RESEARCH ARTICLE

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Expression of Programmed Death-1 Ligands (*PD-L1* and *PD-L2*) in Endometrial Carcinoma: Immunohistochemical Study

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Abstract

Background: On basis of knowledge about the relationship between the immunity and cancer; cancer immunotherapies were introduced. Immune checkpoint regulators rank among the most crucial of those tactics. Programmed Death Ligand-1 (*PD-L1*) and Programmed Death Ligand-2 (*PD-L2*) are 2 ligands of Programmed Death-1 (PD-1); an immune checkpoint regulator. *PD-L1* and *PD-L2* antibodies have been effective in treating a variety of malignancies in clinical trials. Few of these antibodies have been approved for clinical use by the Food and Drug Administration (FDA). The purpose of this study was to assess the immunohistochemical expression of *PD-L1* and *PD-L2* by tumor cells (TC) and tumoral stroma immune cells (IC) in endometrial carcinoma (EC) and their association with the tumor's clinico-pathologic characteristics. **Material and methods:** For 62 EC cases, *PD-L1* and *PD-L2* immunohistochemical expression was examined in the TC and IC. **Results:** Positive TC *PD-L1* (25.8% of cases) was linked to high stromal tumor infiltrating lymphocytes (TILs) and high tumor grade. High TC *PD-L2* (33.9% cases) was associated with non-endometrioid types, high tumor grade, and high FIGO stage. Positive IC *PD-L1* (51.6% of cases) was correlated to non-endometrioid types, high tumor grade, high FIGO stage and high stromal TILs. High IC *PD-L2* expression (14.5% of cases) was associated with lympho-vascular space invasion. Both *PD-L1* and *PD-L2* expression in both TC and IC were found to be directly correlated. Crucially, some of the *PD-L1* negative cases had significant expression of *PD-L2*. **Conclusion:** Our results supported *PD-L1* & *PD-L2* expression in EC, particularly in high grade, high FIGO stage, non-endometrioid and TILs rich tumors, highlighting such cases as candidates for anti- PD-1 therapy. Furthermore, the identification of *PD-L2* positive *PD-L1* negative cases may indicate the combination of *PD-L1* and *PD-L2* testing to nominate cases that may benefit from the PD-1 pathway targeting therapies.

Keywords: Endometrial carcinoma- *PD-L1*- *PD-L2*- Tumor cells- immune cells

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Introduction

Endometrial carcinoma (EC) is the most prevalent cancer of the female genital tract in the US [1]. In Egypt, EC accounts for 31.4% and 22.83% of all malignant gynecologic tumors in different registries [2, 3].

Two different categories are recognized for ECs with distinctive clinico-pathological features and biological behavior. Type I EC is the more common, usually of endometrioid type, low grade and associated with hyper-estrogenic state. While Type II EC is usually of the non-endometrioid type, high grade, not associated with hyper-estrinism and carry a poorer prognosis [4].

Programmed death (PD-1) pathway is a target for cancer immunotherapy since it is a crucial immune response checkpoint. Activated lymphocytes express this co-regulatory receptor, which belongs to the B7-CD28 family [5]. The two ligands of PD-1 are *PD-L1* (B7-H1) and *PD-L2* (B7-DC). Immune responses are inhibited when PD-1 and its ligands cooperate to dampen T-cells [6]. *PD-L1* has been found to be normally expressed in the placenta, while *PD-L2* expression is incredibly low and mostly observed in macrophages and dendritic cells [7].

PD-L1 expression by malignancies and its prognostic significance have been the subject of numerous researches [8]. It has been demonstrated that targeted treatments disrupting the PD-1 and *PD-L1* interaction have anti-tumor effects in a variety of tumors, including melanoma and non-small cell lung cancer [9]. However, in the majority of malignancies, the expression of tumoral *PD-L2* and its clinical consequences have not been thoroughly studied [8].

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Among gynecological malignancies, EC is the most common to overexpress *PD-L1* [10]. Therefore, targeting such pathway appears to be a promising strategy to enhance anti-tumor immune responses.

Limited studies evaluated *PD-L2* expression in EC and reported variable expression levels [11, 12]. *PD-L2* was shown to be significantly expressed in 64.44% of type II ECs in both stromal and epithelial components, primarily in the serous subtype [13].

The purpose of this study was to examine the immunohistochemistry expression of *PD-L1* and *PD-L2* in EC patients and to establish a correlation between these expressions and the tumoral clinico-pathologic parameters.

Materials and Methods

Retrieval Of Cases

Sixty-two paraffin-embedded EC tissue samples from hysterectomy specimens were enrolled in this analytical observational cross-sectional study. They were obtained from the archives of the pathology department at Cairo university during the period from July 2017 till February 2021. The sample size was calculated using EpiCale 2000 statistical calculator.

The exclusion criteria were

- Cases where any data is missing.
- Patients subjected endometrial sampling and not hysterectomy.
- Cases with extensive necrosis or insufficient tumor tissue.

Approval from the Research Ethics Committee (REC) at Faculty of Medicine, Cairo university; REC code: MD 86-2020 was obtained.

Data Collection

The data obtained from the pathology requests and reports for each case included age, histologic diagnosis, histologic grade, and pathologic stage.

Histopathological Evaluation

For histological examination, a single block selected for every case was used to cut a hematoxylin and eosin (H&E) stained slide. After the diagnosis was confirmed, the following features were evaluated:

• Histologic typing based on World Health Organization's (WHO) most recent recommendations [14].

• Histologic grading according to updated International Federation of Gynecology and Obstetrics (FIGO) system [15]. Cases in grades 1 and 2 were grouped as low grade during statistical examination, whereas grade 3 cases were regarded as high grade [12].

• Lympho-vascular space invasion (LVSI) refers to the presence of tumor cells in an endothelium-lined area outside the tumor boundary [16]. It was considered substantial when seen in ≥ 5 vessels, as adopted by WHO 2020 [14] and FIGO staging system 2023 [17].

Tumor Infiltrating Lymphocytes Evaluation

In order to assess tumor infiltrating lymphocytes (TILs), the "International Immuno-Oncology Biomarker Working Group" guidance were applied. Briefly, TILs were assessed within the borders of the tumor, both in the center and within 1 mm from the invasive margin. Only mononuclear infiltrate (lymphocytes, plasma cells and histiocytes) were included. Areas of necrosis, and neutrophilic infiltrate were excluded, as well as perivascular immune infiltrates [18]. Stromal TILs were scored subjectively in 10% increments

Tumors were classified as either High stromal TILs (≥30%) or Low stromal TILs (<30%) [19].

Staging And Risk Stratification

• Case staging was performed using the FIGO staging system 2023 [17] and American joint committee on cancer (AJCC) Staging Manual; eighth edition (2017) [20].

• Risk stratification was performed according to modified European Society For Medical Oncology (ESMO) system [21].

Immunohistochemical Staining

Two sections were cut on positively charged slides. The DAKO Link 48 (AS480) autostainer was used for immunostaining. The primary antibodies utilized were anti-*PD-L1* rabbit monoclonal antibody (clone QR1, acquired from BioSb, USA) and anti-*PD-L2* rabbit polyclonal antibody (obtained from Biossusa, USA).

As a positive control, placenta sections were utilized. A section of tumor tissue was processed as a negative control by using phosphate-buffered saline.

Immunohistochemical Evaluation

Programmed Death-Ligand 1

The expression of *PD-L1* was evaluated independently in tumor cells (TC) and tumor infiltrating immune cells (IC). Only membrane staining, whether partial or complete, of any intensity, was regarded as *PD-L1* expression in TC. Any degree of membrane and/or cytoplasmic staining was regarded as *PD-L1* expression in IC. A cutoff value of 1% was applied to indicate positive *PD-L1* expression [22].

Programmed Death-Ligand 2

Cytoplasmic and/or membranous staining of *PD-L2* in TC and IC was considered as positive expression. Allred score was obtained for each case in TC and IC separately [11]. Such Allred scores represent the sum of the proportion and intensity scores (Table 1). The results

Table 1. Allred Score for *PD-L2* Evaluation [12]

Proportion Score	Positive Cells %	Intensity Score	Intensity
0	0	0	None
1	<1%	1	Weak
2	1% - 10%	2	Intermediate
3	10% - 33%	3	Strong
4	34% - 66%		
5	≥67%		

have been classified as "negative / low" (scores 0–4) and "high" (scores ≥ 5) for statistical analysis [12].

Statistical Methods

Version 25 of the Statistical Package of Social Science (SPSS) software was utilized for statistical analysis. The data was displayed using mean and standard deviation for age and frequency and percentages for other variables. The chi square test was employed to compare the groups. Statistical significance was considered when the P value was ≤ 0.05.

Results

Clinicopathological Parameters

Our study's participants were between 40 and 80 years old, with a mean age of 62. The endometrioid type accounted for 79% of the cases in terms of histologic types. Three serous carcinomas, one clear cell carcinoma, and nine carcinosarcomas constituted the 13 non-endometrioid types (21% of cases). In Table 2, the pathological parameters of the cases under study are displayed.

Immunohistochemical Expression of PD-L1

In our cases, *PD-L1* expression was considered positive in 25.8% of tumor cells and 51.6% of immune cells (Figure 1). We detected a statistically significant relationship (P value=0.001) between TC and IC *PD-L1* expression. In Table 3, the pathologic parameters of the cases categorized by TC and IC *PD-L1* expression are compiled. High stromal TILs and high histologic grade were directly correlated with TC *PD-L1* expression, whereas non-endometrioid histologic type, high histologic grade, high FIGO stage, and high stromal TILs were all directly correlated with IC *PD-L1* expression.

Table 2. The Pathological Parameters of the Cases under Study

Parameter		Number (%)
Histologic Type	Endometrioid	49 (79%)
	Non- Endometrioid	13 (21%)
Histologic Grade	Low Grade	42 (67.7%)
	High Grade	20 (32.3%)
T Stage	T1	45 (72.6%)
	T2	3 (4.8%)
	T3	14 (22.6%)
FIGO Stage	I	30 (48.4%)
	II	16 (25.8%)
	III	13 (21%)
	IV	3 (4.8%)
Depth of Myometrial Invasion	Less Than Half	35 (56.5%)
	More Than Half	27 (43.5%)
Substantial LVSI	Positive	20 (32.3%)
	Negative	43 (67.7%)
Stromal TILs	Low	37 (59.7%)
	High	25 (40.3%)
ESMO Risk	Low	17 (27.4%)
	Intermediate	5 (8.1%)
	Intermediate To High	11 (17.7%)
	High	26 (41.9%)
	Metastatic	3 (4.8%)

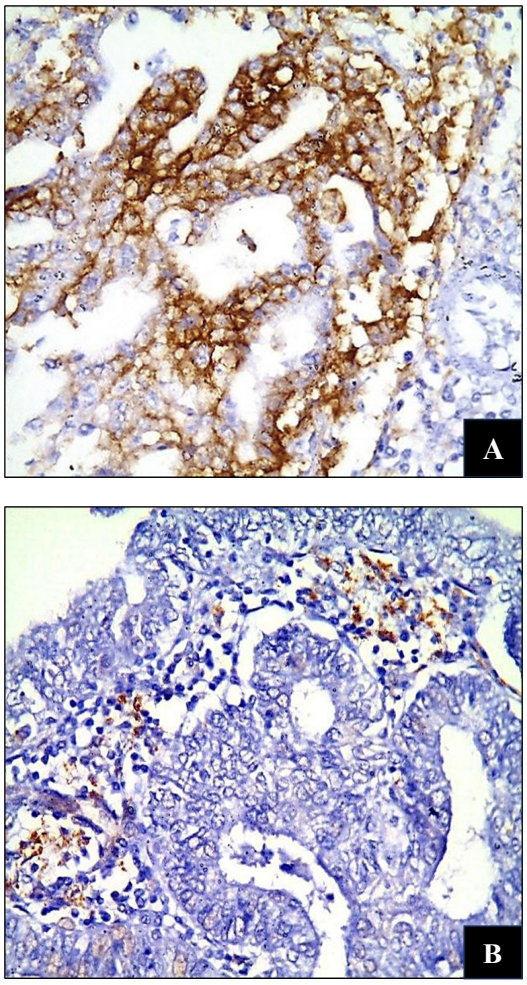

Figure 1. Programmed Death-Ligand 1 Expression In Tumor Cells (A) And Tumor Infiltrating immune cells (B) (X200 original magnification).

Expression of PD-L2

In the present study, TC (33.9% of cases) had greater levels of *PD-L2* expression than IC (14.5% of cases) (Figure 2,3). Nevertheless, the correlation between TC *PD-L2* and IC *PD-L2* expression was significant (P value=0.469). Table 4 summarizes the pathologic characteristics of the cases under study, arranged according to TC and IC *PD-L2* expression. High histologic grade, non-endometrioid histologic type, and high FIGO stage were all directly correlated with TC *PD-L2* expression, whereas substantial LVSI was correlated with IC *PD-L2* expression.

Relationship Between PD-L1 and PD-L2 Expression

Both TC and IC *PD-L1* expression and TC and IC *PD-L2* expression were shown to have statistically significant direct relationships in the current study (Table 5). As

Table 3. The Pathologic Characteristics of Studied Cases Correlated with TC and IC *PD-L1* Expression

Parameter		TC *PD-L1*		P value	IC *PD-L1*		P value
		Positive n (%)	Negative n (%)		Positive n (%)	Negative n (%)	
Histologic Type	Endometrioid	10 (20.4%)	39 (79.6%)	0.059	21 (42.9%)	28 (57.1%)	0.007
	Non- Endometrioid	6 (46.2%)	7 (53.8%)		11 (84.6%)	2 (15.4%)	
Histologic Grade	Low Grade	6 (14.3%)	36 (85.7%)	0.003	15 (35.7%)	27 (64.3%)	0
	High Grade	10 (50%)	10 (50%)		17 (85%)	3 (15%)	
T Stage	T1	11 (24.4%)	34 (75.6%)	0.247	21 (46.7%)	24 (53.3%)	0.446
	T2	2 (66.7%)	1 (33.3%)		2 (66.7%)	1 (33.3%)	
	T3	3 (21.4%)	11 (78.6%)		9 (64.3%)	5 (35.7%)	
FIGO Stage	I	5 (16.7%)	25 (83.3%)	0. 247	10 (33.3%)	20 (66.7%)	0. 016
	II	7 (43.75%)	9 (56.25%)		12 (75%)	4 (25%)	
	III	3 (23.1%)	10 (76.9%)		7 (53.8%)	6 (46.2%)	
	IV	1 (33.3%)	2 (66.7%)		3 (100%)	0 (0%)	
Depth of Myometrial Invasion	Less Than Half	7 (20%)	28 (80%)	0.234	16 (45.7%)	19 (54.3%)	0.29
	More Than Half	9 (33.3%)	18 (66.7%)		16 (59.3%)	11 (40.7%)	
Substantial LVSI	Positive	6 (30%)	14 (70%)	0.603	13 (65%)	7 (35%)	0. 146
	Negative	10 (23.8%)	32 (76.2%)		19 (45.2%)	23 (54.8%)	
Stromal TILs	Low	5 (13.5%)	32 (86.5%)	0.007	15 (40.5%)	22 (59.5%)	0.034
	High	11 (44%)	14 (56%)		17 (68%)	8 (32%)	
ESMO Risk	Low	3 (17.6%)	14 (82.4%)	0.337	7 (41.2%)	10 (58.8%)	0.078
	Intermediate	1 (20%)	4 (80%)		2 (40%)	3 (60%)	
	Intermediate To High	1 (9.1%)	10 (90.9%)		3 (27.3%)	8 (72.7%)	
	High	10 (38.5%)	16 (61.5%)		17 (65.4%)	9 (34.6%)	
	Metastatic	1 (33.3%)	2 (66.7%)		3 (100%)	0 (0%)	

shown in the Table, some of the *PD-L1* negative cases showed *PD-L2* expression.

Discussion

The expression of *PD-L1* and *PD-L2* in EC patients was examined by immunohistochemistry in this study; each was reported in TC and IC independently. 32 (51.6%) and 16 (25.7%) cases respectively, had TC and IC *PD-L1* positive expression.

In the literature, TC *PD-L1* expression rates by EC generally differed widely. Several investigations revealed rates that were lower than ours (17.3%, 8.6%, 15%, 10.2%, and 14%) [11, 22-25]. Additionally, a study on a Middle

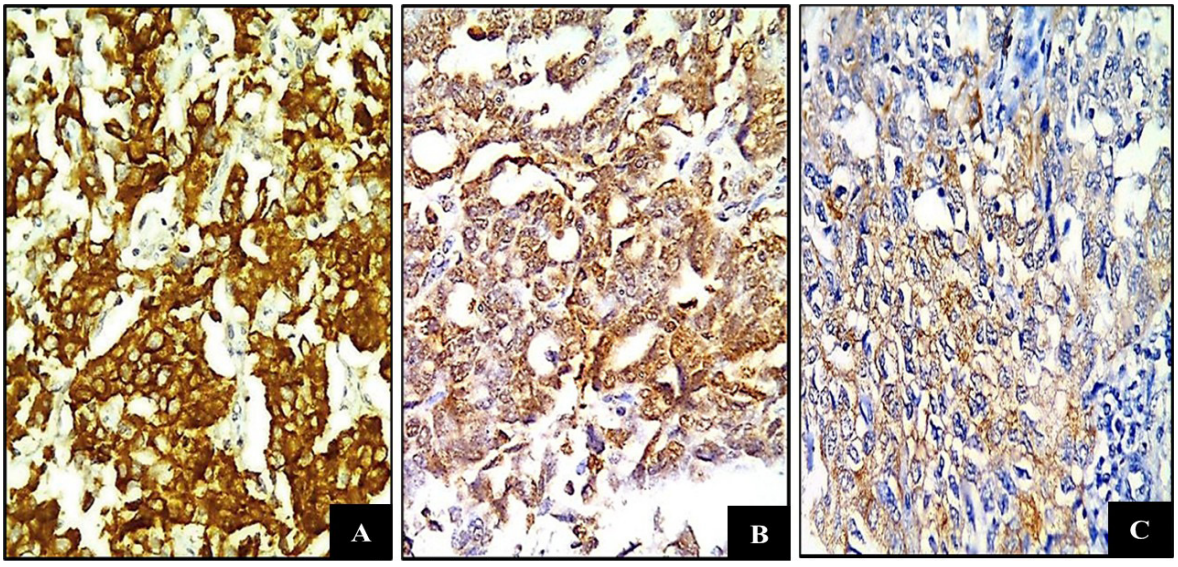

Figure 2. Examples of Strong (A), moderate (B) and weak (C) Tumor Cells Programmed Death-Ligand 2 expression (X200 original magnification)

Table 4. The Pathologic Characteristics of Studied Cases Correlated with TC and IC *PD-L2* Expression

Parameter		TC *PD-L2*		P value	IC *PD-L2*		P value
		High n (%)	Negative / Low n (%)		High n (%)	Negative / Low n (%)	
Histologic Type	Endometrioid	12 (24.5%)	37 (75.5%)	0.002	6 (12.2%)	43 (87.8%)	0.324
	Non- Endometrioid	9 (69.2%)	4 (30.8%)		3 (23.1%)	10 (76.9%)	
Histologic Grade	Low Grade	8 (19%)	34 (81%)	0	5 (11.9%)	37 (88.1%)	0.398
	High Grade	13 (65%)	7 (35%)		4 (20.0%)	16 (80.0%)	
T Stage	T1	14 (31.1%)	25 (83.3%)	0.446	6 (13.3%)	39 (86.7%)	0.577
	T2	2 (66.7%)	1 (33.3%)		0 (0%)	3 (100%)	
	T3	5 (35.7%)	9 (64.3%)		3 (21.4%)	11 (78.6%)	
FIGO Stage	I	5 (16.7%)	25 (83.3%)	0. 028	2 (6.7%)	28 (93.3%)	0. 242
	II	9 (56.25%)	7 (43.75%)		4 (25%)	12 (75%)	
	III	5 (38.5%)	8 (61.5%)		3 (23.1%)	10 (76.9%)	
	IV	2 (66.7%)	1 (33.3%)		0 (0%)	3 (100%)	
Depth of Myometrial Invasion	Less Than Half	10 (28.6%)	25 (71.4%)	0.315	4 (11.4%)	31 (88.6%)	0.432
	More Than Half	11 (40.7%)	16 (59.3%)		5 (18.5%)	22 (81.5%)	
Substantial LVSI	Positive	10 (50%)	10 (50%)	0. 064	6 (30%)	14 (70%)	0.017
	Negative	11 (26.2%)	31 (73.8%)		3 (7.1%)	39 (92.9%)	
Stromal TILs	Low	10 (27%)	27 (73%)	0.166	4 (10.8%)	33 (89.2%)	0.314
	High	11 (44%)	14 (56%)		5 (20%)	20 (80%)	
ESMO Risk	Low	3 (17.6%)	14 (82.4%)	0.088	1 (5.9%)	16 (94.1%)	0.209
	Intermediate	1 (20%)	4 (80%)		0 (0%)	5 (100%)	
	Intermediate To High	2 (18.2%)	9 (81.8%)		1 (9.1%)	10 (90.9%)	
	High	13 (50%)	13 (50%)		7 (26.9%)	19 (73.1%)	
	Metastatic	2 (66.7%)	1 (33.3%)		0 (0%)	3 (100%)	

Eastern population found lower results than ours (18.9%), which may have resulted from the use of tissue microarray in their study [26]. However, many other studies reported higher rates than ours (36.2%, 83%, 83%, 48.4%, 48%, 62.7% and 67%) [12, 27-32].

Likewise, the rates of IC *PD-L1* expression in EC showed a wide variation in the literature. Some studies showed higher rates than ours (60%, 67.8% and 61%) [11, 24, 32] and others showed lower rates (36.2%, 27.7%, 37.3% and 28.8%) [12, 22, 25, 31]. Our rate of IC *PD-L1* expression was very close to what was reported by a meta-analysis involving 12 studies investigating *PD-L1* in endometrial carcinoma cases; 51.39% [33].

The various antibody clones used, using tissue

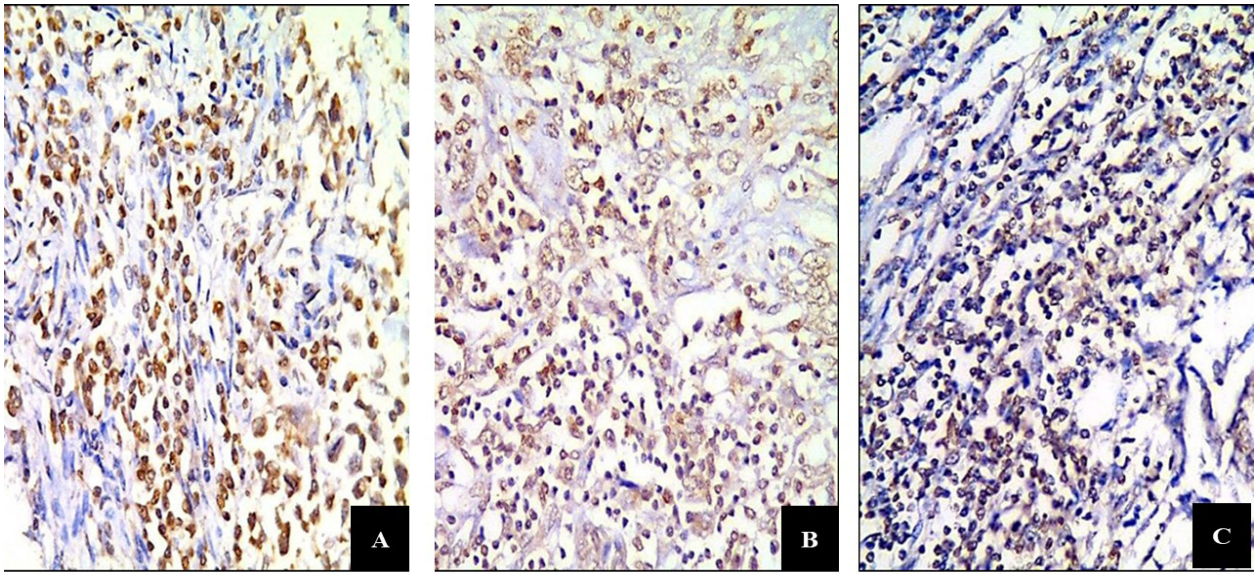

Figure 3. Examples of Strong (A), moderate (B) and weak (C) Immune Cells Programmed Death-Ligand 2 expression (X200 original magnification)

Table 5. Relations between TC and IC *PD-L2* Expression with TC and IC *PD-L1* Expression

		TC *PD-L1*		P value	IC *PD-L1*		
		Positive n (%)	Negative n (%)		Positive n (%)	Negative n (%)	P value
TC PD-L2	High	11(52.4%)	10 (47.6%)	0.001	17 (81%)	4 (19%)	0.001
	Negative / Low	5 (12.2%)	36 (87.8%)		15 (36%)	26 (63.4%)	
IC PD-L2	High	5 (55.6%)	4 (44.4%)	0.027	8 (88.9%)	1 (11.1%)	0.016
	Negative / Low	11 (20.8%)	42 (79.2%)		24 (45.3%)	29 (54.7%)	

microarray or full-face sections, the various cut-offs for *PD-L1* positivity, and the characteristics of the population under study (such as a preponderance of early or advanced stage cases) can all account for such variations.

A statistically significant direct correlation between TC and IC *PD-L1* expression was found in our study (P value=0.001). IC *PD-L1* expression was more prevalent than TC *PD-L1* expression. This was in line with the findings of some [11, 23-25, 32], while others reported the reverse [22, 31, 32].

Our analysis revealed that non-endometrioid cases had a greater rate of TC and IC *PD-L1* expression compared to endometrioid cases. A significant correlation (P value=0.007) was found between the non-endometrioid histological types and the expression of IC *PD-L1*. This result aligned with the majority of the published research [11, 22, 25, 26, 34]. Although Chew et al. [31] similarly found higher TC *PD-L1* expression in non-endometrioid cases, they reported significantly higher IC *PD-L1* expression in endometrioid cases .

Higher TC and IC *PD-L1* expressions were statistically significant in high grade compared to low grade cases in our study; (P values=0.003 & 0.000) respectively. This was compatible with the results of most available studies [9, 11, 12, 22, 25, 31]. Cases categorized as T2 had the highest rates of both TC & IC *PD-L1* expression, although these differences were not statistically significant. Despite the majority of research correlated *PD-L1* expression to FIGO instead of T staging, our findings contradicted those of Siraj et al. [26], who reported T4 stage cases to have the highest levels of *PD-L1* expression .

Although TC *PD-L1* expression was highest in FIGO stage II cases (66.7%), our IC *PD-L1* result, which showed a statistically significant positive expression in high stage cases, was consistent with the majority of studies in the literature [11, 22, 30, 31]. According to our work, there was no statistically significant correlation between the depth of myometrial invasion and *PD-L1* expression. However, cases with more than half of myometrial thickness invasion had higher TC & IC *PD-L1* expression. Similar results were published by Crumley et al. [30], but they were statistically significant (for TC *PD-L1* expression) and Zong et al. [25] (for IC *PD-L1* expression).

In our work, both TC and IC *PD-L1* expression were higher in cases with substantial LVSI, yet with no statistical significance. This conclusion is widely supported by the literature [23, 25, 29, 34]. However, Mo et al. [11] reported high IC *PD-L1* expression in LVSI negative cases. In our study, stromal TILs were associated with statistically significant increases in TC & IC *PD-L1* expression (P values=0.007 and 0.034 respectively. This result showed broad consensus in the literature [22, 24, 25, 30, 31].

According to this study, the highest IC and TC *PD-L1* expression were found in metastatic and high-risk cases with regards to ESMO risk. This was consistent with the findings of the study by Wahba et al. [35], which found that intermediate-high and high-risk cases had increased TCs and TILs *PD-L1* expression, even though no metastatic cases were reported in their work.

The expression of *PD-L2* in TCs and ICs was examined independently in this work. The expression of TC *PD-L2* was high in 21 (33.9%) of our patients. The findings of Mo et al. [11]; Sungu et al. [12] and Marinelli et al. [13] that indicated elevated TC *PD-L2* expression in (37.3%, 27.1%, and 40%) of their cases were in agreement with us . Liu et al. [28] and Vanderstraeten et al. [27], however, observed low or negative rates of TC *PD-L2* expression in the majority of their cases .

In nine of our cases, the expression of IC *PD-L2* was high (14.5%). Higher rates of IC *PD-L2* expression were reported by the majority of the available research; Mo et al. [11] and Sungu et al. [12] observed high IC *PD-L2* expression in 62.7% and 41.5% of their cases, respectively. Such discrepancy can be again explained by the variable antibody clones, IHC protocols and the different cut offs used to differentiate low and high expression and also owing to the different characters of studied EC patients.

The results of our research showed a statistically significant direct relationship between TC and IC *PD-L2* expression (P=0.001), however in contrast to *PD-L1*, the rate of TC *PD-L2* positivity was higher than IC *PD-L2* expression. This concurred with Marinelli et al. [13], who found that the epithelial components were the primary source of *PD-L2* expression and contradicted the findings of Mo et al. [11] and Sungu et al. [12], which showed that *PD-L2* expression was less prevalent in TCs than in ICs.

Non-endometrioid carcinomas had higher frequencies of TC & IC *PD-L2* expression compared to endometrioid subtype; this difference was statistically significant for TC *PD-L2* expression (P value=0.002). These outcomes aligned with the findings of Mo et al. [11]. Higher rates of TC and IC *PD-L2* expression were seen in high grade cases in our assessment; this difference was statistically significant for TC *PD-L2* (P value=0.002). Similarly, Mo et al. [11] found that moderately to poorly differentiated patients had increased *PD-L2* expression . In contrast, Sungu et al. [12] observed that grade I cases had strongly

positive TC & IC *PD-L2* expression .

Controversial findings were published about the relationship between EC T and FIGO staging with *PD-L2* expression. In FIGO stage IV cases, we found statistically significant high expression of TC *PD-L2*, which we also found to be higher in T2 cases. Regarding IC *PD-L2*, the highest expression was reported in T3 and FIGO stage II cases. Mo et al. [11] also reported that *PD-L2* expression (for both TC & IC) was more in higher stage cases (II/III) compared to stage I cases. On the contrary, Sungu et al. [12] reported higher *PD-L2* expression (for both TC & IC) in FIGO I stage cases, rather than other stages (II-III-IV) .

Our findings indicated cases with substantial LVSI expressed more both TC and IC *PD-L2*. A statistically significant relationship was found between IC *PD-L2* expression and LVSI (P value=0.017). According to previous studies, Mo et al. [11] reported that IC *PD-L2* expression was higher in negative LVSI cases while TC *PD-L2* expression was higher in positive LVSI cases . Sungu et al. [12] claimed that both IC and TC *PD-L2* expression was higher in negative LVSI cases.

Similar to our findings on *PD-L1*, subjects with high TIL density also had higher frequencies of TC & IC *PD-L2* positivity. The highest TC and IC *PD-L2* expression were found in our high risk and metastatic cases, respectively, with regard to ESMO risk; however, none of those relationships achieved statistical significance. Revision of the literature revealed that, to our knowledge, no other studies had reported such relations.

Ultimately, our analysis found a strong direct correlation between *PD-L1* and *PD-L2* expression in both TC and IC. Crucially, *PD-L2* positivity was found in *PD-L1* negative cases. Our results are consistent with Yearley et al. [36], who claimed that the expression of *PD-L2* correlated directly with *PD-L1* in several tumor types and that *PD-L2* positivity was reported in cases negative for *PD-L1*. According to Marinelli et al. [13], *PD-L2* was expressed more frequently in EC cell lines than *PD-L1*. Furthermore, *PD-L2* may play a significant role in cancer immune evasion, independent of *PD-L1* status, according to Ok Atılgan et al. [37].

Our investigation is limited by the lack of correlation with patient's survival and prognosis. However, the literature showed a great disagreement regarding the prognostic significance of *PD-L1* & *PD-L2* expression in EC, even though our study and numerous other reports demonstrated that such biomarkers were more prevalent in tumors with poor prognostic factors like high grade and stage and non-endometrioid histological types.

In conclusion, both tumor and immune cells expressed *PD-L1* and *PD-L2* in EC, according to our findings. As potential options for anti-PD-1 pathway targeted therapy, we found that both PD-1 ligands are more highly expressed in non-endometrioid, high grade, high FIGO stage, and high stromal TIL tumors. Furthermore, we found *PD-L2* expression in a few of the *PD-L1* negative cases, indicating the potential use of *PD-L2* testing to identify candidates for anti-PD-1 pathway therapy. This data requires additional clinical trial confirmation.

Author Contribution Statement

All authors contributed equally in this study.

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