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Programmed death ligand I (PDL-1) Expression in renal cell carcinoma: A retrospective cohort study

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Abstract

Background and objectives: Inhibition of programed death -1and programed death ligand-1 pathway enhances antitumor activity of T lymphocytes, therefore, provides a new strategy for tumor treatment utilizing immunotherapy. The aim of this study to assess the frequency of programed death ligand-1 expression in renal cell carcinoma by using the immunohistochemistry and to correlate the results with the clinicopathologic parameters.

Methods: This is a cross sectional retrospective study performed in Duhok City from 2017-2022 on fifty-four formalin fixed paraffin embedded blocks of nephrectomy specimens diagnosed as renal cell carcinoma and collected from central lab of Duhok and some private labs. Two sections were prepared from each block, one section was stained with hematoxylin and eosin for histological analysis and the other one was used for immunohistochemical assessment of programed death ligand-1then the results were correlated with various clinicopathological parameters.

Results: Programed death ligand -1; membranous expression was positive in 19 cases (35.2%) of renal cell carcinoma out of 54 cases. There was no significant correlation between programed death ligand -1 expression and age (p= 0.991), gender (p= 0.272), multifocality (p= 0.607), tumor size (p= 0.796), histological subtype (p= 0.107), tumor stage (p= 0.546), nuclear grade (p= 0.781), surgical margins involvement (p= 0.119) and lymphovascular invasion (p= 0.4), but there was statistically significant correlation with nodal metastases (p= 0.039).

Conclusion: Programmed death ligand -1/ Combined positive score was ≥1 in about one third (35.2%) of renal cell carcinoma cases and this result can be utilized for the provision of immune checkpoint inhibitor (ICIs), regardless the age, gender, histological type, stage, nuclear grade, and the presence of lymphovascular invasion (LVI).

Keywords: PD-1, PDL-1, Renal cell carcinoma

Introduction

Worldwide renal carcinoma ranks twelfth among other cancers and ninth among malignancies in the United States of America.[1]

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Of all human cancers, renal cell carcinoma (RCC) accounts for 1-3%. [2] Globocan data in 2020 showed that there were 400000/ year new cases for both sex and all ages, and 180000 / year death. [3] Kidney cancer is an immunogenic tumor characteristically harbor abundant lymphocyte which make it resistant to chemotherapy and radiation[4], its more common in males than females [5] . Tobacco smoking, alcohol consumption, high body mass index and hypertension are important risk factors.[6] The classical clinical triad of presentation is pain, gross hematuria & palpable abdominal mass.[7] The WHO set a new classification in 2016 that contains 14 subtypes and 4 emerging/provisional entities.[2] Clear cell type is the commonest among others followed by the chromophobe & the papillary type. [8] There is no specific marker for clear cell RCC, but positivity for carbonic anhydrase IX, vimentin and CD10 can aid the diagnosis. [9] The prognosis of RCC depends on staging which includes tumor size, lymph node and distant metastasis in addition to the nuclear grade .[7] Radical nephrectomy remains the standard surgical procedure for most RCCs and 20% to 30% of apparently localized RCCs develop latent metastatic after surgical treatment.[10]

The above facts necessitate the search for new modalities of treatment like the implementation of immunotherapy along with of anti-vascular endothelial growth factor (VEGF) antibodies, VEGF receptor tyrosine kinase inhibitors, mammalian target of rapamycin (mTOR) pathway inhibitors, and immune checkpoint inhibitors (ICIs).[11,12]

Programed death-1(PD-1) was first mentioned in 1992. [12] It binds to two ligands PDL-1(B7-H1) & PDL-2. This binding has a negative regulatory effect on immune response. When PDl-1 is expressed on tumor cell, the T cell immune response to cancer will be inhibited.[13] Normally, PD-1 expressed on the surface of activated CD8 T cell and interacts with PDL-1 on the host normal cells resulting in inhibition of T-cell receptor signaling.[14] Expression of PDl-1 in renal cell carcinoma is correlated with reduced survival. [15] Many common human cancers express PDl-1 like breast carcinoma[16],colorectal[17], gastric[18] and papillary thyroid carcinoma[19]. Inhibition of PD-1-PDL-1 pathway enhances antitumor activity of T lymphocytes and provides a new strategy for tumor treatment utilizing immunotherapy.[17]

The study aimed to assess the frequency of PDL-1 expression in renal cell carcinoma by using the immunohistochemistry and the association with various clinicopathologic parameter.

Materials & methods

This is a cross sectional retrospective study performed in Duhok City from 2017-2022 on fifty-four formalin fixed paraffin embedded blocks of nephrectomy specimens diagnosed as renal cell carcinoma. These blocks and their histopathology reports were retrieved from the central lab in Duhok City and some private labs. Two sections were prepared from each block, one section was stained with hematoxylin and eosin for the purpose of histological analysis & the other one was used for immunohistochemical study of PDL1 expression. The microscopical classification & grading of RCC were performed according to 2016 WHO classification system, and the pathological staging is according to 8th edition of the AJCC. [20]

Immunostaining of 3-4µm formaldehyde- fixed paraffin embedded tissue sections was performed on an automated platform. Antibody used 22C3 (DAKO), For 28-8 / 22C3, automated pre-treatment was carried out at pH 6. At room temperature, primary monoclonal antibodies were incubated for 30 minutes and visualized using the Morsch et al. appropriate DAB-based detection kits and hematoxylin counterstains. In contrast to positive controls used in immunohistochemical studies, negative controls were carried out by removing the main antibody for both pH conditions. Programed death ligand-1 was determined by using a combined positive score (CPS), regardless of staining intensity, any membranous staining in tumor cell was regarded as positive, while any nuclear or cytoplasmic staining of the tumor infiltrating lymphocyte and macrophage was recorded as positive results. Necrotic area was excluded. The CPS was calculated by using the following equation: the total number of positive tumor cell, lymphocytes & macrophages divided by the total number of viable tumor cells, multiplied by 100.[21]

Statistical analysis was done by SPSS (version 28.0) software. PDL-1 was tested against clinicopathological parameters like age, gender, histological type, nuclear grade, tumor size, LVI, surgical margins involvement & the presence of lymph node metastases. Appropriate tests were selected according to available data & P value of ≤ 0.05 was considered statistically significant.

Results

This study included 54 cases of nephrectomy specimens of renal cell carcinoma. The patients aged ranged from 30 to 80 years. The Mean age ± SE= 55.69 ±1.66 years. The peak was in the age group between 60-69 years (Figure 1). Male gender constituted 57.41%.

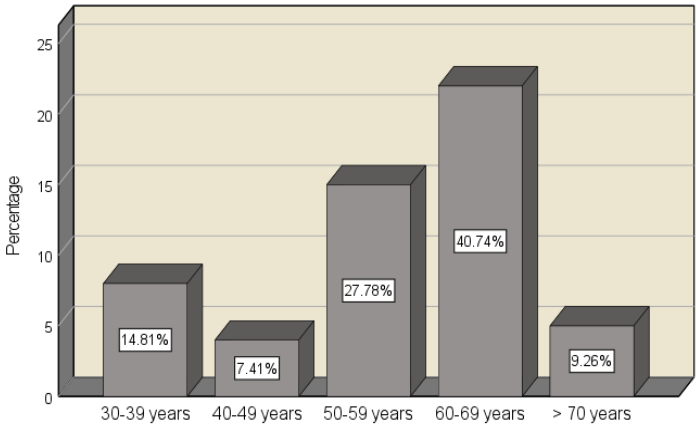

Figure (1): The age groups of the included patients.

Histologically, twenty-seven cases (50%) were classical clear cell carcinoma, 13 (24.1%) were papillary, 10 (18.5%) were chromophobe and 4 (7.4%) were with sarcomatoid differentiation (Figure 2).

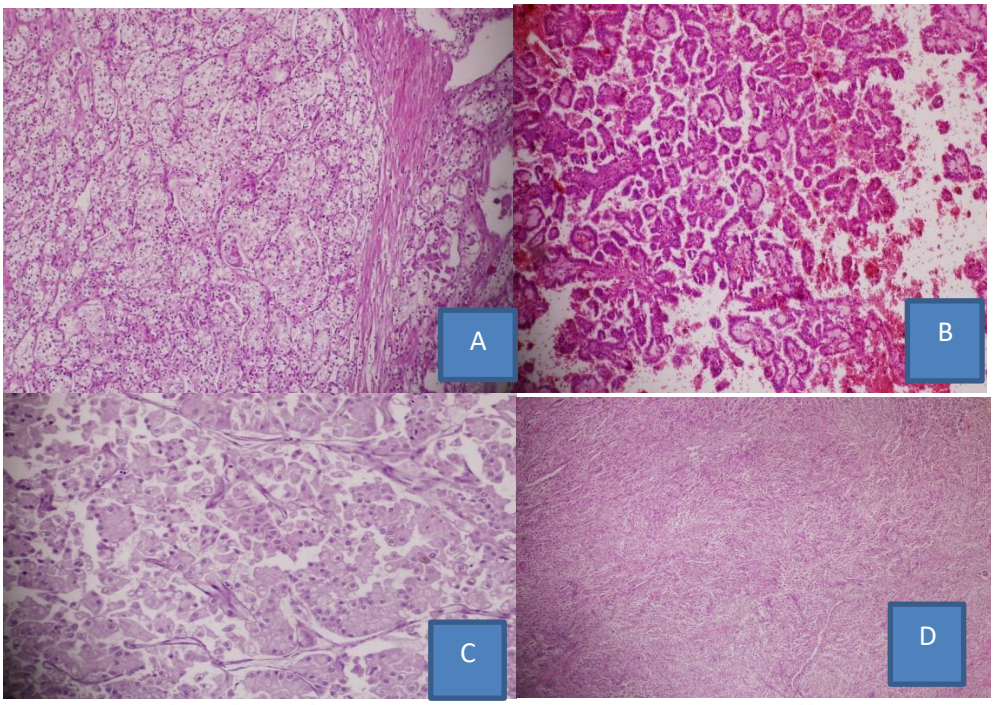

Figure (2): Various histological types; A-CCRCC (H&E x 4), B-Papillary RCC (H&E x10), C-Chromophobe RCC (H&E x10), D- Sarcomatoid RCC (H&E x 4)

The various histologic stage of the enrolled patients is demonstrated in Table (1) and the nuclear grades in Table (2).

Table (1): The histologic stage of disease of the enrolled patients.

PDL-1 expression	Histologic stage *									
	Count and %	T1aN0	T1bN0	T2aN0	T2bN0	T2bN1	T3aN0	T3aN1		p value	Effect size
PDL1/ CPS <1%	Count	12	10	5	4	1	3	0	35	0.546	0.311
	% Within Disease stage	70.6%	62.5%	83.3%	80.0%	50.0%	42.9%	0.0%	64.8%		
PDL1/ CPS ≥1%	Count	5	6	1	1	1	4	1	19		
	% Within Disease stage	29.4%	37.5%	16.7%	20.0%	50.0%	57.1%	100.0%	35.2%		
	Total	17	16	6	5	2	7	1	54		
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%		

*A Fisher exact test was performed for a group association between PDL-1/CPS expression and the histologic stage of the disease indicated no significant association of moderate effect (χ^2 (1, n=54) = 5.208 p=0.546, Cramer's V =0.311).

Table (2): The nuclear grade and its correlation to PDL-1/ CPS expression.

PDL-1 expression	Cancer grade* Count and %	I	II	III	IV	Total	P value	Effect size
PDL1/ CPS <1%	Count	19	12	2	2	35	0.781	0.13
	% Within Cancer grade	67.9%	66.7%	50.0%	50.0%	64.8%		
PDL1/ CPS ≥1%	Count	9	6	2	2	19		
	% Within Cancer grade	32.1%	33.3%	50.0%	50.0%	35.2%		
	Total	28	18	4	4	54		
		100.0%	100.0%	100.0%	100.0%	100.0 %		

*A Fisher exact test was performed for a group association between PDL-1/ CPS expression and the grade of the disease indicated no significant association of small effect (χ^2 (1, n=54) =0.911 p=0.781, Cramer's V=0.13).

The mean tumor size was 60.28±34.5 SD, the largest one was 160 mm, and the smallest is 16 mm. Multifocality was seen in 4 (7.4%) cases. LVI invasion was detected in 7 (12.9%) cases and 3 (5.5%) patients had metastatic nodal disease. Surgical margins involvement was detected in 4 (7.4%) cases. PDL-1/CPS was ≥ 1% in 19 (35.2%) cases and < 1% in the remaining patients (Figure 3).

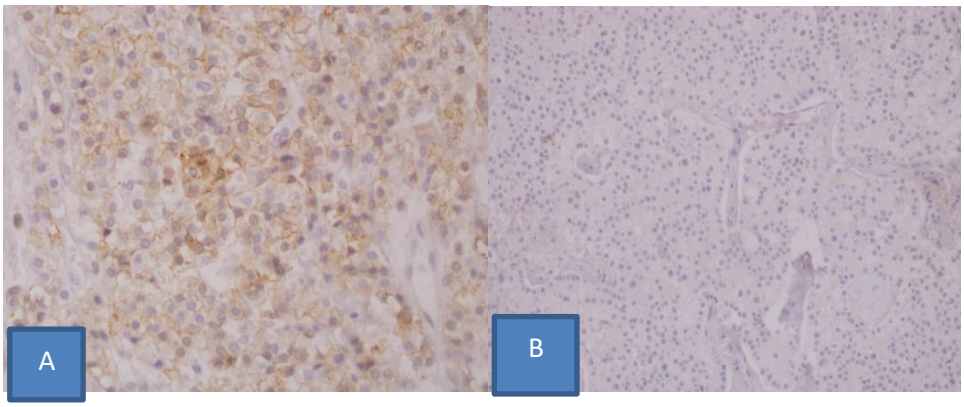

Figure (3): A- Positive PDL-1 expression B- Negative PDL-1 in clear cell RCC (X20)

The statistical correlation between PDL-1/CPS and various clinicopathological parameters as follow; there is no significant correlation between PDL-1/CPS and age (p= 0.991), gender (p= 0.272), multifocality (p= 0.607), tumor size (p= 0.796), histological subtype (p= 0.107), tumor histologic stage (p= 0.546), nuclear grade (p= 0.781), surgical margins involvement (p= 0.119) and LVI (p= 0.4), but there is statistically significant

correlation with nodal metastases (p= 0.039).

Discussion

The predictive value of PDL-1 expression was clearly demonstrated by FAY AP et al/ 2014. [22] High PDL-1 expression has a negative impact on the survival of patients with many of the common human cancers. [23 -25] Programed death ligand-1 expression has a decisive role in immunotherapies.[26] The precise dynamicity between immune check point inhibitor and PD-L1 expression is not well understood yet.[21]

In this study, PDL-1 membranous expression was seen in 19 cases (35.2%) of RCC, this result is similar with the study performed by Walter et al [14] reported 34% and Choueiri et al [27] who found 36% positivity rate. A higher rate was reported by Leite who found positivity rate of 56.5% of their cases[13] and a lower rate of 14% was recorded by Tabriz et al.[28]

Males accounted for 57.41% with the mean age of the patients being (55.69 ±1.66 years) and there was no significant correlation between PDL-1 with age and gender, the results are in keeping with those of Liu Y et al.[29]

There is no significant association between PDL-1 expression & histological subtype, which is in contrast with what Walter et al found that clear cell RCC shows negative results for PDL-1. [14] Further contradicting results were reported in different studies. [27,30,31,32,36]. The discrepancy among studies is probably related to the paucity of non-clear RCC cases, the samples sizes, different reagents, and different standards of positivity evaluation of PDL-1 expression.

The result of this study shows that PDL-1 shows no significant association between histologic stage, grade, size, multifocality, LVI & positive surgical margin, while it shows a significant association with nodal metastasis. The finding is dissimilar with Liu Y et al [29] and Shen et al [31] who found association between tumor size, venous invasion and lymph node metastasis and PDL-1 level were higher in patients with higher stage and poor prognosis.

Robb VA et al [32] finds that PDL-1 expression was associated with poor histological factors such as tumor stage, grade & sarcomatoid component. In keeping with our study, Liu Y et al [29] found that lymph node metastasis correlates significantly with PDL-1 level in RCC. Tabriz et al [28] found no significant association between LVI & sarcomatoid differentiation with PDL-1 staining. On the other hand, Pyo et al [33] reported that PDL-1 expression considerably correlates with worse disease-free survival rate.

Recently, systematic review and meta-analysis demonstrated that anti–PD-1/PD-L1 inhibitors were associated with better overall survival especially in advanced and metastatic cancers compared with conventional therapies.[34]

When there is upregulation PDL-1 expression, sensitivity to PD-1/PDl-1 inhibitor will be increased while elevated levels of PDL-1 expression are associated with poorer outcome.[35,36] But when treated with PD-1/PDL-1 inhibitors, patients with higher level of PDL-1 expression do better in response to immunotherapy.[37]

Conclusion:

Programed death ligand -1/Combined positive score was ≥1 in about one third (35.2%) of renal cell carcinoma cases and this make these patients probable

candidates for beneficial effects of Immune checkpoint inhibitor (ICIs), regardless the age, gender, histological type, stage, nuclear grade, and the presence of lymphovascular invasion. Further research on large groups of patients is required to strengthen the results of this study.

Conflict of interest:

The authors recorded no conflict of interest.

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