The Role of PDL1 Immunostain Expression in RCC: A Clinicopathological Study

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Abstract

Background: The expression of programmed death-ligand 1 (PD-L1) on malignant cells is one method by which the immune system might evade tumorigenesis. PD-1/PD-L1 pathway inhibition improves immunity against tumours. The study aimed to evaluate the statement of PDL1 immunostain in renal cell carcinoma. **Methods:** A case series of research, both prospective and retrospective, was conducted on fifty primary renal cell cancer samples. Hematoxylin and eosin (H and E) stained glass slides were processed from Formalin-fixed and paraffin-embedded (FFPE) blocks and were revised regarding diagnosis. PD-L1 immunohistochemical stain (PD-L1 IHC) was conducted for all cases, PD-L1 IHC 22C3 pharmDx (Dako) monoclonal mouse anti-PD-L1 employing Autostainer Link 48's EnVision FLEX visualization system. **Results:** PDL1 is found to be expressed in 56 %, showing positivity in males mainly in score 3, 63.6%. The frequency of scores in PDL1 expression with histological variant shows statistical significance in score 3 with p- value 0.02. According to the stages, stage 1 was the most common, grades 2 and 3 were strongly positive expressions and showed the left side was strongly positive while the right side was the most common weak positive. **Conclusions:** Positive expression of PDL1 was found in 56% of the study sample. The positivity of the PDL1 score was reported mainly in males. PDL1 score with a histological variant of renal cell carcinoma showed a significant difference among the variants at score 3 with a high prevailing of clear cell carcinoma.

Keywords: Renal Cell Carcinoma, Programmed Cell Death Ligand, Immunohistochemistry.

Introduction

Renal cell carcinoma accounts for 75–80% of adult kidney cancers and 1-3% of all human malignancies.[1] Renal cell carcinoma (RCC) represents approximately 90% of all kidney cancer cases and is the most common solid kidney lesion.[2] In RCC patients, certain biochemical indicators are thought to be sensitive and specific for assessing prognosis.[3] The expression of programmed death-ligand 1 (PD-L1) on malignant cells is one method by which the immune system might evade tumorigenesis. When PD-L1 connects to its T cell receptor, PD-1, it deactivates antitumor T cells.[4] By preventing tumour cells from evading host T-cell responses, suppression of the PD-1/PD-L1 pathway enhances antitumor immunity and presents a novel strategy for tumour immunotherapy.[5] The present study was designed to evaluate the statement of PDL1 immunostain in renal cell carcinoma.

Materials and Methods

Study design: A case series research, both prospective and retrospective, was conducted on fifty primary renal cell cancer samples, which were collected from some teaching hospitals and private laboratories in Mosul City over 7 months extending from November 2022 through May 2023. Biopsy types are collected from patients undergoing the operation of either radical or partial nephrectomy, and then tissue sectioning is done for the histology and immunohistochemistry step.

Histology and immunohistochemistry: Formalin-fixed and paraffin-embedded (FFPE) blocks are used to prepare the slides, which are then stained with hematoxylin and eosin (H and E).[6-8] The slides are reviewed for various criteria such as histological type, grade, and pathological stage. Patient medical records provide additional information like age, sex, and laterality. PD-L1 immunohistochemical stain is conducted on FFPE

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tissues using a specific antibody to identify the PD-L1 protein. The staining and counterstaining procedures are completed using an automated method. PD-L1 expression is assessed based on the degree and percentage of tumour cells exhibiting staining. Different scores are assigned to indicate the level of immunoreactivity. The presence of membranous staining indicates PD-L1 positivity, while cytoplasmic staining alone is considered PD-L1-negative. The evaluation of PD-L1 staining is done at different magnifications to ensure an adequate number of viable tumour cells are present in the sample and to examine PD-L1 expression in different areas of the slide.

The degree and percentage of tumour cells exhibiting membranous or cytoplasmic staining were used to assess PD-L1 expression, and the results were graded as follows: The four immunoreactive states are 0, negative (no immunoreactivity), score 1 weak (5% to less than 25% of cells), score 2 moderate (between 25 and 60% of cells), and score 3 strong (greater than 60% of cells).[9]

Membrane staining is present in PD-L1 positive live tumour cells, regardless of staining intensity or full or partial PD-L1 positivity in the membrane. A tumour cell is considered to be PD-L1-negative if it shows cytoplasmic staining but not membrane staining.

The tumour regions on the slide were examined at lower magnifications (100x) to determine the sufficiency of live tumour cells. This was done by evaluating both PD-L1-staining and non-staining tumour cells to ensure that the sample had at least 100 viable tumour cells. PD-L1 expression in the tumour is examined at a greater magnification (400x), and each section of the slide is assessed separately.

Statistical analysis: IBM's Statistical Package for Society Study (SPSS) for Windows version 25 was used to analyze the data for this descriptive study. The frequency and corresponding percentage of the data were evaluated by the use of the Chi-square test. With a 95% confidence interval, a p-value of 0.05 or less was deemed statistically significant.

Results

PDL1 is found to be expressed in 28/50 (56%), and negatively expressed in (22/50; 44%) with a statistically significant difference (p=0.0001) (Table 1)

Table 1: Frequency of PDL1 Expression of Different PDL1 Score.

PDL Expression	PDL1 Score				*p-value*
	0 N=22	1 N=9	2 N=8	3 N=11	
-Ve	22	-	-	-	*0.0001*
+Ve	-	9	8	11	
P-value	-	-	-	-	

PDL1 expression with age: Patients with scores of 3 (57.36±11.71) and ages 46–74 years had the largest percentage of PD-L1-positive tumours. PD-L1 and age do not statistically significantly correlate (p=0.9) (Table 2).

PDL1 expression with gender: PDL1 expressed positively in males (score 3) with (7/11; 63.6%), and frequency in females (score 1) with (4/9; 44.4%) with no statistical differences PDL1 expression (p=0.8) according to gender (Table 2).

The frequency of scores in positive PDL1 expression with histological variant: has shown statistical significance differences between histopathology variant and strongly positive PDL1 expression in score 3 (p=0.02), with clear cell RCC was (8/11; 72.7%) strongly positive, papillary RCC was most common expression in score 2 (moderate) with 2/8 (25%), and chromophobe was most common in score 3 with (1/11; 9.1%) strongly positive (Table 2).

According to the relation of PDL1 score with stages of renal cell carcinoma: Stage 1 (6/7; 85.7%) cases were mostly moderately positive (score 2) and 3/7 (42.9%) cases in Stage 3 show weak positive. The PD-L1 score and stage have shown non-significant differences (p=0.4) (Table 2).

Table 2: Clinicopathological Parameter among Different PDL1 Score.

Parameters		PDL1 Score				p-value
		0	1	2	3	
Age, mean±SD		53.91±14.62	53.89±13.08	54.75±15.25	57.36±11.71	0.9
Gender, No. (%)	Male	15 (68.2%)	5 (55.6%)	6 (75.0%)	7 (63.6%	0.8
	Female	7 (31.8%)	4 (44.4%)	2 (25.0%)	4 (36.4%)	
	P-value	0.08	0.7	0.1	0.3	
Histopathology variant	Clear cell	16 (72.7%)	9 (100.0%)	6 (75.0%)	8 (72.7%)	0.6
	Papillary	4 (18.2%)	-	2 (25.0%)	2 (18.2%)	
	Chromophore	2 (9.1%)	-	-	1 (9.1%)	
	P-value	0.0001	-	0.1	0.02	
Stage, No. (%)	1	7 (35.0%)	4 (57.1%)	6 (85.7%)	5 (55.6%)	0.4
	2	2 (10.0%)	-	1 (14.3%)	1 (11.1%)	
	3	10 (50.0%)	3 (42.9%)	-	3 (33.3%)	
	4	1 (5.0%)	-	-	-	
	P-value	0.01	0.7	0.05	0.2	
Grade, No. (%)	1	1 (5.0%)	1 (12.5%)	1 (12.5%)	-	0.9
	2	10 (50.0%)	4 (50.0%)	4 (50.0%)	6 (54.5%)	
	3	6 (30.0%)	2 (25.0%)	2 (25.0%)	4 (36.4%)	
	4	3 (15.0%)	1 (12.5%)	1 (12.5%)	1 (9.1%)	
	P-value	0.02	0.3	0.3	0.1	
Laterality, No. (%)	Left	9 (42.9%)	4 (50.0%)	7 (100.0%)	9 (81.8%)	0.02
	Right	12 (57.1%)	4 (50.0%)	-	2 (18.2%)	
	P-value	0.5	1.0	-	0.03	

The relation between PDL1 scores and grade of renal cell carcinoma with 47 cases: Grade 2 and 3 were strongly positive expressions with (6/11; 54.5%) and (4/11; 36.4%) respectively. This refers to increased expression with grade although the p-value was statistically non-significant, and grade 4 showed equal positivity in different scores (Table 2). According to the laterality of RCC with PDL1 score: The left side was strongly positive in PDL1 expression at score 3 (9/11; 81.8%) while the right side was most common with weak positive PDL1 expression at score 1 (4/8; 50%). PDL1 expression was statistically significant with p-value 0.02 (Table 2).

Discussion

In the present study, PDL1 is found to be expressed in 28/50 (56 %), according to the previous study in Brazil and Egypt in which, PD-L1 expression was similar results to our study.[10,11] The research worked in Iran and Germany reported lower results in PDL1 expression than this study.[12,13] PDL1 expression reported in India and the USA shows in between results of previous studies.[14,15] This variability in the results of PDL1 expression in the mentioned studies is due to different sample sizes and the source of the use of antibodies.

In the current study, the highest proportion of PD-L1-positive tumours occurred in patients at the age of (46-74 years; 57.36±11.71) in score 3. Similar results were reported in Egypt, Iran, and India studies which showed higher expression of PDL1 in the fifth decade of life.[11,12,16]

Regarding the gender distribution according to PDL1 expression, the present study showed that the PD-L1 positivity in males more than in females mainly in score 3 with non-significant statistical difference. Correspondingly, PD-L1 positivity was not correlated with gender.[17,18] However, the most comprehensive meta-analysis by Lu *et al.*[19], reported that the PD-L1 over-expression was more prevalent in women in contrast to the current result.[19] On the other hand, PD-L1 positivity was not correlated with gender,

The immune staining for PD-L1 differed considerably amongst subtypes of renal carcinoma. Results of this study show statistical significance between histopathology variant and strongly positive PDL1 expression in score 3 with p- value 0.02, with clear cell RCC (72.7%) strongly positive, papillary RCC was most common expression in score 2 (moderate) with (25.0%), and chromophobe was most common in score 3 with (9.1%) strongly positive. According to research conducted by Chandrasekaran *et al.*[14], and Elkhodary *et al.*[11], have found that clear cell RCCs had greater PD-L1 positive rates than other renal tumour subtypes, none of the histological variants revealed a significant relationship between PDL1 expression or outcome. They may have been due to the small patient sample size and the variety of histological subtypes.[11,14] Thompson *et al.*[20] and Iacovelli *et al.*[21], found that RCCs with the clearest cell composition had PD-L1 expression, and tumours with higher levels of the protein had a worse prognosis.[20,21]

PD-L1 was expressed in clear cells RCC subtype and the earlier research claimed that PD-L1 was particularly related to occasional clear cell RCC. These findings lend credence to the idea that, despite hypoxia-inducible factor degradation brought on by the presence of an active VHL protein, alternative carcinogenic pathways exist in clear cell RCC and result in PD-L1 overexpression. Alternative pathways, such as the MAP kinase and PI3K-AKT-mTOR pathways implicated in clear cell RCC oncogenesis, may be used by tumours without VHL inactivation to bypass VHL safeguards. It has already been noted that these other mechanisms can increase PD-L1 expression.[21]

Tabriz *et al.*[12], Möller *et al.*[13], and Lee *et al.*[22], with research revealed that PD-L1 expression was greater in non-clear cell RCCs than in clear cell RCCs.[12,13,22]

Concerning the relation of PDL1 score with stages of renal cell carcinoma, in the present study, only stage 1 showed a significant association with moderate positive expression. One of the first to describe PD-L1 expression in RCC was Thompson *et al.*[23], PD-L1 expression was linked to aggressive characteristics such as greater TNM stage in one research of 196 patients, as well as an increased risk of cancer-specific death.[23]

Patients with RCC who exhibit PD-L1 in their tumours are more likely to have advanced tumour stages and less favourable clinical outcomes.[17] The PDL1 was positive commonly stage III followed by stage II and I.[16] Half of the patients held tumour stage 2.[24] Tabriz *et al.*[12], found that PDL-1 staining was not substantially correlated with tumor stage.[12] This discrepancy with the current study is due to bias in sample collection.

The more prominent the PD-L1 expression was seen, the higher the nuclear grade. This suggests that to more precisely anticipate the therapeutic impact of immune checkpoint inhibitors, PD-L1 expression may need to be evaluated in metastases.[14]

The current study revealed that grades 2 and 3 were strongly positive expressions although the association is statistically not significant. Similar studies have found, that PDL1 was positive commonly in grades 2 and 3 followed by stage 1.[16]

The differentiation grade of the investigated malignancies was substantially correlated with a gradual rise in PD-L1 positive.[25] However, some studies showed PDL1 positive tumor cells had higher nuclear grade yet all with no statistical significance.[11]

The research found that RCC-CC patients had PD-L1 expression and that positive expression was associated with a higher Fuhrman nuclear grade.[10]

In contrast, according to research by Xu *et al.*[26], patients with RCC who had higher levels of PD-L1 had larger tumours; were more advanced, and had lower nuclear grades.[26]

In the present study, left-side renal cell carcinoma was strongly positive in a statistically significant way. Contrary to other studies, no statistically significant association of PD-L1 with laterality of the renal cell carcinoma.[27,28]

Treatment and post-treatment complications could be as challenging as usual with another type of cancer.[29,30]

The limitations of the present study include a small sample

size, which affects the generalizability of the results. Being a retrospective study, the results biased limit the ability to draw causal inferences. Other biomarkers were neglected and the study was based on PDL1 immunostain expression in RCC which might be overlooked and other markers need to be included to characterize the type of cancer. The findings do not express other populations and lack external validity.

Conclusion

In a study sample, 56% of participants showed positive expression of PDL1. The positivity of the PDL1 score was predominantly observed in males. There was a significant difference in PDL1 scores among different histological variants of renal cell carcinoma, particularly in score 3, where clear cell carcinoma was most prevalent. The findings could improve the patient's care and decision in clinical settings, follow-up disease progression and response to therapy advancing the field of personalized medicine and minimizing overall adverse effects.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Barth DA, Slaby O, Klec C, Juracek J, Drula R, Calin GA, Pichler M. Current concepts of non-coding RNAs in the pathogenesis of non-clear cell renal cell carcinoma. Cancers. 2019; 11(10): 1580. doi: https://doi.org/10.3390/cancers11101580.
2. Ljungberg B, Albiges L, Abu-Ghanem Y, et al. European Association of Urology Guidelines on Renal Cell Carcinoma: The 2019 Update. Eur Urol. 2019; 75(5): 799-810. doi: https://doi.org/10.1016/j.eururo.2019.02.011.
3. Moradi Tabriz H, Nazar E, Ahmadi SA, Azimi E, Majidi F. Survivin and Her2 Expressions in Different Grades of Urothelial Neoplasms of Urinary Bladder. Iran J Pathol. 2021; 16(2): 154-61. doi: https://doi.org/10.30699/ijp.2020.130859.2447.
4. Jilaveanu LB, Shuch B, Zito CR, et al. PD-L1 Expression in Clear Cell Renal Cell Carcinoma: An Analysis of Nephrectomy and Sites of Metastases. J Cancer. 2014; 5(3): 166-72. doi: https://doi.org/10.7150/jca.8167.
5. Walter B, Gil S, Naizhen X, Kruhlak MJ, Linehan WM, Srinivasan R, Merino MJ. Determination of the Expression of PD-L1 in the Morphologic Spectrum of Renal Cell Carcinoma. J Cancer. 2020; 11(12): 3596-603. doi: https://doi.org/10.7150/jca.35738.
6. George N, Sasikala K. Histopathological Changes in Skin and Subcutaneous Tissues at Ligature Site in Cases of Death Due to Hanging and Strangulation. Journal of Indian Academy of Forensic Medicine. 2022; 44(1): 74-78. Available from: https://jiafm.in/index.php/jiafm/article/view/7.
7. Kadavkar SM, Bansude ME, Umbare RB, Dode CR. A Histopathological Study of Vital Organs in Deaths due to Burns. Journal of Indian Academy of Forensic Medicine. 2022; 44(2): 78-85. Available from: https://jiafm.in/index.php/jiafm/article/view/31.
8. Abdullah SI, Al-Bayti AAH, Salih MJ, Merkhan MM. Histological and Biochemical Changes Associated with Blocking of Serotonin Receptor. Tropical Journal of Natural Product Research. 2022; 6(8): 1189-92. doi: https://doi.org/10.26538/tjnpr/v6i8.4.
9. Chipollini J, da Costa WH, Werneck da Cunha I, et al. Prognostic value of PD-L1 expression for surgically treated localized renal cell carcinoma: implications for risk stratification and adjuvant therapies. Ther Adv Urol. 2019; 11: 1756287219882600. doi: https://doi.org/10.1177/1756287219882600.
10. 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: https://doi.org/10.1186/s13000-015-0414-x.
11. Elkhodary HS, Nasr KE, Ahmed SH, Shakweer MM, Ezz-Eldin MMA. Clinicopathological correlation and prognostic value of PD-L1 expression in renal cell carcinoma. Immunopathol Persa. 2022; 8(2): e30329. doi: https://doi.org/10.34172/ipp.2022.30329.
12. Tabriz HM, Nazar E, Akhlaghi N, Javadi AE. Expression of Programmed Death-1 Ligand in Renal Cell Carcinoma and Its Relationship with Pathologic Findings and Disease-Free Survival. Nephrourol Mon. 2022; 14(4): e127476. doi: https://doi.org/10.5812/numonthly-127476.
13. Möller K, Fraune C, Blessin NC, 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(12): 2493-503. doi: https://doi.org/10.1007/s11255-021-02841-7.
14. Chandrasekaran D, Sundaram S, N K, R P. Programmed Death Ligand 1; An Immunotarget for Renal Cell Carcinoma. Asian Pac J Cancer Prev. 2019; 20(10): 2951-57. doi: https://doi.org/10.31557/apjcp.2019.20.10.2951.
15. Joseph RW, Millis SZ, Carballido EM, et al. PD-1 and PD-L1 Expression in Renal Cell Carcinoma with Sarcomatoid Differentiation. Cancer Immunol Res. 2015; 3(12): 1303-07. doi: https://doi.org/10.1158/2326-6066.cir-15-0150.
16. Kumar B, Ghosh A, Datta C, Pal DK. Role of PDL1 as a prognostic marker in renal cell carcinoma: a prospective observational study in eastern India. Ther Adv Urol. 2019; 11: 1756287219868859. doi: https://doi.org/10.1177/1756287219868859.
17. Choueiri TK, Fay AP, Gray KP, et al. PD-L1 expression in nonclear-cell renal cell carcinoma. Ann Oncol. 2014; 25(11): 2178-84. doi: https://doi.org/10.1093/annonc/mdu445.
18. Zhao Y, Shi Z, Xie Y, Li N, Chen H, Jin M. The association between PD-1 / PD-L1 expression and clinicopathological features in sarcomatoid renal cell carcinoma. Asian J Surg. 2024; 47(1): 163-68. doi: https://doi.org/10.1016/j.asjsur.2023.06.065.

19. Lu Y, Song Y, Xu Y, et al. The prevalence and prognostic and clinicopathological value of PD-L1 and PD-L2 in renal cell carcinoma patients: a systematic review and meta-analysis involving 3,389 patients. Ther Adv Urol. 2020; 9(2): 367-81. doi: https://doi.org/10.21037/tau.2020.01.21.
20. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, Kwon ED. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res. 2007; 13(6): 1757-61. doi: https://doi.org/10.1158/1078-0432.ccr-06-2599.
21. Iacovelli R, Nolè F, Verri E, et al. Prognostic Role of PD-L1 Expression in Renal Cell Carcinoma. A Systematic Review and Meta-Analysis. Target Oncol. 2016; 11(2): 143-48. doi: https://doi.org/10.1007/s11523-015-0392-7.
22. Lee Z, Jegede OA, Haas NB, et al. Local Recurrence Following Resection of Intermediate-High Risk Nonmetastatic Renal Cell Carcinoma: An Anatomical Classification and Analysis of the ASSURE (ECOG-ACRIN E2805) Adjuvant Trial. J Urol. 2020; 203(4): 684-89. doi: https://doi.org/10.1097/ju.0000000000000588.
23. Thompson RH, Gillett MD, Cheville JC, et al. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A. 2004; 101(49): 17174-79. doi: https://doi.org/10.1073/pnas.0406351101.
24. Mohamed AH, Abdullahi IM, Eraslan A, Mohamud HA, Gur M. Epidemiological and Histopathological Characteristics of Renal Cell Carcinoma in Somalia. Cancer Manag Res. 2022; 14: 1837-44. doi: https://doi.org/10.2147/cmar.s361765.
25. Tziakou P, Theodoropoulos G, Tsiambas E, et al. Impact of PD-L1 Protein Expression on Renal Cell Carcinoma Histo-differentiation. Anticancer Res. 2021; 41(8): 3809-13. doi: https://doi.org/10.21873/anticanres.15173.
26. Xu F, Xu L, Wang Q, An G, Feng G, Liu F. Clinicopathological and prognostic value of programmed death ligand-1 (PD-L1) in renal cell carcinoma: a meta-analysis. Int J Clin Exp Med. 2015; 8(9): 14595-603. Available from: https://pubmed.ncbi.nlm.nih.gov/26628942.
27. Ning XH, Gong YQ, He SM, 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(2): 3355-63. doi: https://doi.org/10.18632/oncotarget.13765.
28. Chang K, Qu Y, Dai B, et al. PD-L1 expression in Xp11.2 translocation renal cell carcinoma: Indicator of tumor aggressiveness. Sci Rep. 2017; 7(1): 2074. doi: https://doi.org/10.1038/s41598-017-02005-7.
29. Zelić M, Petrović LM, Pavlović D. Swallowing disorders during and after the treatment of larynx cancer. Medicinski časopis. 2021; 55(1): 33-39. doi: https://doi.org/10.5937/mckg55-31139.
30. Merkhan MM, Faisal IM, Alsaleem DZ, Shindala OM, Almukhtar HM, Thanoon IA. Immunodepressant and oxidant potential of standard leukaemia drug regimen. International Journal of Research in Pharmaceutical Sciences. 2020; 11(4): 5608-14. doi: https://doi.org/10.26452/ijrps.v11i4.3199.