction	1	50.0	1	50.0	
T Stage	T1	1	50.0	1	50.0	0.567
	T2	1	25.0	3	75.0	
	T3	12	63.2	7	36.8	
	T4	2	50.0	2	50.0	
N Stage	N0	9	52.9	8	47.1	0.811
	N1	3	50.0	3	50.0	
	N2	4	66.7	2	33.3	
Grade	Well differentiated	3	75.0	1	25.0	0.251
	Moderately diff.	11	47.8	12	52.2	
	Poorly diff.	2	100	-	-	
Intra epithelial CD8	High	4	80.0	1	20.0	0.220
	Low	12	50.0	12	50.0	
Stromal CD8/CD3 ratio	Low (<50%)	6	35.3	11	64.7	0.010*
	High (=>50%)	10	83.3	2	16.7	
Lympho/stroma (%)	Low	-	-	4	100	0.024*
	Moderate	13	72.2	5	27.8	
	High	3	42.9	4	57.1	
*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 levels.						

Table 5: Statistical relations between intraepithelial CD8 lymphocytes and patients clinic-pathological data

		Intra epithelial CD8				P value
		High		Low		
		No	%	No	%	
Age (years)	<50years	-	-	4	100	0.019*
	50---59	3	42.9	4	57.1	
	60---69	-	-	14	100	
	=>70years	2	50.0	2	50.0	
Gender	Male	1	6.3	15	93.8	0.082
	Female	4	30.8	9	69.2	
Tumor size	<3x3cm	1	11.1	8	88.9	0.106
	3x3---	-	-	6	100	
	4x4---	1	12.5	7	87.5	
	=>5x5cm	3	50.0	3	50.0	
Tumor infiltrating lymphocytes K-M	Patchy/focal	2	11.8	15	88.2	0.384
	Band like	3	30.0	7	70.0	
	Distraction	-	-	2	100	
T Stage	T1	-	-	2	100	0.657
	T2	1	25.0	3	75.0	
	T3	4	21.1	15	78.9	
	T4	-	-	4	100	
N Stage	N0	2	11.8	15	88.2	0.485
	N1	1	16.7	5	83.3	
	N2	2	33.3	4	66.7	
Grade	Well differentiated	2	50.0	2	50.0	0.058
	Moderately diff.	2	8.7	21	91.3	
	Poorly diff.	1	50.0	1	50.0	
Intra epithelial CD3	High	4	25.0	12	75.0	0.220
	Low	1	7.7	12	92.3	
Stromal CD8/CD3 ratio	Low (<50%)	1	5.9	16	94.1	0.054
	High (=>50%)	4	33.3	8	66.7	
Lympho/stroma (%)	Low	-	-	4	100	0.158
	Moderate	5	27.8	13	72.2	
	High	-	-	7	100	
*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 levels.						

Table 6: Statistical relations between lymphocytes/ stromal ratio and patient's clinic-pathological data

		Lympho/stroma (%)						P value
		Low		Moderate		High		
		No	%	No	%	No	%	
Age (years)	<50years	1	25.0	2	50.0	1	25.0	0.582
	50---59	1	14.3	6	85.7	-	-	
	60---69	2	14.3	8	57.1	4	28.6	
	=>70years	-	-	2	50.0	2	50.0	
Gender	Male	4	25.0	9	56.3	3	18.8	0.144
	Female	-	-	9	69.2	4	30.8	
Tumor size	<3x3cm	2	22.2	5	55.6	2	22.2	0.074
	3x3---	-	-	2	33.3	4	66.7	
	4x4---	2	25.0	5	62.5	1	12.5	
	=>5x5cm	-	-	6	100	-	-	
Tumor infiltrating lymphocytes K-M	Patchy/focal	3	17.6	12	70.6	2	11.8	0.092
	Band like	1	10.0	6	60.0	3	30.0	
	Distraction	-	-	-	-	2	100	
T Stage	T1	-	-	-	-	2	100	0.203
	T2	-	-	3	75.0	1	25.0	
	T3	3	15.8	12	63.2	4	21.1	
	T4	1	25.0	3	75.0	-	-	
N Stage	N0	2	11.8	9	52.9	6	35.3	0.149

	N1	-	-	6	100	-	-	
	N2	2	33.3	3	50.0	1	16.7	
Grade	Well differentiated	-	-	3	75.0	1	25.0	0.690
	Moderately diff.	4	17.4	13	56.5	6	26.1	
	Poorly diff.	-	-	2	100	-	-	
Intra epithelial CD3	High	-	-	13	81.3	3	18.8	0.024*
	Low	4	30.8	5	38.5	4	30.8	
Intra epithelial CD8	High	-	-	5	100	-	-	0.158
	Low	4	16.7	13	54.2	7	29.2	
Stromal CD8/CD3 ratio	Low (<50%)	3	17.6	8	47.1	6	35.3	0.132
	High (=>50%)	1	8.3	10	83.3	1	8.3	
*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 levels.								

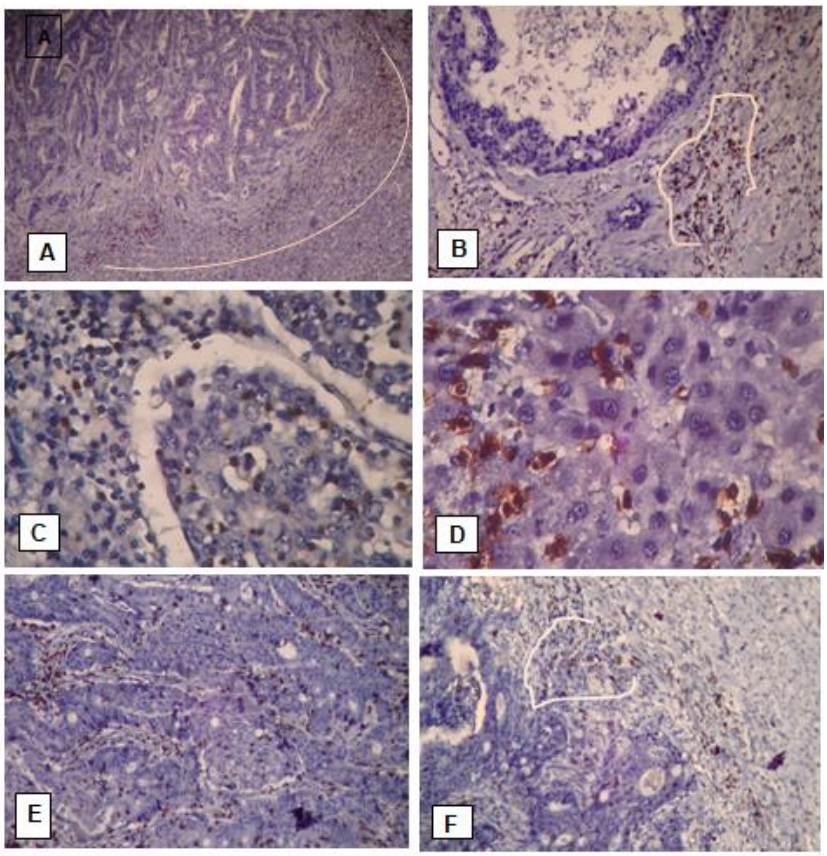

Figure 1: Immunohistochemical stain; A: low power view; CD3 stain showing Band like infiltration at tumor margin (K-M)2; B: medium power view showing stromal infiltrating CD3 cells(moderate infiltration); C: Medium power view show Increased Intraepithelial CD8 cells; D: high power view show Increased intraepithelial CD3 cells; E: Medium power view show low stroma/tumor ratio with moderate CD3 infiltration; F: low power view showing Patchy marginal CD3 cells infiltrate (K-M)1.

DISCUSSION

Colorectal cancer is the most common gastrointestinal tract cancer worldwide. In Iraq, a low CRC incidence rate but with a steady increase overtime. It was the seventh top cancers (Al D. *et al.*, 2018) in our study the age group ranged from 50-70 years with most cases between 60-70 year, there was slight Male predominance (55%) to female ratio varied from 1.17:1 to 1.28:1. which was similar to other studies by (Khalil *et al.*, 2018), (Al D. *et al.*, 2018). About 40%-46% of cases were diagnosed in the age group of 40-59 years, and the tumor size ranged from≤ 3 to5 cm,65% of the cases diagnosed as stage III similar to (Khalil *et al.*, 2018).

In this study, we tried to compare the relation of two validated measures of the tumor inflammatory cell infiltrate-the KM grade and the immunohistochemical estimation of CD3, CD8 cell ratio with tumor stage and grade. There was no significant association between clinicopathological parameters and KM grade and stromal lymphocytes similar to (Fuchs *et al.*, 2020), (Wu *et al.*, 2020). This may be due to heterogeneous infiltration of T-cells in association with tumor microenvironment, immune status and tumor grade which demonstrated firstly in Lynch syndrome (Fuchs *et al., 2020*). Although an increase in KM grade is associated with an increase in the density of tumor-infiltrating T-lymphocytes by

(Richards *et al.,* 2012). We did not found such relation in our study ,cause most our cases were moderately differentiated adenocarcinoma; more cases with variation in grade and histological subtype may show different results. The significant association between patients age and intraepithelial CD8 cells was similar to (Fuchs *et al.,* 2020); that may due to the progressive deterioration of the immune system that occurs with aging, remain an important trigger in the development of many tumors (Kavvadas *et al.,* 2022), in addition to chronic inflammation of the colonic mucosa with ongoing molecular-structural remodeling (Yamauchi *et al.,* 2018).

Significant Relation between Lymphocytes/Stroma and Intraepithelial Lymphocytes

There was significant relation between stromal CD3 lymphocytes and tumor stage; most of tumor infiltrating cells was of T-cell, which included in body defense against cancer cells. The components of the extracellular matrix (ECM), through their specific biochemical properties, can regulate the migration of immune cells toward the development of cancer (Zadka *et al.,* 2021). This disagree with (Yamauchi M *et al.,* 2018) were negative relation found between CD3 stromal cells and TNM staging, may due to the heterogeneous pattern of the intraepithelial lymphocytic infiltration within the numbers of inspected fields and the variation between patient's immune state. Also significant relation found between stromal CD3/CD8 ratio and tumor size and the intraepithelial lymphocytes similar to (Zadka *et al.,* 2021), this may be due to the positive relation between inflammation, inflammatory mediators, and cancer which expressed by CD3 positivity as a pan T-cells marker where CD8 is specific for cytotoxic T-cells which involved in body defiance against cancer cells, this relation cannot be seen by routine H&E stain, so the automated calculation of cells and Immunoscore has recently been refined to reflect a cumulative score based on the density of the overall mature CD3+ T-lymphocyte population in addition to the CD8+ T-lymphocyte population within the tumor invasive margin and tumor core, and has been validated as a prognostic marker with superior prognostic ability compared to TNM staging in colorectal cancer (Mlecnik *et al.,* 2011), (Anitei *et al.,* 2014), (Galon *et al.*, 2014).

The relative difference in the prognostic value of the inflammatory cell infiltrate may be explained by the components of the immune response. Whereas the KM grade provides a measure of the overall, generalized inflammatory cell infiltrate, the Immunohistochemistry measures the host adaptive T-lymphocytic response to cancer (Park *et al.,* 2016). (Vayrynen *et al.,* 2013), (Richards *et al.,* 2014), they suggested that the presence of a tumor-associated stroma precludes effective infiltration of the tumor microenvironment by an antitumor immune response, this finding was confirmed by (Roxburgh *et al.,* 2012), (Hartmann *et al.,* 2014), (Mei *et al.,* 2014).

STATISTICAL ANALYSIS

Analysis of data was carried out using the available statistical package of SPSS-27 (Statistical Packages for Social Sciences- version 27). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different means (quantitative data) was tested using Students-t-test for difference between two independent means or ANOVA test for difference among more than two independent means. The significance of difference of different percentages (qualitative data) was tested using Pearson Chi-square test (χ^2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05 (Biostatistics 2010), (Biostatistics 2013), (Celentano *et al.,* 2019).

CONCLUSION

- The relation between classical TNM staging and tumor infiltrating lymphocytes are independent; each one of them measures different aspect of cancer cells behavior, and both of them are important for patient prognosis.
- There is variation in tumor infiltrating lymphocytes, intraepithelial lymphocyte (CD3, CD8) which clarified by immune histochemical method.
- Automated calculation method (immunoscore) is superior.

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