RESEARCH ARTICLE

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# *PD-L1* Immunohistochemical Expression in Endometrial Carcinoma: Egyptian Cross-Sectional Study

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## Abstract

**Objective:** Endometrial carcinoma (EC) is the most common cancer of the female genital tract. According to the recently evolved strategies of cancer immunotherapy, immune checkpoints inhibitors are one of the most crucial strategies. Programmed Death Ligand 1 (PD-L1) is an important immune checkpoint regulator. *PD-L1* antibodies have shown efficacy in clinical trials of some malignancies. Some of these antibodies have been approved for clinical usage by the Food and Drug Administration (FDA). **Methods:** This retrospective study included a total of 100 ECs, collected from archived, formalin-fixed, paraffin-embedded tissue blocks of hysterectomy specimens of Egyptian females. The samples were immunohistochemically analyzed for *PD-L1* expression (in both tumor cells; TCs and tumor infiltrating leucocytes; TILs) by a semiquantitative score (0 to 4), with cutoff points of (0: <1% of the cells, 1: 1% to 4%, 2: 5% to 9%, 3: 10% to 49%, and 4: ≥ 50%). Membranous staining only was considered positive. **Results:** *PD-L1* was highly expressed in ECs (67% TCs+ and 61% TILs+), with statistically significant relationships with age, lympho-vascular space invasion (LVSI) and TILs score (P = 0.006, 0.016 and <0.005 respectively). However, no statistically significant relationships were detected between *PD-L1* expression and the following parameters: histological type, histological grade, pathological stage (pT) or FIGO stage, myometrial, cervical, adnexal/serosal, parametrial involvements and nodal metastasis, as well as ESMO risk stratification system. Moreover, statistically significant relationships were achieved when correlating TILs score with tumor grade and LVSI (P = 0.034 and 0.012 respectively). Also, comparing endometrial hyperplasia (EH) *PD-L1* and TCs PDL1 median scores achieved statistically significant relationship (P = 0.001). **Conclusion:** Our results concluded that *PD-L1* expression was greater in both TCs and TILs in a subgroup of patients that have advanced age, LVSI and are TILs-rich, identifying them as potential candidates for anti-PD-1/*PD-L1* immunotherapy.

**Keywords:** Endometrial carcinoma- PD-L1- immunohistochemistry- tumor cells- TILs

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## Introduction

EC is the most common gynecologic cancer in the U.S. The incorporation of molecular testing using next-generation sequencing (targeted POLE sequencing) has been increasingly used in the last decades, in addition to the histopathological and immunohistochemical studies (with surrogate markers for all cases as MSH6, PMS2 and p53) to augment the diagnosis of EC and has been increasingly demanded by the oncologist for better targeted treatment and prognosis [1, 2]. But, unfortunately the health system in the developing countries doesn't support these high-cost tests. In Egypt, EC accounts for 22.83% of all malignant female genital tract (FGT) tumors according to Cancer Pathology Registry, National Cancer Institute, Cairo University [3] and according to the Pathology-based Cancer Registry at the Ain-Shams Faculty of Medicine, 31.4% of all malignant FGT tumors were ECs [4].

Cancer immunotherapy with checkpoint inhibitors has been changing the face of oncology treatments for several tumors, and EC may not be an exception [5]. Additional research is required to identify immune targets and patient type most likely to benefit from this treatment [6].

The programmed death (PD) 1 pathway is a major immune response checkpoint expressed by activated lymphocytes, having two known ligands, *PD-L1* and PD-L2. *PD-L1* appears to be up-regulated in multiple solid malignancies [7], with demonstrated clinical responses by using anti-PDL1 monoclonal antibodies that shown a significantly improved survival in several clinical settings [8], and has been more advantageous than conventional therapies of advanced and metastatic cancers especially those with high *PD-L1* expression. Not only tumor cells,

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but also immune cells express PD-L1, which has clinical implications [9].

Some studies investigated *PD-L1* immunohistochemical (IHC) expression in ECs microenvironment. They showed variable rates of expression within the tumor cells and the tumor infiltrating leucocytes (TILs), concluded that *PD-L1* can be used as an independent biomarker for poor prognosis [10] and suggested it as a potential target for immunotherapy in some ECs with strong immune reaction [11]. The aim of this study was to evaluate *PD-L1* immunohistochemical expression in ECs TCs and TILs, and to correlate this expression with tumor type, grade, stage, extent of TILs and other available clinical and pathological parameters.

## Materials and Methods

This is a retrospective cross sectional analytical study that included 100 stored, formalin-fixed, paraffin-embedded (FFPE) tumor tissue blocks of (EC) cases (from total or subtotal hysterectomy, ± bilateral or unilateral salpingo-oophorectomy specimens ± pelvic lymphadenectomy), that were collected from the archives of Pathology department, Faculty of Medicine, Cairo University during the period from April, 2017 till August, 2019.

The study was approved by the Medical Ethics Committee of the Pathology Department, Faculty of Medicine, Cairo University.

The data collected from the pathology reports of the EC cases included age at time of diagnosis, extent of myometrial invasion, presence of tumor involvement of cervix, serosa, adnexa and parametrium, nodal metastasis (if available) and lympho-vascular space involvement (LVSI).

Inclusion criteria: Tumor sections from hysterectomy specimens with sufficient material and available essential clinical and pathological data.

*Exclusion criteria*

Tumor sections from endometrial curette or pipelle biopsy, cases received neo-adjuvant therapy, cases with missing essential clinical and pathological data, and necrotic or scarce samples (<100 cells).

*Histopathological Assessment*

Each paraffin block was re-cut by rotatory microtome at 4 μm thickness then mounted on a glass slide and stained by hematoxylin and eosin (HandE) for routine histopathological examination which included: Histological classification according to the latest WHO recommendations [2].

Histological grading according to FIGO grading system (Zhou et al., 2018); Pathological staging according to FIGO staging system (FIGO Committee on Gynecologic Oncology, 2014) and the 8th edition of the American Joint Committee on Cancer's AJCC Cancer Staging Manual (Powéll et al., 2017).

*Tumor infiltrating leucocytes (TILs) scoring*

In this study, we manually scored TILs (combined stromal and intra-tumoral: as stromal TILs are superior and more reproducible parameter, so scoring intra-tumoral TILs does not add to the information provided by stromal TILs since they usually parallel stromal TILs) in a subjective 10% increments based on pathologists' visual estimation of TIL density based on example images (Salgado et al., 2015), rounding it up to the nearest 5–10%. Areas to be included and to be excluded in the TILs score evaluation were guided by Hendry et al., 2017 work. Tumors were defined as High-TILs (≥30%) or Low-TILs (<30%), based on the evaluation done by Tomioka et al. in 2018 on triple negative breast cancer.

*PD-L1 Immunohistochemical Staining and Evaluation*

Paraffin sections were cut at 3-4 μm thickness and mounted on positively charged slide. IHC was performed using an automated staining system (Dako autostainer link 48), with monoclonal rabbit antibodies against *PD-L1* (clone RBT-PDL1, dilution 1:100; Bio SB, Santa Barbara, CA, USA). Human tonsil FFPE tissue sections with *PD-L1* antibody were used as positive controls according to the manufacturer recommendations, with each run of IHC staining, while negative controls were tissue sections not treated with the *PD-L1* antibodies. *PD-L1* expression was defined as any convincing partial or complete membranous staining in viable tumor cells (TCs), and membranous and/or cytoplasmic staining in TILs. We determined the percentage of positive TCs and TILs separately. Semiquantitative scoring was adopted as follows:

0: <1% of the cells, 1: 1% to 4%, 2: 5% to 9%, 3: 10% to 49%, and 4: ≥ 50%. The cutoff for *PD-L1* positive staining was set at 1%. The cutoff for strong positivity was set at ≥ 50% for TCs and ≥ 10% for TILs [12].

*Statistical Analysis*

The previously mentioned clinical, histopathological and immunohistochemical data were entered on Microsoft excel 2013 and then transferred to the Statistical Package of Social Science (SPSS) Software program, version 25 to be statistically analyzed. Numerical data were checked for normality and were statistically described in terms of mean (±standard deviation) or median (range) as appropriate. Categorical data were described as numbers and percentages. Comparison between 2 numerical variables was done using Student t-test if normally distributed and Mann-Whitney U test if not normally. Comparison between more than 2 variables was done using Analysis of variance (ANOVA) with Bonferroni adjustment if normally distributed and Kruskal Wallis test if not normally distributed. Spearman correlation coefficient was calculated for EH- PDL1 scoring and PDL1 score in TCs. When comparing categorical data, Chi square test or Fisher's exact test were performed as appropriate. Logistic regression analysis was used with Forward LR variable selection method and included all significant variables on the univariate analyses. Odds ratios with 95% confidence interval were calculated for the significant variables in the final step of the logistic regression. P-value is always 2 tailed and set significant at <0.05 level.

## Results

This study included 100 EC cases, their clinicopathological data are summarized in Table 1.

About two-thirds of EC cases (n=67) showed positive membranous TCs *PD-L1* expression, with about two-thirds of the positive cases had *PD-L1* strong positivity (as shown in Figures 1 and 2), which had statistically significant correlation with two variables on the univariate level of analysis (age and LVSI) as shown in Table 2, while multivariate final model of analysis revealed that only age was significant (P-value = 0.007,

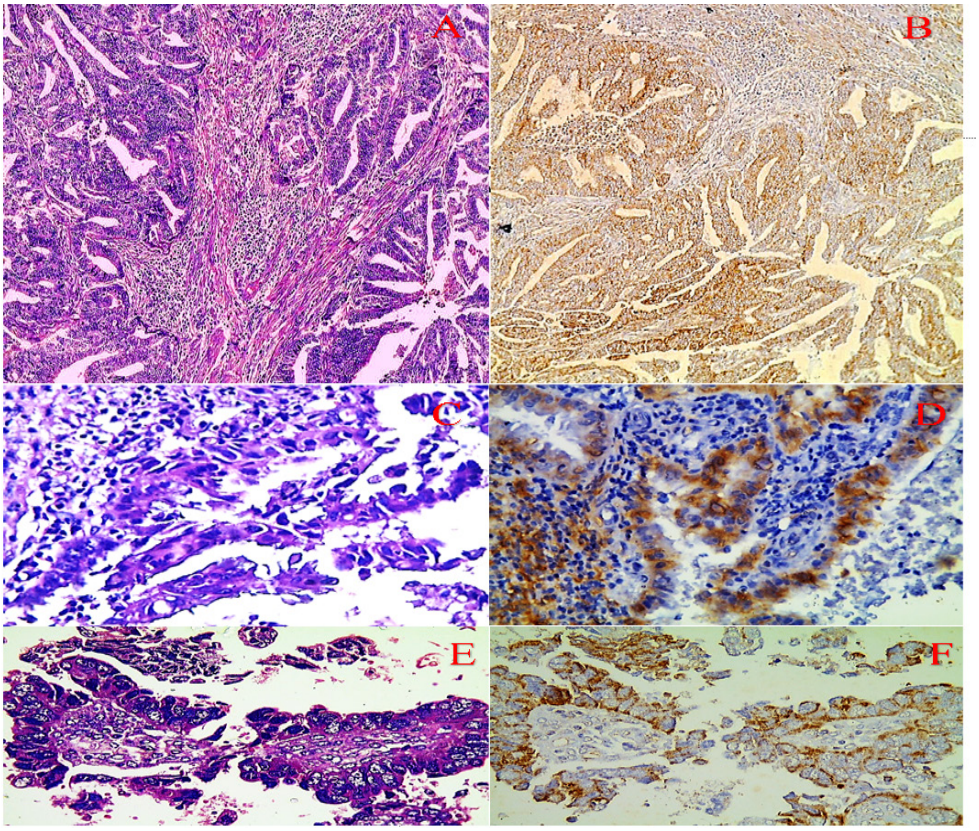


Figure 1. Endometrial Endometrioid Adenocarcinoma NOS, Grade II (A & B), endometrial CCC NOS (C & D), endometrial serous carcinoma NOS (E & F), showing high TIL count (in A & C), score 3 TCs PD-L1 positive membranous staining (B, D & F) and a positive TILs PD-L1 staining (in B & D) (A, C & E: H & E stain; A x100, C & E x 400 original magnification) (B, D & F: IHC stain; B x100, D & F x 400 original magnification).

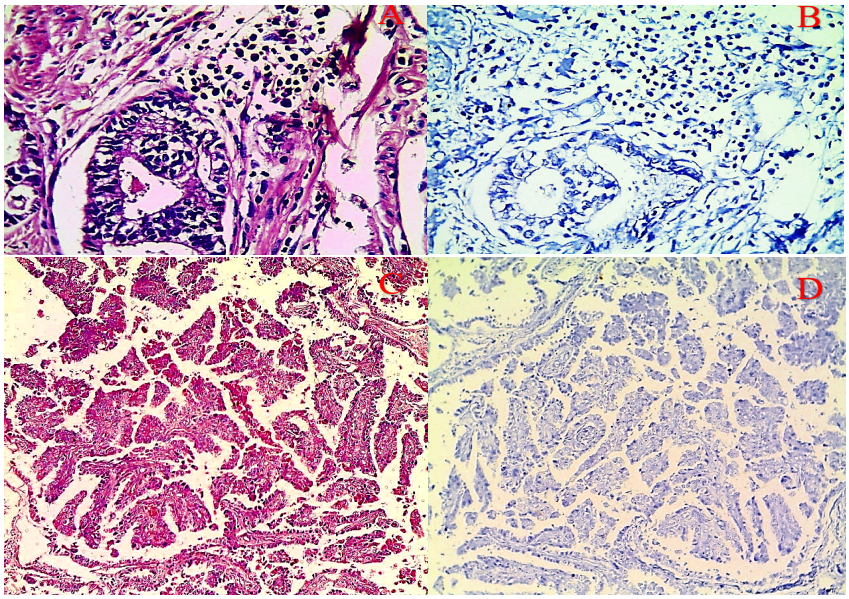


Figure 2. Endometrial Endometrioid Adenocarcinoma NOS, grade II, with low TIL count (A), & endometrial serous carcinoma NOS, with scarce TILs (C), showing negative TCs PD-L1 staining in the malignant glands and negative ICs PD-L1 staining in TILs (B & D) (A & C: H & E stain; A x 400 & C x100 original magnification) (B & D: IHC stain; B x 400 & D x100 original magnification).

Table 1. Clinico-Pathologic Characteristics of Endometrial Carcinoma Patients (n=100) & *PDL1* Expression

| | | N | (%) |
|---|---|---|---|
| Age (years), mean (SD) | | 60.9 | -9.1 |
| Histologic type | Endometrioid | 79 | -79 |
| | PSC | 6 | -6 |
| | CCC | 6 | -6 |
| | Carcinosarcoma | 7 | -7 |
| | Dedifferentiated | 2 | -2 |
| Grade | I | 21 | -21 |
| | II | 46 | -46 |
| | III | 33 | -33 |
| FIGO Stage | I | 69 | -69 |
| | II | 12 | -12 |
| | III | 18 | -18 |
| | IV | 1 | -1 |
| T Stage | T1 | 71 | -71 |
| | T2 | 14 | -14 |
| | T3 | 15 | -15 |
| Myometrial invasion | None | 5 | -5 |
| | < ½ | 50 | -50 |
| | > ½ | 45 | -45 |
| Cervical involvement | Absent | 81 | -81 |
| | Present | 19 | -19 |
| Serosal/Adnexal involvement | Absent | 88 | -88 |
| | Present | 12 | -12 |
| Parametrial involvement | Absent | 93 | -93 |
| | Present | 7 | -7 |
| Nodal Metastasis | Absent | 17 | -17 |
| | Present | 6 | -6 |
| | Not assessed | 77 | -77 |
| LVSI | Absent | 71 | -71 |
| | Present | 29 | -29 |
| ESMO | Low | 33 | -33 |
| | Intermediate | 12 | -12 |
| | Intermediate-high | 11 | -11 |
| | High | 43 | -43 |
| | Metastatic | 1 | -1 |
| $PDL_1$TC score, median (range) | | 3 | (0-4) |
| | Negative (0) | 33 | -33 |
| | Positive (1-4) | 67 | -67 |
| | 1 | 7 | -7 |
| | 2 | 4 | -4 |
| | 3 | 10 | -10 |
| | 4 | 46 | -46 |
| EH (n=19) | Without atypia | 5 | -26.3 |
| | With atypia | 14 | -73.7 |
| $PDL_1$ EH (%), (n=19) median (range) | | 50 | (0-80) |
| | Negative | 5 | -26.3 |
| | Positive (≤ 50) | 9 | -47.4 |
| | Strongly positive (>50) | 5 | -26.3 |

PSC, papillary serous carcinoma; CCC, clear cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics; LVSI, Lympho-vascular space invasion; ESMO, European Society for Medical Oncology; PDL, Programmed death-ligand; TC, tumor cells; TILs, tumor-infiltrating lymphocytes; EH, endometrial hyperplasia

with odds ratio of 1.074).

Analytical correlations of TCs *PD-L1* expressions in EC cases with the rest of clinicopathological variables revealed statistically insignificant correlations between TCs *PD-L1* expressions and histological type, histological grade, pathological T stage, FIGO stage, myometrial invasion, cervical, adnexal/serosal, parametrial involvements and nodal metastasis, as well as ESMO risk stratification system (Table 2).

The correlation between TCs *PD-L1* expression scores in the 19 EC cases showing nearby EH, and its expression scores in the EHs (as shown in Table 1) using Spearman correlation test showed a statistically significant relationship (P-value <0.001). Applying the correlation using Mann-Witney U test (correlating the median of expression of the 2 groups) also showed a statistically significant relationship.

Score of *PD-L1* positive membranous expression in the TILs ranged from 0-95%, with a median of 5%. About two-thirds of EC cases (n=61) showed positive membranous *PD-L1* expression in the TILs (as shown in Figure 1), with the majority of the positive cases (49 cases) having *PD-L1* strong positivity, which had statistically significant correlation with age and LVSI on the univariate level of analysis (P-value= 0.006 and 0.016, respectively) as shown in table (3). So, Multivariate analysis was done and both of them were significant on its final model (P-value = 0.007 and 0.015, with odds ratio of 1.076 and 3.710, respectively). More than three-quarters of combined TILs-high (32 out of 41) and TILs-low (29 out of 38) cases showed positive *PD-L1* expression of their TILs, while all the cases with nearly absent TILs corresponded to those with negative TILs *PD-L1* expression as shown in Figure 3), and that showed a highly statistically significant correlation between TILs *PD-L1* Expression and TILs Score in all EC cases (P-value > 0.001).

Moreover, near total EC cases with positive TCs *PD-L1* expression showed a concurrent positive *PD-L1* expression of their TILs (n=59; 96.7%). At the same time, the majority of cases with negative TCs *PD-L1* expression showed a concurrent negative TILs *PD-L1* expression (n=31; 93.9%), with a highly statistically significant correlation (P-value > 0.001).

Analytical correlations of TILs *PD-L1* expressions in EC cases with the rest of clinicopathological variables revealed statistically insignificant correlations between TCs *PD-L1* expressions and histological type, histological grade, pathological T stage, FIGO stage, myometrial invasion, cervical, adnexal/serosal, parametrial involvements and nodal metastasis, as well as ESMO risk stratification system (Table 3).

## Discussion

In our studied cases, the extent of TILs, 79 cases had appreciable TILs, with 38 cases showed high TIL scores (>30%), keeping with the literature classifying EC as an immunogenic malignancy [13]. TILs score in the studied EC cases ranged from 0-95%, with a median percentage of 15%. This was near to Chavez et al. [11] study Figures

Table 2. Correlations of TCs *PD-L1* IHC Expression & Clinico-Pathologic Characteristics of Endometrial Carcinoma Patients (n=100)

| | | PD-L1 TCs | | | | |
|---|---|---|---|---|---|---|
| | | Negative (n=39) | | Positive (n=61) | | |
| | | n | (%) | n | (%) | P-value |
| Age (years), mean (SD) | | 57.7 | -10 | 62.9 | -7.9 | 0.006 [a] * |
| Histologic type | Endometrioid | 31 | -39.2 | 48 | -60.8 | 0.924 [b] |
| | Other types | 8 | -38.1 | 13 | -61.9 | |
| Grade | I | 11 | -52.4 | 10 | -47.6 | 0.955 [b] |
| | II | 15 | -32.6 | 31 | -67.4 | |
| | III | 13 | -39.4 | 20 | -60.6 | |
| | Low (I-II) | 26 | -38.8 | 41 | -61.2 | 0. 305 [b] |
| | High (III) | 13 | -39.4 | 20 | -60.6 | |
| FIGO Stage | I | 29 | -42 | 40 | -58 | 0.648 [b] |
| | II | 4 | -33.3 | 8 | -66.7 | |
| | III-IV | 6 | -31.6 | 13 | -68.4 | |
| T Stage | T1 | 29 | -40.8 | 42 | -59.2 | 0.688 [b] |
| | T2 | 4 | -28.6 | 10 | -71.4 | |
| | T3 | 6 | -40 | 9 | -60 | |
| Myometrial invasion | None | 2 | -40 | 3 | 60.0) | 0.812 [b] |
| | <1/2 | 21 | -42 | 29 | 58.0) | |
| | >1/2 | 16 | -35.6 | 29 | -64.4 | |
| Cervical involvement | Absent | 32 | -39.5 | 49 | -60.5 | 0.830 [b] |
| | Present | 7 | -36.8 | 12 | -63.2 | |
| Serosal/Adnexal involvement | Absent | 35 | -39.8 | 53 | -60.2 | 0.761 [c] |
| | Present | 4 | -33.3 | 8 | -66.7 | |
| Parametrial involvement | Absent | 35 | -37.6 | 58 | -62.4 | 0.427 [c] |
| | Present | 4 | -57.1 | 3 | -42.9 | |
| Nodal Metastasis (n=23)** | Absent | 9 | -52.9 | 8 | -47.1 | 0.179 c |
| | Present | 1 | -16.7 | 5 | -83.3 | |
| LVSI | Absent | 33 | -46.5 | 38 | -53.5 | 0.016 [b] * |
| | Present | 6 | -20.7 | 23 | -79.3 | |
| ESMO | Low-Intermediate | 21 | -46.7 | 24 | -53.3 | 0.197 [c] |
| | Intermediate-High | 2 | -18.2 | 9 | -81.8 | |
| | High | 16 | -36.4 | 28 | -63.6 | |
| PD-L1 EH | Median (range) | 0 (0.0-0.0) | | 50 (0.0-80.0) | | 0.001[d] * |
| | Correlation Coefficient | (r) = 0.822 e | | | | <0.001* |

FIGO, International Federation of Gynecology and Obstetrics; LVSI, Lymphovascular space invasion; ESMO, European Society for Medical Oncology; NA, Not applicable; ** 77 patients were not assessed for nodal Mets and were excluded from analysis; *, Statistically significant at <0.05 level; [a], Student's independent t-test; [b], Chi Square test; [c], Fisher's Exact test; [d], Mann-Witney U test; [e], Spearman correlation coefficient

in 2019 that showed a median percentage of TILs of 20% (range 0-100%).

In our current study, the higher TILs score in EC cases was associated with more frequently detected LVSI; 58.6% of TILs-high ECs showed LVSI in the vicinity of the tumor, and this achieved a statistically significant correlation (P = 0.012). To our knowledge, this correlation was not considered in other comparative studies on ECs. However, revising literature denoted their association, as higher TILs density (tumor-associated macrophage density) was correlated with aggressive behavior as LVSI and LN metastasis [14].

In this study, positive TCs *PD-L1* expression was reported in 67 cases, with strong positivity in 46% of cases. The rates of TCs *PD-L1* expression varied greatly in the literature, ranging from 8.6% [12], up to 48.4% [15]. Although the study by Al-Hussaini et al. [16] (66.7%) was done on undifferentiated ECs, but their results were close to our study results. In the contrary, Siraj et al. [17] study in 2021 was done on Middle Eastern population, but showed marked lower results than ours, which could be due to usage of tissue microarray (TMAs). This difference

Table 3. Correlations of TILs *PD-L1* IHC Expression & Clinico-pathologic Characteristics of Endometrial Carcinoma Patients (n=100)

| | | *PD-L1* TILs % | | | | |
|---|---|---|---|---|---|---|
| | | Negative (n=39) | | Positive (n=61) | | |
| | | n | (%) | n | (%) | P-value |
| Age (years), mean (SD) | | 57.7 | -10 | 62.9 | -7.9 | 0.006 [a] * |
| Histologic type | Endometrioid | 31 | -39.2 | 48 | -60.8 | 0.924 [b] |
| | Other types | 8 | -38.1 | 13 | -61.9 | |
| Grade | Low (I-II) | 26 | -38.8 | 41 | -61.2 | 0.955 [b] |
| | High (III) | 13 | -39.4 | 20 | -60.6 | |
| FIGO Stage | I-II | 33 | -40.7 | 48 | -59.3 | 0.461 [b] |
| | III-IV | 6 | -31.6 | 13 | -68.4 | |
| T Stage | T1 | 29 | -40.8 | 42 | -59.2 | 0.688 [b] |
| | T2 | 4 | -28.6 | 10 | -71.4 | |
| | T3 | 6 | -40 | 9 | -60 | |
| Myometrial invasion | Non to <1/2 | 23 | -41.8 | 32 | -58.2 | 0.523 [b] |
| | > 1/2 | 16 | -35.6 | 29 | -64.4 | |
| Cervical involvement | Absent | 32 | -39.5 | 49 | -60.5 | 0.830 [b] |
| | Present | 7 | -36.8 | 12 | -63.2 | |
| Serosal/Adnexal involvement | Absent | 35 | -39.8 | 53 | -60.2 | 0.668 [b] |
| | Present | 4 | -33.3 | 8 | -66.7 | |
| Parametrial involvement | Absent | 35 | -37.6 | 58 | -62.4 | 0.427 [c] |
| | Present | 4 | -57.1 | 3 | -42.9 | |
| Nodal metastasis (n=23) ** | Absent | 9 | -52.9 | 8 | -47.1 | 0.179 [c] |
| | Present | 1 | -16.7 | 5 | -83.3 | |
| LVSI | Absent | 33 | -46.5 | 38 | -53.5 | 0.016 [b] * |
| | Present | 6 | -20.7 | 23 | -79.3 | |
| ESMO | Low-Intermediate | 21 | -46.7 | 24 | -53.3 | 0.197 |
| | Intermediate-High | 2 | -18.2 | 9 | -81.8 | |
| | High | 16 | -36.4 | 28 | -63.6 | |

FIGO, International Federation of Gynecology and Obstetrics; LVSI, Lymphovascular space invasion; ESMO, European Society for Medical Oncology; NA, Not applicable; ** 77 patients were not assessed for nodal Mets and were excluded from analysis; * Statistically significant at <0.05 level; [a], Student's independent t-test; [b], Chi Square test; [c], Fisher's Exact test

between various studies can be generally explained by the different clones of the used antibodies, the use of full-face sections versus TMAs (TMAs may not represent the whole tumor spectrum, resulting in false negativity or false high positivity due to intra-tumoral heterogeneity), and the composition of the studied population itself affecting the rate of *PD-L1* expression. In addition, the difference between the observers may be a further factor.

In our study, the relatively high rate of TCs *PD-L1* expression can be explained by the use of full-face sections covering the heterogeneous nature of *PD-L1* expression, inclusion of a wide variety of EC histotypes (high-grade endometrioid and type II EC), as well as, the low cut-off of positivity (1%).

Concerning the TILs *PD-L1* expression, it was positive in 61 cases of our cases, with the majority of cases (49%) had *PD-L1* strong positivity (≥ 10% of TILs were positively stained). As reported in the literature about rates of TCs *PD-L1* expression, similarly TILs *PD-L1* expression rates had a wide range, ranging from 27.7% [12], up to 67.8% [18], with the latter being very close to ours.

The rate of TILs *PD-L1* expression in our study (61%) was slightly lower than TCs *PD-L1* expression (67%), in agreement with the studies done by kir et al. [18], Pasanen et al. [12] and Zong et al. [19]. However, Pasanen in 2021 found that *PD-L1* expression was higher in immune cells (27.7%) than in tumor cells (8.6%), in contrast to our study. This can be explained by the bigger study sample (842 patients) and using TMA.

In the 100 EC cases of our study, 19 cases showed nearby areas of EH; the all 14 cases (73.7%) that showed positive membranous *PD-L1* expression were EH with atypia, while only 5 (26.3%) had negative *PD-L1* expression and 4 of them were EH without atypia. This showed a statistically significant, strong relationship with TCs *PD-L1* expression scores, by using Spearman correlation test (P<0.001) and Mann-Witney U test (correlating the median of expression of the 2 groups) (P = 0.001). This was consistent with Antomarchi et al. [20] study in 2019 that found unchanged *PD-L1* gene expression by (real-time PCR) in the hyperplasia group,

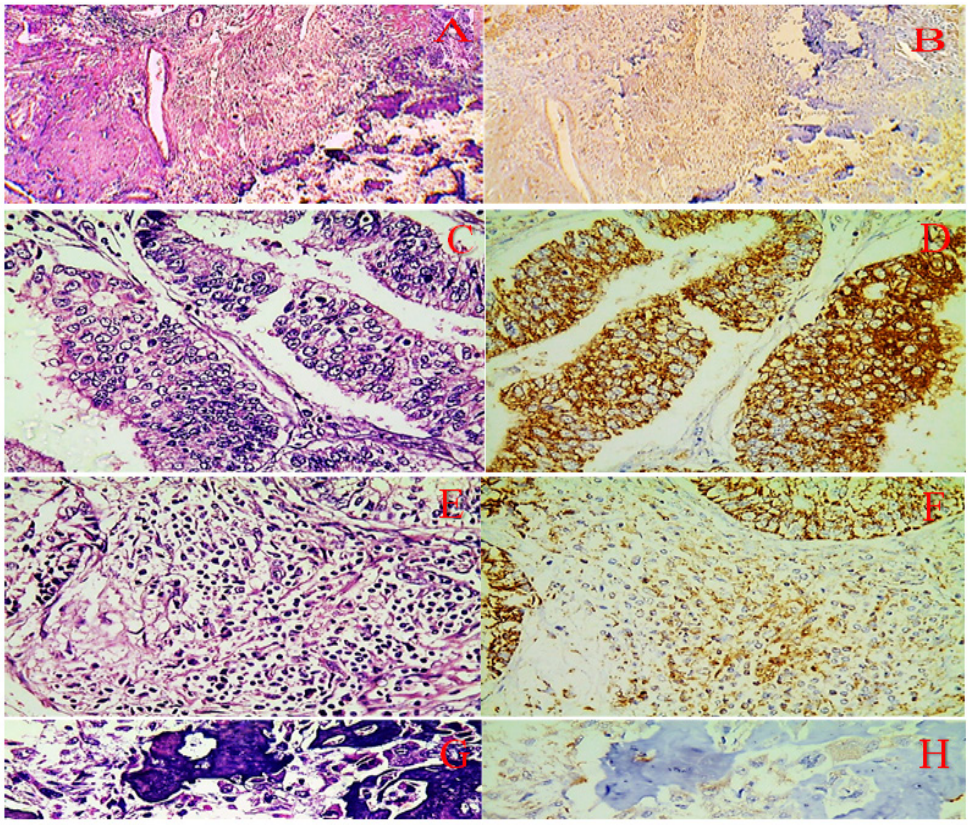


Figure 3. (A & B) A case of endometrial carcinosarcoma NOS, showing carcinomatous (endometrioid) & sarcomatous (heterologous cartilaginous) components, with high TIL count (A: H&E & B: IHC stains; x 40 original magnification). (C & D) Strong positive TCs *PD-L1* membranous staining in carcinomatous component (H & E and IHC stains; x 400 original magnification). (E & F) Strong positive TILs PD-L1 membranous staining (H & E and IHC stains; x 400 original magnification). (G & H) The sarcomatous component showing a strong positive TCs *PD-L1* membranous staining (H & E and IHC stains; x 400 original magnification).

while it was significantly increased in all tumor groups. Also, Chew et al. [21] in 2020 found all 32 non-neoplastic *PD-L1* negative endometrial samples, while the 59 ECs showed *PD-L1* positivity in 72.7% of TILs and 28.8% of TCs, with statistically significant correlations (P <0.001 and = 0.001 respectively), in concordance with our study.

In our study, cases with positive TCs and TILs *PD-L1* expressions showed a slightly higher mean of age (62.9) compared to those with negative *PD-L1* expression (57.7), that achieved a statistically significant correlation both on the univariate level of analysis (P= 0.006) and on the final model of multivariate analysis (P= 0.007). This support the fact that age is an important independent prognostic factor in ECs proved in the literature. In agreement with our study result, Sungu et al. [10] study in 2019 also found a significant difference between TCs *PD-L1* scores and age (P = 0.013), with a higher median age in positive than in negative cases. Chew et al. [21] study in 2020 showed that TCs *PD-L1* expression was higher in cases over 60 years compared to younger patients (43.5% vs. 19.4%, P = 0.047), yet TILs *PD-L1* expression had statistically insignificant difference between these age groups (P = 0.432). On the contrary, Siraj et al. [17] study in 2021 found statistical insignificant association with age (0.5387).

In our study, both TCs and TILs *PD-L1* expressions were associated with LVSI, as 23/61 positive TCs and TILs *PD-L1* cases had LVSI and 33/39 negative TILs *PD-L1* cases lacked LVSI, which achieved a statistically significant correlation (P of both = 0.016), with only TILs *PD-L1* expression came out to be significant on the final model of multivariate analysis (P = 0.015).

These findings were in agreement with the study by Li et al. [22] in 2018 (P = 0.019 for TILs *PD-L1* expression) and Zong et al. [19] in 2021 (P = 0.019 for TCs *PD-L1* expression) that showed a significant association between *PD-L1* expression and LVSI. Crumley et al. [23] study in 2019 of 132 grade II, MMR-intact, endometrioid ECs cases, also found that TCs *PD-L1* expression was associated with LVSI (P = 0.001). However, Sungu et al. [10] in 2019 (P = 0.359 and 0.116 respectively) and Mo et al. [24] in 2016 (P = 0.764 and 0.427 respectively) found no difference between LVSI and TCs and TILs *PD-L1* expressions. Also, Tawadros and Khalafalla, [15] in 2018 (P = 0.439) found no significant correlation between TCs *PD-L1* expression and LVSI.

Concerning TILs scores in our studied cases, 38% were TILs-high and 41% were TILs-low. They showed higher rate of TCs and TILs *PD-L1* positivity (71.1%, 76.3% with TCs and 78% with TILs, respectively), than in cases with nearly absent TILs (38.1%), and this revealed statistically significant correlation (P <0.005). This was consistent with Pasanen et al. [12] in 2020, who found that ECs with moderate-abundant T-cell density had TCs and TILs

*PD-L1* positivity (10.6% and 36.6% respectively) more frequently than ECs with scarce T-cells, with statistically significant correlation (P <0.001). However, Crumley et al. [23] in 2019 found that TCs *PD-L1* expression was not associated with significant differences in CD3+ or CD8+ TILs, but PD-L1-positive cases had higher TILs scores.

The previously discussed three variables (age, LVSI and TILs score) were the only ones that showed statistical significance with both TCs and TILs *PD-L1* expressions in our study. Analytical correlations of both TCs and TILs *PD-L1* expressions with the rest of variables revealed statistically insignificant correlations.

Regarding the histological types, our study showed no statistical difference in TCs or TILs *PD-L1* expressions between endometrioid and non-endometrioid ECs (P of both = 0.924), yet, endometrioid ECs showed higher rate of expression (78.7% of positive cases). This was compatible with what was reported by Sungu et al. [10] in 2019 (P = 0.061 and 0.791 respectively) and Chew et al. [21] in 2020 (P = 0.382 and 0.746 respectively), who found no statistically significant difference between histological types and TCs and TILs *PD-L1* scores (P = 0.061 and 0.791 respectively). However, the latter found higher TCs *PD-L1* positivity in non-endometrioid ECs, while TILs *PD-L1* positivity was higher in endometrioid ECs. In the contrary, this finding disagreed with most of reported studies [17, 19, 12, 22, 24] that found statistically significant difference between histological types and TILs +/- TCs *PD-L1* expression. This can be explained by the large cohort of studied cases and usage of TMA.

Our studied cases showed no statistical difference between TCs or TILs *PD-L1* expressions in either high or low-grade ECs (P = 0. 305 and 0.955 respectively), yet, low-grade ECs showed higher rate of expression (67.2% of positive cases). This finding agreed only partially with few studies; TILs *PD-L1* expression in Zong et al. [19] study in 2021 and TCs *PD-L1* expression in Pasanen et al. [12] study in 2020. Dissimilarly, most of reported studies [19, 21, 12, 10, 24] observed a significant correlation between the histological grade and *PD-L1* expression (either in TCs or TILs), with higher frequency of expression with high-grade ECs. This difference can be due to usage of large cohort of cases, polyclonal antibodies, and grouping of grade II cases with grade III instead of grade I in some studies.

Regarding FIGO stage, our study results showed no statistical difference between TCs or TILs *PD-L1* expressions in either stage groups (IandII versus IIIandIV) (P = 0. 305 and 0.955 respectively), yet, stage IandII group of ECs showed higher rate of expression (78.7% of positive cases). This was in concordance to Zong et al. [19] study in 2021 that also found statistically insignificant differences (P = 0.316 and 0.315 with TILs and TCs respectively), also Chew et al. [21] found no statistically significant difference (P = 0.512), and Mo et al. [24] (p= 0.171 and 0.315 with TILs and TCs respectively). Dissimilarly, Pasanen et al. [12] who also compared advanced- stage (III and IV) disease with early-stage (I and II) disease, found statistically significant correlations with both TCs and TILs *PD-L1* expressions (P = 0.016 and 0.037 respectively). Also, Sungu et al. [10] study in 2019 found statistically significant correlations between FIGO stage and both TCs and TILs *PD-L1* expressions (P=0.004 and 0.046 respectively).

Regarding the T stage, cases classified as T1 showed the highest rates of TCs and TILs *PD-L1* expression (68.9% for both), but with no statistically significant correlations (P = 0.688 for both). This was in concordance to Siraj et al. [17] study in 2021 that also found statistically insignificant difference (P = 0.0570). However, Crumley et al. [23] study in 2019 stated that advanced tumor stage was associated with *PD-L1* expression with a P-value of 0.012.

Concerning the extent of myometrial invasion (MI), cases with >50% MI showed higher rate of TCs and TILs *PD-L1* positivity (64.4% for both), but these results were statistically insignificant (P = 0.523 for both), similar to a study performed by Siraj et al. [17] (P = 0.0741) and by Tawadros and Khalafalla, [15] in 2018 (P = 0.384). The same findings yet with statistically significant difference were reported by Zong et al. [19] (P = 0.009 for TILs *PD-L1* expression), and Crumley et al. [23] (p= 0.002 for TCs *PD-L1* expression in 132 MMR-intact, grade II, endometrioid ECs).

Our studied cases with cervical involvement by the tumor showed higher rate of TCs and TILs *PD-L1* expressions (63.2% for both), but with statistically insignificant difference (P = 0.830 for both). Also, our study documented cases with serosal/ adnexal involvement by the tumor showed higher rates of TCs and TILs *PD-L1* expressions (66.7% for both), but with no statistically significant difference (P = 0.761 and 0.668 respectively). Our study results showed a slightly higher rate of TCs and TILs *PD-L1* expressions in cases without parametrial involvement (57.1% for both), also with statistically insignificant difference (P = 0.427 for both).

To our knowledge, cervical, serosal/adnexal or parametrial invasions were not investigated research issues to be compared with our study results as regards *PD-L1* expression. However, the revised literature confirmed the prognostic role of cervical stromal invasion being correlated with EC recurrence [25] and of positive peritoneal cytology being associated with poor prognosis and decreased survival outcomes [26].

Our studied cases with positive nodal metastasis came out to have higher rate of TCs and TILs *PD-L1* expressions (83.3% for both), but with statistically insignificant difference (P = 0.179 for both). This was in concordance to Crumley et al. [23] study in 2019 that found no significant differences in pelvic or para-aortic LN metastases with *PD-L1* expression (P = 0.105 and 0.156, respectively). On the contrary, Siraj et al. [17] in 2021 (P = 0.0172 for TCs in 440 ECs), and Tawadros and Khalafalla, [15] in 2018 documented statistically significant correlations with LN metastases.

Concerning ESMO risk stratification system, our studied intermediate-high and high- risk cases showed the highest rate of TCs and TILs *PD-L1* expressions (81.8% and 63.6%, respectively), but with statistically insignificant difference (P = 0.197 for both). By revising the literature, this correlation was not evaluated by other comparative studies. However, the revised literature confirmed the prognostic role of ESMO risk stratification

system in the prediction of recurrence, LN status and survival [27].

To sum up, we concluded that both TCs and TILs *PD-L1* expressions in our study achieved significant correlations with patient's advanced age, LVSI and TILs score. Those variables can stratify candidates that can benefit most from anti-PD-1/*PD-L1* immunotherapy, or further high-cost molecular investigations. Also, it can be used as a predictor of LN and distant metastases, with its higher statistically significant IHC TCs *PD-L1* expression in ECs and EH with atypia, than in EH without atypia, confirming its important role in tumor invasiveness and progression, making it an important prognostic and therapeutic marker (theragnostic).

This study is limited by its modest size and lack of correlation with patient's prognosis, particularly disease recurrence and survival. One important limitation of this study is the lack of correlation with EC molecular subtyping due to fund limits.

## Author Contribution Statement

All the authors contributed efficiently to the research and approved the manuscript. MGW shared in study design and sample collection. MGW and MES shared in writing the manuscript. SSN shared by reviewing study methods and performed statistical analysis of the study raw data. MGW interpreted and analyzed the results. All authors shared in revising and approving the final version of the manuscript.

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*Conflicts of interest*

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