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Clinicopathological correlation and prognostic value of PD-L1 expression in renal cell carcinoma

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Abstract

Introduction: The expression of programmed cell death-ligand 1 (PD-L1) and its correlation with the prognosis of renal cell carcinoma (RCC) remains controversial.
Objectives: This study aimed to study PD-L1 expression in tumor cells and tumor-infiltrating lymphocytes (TILs) in patients with RCC and its association with clinicopathological factors and survival outcomes.
Patients and Methods: PD-L1 expression in tumor cells and TILs was analyzed using immunohistochemistry (IHC) from patients with histologically proven RCC.
Results: PD-L1 was positive in tumor cells for 55.8% of patients. PDL-1 expression in TIL was reported in 31.2 % of patients. Patients with PDL1 positive tumor cells had higher median tumor size ($P=0.07$), higher nuclear grade ($P=0.56$), and higher lymphovascular invasion (LVI) ($P=0.23$). Patients with PDL1 positive TILs were significantly associated with larger median pathological tumor size ($P=0.02$), higher probability of renal fat invasion ($P=0.001$), higher nuclear grade ($P=0.05$), higher probability of positive margin ($P=0.02$), positive LVI ($P=0.03$), higher pathological T stage ($P=0.0004$); whereas patients with PDL-1 negative TILs had earlier stage at presentation (stage I-II) ($P=0.004$). There was no statistically significant difference in disease-free survival (DFS), progression-free survival (PFS), or overall survival (OS) for PD-L1 expression of tumor cells and TILs.
Conclusion: PDL1 positivity in TILs and not in tumor cells was significantly associated with more aggressive features, and higher stage. No association was found with DFS, PFS, or OS. These data suggest that PD-L1 expression of TILs in RCC tumors contributes to cancer aggressiveness.

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Introduction

Renal cell carcinoma (RCC) accounts for about 2% of cancer diagnosis and deaths globally (1). It is considered the seventh most common form of neoplasm in the developed world (2).

Renal cell tumors represent a group of histologically and molecularly heterogeneous diseases. The histologic classification of RCC has significantly changed in the last few decades, however several new entities were added based on either pathologic features or distinctive molecular alterations (3).

The major subtypes are clear cell RCC (ccRCC) representing 65–70% of all RCC, papillary RCC (PRCC) 15–20%, and chromophobe RCC (ChRCC) 5–7% (3).

RCC is considered as an immunogenic cancer, with pathologic specimens harboring a high number of tumor-infiltrating lymphocytes (TILs) which are considered manifestations of host immune reactions against cancers (4,5).

PD-1 is a cell surface glycoprotein within the B7 family of T cell costimulatory molecules, which it was first described by Ishida et al in 1992 (6). PDL1, when bound to PD1 protein, leads to downregulation of activated T cells (7). It was suggested that approximately 30% of malignant tumor cells, including RCC among other tumors, express programmed cell death-ligand 1 (PD-L1) which closely associate with the prognosis of the patients (8-10).

The expression of PDL-1 is currently being investigated as an important prognostic and predictive biomarker; however, it is still not validated alone determining which patients should be offered PD-1/L1 blockade therapy (11, 12).

Key point

PD-L1 expression is a novel marker in most tumors. Its role in RCC is controversial. We analyzed retrospectively PD-L1 expression in tumor and TILs. PD-L1 expression in TILs was associated with aggressive tumor features but could not be correlated with survival outcomes.

Objectives

This study aimed to study PD-L1 expression in tumor cells and TILs in patients with RCC and its association with clinicopathological factors and survival outcomes.

Patients and Methods

Study design

This study included patients diagnosed with RCC, presented at the department of clinical oncology, Ain Shams university hospitals in the period from January 2016- December 2019.

Eligible patients had pathologically confirmed RCC. Whereas patients who had second primary malignancy, inadequate or insufficient tissue samples were excluded.

Tissue collection

This study included 43 specimens of formalin-fixed and paraffin-embedded tumor sections. Cases were retrieved from the archives of the Pathology department and clinical oncology department of Ain Shams University hospitals, Cairo, Egypt. The cases included in this study were selected to have sufficient representative tissues for evaluation. The clinicopathologic variables such as gender, age, maximal tumor size, tumor histology and grade, tumor location, tumor stage and the status of the resection margin were reviewed retrospectively based on medical records.

Grading was conducted based on the 2012 International Society of Urologic Pathologists (ISUP) grading system for ccRCC and pRCC that has been adopted by the World Health Organization (WHO) (3,13).

Immunohistochemical staining

Immunohistochemistry (IHC) staining was conducted on the paraffin-embedded tissue sections with a labelled streptavidin- biotin-peroxidase complex technique using a rabbit monoclonal antibody for PD-L1 (CD274molecule) (catalog number: cell signaling, 13684; dilution: 1/200). Antigens were retrieved by microwaving in citrate buffer for 20 minutes for PDL-1.

Immunohistochemical analysis

Only tumor cells with membranous positivity were considered positive for PD-L1. Cytoplasmic positivity was disregarded. Tumor cells were quantified by evaluating the ratio of stained and unstained cells (number of PD-L1positive tumor cells /number of all tumor cells). Expression in ≥5% of tumor cells is considered the cut off value for positive expression (14).

A modified scoring based on Möller et al (15) for evaluation of PD-L1 expression on tumor cell membrane was determined semi-quantitatively on a 0+ to 3+ scale; (0+: Negative immunostaining; 1+: any degree of membranous staining that reached the cut off value ≥5% of tumor cells but <10%, 2+, moderately to intensely positive membranous staining in ≥10% of tumor cells however <50%, 3+, intensely positive membranous staining in ≥50% of tumor cells.

PDL-1 immuno-expression on TIL was considered negative if no staining and positive if cells are stained.

Statistical methods

Baseline characteristics of patients and tumor pathological features are expressed as absolute values, mean, and median when appropriate. Correlations between PD-L1 expression and the clinical and pathologic features were evaluated employing chi-square test (χ^2). Overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS) were estimated using the Kaplan-Meier method. The duration of follow-up was calculated from the date of surgery or biopsy to the date of death or last known follow-up. DFS for non-metastatic patients was calculated from the date of surgery/biopsy to the date of recurrence/death whichever comes first. While PFS for metastatic patients was calculated from the date of surgery/biopsy to the date of progression/death whichever comes first. OS was calculated from the date of surgery/biopsy to death due to any reason. Results were shown as *P* value where $P < 0.05$ was considered statistically significant.

Results

Patients' characteristics

This study included 43 patients with tissue diagnosis of RCC. Patients had a median follow up of 22.9 months (4.8-62.1 months).

Patients had a median age of 53 years with a male predominance (62.8%). Most of the patients had an Eastern Cooperative Oncology Group (ECOG) performance status (16) of 1 at presentation. Patients' characteristics are shown in Table 1.

Outcome measures

PDL-1 was detected by IHC both in tumor cells and TILs, PD-L1 was positive in tumor cells for 24 patients (55.8%) while 19 patients (44.2%) had negative PD-L1 in tumor cells.

In the PD-L1 positive group of tumor cells, 10 (41.67%) had strong positive score (3+) as represented in Figure 1A and 1B, seven (29.16%) had moderate positive score (2+) and seven patients (29.16%) had weak positivity (1+).

As for PDL-1 expression in TIL, positivity was reported in 31.2 % of patients (n=13) with high level of TILs expression as presented in Figure 2.

Correlation between PD-L1 in tumor cells and clinicopathological factors

In correlation with clinical and pathological factors, patients with PDL1 positive tumor cells had higher median tumor size ($P=0.07$), higher nuclear grade ($P=0.56$), higher lymphovascular invasion (LVI) ($P=0.23$) all with no statistical significance as presented in Table 2.

Table 1. Patients' characteristics

Characteristics	
Age (y)	
Mean	52.2 (± 12.08)
Median	53
Gender	
Male	27 (62.8%)
Female	16 (37.2%)
ECOG performance status[a]	
0	3 (7.0%)
1	32 (74.4%)
2	4 (9.3%)
3	3 (7.0%)
4	1 (2.3%)
Presenting symptom	
Loin Pain	17 (42.5%)
Hematuria	11 (26.8%)
Accidental	4 (9.8%)
Others	11 (25.5%)
Surgery[b]	
Radical nephrectomy	26 (60.4%)
Partial nephrectomy	1 (2.3%)
Cytoreductive surgery	14 (32.6%)
Biopsy only	2 (4.7%)
Histological subtype	n=43
Clear cell	27 (62.8%)
Papillary	9 (20.9%)
Chromophobe	6 (14.0%)
Oncocytic	1 (2.3%)
Nuclear grade	n=43
Grade 1	3 (7.0%)
Grade 2	24 (55.8%)
Grade 3	12 (27.9%)
Grade 4	0 (0.0%)
Not reported	4 (9.3%)
TNM stage[c]	
I	9 (20.9%)
II	8 (18.6%)
III	10 (23.3%)
IV	16 (37.2%)
Pathological stage[d]	n=41
PT stage	
T1	11 (26.8%)
T2	13 (31.7%)
T3	15 (36.6%)
T4	2 (4.8%)
PN stage	
N0	3 (7.3%)
N1	2 (4.9%)
Nx	36 (87.8%)
M stage	
M0	27 (65.85%)
M1	14 (34.15%)
Site of metastasis	
Lung	11 (25.6%)
Liver	5 (11.6%)
Bone	5 (11.6%)
Others (Non-regional lymph nodes)	4 (9.3%)
Margin	n=41
Negative	37 (90.2%)
Positive	3 (7.3%)
Not reported	1 (2.4%)
LVI	n=41
Absent	23 (56.1%)
Present	9 (21.95%)
Not reported	9 (21.95%)
Renal fat invasion	n=41
Absent	20 (48.78%)
Present	18 (43.90%)
Not reported	3 (7.32%)

[a] ECOG performance status: scale from 0-5; [B] radical nephrectomy for non-metastatic patients and cytoreductive surgery for metastatic patients, [c] staging according to AJCC 8th edition; [d] Pathological stage for patients who had surgery (n=41).

Correlation between PD-L1 in TILS and clinicopathological factors

When correlating PDL1 in TIL with clinicopathological factors, patients with PDL1 positive TILs were significantly associated with larger median pathological tumor size ($P=0.02$), higher probability of renal fat invasion ($P=0.001$), higher nuclear grade ($P=0.05$), higher probability of positive margin ($P=0.02$), positive LVI ($P=0.03$), higher pathological T stage ($P=0.0004$), whereas patients with PDL1 negative TILs had an earlier stage at presentation (stage I-II) ($P=0.004$) as shown in Table 3.

Survival analysis

Correlations were conducted for 2-year DFS for non-metastatic patients, PFS for metastatic patients and OS for both metastatic and non-metastatic patients in PD-L1 positive and negative groups both in tumor cells and TILs. Median survival was calculated summarized in Table 4.

In non-metastatic patients, the 2-year DFS was 75.1% and median survival was not reached. When correlating 2-year DFS with PD-L1 of tumor cells, PDL-Tumor

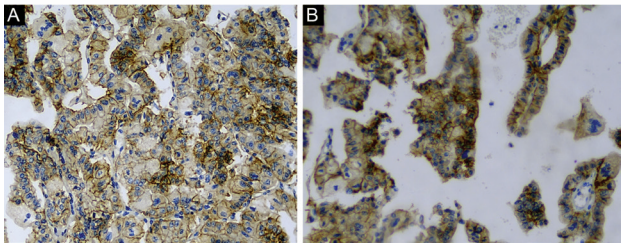

Figure 1. (A) A case of clear cell renal cell carcinoma with moderate to strong membranous immunostaining of PDL-1 (score 3+) (PDL-1 ×200). **(B).** A case of papillary RCC with strong PDL1 expression (score 3+) (PDL1 ×200).

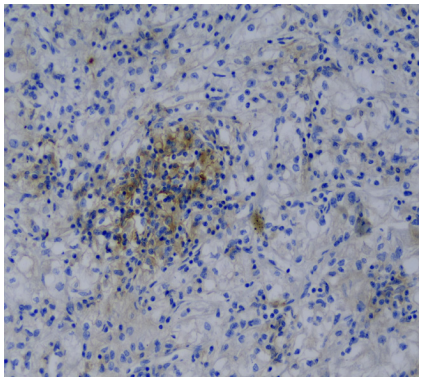

Figure 2. A case of clear cell RCC with negative PDL-1 expression and high expression in TIL (PDL1×200).

negative had 2-year DFS 70% while PDL-Tumor positive was 79.92% ($P=0.6106$). In PD-L1 TILs, 2-year DFS of PDL-TIL negative was 78% versus 62.8% for PDL-TIL positive ($P=0.5101$).

When comparing OS in non-metastatic patients, no statistical significance could be found. Median OS was not reached for PD-L1 positive tumor cells versus 47.7 months for PD-L1 negative ($P=0.0701$). The 2-year OS in PD-L1 tumor cells negative was 89% versus 100% for PD-L1 tumor cells positive as shown in Figures 3 and 4.

PD-L1 positive TILs median OS was not reached versus 47.6 months for PD-L1 negative TILs ($P=0.3684$). The 2-year OS in PD-L1 TILs negative 93.2% versus 100% for PD-L1 TILs positive.

As for metastatic patients, the median PFS was 18.7 months for the whole group. Median PFS in PD-L1 negative tumor cells was 8.5 months versus 28.6 months for PD-L1 positive patients ($P=0.3046$).

Median PFS in PD-L1 negative TILs was 10.6 months versus 28.6 months for PD-L1 positive TILs ($P=0.94$).

No statistical difference was also detected in metastatic patients as regards OS. The median survival was not reached in PD-L1 positive tumor cells versus 12.5 months in the PD-L1 negative group ($P=0.33$). For PD-L1 positive TILs, median survival was 12.5 months versus 38.8 months for the PD-L1 negative TILs group ($P=0.9390$) a shown in Figures 5 and 6.

Table 2. Relationship between PDL1-Tumor and clinicopathological factors

Category	PD-L1 tumor cells negative	PD-L1 tumor cells positive	*P* value
Age (years)			
50	4 (9.3%)	11 (25.6%)	0.1161
≥ 50	15 (34.9%)	13 (30.2%)	
Gender			
Male	8 (18.6%)	8 (18.6%)	0.5592
Female	11 (25.6%)	16 (37.2%)	
ECOG PS			
0-1	15 (34.9%)	20 (46.5%)	0.7168
2-4	4 (9.3%)	4 (9.3%)	
Pathological features			
T size (cm)			
Median	7.20	8.75	0.0756
Renal fat invasion			
Positive	8 (21.1%)	10 (26.3%)	0.5573
Negative	7 (18.4%)	13 (34.2%)	
Histology			
Clear cell	12 (27.9%)	15 (34.9%)	
Chromophobe	2 (4.7%)	4 (9.3%)	0.6706
Papillary	4 (9.3%)	5 (11.6%)	
Oncocytic	1 (2.3%)	0 (0.0%)	
Nuclear grade			
1	1 (2.3%)	2 (4.7%)	
2	11 (25.6%)	13 (30.2%)	0.5644
3	4 (9.3%)	8 (18.6%)	
4	0 (0.0%)	0 (0.0%)	
Positive margin			
Present	1 (2.5%)	2 (5%)	1.0000
Absent	16 (40.0%)	21 (52.5%)	
LVI			
Present	2 (6.2%)	7 (21.9%)	0.2349
Absent	12 (37.5%)	11 (34.4%)	
Pathological (TNM) stage			
PT stage			
1	7 (17.1%)	4 (9.8%)	
2	4 (9.8%)	9 (22.0%)	0.3741
3	6 (14.6%)	9 (22.0%)	
4	1 (2.4%)	1 (2.4%)	
PN stage			
0	2 (4.9%)	1 (2.4%)	0.3325
1	0 (0%)	2 (4.9%)	
Stage			
1	5 (11.6%)	4 (9.3%)	
2	2 (4.7%)	6 (14.0%)	0.7068
3	3 (7.0%)	7 (16.3%)	
4	9 (20.9%)	7 (16.3%)	

Abbreviations: PS, performance status; PD-L1, programmed cell death-ligand 1; ECOG, Eastern Cooperative Oncology Group; LVI, lymphovascular invasion;

Discussion

PD-1 and PD-L1 are promising targets for immunotherapeutic approaches, and they are considered novel markers with potential prognostic value in RCC (17).

Currently, the treatment landscape in mRCC is shifting back towards immuno-oncology agents such as immune checkpoint inhibitors (18,19), which have been shown to have a good response and improve OS in mRCC patients (20).

Clinical trials are currently investigating the role of adjuvant immune checkpoint inhibitors or other novel drugs and testing the possibility of improving the prognosis of patients with RCC at higher risk of disease recurrence or progression after nephrectomy (iMmotion 010, Checkmate-914) (21,22). Recently, results of Keynote-564 showed significant improvement in DFS for adjuvant pembrolizumab as compared with placebo (23). Thus, the expression of PDL-1 is currently being investigated as

Table 3. Relationship between PDL1-TILs and clinicopathological factors

Category	PD-L1 TILs negative	PD-L1 TILs positive	*P* value
Age (years)			
50	11 (25.6%)	4 (9.3%)	1
≥50	19 (44.2%)	9 (20.9%)	
Gender			
Male	19 (44.2%)	8 (18.6%)	0.9120
Female	11 (25.6%)	5 (11.6%)	
ECOG PS			
0-1	26 (60.5%)	9 (20.9%)	0.1823
2-4	4 (9.3%)	4 (9.3%)	
Pathological features			
T size (cm)			
Median	7.20	9.00	0.0211
Renal fat invasion			
Positive	7 (38.9%)	11 (61.1%)	0.0016
Negative	18 (47.4%)	2 (5.3%)	
Histology			
Clear cell	17 (39.5%)	10 (23.3%)	0.2997
Chromophobe	6 (14%)	0 (0.0%)	
Papillary	6 (14%)	3 (7%)	
Oncocytic	1 (2.3%)	0 (0.0%)	
Nuclear grade			
1	3 (7.7%)	0 (0.0%)	0.05
2	18 (46.2%)	6 (15.4%)	
3	6 (15.4%)	6 (15.4%)	
4	0 (0.0%)	0 (0.0%)	
Positive margin			
Present	0 (0.0%)	3 (100.0%)	0.028
Absent	27 (67.5%)	10 (25.0%)	
LVI			
Present	3 (33.3%)	6 (66.7%)	0.034
Absent	18 (56.2%)	5 (15.6%)	
Pathological (TNM) stage			
PT			
1	11 (26.8%)	0 (0.0%)	0.0004
2	11 (26.8%)	2 (4.9%)	
3	5 (12.2%)	10 (24.4%)	
4	1 (2.4%)	1 (2.4%)	
PN			
0	2 (4.9%)	1 (2.4%)	0.8459
1	1 (2.4%)	1 (2.4%)	
Stage			
1	9 (20.9%)	0 (0.0%)	0.0041
2	8 (18.6%)	0 (0.0%)	
3	4 (9.3%)	6 (14.0%)	
4	9 (20.9%)	7 (16.3%)	

Abbreviations: PS, performance status; PD-L1, programmed cell death-ligand 1; ECOG, Eastern Cooperative Oncology Group; LVI, lymphovascular invasion; TILs, tumor-infiltrating lymphocytes.

an important prognostic and predictive biomarker (14); however, it is still not validated alone to determine which patients should receive PD-1/L1 blockade therapy (12).

The current study aimed at identifying PD-L1 expression both on tumor cells and immune cells in patients diagnosed with RCC and correlate this to tumor characteristics and prognosis.

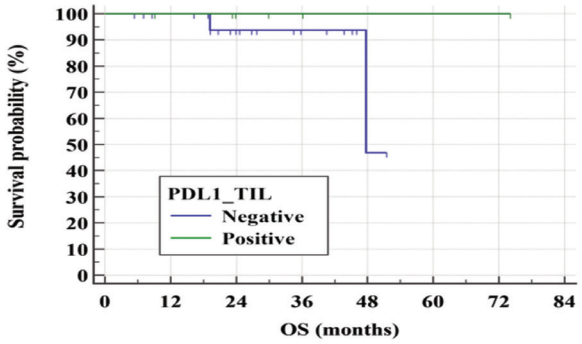

Figure 3. Kaplan-Meier cure comparing OS in PD-L1 TILs positive versus negative group in non-metastatic patients.

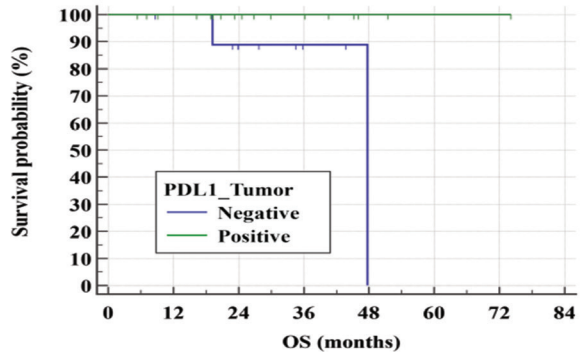

Figure 4. Kaplan-Meier cure comparing OS in PD-L1 tumor cells positive versus negative group in non-metastatic patients.

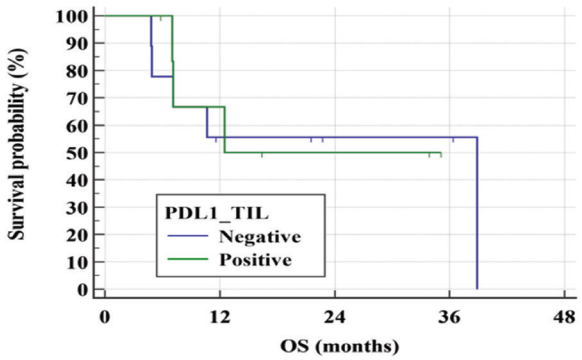

Figure 5. Kaplan-Meier cure comparing OS in PD-L1 TILs positive versus negative group in metastatic patients.

PD-L1 positivity was recorded at 55% on the tumor cells and 30.2% on the immune cells in this study population.

Studies that investigated PD-L1 expression by IHC have reported positivity rates ranging from 5 to 57% for tumor cells (18,24) and from 8 to 75% for TILs (25,26).

Reported levels of PD-L1 have been very broad. In a study of 306 patients, PD-L1 positive expression was seen in 23% of cases (27). Additionally, in another study of 346 RCC patients, PD-L1 positivity in tumor cells was found in 14.9% of patients and PD-L1 expression in TILs was observed in 18.2% of patients (25).

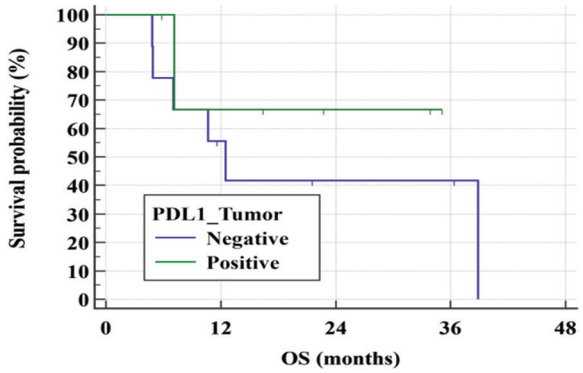

Figure 6. Kaplan-Meier cure comparing OS in PD-L1 tumor cells positive versus negative group in metastatic patients.

This great variation may be related to differences in PD-L1 expression between RCC subtypes. The existing data regarding this subject are conflicting. A recent study reported lower rates of PD-L1 expression in clear cell compared to papillary (0–16% versus 27–32%) or in chromophobe RCC (0% versus 35%) (28,29). Similarly, in a meta-analysis, a significant difference in expression between clear cell and non-clear cell histology was detected (30). However, there are also studies showing higher PD-L1 positivity rates in clear cell RCCs than in other renal tumor subtypes (31,32).

In our study, the study population included all histological subtypes of RCC, most of the patients 62.7% (n=27) had clear cell histological subtype while 20.9% (n=9) had papillary RCC, 13.9% (n=6) had chromophobe subtype and only one patient had oncocytic subtype. All of which had no significant association with PD-L1 expression and outcome. The small population and heterogeneity in histological subtypes may have affected our results. Our study also included both non-metastatic and metastatic patients. Most of the metastatic patients underwent cytoreductive surgery and hence metastatic sites were not biopsied. Histological diagnosis and PD-L1 testing were conducted on the tissue of the primary tumor.

Heterogeneity in RCC has been described by Gerlinger et al (33) when several tumor biopsies were obtained from different regions of the primary tumor and site of metastasis. Results have shown heterogeneity of PD-L1expression both within the primary tumor and between primary tumors and metastasis.

Similarly, a recent analysis has reported discordance in the expression of PD-L1 between primary tumors and metastasis (20.8%), suggesting heterogeneity in PD-L1 expression within the same patient (26). Another study also came to the same conclusion and suggested that employing PD-L1 as a predictive biomarker for PD-1 blockade may require analysis of metastatic lesions (34).

The type of technique used for the assessment of PD-L1 expression is still not standardized. Different techniques are utilized in different studies. In a recent meta-analysis, several studies conducted IHC on tumor tissue while others used ELISA in the serum of affected patients. When the analysis was limited to studies utilizing IHC, a marked difference in the risk of death related to the increased expression of PD-L1 was seen (risk of death 2 compared to 1.81) (30).

When the IHC technique is used, the cutoff and the type of monoclonal antibody employed harbors another controversy. Several studies conducted different antibodies (e.g., Dako, Leica platform, Ventana Medical System) and different cutoff values which had a range between 5 and 10 %, whereas other studies performed the H-scores that is calculated based on both the percentage of positive cells and the expression score evaluated by a scale ranging from 0 to 3+. Up till now, no validated method or optimal cut-off definition for PD-L1 IHC was observed (35).

All these factors along with the small sample size may have contributed to the difficulty in interpretation of our results regarding PD-L1 expression and thus rendering the comparison with other existing data inaccurate.

When correlating the PD-L1 tumor expression with clinical and pathological factors, we could not detect a statistically significant difference. Patients with PDL1 positive tumor cells had higher median tumor size ($P=0.07$), higher nuclear grade ($P=0.68$), higher LVI ($P=0.23$); yet all with no statistical significance.

This could be attributed to heterogeneity in our study population where different histological subtypes were included. Moreover, our population included both metastatic and non-metastatic patients. This diversity in the study population may have affected the accuracy of results.

Several studies reported that in ccRCC, expression of PD-L1 is strongly correlated with aggressive features and prognosis (10, 30,36). In a study that included patients with pRCC, no significant association was found between PD-L1 expression and all clinicopathological factors (37).

Moreover, in a cohort of 81 chRCC patients, PD-L1 positivity was not associated with tumor aggressiveness. It was suggested that neither PD-1 positivity in inflammatory cells nor PD-L1 positivity in the tumor had an impact on the natural course of a chRCC tumor (38).

Regarding survival analysis in our study, PD-L1 expression of tumor cells was not significantly associated with prognosis, since no statistically significant difference in DFS, PFS, and OS for metastatic and non-metastatic groups when analyzed separately, was detected.

PD-L1 expression has been investigated as a prognostic factor with great controversy. Most studies showed that PD-L1 positivity is associated with worse prognosis and survival(15,24,30,32). However, other studies could not find a significant association with prognosis (35-41).

On the contrary of PD-L1 in tumor cells, PDL1 positive TILs in our study were significantly associated with larger pathological tumor size ($P=0.02$), higher probability

of renal fat invasion ($P=0.001$), higher nuclear grade ($P=0.05$), higher probability of positive margin ($P=0.02$), positive LVI ($P=0.03$), higher pathological T stage ($P=0.0004$); whereas patients with PDL1 negative TILs had an earlier stage at presentation (stage I-II) ($P=0.004$).

RCC is known to be an immunogenic tumor and it was found to be highly associated with infiltrating immune cells mainly T-cells. The presence of TILs in solid tumors has been correlated with improved outcomes in retrospective studies of different tumor types, including melanoma and colorectal carcinoma (42-44). However, unlike other tumors, increased TILs in RCC tumors were found to confer a poor prognosis (25,45). These data suggest the hypothesis that immune cells within the renal tumor microenvironment contribute to facilitating tumor progression and thus worse prognosis.

Similar to our study, high levels of tumor-infiltrating immune cells, particularly CD8+ T cells, have been associated with adverse features, possibly due to an impairment of antitumor immune responses (46).

Previous studies have shown that PD-1 TILs positivity in ccRCC was considered as an independent prognostic indicator for OS. Thompson et al, described a strong association of adverse prognostic features as well as OS in patients with positive PD-L1 expression in both tumor cell membrane and TILs (46). However, Abbas et al found no significant association with survival parameters (47). Furthermore in another study, PD-L1 status was associated with parameters of aggressiveness but was not proven to be a significant independent prognostic biomarker (36).

In our study, no statistically significant difference was detected in the prognosis of patients with PD-L1 positive expression of TILs compared to PD-L1 negative TILs. Interestingly, a study which evaluated PD-L1 mRNA level in RCC tumors employing the RNA-seq approach reported that patients with low-expression of PD-L1 mRNA level had more aggressive disease than those with high PD-L1 mRNA expression. Thus, a higher PD-L1 mRNA level in RCC seemed to be associated with a favorable outcome in these patients (48). Similarly, in another study, the mRNA expression of PD-L1 in primary nephrectomy specimens revealed no significant association with unfavorable clinical parameters and a positive correlation with patient survival was found (HR=0.59, $P=0.006$) (49).

Although PD-L1 TILs positive group in our patients was associated with significantly more aggressive features, mean OS for PD-L1 positive TILs was 57 months versus 41 months with an insignificant statistical difference (p=0.88).

Giraldo et al (50) suggested a heterogeneity in the composition of the immune microenvironment among patients and tumor types. They have demonstrated that patients with high tumor infiltration of the $CD8^+$ T-cell population had a good prognosis. Similarly, Nakano et al (46), showed that TILs with high $CD8^+$ T cell content were associated with improved survival among patients with advanced RCC. Further studies however are needed to identify the role of other immune cells and their effect on prognosis.

When we compared OS in both non-metastatic and metastatic patients, no statistical significance could be found both for PD-L1 in tumor cells and TILs groups.

Conclusion

There is still controversy about the role of PD-L1 as a prognostic factor in RCC. PDL1 positive TILs were significantly associated with larger tumor size, higher nuclear grade, more aggressive features, and higher stage.

Limitations of the study

Our study is considered the first study to report PD-L1 expression in RCC among the Egyptian population. Our study had several limitations as it is a retrospective analysis which may have resulted in selection bias. Moreover, our study sample is small which may not be representative of the whole population.

There was no statistically significant difference in DFS, PFS, or OS in patients with PD-L1 positive and negative both in tumor cells and immune cells. Further studies with a larger sample size are warranted to determine the prognostic and predictive value of PD-L1 in RCC patients.

Authors' contribution

All authors contributed to the idea and the study design. Data collection was done by HS and was supervised by MMAE, MMS. Whereas, MMS carried out the IHC analysis of specimens and the grading and scoring of PD-L1 expression level. Data analysis was conducted by HS and MMAE, while all authors contributed equally to data interpretation. Manuscript drafting was carried out by HS and MMAE, while SHA, MMS and KEN have revised the manuscript. All authors accepted the final form of the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The study was carried out with the approval of the faculty of medicine, Ain Shams university ethical committee with approval number FWA000017585. The study was based on data collection and immunohistochemical analysis of positively charged slides prepared from paraffin blocks so informed consent was not applicable to our study. This study was extracted from MD thesis of Hoda Sayed registered at faculty of medicine, Ain Shams university. Additionally, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71:209-49. doi: 10.3322/

caac.21660.
2. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, et al. SEER Cancer Statistics Review, 1975–2017, National Cancer Institute. Bethesda (MD): National Cancer Institute. https://seer.cancer.gov/csr/1975_2017/. Accessed April 28, 2021
3. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours. Eur Urol. 2016;70:93-105. doi: 10.1016/j.eururo.2016.02.029/
4. Van den Hove LE, Van Gool SW, Van Poppel H, Baert L, Coorevits L, et al. Phenotype, cytokine production and cytolytic capacity of fresh (uncultured) tumour-infiltrating T lymphocytes in human renal cell carcinoma. Clin Exp Immunol. 1997;109:501-9. doi: 10.1046/j.1365-2249.1997.4771375.x.
5. Bromwich EJ, McArdle PA, Canna K, McMillan DC, McNicol AM, Brown M, et al. The relationship between T-lymphocyte infiltration, stage, tumour grade and survival in patients undergoing curative surgery for renal cell cancer. Br J Cancer. 2003;89:1906-8. doi: 10.1038/sj.bjc.6601400.
6. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11:3887.
7. Afreen S, Dermime S. The immunoinhibitory B7-H1 molecule as a potential target in cancer: Killing many birds with one stone. Hematol Oncol Stem Cell Ther. 2014;7:1-17. doi: 10.1016/j.hemonc.2013.09.005.
8. Ross K, Jones RJ. Immune checkpoint inhibitors in renal cell carcinoma. Clin Sci. 2017;131:2627-42. doi: 10.1042/CS20160894.
9. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A. 2002;99:12293-7. doi: 10.1073/pnas.192461099
10. Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS,et al. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci USA. 2004;101:17174-9. doi: 10.1073/pnas.0406351101.
11. Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: Meta-analysis. BMJ. 2018;362:3529. doi: 10.1136/bmj.k3529.
12. Meng X, Huang Z, Teng F, Xing L, Yu J. Predictive biomarkers in PD-1/PD-L1 checkpoint blockade immunotherapy. Cancer Treat Rev. 2015;41:868-876. doi: 10.1016/j.ctrv.2015.11.001
13. Delahunt B, Eble JN, Egevad L, Samaratunga H. Grading of renal cell carcinoma. Histopathology. 2019;74:4-17. doi: 10.1111/his.13735.
14. Choueiri TK, Fay AP, Gray KP, Callea M, Ho TH, Albiges L, et al. PD-L1 expression in nonclear-cell renal cell carcinoma. Ann Oncol. 2014;25:2178-2184. doi: 10.1093/annonc/mdu445.
15. Möller K, Fraune C, Blessin NC, Lennartz M, Kluth M, Hube-Magg C, et al. Tumor cell PD-L1 expression is a strong predictor of unfavorable prognosis in immune checkpoint therapy-naive clear cell renal cell cancer. Int Urol Nephrol. 2021;53:2493-503. doi: 10.1007/s11255-021-02841-7
16. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649-55.
17. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA,et al. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clin Cancer Res. 2013;19:462-8. doi: 10.1158/1078-0432.CCR-12-2625.
18. Stenzel PJ, Schindeldecker M, Tagscherer KE, Foersch S, Herpel E, Hohenfellner M, et al. Prognostic and Predictive Value of Tumor-infiltrating Leukocytes and of Immune Checkpoint Molecules PD1 and PDL1 in Clear Cell Renal Cell Carcinoma. Transl Oncol. 2020;13:336-45. doi: 10.1016/j.tranon.2019.11.002.
19. Bedke J, Stühler V, Stenzl A, Brehmer B. Immunotherapy for kidney cancer: Status quo and the future. Curr Opin Urol. 2018;28:8-14. doi: 10.1097/MOU.0000000000000466.
20. McDermott DF, Drake CG, Sznol M, Choueiri TK, Powderly JD, Smith DC, et al. Survival, durable response, and long-term safety in patients with previously treated advanced renal cell carcinoma receiving nivolumab. J Clin Oncol. 2015;33:2013-2020. doi: 10.1200/JCO.2014.58.1041.
21. Bex A, Russo P, Tomita Y, Grünwald V, Ramirez LM, McHenry BM et al. A phase III, randomized, placebo-controlled trial of nivolumab or nivolumab plus ipilimumab in patients with localized renal cell carcinoma at high-risk of relapse after radical or partial nephrectomy (CheckMate 914). J Clin Oncol. 2020;38:TPS5099. doi: 10.1200/jco.2020.38.15_suppl.tps5099.
22. Uzzo R, Bex A, Rini BI, Albiges L, Suarez C, Donaldson F,et al. A phase III study of atezolizumab (atezo) vs placebo as adjuvant therapy in renal cell carcinoma (RCC) patients (pts) at high risk of recurrence following resection (IMmotion010). J Clin Oncol. 2017;35:TPS4598. doi: 10.1200/jco.2017.35.15_suppl.tps4598.
23. Choueiri TK, Tomczak P, Park SH, Venugopal B, Ferguson T, Chang YH, et al. Adjuvant Pembrolizumab after Nephrectomy in Renal-Cell Carcinoma. N Engl J Med. 2021;385:683-94. doi: 10.1056/nejmoa2106391.
24. Leite KR, Reis ST, Junior JP, Zerati M, Gomes Dde O, Camara-Lopes LH, Srougi M. PD-L1 expression in renal cell carcinoma clear cell type is related to unfavorable prognosis. Diagn Pathol. 2015;10:189. doi: 10.1186/s13000-015-0414-x.
25. Carlsson J, Sundqvist P, Kosuta V, Fält A, Giunchi F, Fiorentino M, et al. PD-L1 expression is associated with poor prognosis in renal cell carcinoma. Appl Immunohistochem Mol Morphol. 2020;28:213-20. doi: 10.1097/PAI.0000000000000766.
26. Callea M, Albiges L, Gupta M, Cheng SC, Genega EM, Fay AP,et al. Differential expression of PD-L1 between primary and metastatic sites in clear-cell renal cell carcinoma. Cancer Immunol Res. 2015;3:1158-64. doi: 10.1158/2326-6066.CIR-15-0043.
27. Thompson RH, Kuntz SM, Leibovich BC, Dong H, Lohse CM, Webster WS, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res. 2006;66:3381-5. doi: 10.1158/0008-5472.CAN-05-4303.
28. Lee HJ, Shin DH, Lee YJ, Lee SJ, Hwang CS, Kim A, et al. PD-L1 expression and infiltration by CD4+ and FoxP3+ T cells are increased in Xp11 translocation renal cell carcinoma and indicate poor prognosis. Histopathology. 2020;76:714-21. doi: 10.1111/his.14047.
29. Walter B, Gil S, Naizhen X, Kruhlak MJ, Linehan WM, Srinivasan R, et al. Determination of the expression of PD-L1 in the morphologic spectrum of renal cell carcinoma. J Cancer. 2020;11:3596-603. doi: 10.7150/jca.35738.
30. Iacovelli R, Nolè F, Verri E, Renne G, Paglino C, Santoni M, et al. Prognostic Role of PD-L1 Expression in Renal Cell Carcinoma. A Systematic Review and Meta-Analysis. Target Oncol. 2016;11:143-8. doi: 10.1007/s11523-015-0392-7.
31. Chandrasekaran D, Sundaram S, Kadhiresan N, Padmavathi R. Programmed Death Ligand 1; An immunotarget for renal cell carcinoma. Asian Pacific J Cancer Prev. 2019;20:2951-7. doi: 10.31557/APJCP.2019.20.10.2951.
32. Shin SJ, Jeon YK, Kim PJ, Cho YM, Koh J, Chung DH,et al.

Clinicopathologic Analysis of PD-L1 and PD-L2 Expression in Renal Cell Carcinoma: Association with Oncogenic Proteins Status. Ann Surg Oncol. 2016;23:694-702. doi: 10.1245/s10434-015-4903-7.

33. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. N Engl J Med. 2012;366:883-92. doi: 10.1056/nejmoa1113205
34. Jilaveanu LB, Shuch B, Zito CR, Parisi F, Barr M, Kluger Y, et al. PD-L1 expression in clear cell renal cell carcinoma: An analysis of nephrectomy and sites of metastases. J Cancer. 2014;5:166-172. doi: 10.7150/jca.8167.
35. Jang A, Sweeney PL, Barata PC, Koshkin VS. PD-L1 Expression and Treatment Implications in Metastatic Clear Cell Renal Cell Carcinoma: A Systematic Review. Kidney Cancer. 2021;5:31-46. doi: 10.3233/KCA-200108.
36. Abbas, M; Steffens, S; Bellut, M; Eggers, H; Großhennig, A, BeckerJ U et al. Intratumoral expression of programmed death ligand 1 (PD-L1) in patients with clear cell renal cell carcinoma (ccRCC). Med Oncol 2016 337. 2016;33:1-7. doi: 10.1007/S12032-016-0794-0.
37. Motoshima T, Komohara Y, Ma C, Dewi AK, Noguchi H, Yamada S, et al. PD-L1 expression in papillary renal cell carcinoma. BMC Urol. 2017;17:1-6. doi: 10.1186/s12894-016-0195-x.
38. Erlmeier F, Hartmann A, Autenrieth M, Wiedemann M, Ivanyi P, Steffens S, et al. PD-1/PD-L1 expression in chromophobe renal cell carcinoma: An immunological exception? Med Oncol. 2016;33:1-6. doi: 10.1007/s12032-016-0833-x.
39. Kim SH, Park WS, Park EY, Park B, Joo J, Joung JY, et al. The prognostic value of BAP1, PBRM1, pS6, PTEN, TGase2, PD-L1, CA9, PSMA, and Ki-67 tissue markers in localized renal cell carcinoma: A retrospective study of tissue microarrays using immunohistochemistry. PLoS One. 2017;12:e0179610. doi: 10.1371/JOURNAL.PONE.0179610.
40. Jikuya R, Kishida T, Sakaguchi M, Yokose T, Yasui M, Hashizume A,et al. Galectin-9 expression as a poor prognostic factor in patients with renal cell carcinoma. Cancer Immunol Immunother. 2020;69:2041-51. doi: 10.1007/s00262-020-02608-6.
41. Dogan HT, Kiran M, Bilgin B, Kiliçarslan A, Sendur MA, Yalçin B, B, et al. Prognostic significance of the programmed death ligand 1 expression in clear cell renal cell carcinoma and correlation with the tumor microenvironment and hypoxia-inducible factor expression. Diagn Pathol. 2018;13:1-9. doi: 10.1186/s13000-018-0742-8.
42. Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, McCarthy SW, et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. J Clin Oncol. 2012;30:2678-83. doi: 10.1200/JCO.2011.37.8539.
43. Ogino S, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clin Cancer Res. 2009;15;6412-20. doi: 10.1158/1078-0432.CCR-09-1438.
44. Erdag G, Schaefer JT, Smolkin ME, Deacon DH, Shea SM, Dengel LT, et al. Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. Cancer Res. 2012;72:1070-80. doi: 10.1158/0008-5472.CAN-11-3218.
45. Webster WS, Lohse CM, Thompson RH, Dong H, Frigola X, Dicks DL, et al. Mononuclear cell infiltration in clear-cell renal cell carcinoma independently predicts patient survival. Cancer. 2006;107:46-53. doi: 10.1002/cncr.21951.
46. Nakano O, Sato M, Naito Y, Suzuki K, Orikasa S, Aizawa Met al. Proliferative activity of intratumoral CD8+ T-lymphocytes as a prognostic factor in human renal cell carcinoma: Clinicopathologic demonstration of antitumor immunity. Cancer Res. 2001;61:5132-5136.
47. Abbas M, Steffens S, Bellut M, Becker JU, Großhennig A, Eggers H, et al. Do programmed death 1 (PD-1) and its ligand (PD-L1) play a role in patients with non-clear cell renal cell carcinoma? Med Oncol. 2016;33(6):59. doi: 10.1007/s12032-016-0770-8.PMID: 27165272.
48. Ning XH, Gong YQ, He SM, Li T, Wang JY, Peng SH, et al. Higher programmed cell death 1 ligand 1 (PD-L1) mRNA level in clear cell renal cell carcinomas is associated with a favorable outcome due to the active immune responses in tumor tissues. Oncotarget. 2017;8:3355-63. doi: 10.18632/oncotarget.13765.
49. Peters I, Tezval H, Kramer MW, Wolters M, Grünwald V, Kuczyk MA, et al. [Implications of TCGA Network Data on 2nd Generation Immunotherapy Concepts Based on PD-L1 and PD-1 Target Structures]. Aktuelle Urol. 2015;46:481-5. doi: 10.1055/s-0041-106169.
50. Giraldo NA, Becht E, Pages F, Skliris G, Verkarre V, Vano Y, et al. Orchestration and prognostic significance of immune checkpoints in the microenvironment of primary and metastatic renal cell cancer. Clin Cancer Res. 2015;21:3031-40. doi: 10.1158/1078-0432.CCR-14-2926.