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Immunohistochemical Evaluation of PD-L1 Expression in Colonic Adenocarcinoma

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Abstract

Background: **Colon carcinoma is one of the most common carcinomas worldwide. It is the third most diagnosed cancer and the third most common cause of cancer-related death in both men and women worldwide. PD-L1 plays major roles in physiological and pathological immune modulation, including tumor microenvironment.** ***Aim:*** **This study aimed to evaluate the immunohistochemical expression of PD-L1 in colonic adenocarcinoma, and its association with clinicopathologic prognostic factors.** ***Materials and Methods:*** **This analytical cross-sectional study was conducted at the department of Pathology of Suez Canal University Hospital and included 44 cases that were retrieved from the archived paraffin-embedded blocks at the department with their corresponding medical records, for immunohistochemical expression of PD-L1 and its role in prognosis.** ***Results:*** **PD-L1 expression was high in 64% of cases, and low in 25% of cases, while 11% of cases were negative. Tumor showed statistically significant association with grading, tumor infiltrating lymphocytes, neutrophilic infiltration, tumor budding, desmoplasia, and tumor necrosis. By following up the cases to assess prognosis of the disease and its impact on the 5-year survival rate, it was found that 54.5% of the studied group showed 5-year survival while 45.5% of patients were died.** ***Conclusions:*** **This study provides that PD-L1 expression is statistically associated with aggressive tumor characteristics of colonic adenocarcinoma. These findings suggest that PD-L1 expression may serve as an independent prognostic marker in colon tumors, with consideration given to the heterogeneity of the included studies. This information could be valuable in identifying patients likely to benefit from treatment with PD-1/PD-L1 blockers.**

Keywords: Colonic adenocarcinoma, PD-L1, Immunotherapy

Introduction

Colonic cancer (CA) is considered the third most common cancer worldwide, after lung and breast cancer. In Egypt, and according to the National Cancer Institute registry at Cairo University, colonic adenocarcinoma (CA) accounts for 6.5 % of all malignant tumors [(13)]. In the years

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2002-2003, Colonic cancer was the 6th most documented tumor. Male to female ratio is 4.2: 3.8. In addition, colonic cancer is detected in 14.0 % of all colonoscopies in Egypt [6].

Colonic cancer (CA) has been treated with multimodal therapies as typical first line therapies [14]. Despite recent advances in both diagnosis and treatment, recurrence and metastasis are primary causes of therapy failure [1]. The prognosis of CA is still poor, especially in advanced stages. The tumor microenvironment has recently gotten a lot of interest, and studies of the interactive relationship between tumor cells and the immune system have gotten a lot of attention [14]. Tumors are rarely spontaneously rejected due to their capacity to establish an immunosuppressive microenvironment by stimulating immunological checkpoints, including PD-L1 [10].

PD-L1 is one of the co-stimulatory factors found on various types of tumor cells and in immune cells, including activated B cells, T cells, macrophages, and dendritic cells [25]. In addition, PD-L1 is expressed on tumor cells in a variety of cancers [21]. It acts via the PD-1/PD-L1 transduction pathway and inhibits T cell proliferation and differentiation. It also blocks signal transduction and the secretion of different cytokines, causing cancer cell invasion and metastasis. Its expression indicates a weaker host immune response and a bad prognosis in different types of cancer [24].

Recently, Immunotherapies that target PD-L1 and PD-1 inhibitors have become standard treatments for many cancers [14, 20]. However, research assessing the prognostic value of PD-L1 expression in CA remains controversial [23]. Yet in CA, there are no selection criteria that may consistently detect subgroups of patients whose tumors will respond to existing immunotherapies [14].

Aim

To evaluate Immunohistochemical expression of PD-L1 in Colonic cancer (CA), and its correlation with clinicopathologic prognostic factors and the 5-year survival rate.

Materials and methods:

This cross-sectional analytic study included 44 formalin-fixed paraffin-embedded [FFPE] blocks of hemi- and total colectomy specimens diagnosed as colonic adenocarcinoma, archived in Pathology laboratory, of patients that underwent surgical excision by colectomy without previous chemotherapy or radiation. The clinical and pathological data were obtained from medical records and Pathology reports including age at diagnosis, stage, histological subtype, and tumor grade. The 5–year survival rate was obtained from the archived files of patients at Oncology department, Suez Canal University Teaching Hospitals from January 2012 to December 2016.

All specimens were stained first by H&E and reviewed to confirm the diagnosis of colonic Adenocarcinoma, ensure sufficient material for inclusion, and confirm the tumor histologic type, grade, stage, degree of infiltration, lymphovascular invasion, perineural invasion, tumor budding, perforation, and state of margins. The histologic classification was evaluated based on the WHO [12]. The pathological staging of the tumor was evaluated based on the AJCC and UICC1 TNM staging system [3].

Immunohistochemical staining

The slides were then immune-stained with rabbit monoclonal human anti-PD-L1 antibody (Quartett Immunodiagnostics, Berlin, Germany) 7ml (QR001, Rb, 7mL RTU, Item number: P-P001-70) and using positively charged slides. The detection kit ProTaqs® Essential with DAB (Cat. No. 300120200) was used. We used the antibodies according to manufacturer instructions. For antigen retrieval, slides were brought to a boil in 10 ml Tris/EDTA buffer (pH 9.0) maintained at Microwave at full power for 20 minutes. A section of human tonsil tissue was used as a positive and negative control for each staining run (positive lymphocytes and negative epithelial cells).

Evaluation of PD-L1 immunohistochemical staining

Immunohistochemically stained tissue sections were examined by light microscopy. PD-L1 expression was calculated using combined positive score (CPS). Combined Positive Score is defined as the number of positive tumor cells, lymphocytes and macrophages (monomorphic cells), divided by the total number of viable tumor cells multiplied by 100. Where minimum of 100 viable tumor cells must be present for evaluation. Tumor cells with partial or complete membrane staining are included. Immune cells with both membranous or cytoplasmic staining of lymphocytes and macrophages are included. A cutoff ≥1

was used for interpretation of combined positive score. Positive cases were further scored by a two-tiered system into: low PD-L1 expression (≥1–49) and high PD-L1 expression (≥50) [17].

Statistical Data Analysis

All statistical analyses were performed using the statistical package for social science (SPSS) version 25m, being statistically significant at a p-value less than 0.05. Descriptive statistics were applied in numerical form (mean + SD or percentages) to describe the quantitative variables. Tables, charts and diagrams were used to describe the quantitative and qualitative variables. Associations between variables were tested for significance by using the Fisher's exact test and the Pearson correlation, in expressing categorical variables.

Ethical considerations

All required criteria were fulfilled.

Results

Demographic data of patients

44 patients were included in this study. The patients' ages ranged from

17 to 77 years with mean age; 58.93 +/- 12.64 years. 26 (59.1% of patients were ≥ 60 years and 18 (40.9%) of patients were < 60 years. 25 (56.80%) were males and 19 (43.20%) were females (Table 1)

Table (1): Clinical characters of patients (n=44)

Variable		Mean /frequency N (%)
Age (years)		Mean = 58.93 +/- 12.64 years
Age categories	≥ 60 years	26 (59.1%)
	< 60 years	18 (40.9%)
Gender	Male	25 (56.80%)
	Female	19 (43.20%)

Table (2): Histopathological characters of cases (n=44)

Variable		Frequency (%)
Site	Right-sided colon	35 (79.6 %)
	Transverse colon	6 (13.6 %)
	Left-sided colon	3 (6.8 %)
Histologic type	Infiltrating adenocarcinoma	27 (61.4 %)
	Infiltrating mucinous adenocarcinoma	9 (20.4 %)
	Infiltrating adenocarcinoma with mucinous differentiation	8 (18.2 %)
Grading	Grade I	5 (11.4%)
	Grade II	17 (38.6 %)
	Grade III	22 (50 %)
Desmoplasia	Mild	26 (59 %) 13
	Moderate	(29.5 %)
	Marked	5 (11.5 %)
LVI	Yes	10 (22.7%)
	No	34 (77.3 %)
PNI	Yes	6 (13.6%)
	No	38 (86.4 %)
TILs	Mild	33 (75 %)
	Moderate	11 (25 %)
Neutrophilic Infiltrate	Mild	30 (68.2%)
	Moderate	14 (31.8 %)
Tumor necrosis	Yes	16 (63.6 %)
	No	28 (13 %)
Tumor budding	High	25 (56.8%)
	Low	19 (43.2 %)
pT stage	pT2	3 (6.8%)
	pT3	30 (68.2 %)
	pT4	11 (25%)
N stage	No	17 (36.6 %)
	N1	15 (34.2%)
	N2	12 (27.4 %)
TNM stage	Stage I/II	17 (38.6 %)
	Stage III	27 (61.4 %)

Histopathological assessment

Regarding the site of primary tumor origin, 35 (79.6 %) were in the right side of the colon, 6 (13.6 %) were in the transverse colon and only 3 (6.8 %) in the left side of colon. The mean size of the tumors was 6.76 ±3.19 cm, with a minimum of 2cm, a maximum of 20 cm, and range of 18 cm. Regarding the histologic type of the tumor, 27 (61.4 %) were infiltrating adenocarcinoma, 9 (20.4 %) were infiltrating mucinous adenocarcinoma and 8 (18.2 %) were infiltrating adenocarcinoma with mucinous differentiation. The tumors were subdivided according to differentiation grades into 5 (11.4 %) well differentiated, 17 (38.6 %) moderately differentiated and 22 (50 %) poorly differentiated. Lympho-vascular invasion was detected in 10 (22.7%) and peri-neural invasion was present in 6 (13.6%) of cases. Regarding the density of tumor infiltrating lymphocyte, 33 (75 %) were mild and 11 (25 %) were moderate. Regarding neutrophilic infiltrate, 30 (68.2%) showed mild neutrophilic infiltrate and 14 (31.8 %) showed moderate neutrophilic infiltrate. Regarding tumor budding, 25 (56.8%) showed high tumor budding and 19 (43.2 %) showed low tumor budding. Regarding desmoplastic reaction, 26 (59 %) showed mild desmoplasia, 13 (29.5 %) showed moderate desmoplasia, 5 (11.5 %) showed marked desmoplasia. Regarding tumor necrosis, 16 (36.4 %) showed tumor necrosis. The primary tumor (pT) staging of the tumor involved, 30 (68.2 %) being classified as pT3, 11 (25%) as pT4, and 3 (6.8%) as pT2. Regarding lymph node metastasis 17 (36.6 %) were classified as N0, 15 (34.2%) as N1, and 12 (27.4 %) as N2. 3 (6.8%) had extra-nodal extension, and 12 (27.3%) were positive for tumor deposits. According to TNM Staging the tumors were classified as 17 (38.6 %) stage I/II, 27 (61.4 %) as stage III (Table 2). Regarding the 5-year survival rate, 24 (54.5%) of the studied group showed 5-year survival while 20 (45.5%) patients were died (Table 3)

Table 3: *The 5-year survival rate of patients (n=44)*

	Variable	**Frequency (%)**
5-year survival	Alive	24 (54.5%)
	Dead	20 (45.5%)

Immunohistochemical assessment

We found that 39 cases (89%) were positive for PD-L1, 28 cases (64%) of the positive ones showed high expression of PD-L1 (CPS ≥50), and 11 cases (25%) of the positive ones showed low expression of PD-L1 (CPS ≥1–49) (Figure 1), while 5 cases (11%) were negative for PD-L1 expression (CPS <1). There was no statistically significant correlation between PDL1 expression and gender or age of patients. High expression of PDL-1 was more in grade III than grade I and II (Figure 2-4). The difference was statistically significant (p=0.017). However, there was no statistically significant difference in expression of PDL-1 according to histologic type, pT-staging, TNM staging or grade. There was no statistically significant difference in expression of PDL-1 according to tumor deposits, lymph node metastasis, extra-nodal extension, lympho-vascular invasion, or peri-neural invasion. High expression of PDL-1 was more in moderate tumor infiltrating lymphocytes, moderate neutrophilic infiltrate, high tumor budding, marked desmoplasia and tumor necrosis. The difference was statistically significant (p=

0.015, 0.003, 0.001, 0.05 & 0.035 respectively). There was a strong positive correlation between PD-L1 expression and tumor size (r=1), but it is not statistically significant (p=0.124) (Table 4). There was no statistically significant difference in expression of PDL-1 according to 5-year survival rate (Table 5).

Table (4): Association of PD-L1 expression with clinico-pathological features (n=46).

		PD-L1			Total	X^2	p-value
		-VE	Low	High			
Gender	Male	4	5	16	25	1.675 [a]	0.433
	Female	1	6	12	19		
Age	<60	1	6	11	18	1.781 [a]	0.410
	≥60	4	5	17	26		
Site	Right side colon	3	10	22	35	5.072 [a]	0.285
	Transverse colon	2	1	3	6		
	Left sided colon	0	0	3	3		
Histologic type	Infiltrating adenocarcinoma	2	5	20	27	4.892 [a]	0.299
	Infiltrating mucinous adenocarcinoma	2	4	3	9		
	Infiltrating adenocarcinoma with mucinous differentiation	1	2	5	8		
Grading	Grade 1	0	2	3	5	12.081[a]	0.017*
	Grade 2	4	7	6	17		
	Grade 3	1	2	19	22		
Desmoplasia	Mild	5	9	12	26	9.341 [a]	0.05*
	Moderate	0	2	11	13		
	Marked	0	0	5	5		
LVI	Yes	0	1	9	10	4.049 [a]	0.132
	No	5	10	19	34		
Perineural invasion	Yes	0	0	6	6	3.970 [a]	0.137
	No	5	11	22	38		

TILs	mild	5	11	17	33	8.381 [a]	0.015*
	Moderate	0	0	11	11		
Neutrophilic infiltrate	Mild	5	11	14	30	11.733 [a]	0.003*
	Moderate	0	0	14	14		
Tumor necrosis	Yes	0	2	14	16	6.679 [a]	0.035*
	No	5	9	14	28		
Tumor budding	High	0	0	25	25	33.083[a]	0.001*
	Low	5	11	3	19		
LN metastasis	No	2	6	9	17	2.239 [a]	0.674
	N1	1	3	11	15		
	N2	2	2	8	12		
Extranodal extension	No	0	0	3	3		
	Yes	5	11	25	41		
pT staging	pT2	0	0	3	3	2.780 [a]	0.595
	pT3	3	9	18	30		
	pT4	2	2	7	11		
TNM staging	Stage I/II		6	9	17	1.676 [a]	0.433
	Stage III		5	19	27		

[a] values are based on Fisher`s exact test.

Statistical significance at p < 0.05.

** Statistically significant*

Table (5): - *Correlation between PDL-1 and 5-year survival rate (n=44).*

		PD-L1			Total	X2	*p-value*
		Negative	**Low expression**	**High expression**			
5 year survival	**Alive**	3	3	18	24	4.431	*0.109*
	Dead	2	8	10	20		

Values are based on Fisher`s exact test.

Statistical significance at p < 0.05.

***** Statistically significant*

Discussion

Colon cancer poses a significant global health challenge, representing 7.9% of all new cancer cases and 8.6% of all cancer-related deaths (Cancer Statistical Information: Colorectal Cancer, 2022). Recently, immune checkpoint inhibitors have emerged as a promising treatment strategy for colorectal cancer by modulating the immune system [(14)]. PD-L1 plays a crucial role in the tumor microenvironment, influencing tumor immune evasion mechanisms. Current cancer research focuses on disrupting the PD-1/PD-L1 pathway to combat tumor immune evasion through immunotherapy. However, the relationship between PD-L1 expression, clinicopathological features, and prognosis in colon cancer patients lacks a clear consensus [(19)].

Our study, a cross-sectional analysis, included 44 colon resection specimens diagnosed as colon adenocarcinoma, archived at the pathology laboratory and oncology department of Suez Canal University Hospital from January 2012 to December 2016. The study aimed to assess the immunohistochemical expression of PD-L1 in colon adenocarcinoma and its correlation with various clinicopathological prognostic factors and 5-year survival rates. The average age of patients was 58.93 ± 12.64 years, with over half of the patients (59.1%) aged 60 years or older, while 40.9% were younger than 60, regardless of expression intensity.

Among the positive cases (39 cases), 28 (64%) displayed low CPS (≥1-49), whereas 11 (25%) showed high CPS (≥50). In contrast to our study, Lang-Schwarz et al. (2021) utilized TPS, IC, and CPS scoring, reporting completely negative PD-L1 in 182 cases (52.8%). TPS and IC were positive in 163 cases (47.2%); TPS was positive in 61 cases (17.6%), and IC was positive in 157 cases (45.2%). However, they did not show the results of cases recorded by CPS.

Our study found no correlation between PD-L1 and demographic characteristics such as age, sex, or tumor location. This finding is consistent with previous studies by Secinti et al. (2022) and Wang et al. (2020).

However, Rosenbaum et al. (2016) and Husain et al. (2021) have shown that PD-L1 expression is significantly associated with older age at resection. Additionally, Rosenbaum et al. (2016), Eriksen et al. (2019), and Lang-Schwarz et al. (2021) reported that PD-L1 expression is significantly associated with female gender.

Lang-Schwarz et al. (2021) reported that overall PD-L1 positivity with the QR clone was significantly correlated with right colon cancer.

Regarding the correlation between PD-L1 expression and T stage, our results showed that tumor size and extension into the colon wall of PD-L1-positive cases and PD-L1 negativity were similar. Although there was a strong positive correlation between PD-L1 value and tumor size, it was not statistically significant.

Regarding lymph node metastasis, our results showed no statistically significant correlation between PD-L1 expression and lymph node metastasis, extranodal spread, or tumor deposition. This finding is consistent with the results reported by Droeser et al. (2013), Masugi et al. (2016), and Rosenbaum et al. (2016).

Our study found that high PD-L1 expression was more common in grade III tumors than in grade I and II tumors, and the difference was statistically significant. This finding is similar to previous studies

by Rosenbaum et al. (2016), Lee et al. (2019), Lang-Schwarz et al. (2021), Ntomi et al. (2021), and Secinti et al. (2022). However, Wang et al. (2020) reported a negative correlation between PD-L1 expression and tumor differentiation, while Shan et al. (2019) reported no significant difference in PD-L1 expression between different histological grades.

Our study examined the correlation between PD-L1 score and the histological type of colon cancer. We found that there was no statistically significant correlation between PD-L1 score and the histological type of colon cancer. Similar results were reported by Secinti et al. (2022), who also found no correlation between PD-L1 expression and histological type, particularly mucinous differentiated tumors. However, Lang-Schwarz et al. (2021) reported a negative correlation between PD-L1 expression and mucinous tumors ($p = 0.050$).

We also found no statistically significant relationship between PD-L1 expression and lymphovascular invasion. This is consistent with the results reported by Rosenbaum et al. (2016), where lymphovascular invasion was not statistically associated with PD-L1 expression. However, Secinti et al. (2022) reported that PD-L1 expression was higher in LVI-positive cases than in LVI-negative cases (43.5% and 25.9%, respectively), although the relationship was not statistically significant ($P = 0.065$). The authors suggested that the lack of significant correlation between PD-L1 expression and LVI may be due to sampling limitations or difficulty distinguishing true LVI invasion from artifacts.

Regarding perineural invasion (PNI), we found no statistically significant relationship between PD-L1 expression and perineural invasion. This is consistent with the results reported by Rosenbaum et al. (2016), who also found no significant association between PD-L1 expression and PNI. However, Ntomi et al. (2021) and Secinti et al. (2022) both reported a significant relationship between PD-L1 expression and PNI.

We found that high PD-L1 expression was more common in tumors with moderate tumor-infiltrating lymphocytes (TILs), and this difference was statistically significant ($p = 0.015$). This finding is consistent with the results reported by Rosenbaum et al. (2016), Lang-Schwarz et al. (2021), and Secinti et al. (2022), where a statistically significant relationship between PD-L1 expression and TILs was observed. In contrast, Cho et al. (2011) reported that PD-L1-positive tumor tissues were associated with low-density tumor-infiltrating lymphocytes (TIL).

We also found that high PD-L1 expression was more frequent in tumors with high tumor budding and differentiation. This was statistically significant ($p = 0.001$). This is similar to the results reported by Secinti et al. (2022), where a significant relationship was found between tumor growth and PD-L1. PD-L1 positive cases had higher bud scores ($P=0.023$). However, Lang-Schwarz et al. (2021) found that PD-L1 positivity was significantly correlated with low tumor budding ($p = 0.044$).

The following study found that there was no significant difference in PD-L1 expression based on 5-year survival, which is consistent with the results of Eriksen et al. (2019). They observed high PD-L1 expression in only 6% of colon tumors, which was not associated with survival. These findings suggest that PD-L1 positivity may not be a reliable biomarker for predicting tumor aggressiveness or metastatic potential in certain cancer types. Other factors, such as tumor stage

and molecular subtyping, are likely to play a more important role in predicting clinical outcome and guiding treatment decisions.

In another study by Enkhsaikhan et al. (2018), PD-L1 expression did not significantly differ in disease-free survival (DFS) (5-year DFS rate was 77.9% in patients with PD-L1 expression vs. 78.1% in patients with negative PD-L1 expression). However, PD-L1-positive patients had a significantly lower overall survival (OS) than PD-L1-negative patients (5-year OS rate of 76.7% in PD-L1-positive patients compared with 93.2% in PD-L1-negative patients).

Meanwhile, Li et al. (2016) studied a large group of patients from The Cancer Genome Atlas (TCGA) database and found an association between high expression of PD-1 and PD-L1 and improved overall survival. Overall, the studies discussed earlier had greater variability. This may be due to the timing of patient recruitment, the presence of underlying clinical confounders such as tumor stage and use of adjuvant chemotherapy, and the retrospective nature of these studies that do not allow us to establish a direct cause-and-effect relationship between PD-1/PD-L1 and poor survival.

Additionally, other factors include the use of different monoclonal antibodies with different affinities, different staining procedures, differences between fresh and paraffin-embedded samples, differences in the amount of tissue available for PD-L1 assessment (e.g., tissue microarray versus whole tumor), and variations in scoring PD-L1 expression on inflammatory cells, as well as differences in scoring algorithms with different thresholds. Therefore, specific conclusions cannot be drawn from the current literature and the prognostic value of PD-L1 expression in colon cancer remains controversial.

Conclusion

PD-L1 expression may serve as an independent prognostic marker in colon tumors, with consideration given to the heterogeneity of the included studies. This information could be valuable in identifying patients likely to benefit from treatment with PD-1/PD-L1 blockers and may lead to improved patient outcomes through more targeted and effective pharmacological interventions. Further studies are needed to address the heterogeneity of the studies included, to explore the relationship between PD-L1 expression and other clinical factors not found to have a statistically significant correlation in this study. Clinical trials, with PD-1/PD-L1 blockers and other pharmacological agents, are needed to determine their efficacy and optimal dosages and to investigate the correlation between PD-L1 expression and response to immunotherapy treatments, such as PD-1/PD-L1 blockers.

Declaration of Conflicting Interests

Authors declared no conflicts of interest in the research, authorship or publication of this article.

Ethics approval and consent to participate

All the available data and pathological samples obtained from archives and Pathology laboratory reports were segregated from material for keeping confidentiality. Paraffin blocks were not entirely used up. Proper disposal of amount left, and containers of carcinogenic materials was considered. Approval of the ethical committee to the final protocol was obtained.

Availability of data and materials

All data generated or analyzed during this study are included in the article.

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