PD-L1 Expression in Bladder Cancer and Correlation with Tumor Grade, Stage and Outcome

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Abstract

Objectives: The aim of this study was to evaluate the expression of programmed death-ligand (PD-L1) in bladder cancer cases using immunohistochemistry, and to determine whether an association exists between the level of expression and tumor grade, stage, and outcome. This study also looked at the available clinicopathological features of bladder cancer among Omanis.

Methods: This was a retrospective cohort study of patients using archived samples that was subjected to prospective analysis. All patients diagnosed and treated for bladder cancer in Sultan Qaboos University Hospital (SQUH) from January 2006 to December 2017 and followed-up for at least one year were included. Clinical information including age, sex, any risk factors, and stage at diagnosis were obtained from the medical records. PD-L1 testing using immunohistochemistry was performed on formalin fixed paraffin embedded tissue blocks. Scoring of PD-L1 expression by tumor cells was done independently by two pathologists. Positivity was defined using two different cut-off values (≥ 5% and ≥ 25%) of tumor cells showing membrane or cytoplasmic staining. Outcome was divided into two categories either no recurrence at last follow-up or recurrence/disease progression/death.

Results: There was a total of 68 cases, 72.1% were males and 27.9% were females with an age range of 35 to 89 years (mean = 65.32 and median = 66). Majority were diagnosed with pT2 stage disease (38.8%) followed by pT1 stage disease (32.8%). Hematuria was the most common presentation (58.7%). Invasive urothelial carcinoma appeared in 79.4% of patients and 83.8% were of high grade. PD-L1 testing were performed on 63 cases, five were excluded due to non-availability of tissue blocks. PD-L1 was positive in 44% of cases using cut-off value of 5%, however it dropped to 30% using the cut-off value of 25%. Using a cut-off value of 5%, it was significantly associated with tumor grade (p = 0.033), but this was not significant using cut-off value of 25% (p = 0.250). No statistically significant association was found between PD-L1 expression and outcome using both cut-off values (p = 1.000 for both) and stage at diagnosis (p = 0.798 and p = 0.102).

Conclusion: This study showed that at a cut-off value of ≥ 5%, 44% of cases of bladder cancer were PD-L1 positive. There was a significant association between PD-L1 expression in bladder cancer and tumor grade. On the other hand, no statistically significant association was found between tumor stage and outcome. These results indicate potential benefit for patients of high tumor grade with anti-PD-L1 immunotherapy. In addition, this study highlights the clinicopathological features of bladder cancer among Omani patients and shows comparable results to such data worldwide. Expanding the sample size by including other health institutions in Oman will add more strength to this study.

Keywords: Bladder Cancer; PD-L1; PD-L1 Inhibitors.

Introduction

Bladder cancer is a complex disease associated with high morbidity and mortality rates if not treated optimally[1,2] . There are about 380000 new cases and 150000 deaths per year worldwide[1,3] . According to Cancer Incidence in Oman 2011, the incidence of bladder cancer per 100,000 per year is 2.7 and 0.8 for males and females respectively[4] . While generally advanced disease is best treated with systemic cisplatin-based chemotherapy, immunotherapy is emerging as a viable salvage treatment for patients in whom first-line chemotherapy cannot control the disease. This is done by using monoclonal antibodies (mAbs) targeting Programmed death (PD-1) or Programmed death-ligand 1(PD-L1) that can block the PD-1/PD-L1 pathway and activate T cells to do its functions[5] .

Programmed death-ligand 1 (PD-L1) is a transmembrane protein whose major role is to inhibit immune cells mainly activated T cells. PD-L1 is expressed in a wide range of human tissues including placenta, heart, pancreas, spleen, lymph node, and thymus, while it is absent in brain and kidney tissue[6] . There is a specific receptor that can be ligated by PD-L1 which called programmed death receptor 1 (PD-1) which is expressed on CD4+ and CD8+ T cells, monocytes, natural killer T cells, B cells, and dendritic cells [5] . In normal tissues, PD-1 signaling in T cells regulates immune responses to decrease damage to adjacent tissue and play a major role in the development of autoimmunity by inducing tolerance to self-antigens. Binding of PD-L1 to PD-1 leads to the inhibition of T cell activation through induction of apoptosis, reduction of proliferation, and inhibition of cytokine secretion[7] . PD-L1 is found on the surface of tumor cells and antigen presenting cells [5] . The mechanism of expression of PDL-1 on tumor cells is related to cancer immune-editing process which has three phases: elimination, equilibrium and escape. During the first phase which is elimination phase, cancer cells are recognized and destroyed by immune cells before they become detectable clinically. If tumor cells survive the elimination phase, they enter the equilibrium phase in which editing of tumor immunogenicity occurs and the outgrowth of the tumor cells is suppressed. However, a variant of tumor cells that are not recognized by the immune system may arise and therefore they enter the escape phase during which many changes on the surface antigens and production of immunosuppressive molecules and cytokines that are activated by tumor cells occur and result in clinically evident disease.(8)

PD-1/PD-L1 pathway targeting mAbs have been used in the treatment of different types of malignancies including melanoma, NSCLC, RCC, bladder cancer, CRC, and gastric cancer. The overall response rates achieved was 16%–100% .[7]

Atezolizumab which is Programmed death-ligand 1(PD-L1) inhibitor has been FDA-approved as second-line therapy for advanced bladder cancer.[9] Increased levels of PD-L1 expression on immune cells have been associated with increased response.[10,11] PDL-1 expression as detected by Immunohistochemistry (IHC) seems to be the best currently available biomarker and may be indicative of a dose–response relationship between PD-L1 expression and drug efficacy.[12] The detection of PD-L1 by IHC may be difficult at times because of tumor histology, and the staining can be either focal or diffuse. Hence assessment of PD-L1 and selection of the optimum site for biopsy for assessing PD-L1 expression status remains challenging.[5] PD-L1 overexpressed in bladder tumor cells has been linked to higher clinical stage and reduced disease-free survival rates, and a positive correlation between PD-L1 overexpression and cancers developing lymph node metastasis and loco-regional failure has been shown.[13] Testing for PD-L1 using immunohistochemistry will guide the selection of patients who will be treated by immunotherapy and it can be used to evaluate whether the patient will benefit from cancer immunotherapy or not to avoid unnecessary toxicity from this immunotherapy.[5]

The aim of this study was to evaluate the expression of programmed death-ligand (PD-L1) in bladder cancer cases by using immunohistochemistry, and to determine whether an association exists between the level of expression and tumor grade, stage, and outcome. This study also looked at the available clinicopathological features of bladder cancer among Omanis.

Methods

This was a retrospective cohort study of patients using archived samples that was subjected to prospective analysis. This study included all patients diagnosed and treated for bladder cancer in Sultan Qaboos University Hospital (SQUH) from the period between January 2006 to December 2017 with at least one-year follow-up. Patient with no sufficient follow up or those with no sufficient tissue block were excluded from PD-L1 testing. Patients list was obtained from the hospital information system (HIS) using these key wards : urothelial carcinoma, bladder cancer. Then a data collection sheet was used to collect data for each patient which includes age, sex, any risk factors, stage at diagnosis and outcome from hospital medical records. To protect and ensure patient privacy, each patient was given a unique serial number instead of using the patient hospital medical record number. The Outcome was divided into two categories either no recurrence at last follow up or recurrence/disease progression/death.

PD-L1 testing using immunohistochemistry was performed on formalin fixed paraffin embedded tissue blocks. H&E slides and tissue blocks of included cases were retrieved. Then they were reviewed, and different histological tumor areas of interest were marked. Tissue microarray sections were used to prepare the slides for PD-L1 testing. Testing was done using the following antibody (anti PD-L1 antibody, Product code : Ab205921, Supplier: Abcam plc, Positive control :placenta , tonsil).

Scoring of PD-L1 expression by tumor cells was done independently by two pathologists. For cases with discrepancy, double-head microscope discussion was done to reach consensus. Positivity was defined using two different cut-off values (≥ 5% and ≥ 25%) of tumor cells showing membrane or cytoplasmic staining. IBM SPSS statistics 22 program was used for result analysis. Chi-squared test is used to assess correlation

Ethical approval: Ethical approval was obtained from Medical research ethics committee (MREC), college of medicine & health sciences, Sultan Qaboos University.

Results

A total of 68 cases were included, 72.1% were males and 27.9% were females with an age range of 35 to 89 years (mean = 65.32 and median = 66). Hematuria was the most common presentation (58.7%). Other forms of presentations are incidental (22.20%), lower urinary tract symptoms other than hematuria (15.90%) and flank/pack pain (3.20%). Very limited information was available regarding bladder cancer risk factors. For instance,10.3% of patients were smoker but unfortunately no data available for the rest of included patient.

Majority of patients were diagnosed with pT2 stage disease (38.8%) followed by pT1 stage disease (32.8%) while minority were diagnosed with pT4 stage disease (4.50%). Rest of patients had either pTa (13.40%) or pT3 (10.40%) stage at diagnosis. Invasive urothelial carcinoma appeared in 79.4% of patients and 83.8% were of high grade. Apart from invasive urothelial carcinoma, the following morphology were identified in descending order: non-invasive urothelial carcinoma (13.2%), squamous cell carcinoma (5.90%) and one case with carcinosarcoma (1.50%). In addition, among those with invasive urothelial carcinoma, three patients had also coexisting prostatic adenocarcinoma, one had HNPCC associated rectal cancer and one with cervical cancer.

PD-L1 testing were performed on 63 cases, five were excluded due to non-availability of tissue blocks. PD-L1 was positive in 44% of cases, using cut-off value of 5%, however it dropped to 30% using the cut-off value of 25% (fig1). PD-L1 expression is significantly associated with the tumor grade (at ≥5%) p=0.033 while it is insignificantly associated with tumor grade at ≥25% p=0.25 (table1). PD-L1 were not perform on cases with bladder SCC as those cases were excluded due to unavailable tissue.

In addition, no statistically significant association was found between PD-L1 expression and outcome using both cut off values (p value=1.000 for both) and stage at diagnosis (p values 0.798 and 0.102).

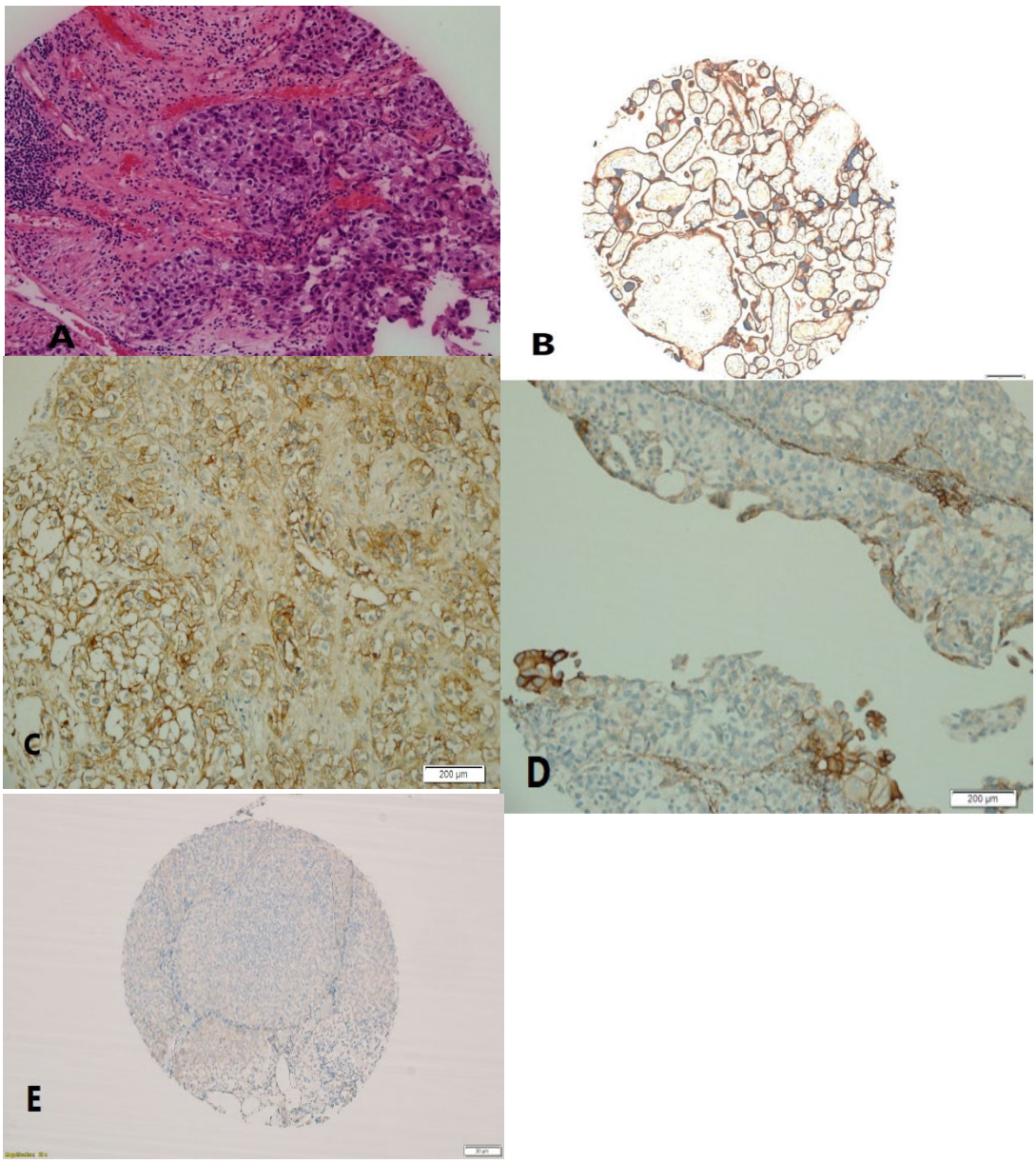

Figure 1: A, Tissue microarray section of urothelial carcinoma. B, PD-L1 control (placental tissue).C, PDL1 expression in tumor cells showing cytoplasmic/membranous positivity in 100% of tumor cells while it is 20% in D. E, Complete negativity for PD-L1 in tumor cells.

Table 1: PD-L1 Positive cases in high grade tumors.

	PD-L1 expression ≥5%	PD-L1 expression ≥25%
Positive	27 (49.1%)	18 (34%)
Negative	26 (50.9%)	35 (66%)
Total	53 (100%)	53 (100%)

Discussion

This study shows that the majority of the patient with bladder cancer were male and the most common clinical presentation is hematuria. These results were comparable to other studies done in Yemen [12] Pakistan[13] and other places across the world. Also noted that majority of patient were first diagnosed with p T2 stage followed by p T1 stage while a minority were diagnosed with p T4 stage Moreover, This study showed a significant association between PD-L1 expression and tumor grade as most of tumors that express PD-L1 are of high grade. The same results were also observed in variable studies[14-16]. The association between expression of PD-L1 and both tumor pathological T stage and outcome was also investigated in this study. While a positive trend was observed between the expression of PD-L1 on tumor cells and both high pathological T stage and worse outcome, there was no statically significant association found, which may be explained by the small sample size. On the other hand, several studies have reported a significant association between higher expression of PD-L1 and worse outcome[15-17]. A meta-analysis, including 27 studies and 4032 patients concluded that PD-L1 expression in bladder cancer tumor cells is associated with muscle invasion and shortening of the overall survival[15]. In other words, PD-L1 expression on tumor cells can be used as an indicator for tumor aggressiveness and hence may predict the usefulness of immunotherapy[16] . It is thought that the lack of large sample size in this study as well as the late presentation with advanced disease may have contributed to the lack of significant results as will be discussed shortly.

The status of PD-L1 can be used to direct the use of PD-L1 Inhibitors as tumors with increased expression are more likely to respond to anti-PD-L1 therapy [14-16] . The first PD-L1 inhibitor found to be active in bladder cancer was Atezolizumab that was approved by food and drug administration in 2016. It was approved for locally advanced or metastatic urothelial carcinoma (15-17). In 2017, other PD-L1 inhibitors were also approved such as Pembrolizumb, Nivolumab and Durvalumb (16). Findings from a study that evaluate PD-L1 expression in tumor cells from bladder cancer as well as metastatic site showed homogenous positivity which suggest that testing of PD-L1 can be done from either site as they have similar biological behavior [15].

This study had several limitation and challenges. The number of cases was limited and some of the patient were treated outside Oman for which proper documentation and material were not available. Consequently, this would have affected the process of statistical analysis and made it not possible to draw conclusions based on limited number of patients. Moreover, there are different PD-L1 antibodies in the market with variable staining platform, scoring guidelines and positivity definition which make it some time harder to compere results with other studies[18]. In this study, as explained in the method section, positivity was defined using two different cut-off values (≥ 5% and ≥ 25%) of tumor cells showing membrane or cytoplasmic staining[19]. However, this is the first study that investigate PD-L1 expression in bladder cancer among Omanis which represent a strength to it.

For future, this study can be expanded to include bladder cancer patients from other institutions within Oman. Also, additional task of looking at PD-L1 expression in tumor infiltrating immune cells (ICs) along with tumor cells is to be considered because available data is suggesting that PD-L1 expression in ICs is a predictor for better overall survival even without PD-L1 inhibitor treatment [15,20].Overall, PD-L1 expression in bladder cancer

is a developing area with many uncertainties. Standardization of the testing and result scoring will make understanding it easier and more effective.

Conclusion

This study showed that 44% of bladder cancer cases were PD-L1 positive. There was a significant association between PD-L1 expression in bladder cancer and tumor grade. On the other hand, no statistically significant association was found between tumor stage and outcome. These results indicate potential benefit for patients of high tumor grade with anti-PD-L1 immunotherapy. In addition, this study highlights the clinicopathological features of bladder cancer among Omani patients and shows comparable results to such data worldwide.

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References

1. Kim J, Akbani R, Creighton CJ, Lerner SP, Weinstein JN, Getz G, et al. Invasive Bladder Cancer: Genomic Insights and Therapeutic Promise. Clin Cancer Res Off J Am Assoc Cancer Res. 2015 Oct 15;21(20):4514–24.

2. Ashish M, Noah M, Jason A, et al. Bladder cancer. The Lancet, Volume 388, Issue 10061, 3–9 December 2016, Page 2742. https://doi.org/10.1016/S0140-6736(16)30512-8.

3. The Cancer Genome Atlas research network. Comprehensive molecular characterization of urothelial bladder carcinoma , Nature 507,315–322 (20 March 2014). doi:10.1038/nature12965.

4. Cancer incidence in Oman [Internet]. [cited 2017 April 20]. Available from: http:/www.moh.gov.om.

5. Wang, X., Teng, F., Kong, L. and Yu, J. (2016) 'PD-L1 expression in human cancers and its association with clinical outcomes', OncoTargets and Therapy, 9, pp. 5023–5039. doi: 10.2147/OTT.S105862.

6. Blank, C., Gajewski, Æ. T. F. and Tumor, P. Æ. T. Æ. (2005) 'Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion : implications for tumor immunotherapy', 1, pp. 307–314. doi: 10.1007/s00262-004-0593-x.

7. Manuscript, A. (2016) 'NIH Public Access', 21(1), pp. 24–33. doi: 10.1016/j.molmed.2014.10.009.Human.

8. Mittal, D., Gubin, M. M., Schreiber, R. D. and Smyth, M. J. (2015) 'HHS Public Access', pp. 16–25. doi: 10.1016/j.coi.2014.01.004.New.

9. Inman BA, Longo TA, Ramalingam S, Harrison MR. Atezolizumab: a PD-L1 blocking antibody for bladder cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2016 Nov 30.

10. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. The Lancet. 2016 May 13;387(10031):1909–20.

11. J. Bellmunt, T. Powles, N J. Vogelzang. A review on the evolution of PD-1/PD-L1 immunotherapy for bladder cancer: The future is now. March 2017 Volume 54, Pages 58–67 http://dx.doi.org/10.1016/j.ctrv.2017.01.007.

12. Al-Samawi AS, Aulaqi SM. Urinary bladder cancer in yemen. Oman Med J. 2013; 28(5): 337-40[DOI][PubMed]

13. aved Altaf et al. (2017), Clinicopathological features of Bladder Tumors in a Single Institution in Hyderabad, Sindh, Pakistan. Int J clinical & case studies. 1:1, 22-29. DOI: 10.25141/2472-102X-2017-1.0022

14. Kawahara, T., Ishiguro, Y., Ohtake, S., kato, I., Ito, Y., Itom H., et al. PD-1 and PD-L1 are more highly expressed in high-grade bladder cancer than in low-grade cases: PD-L1 might function as a mediator of stage progression in bladder cancer. BMC Urol 18, 97 (2018). https://doi.org/10.1186/s12894-018-0414-8

15. Ding, X., Chen, Q., Yang, Z., Li, J., Zhan, H., Lu, N., Chen, M., Yang, Y., Wang, J., & Yang, D. (2019). Clinicopathological and prognostic value of PD-L1 in urothelial carcinoma: a meta-analysis. Cancer management and research, 11, 4171–4184. https://doi.org/10.2147/CMAR.S176937

16. Seranio N, Malkowicz SB, Aguarin L, Dorsey JF, Christodouleas J, et al. (2019) Predicting bladder cancer responses to PD-L1 inhibitors? A case report and overview for the busy clinician. J Transl Sci 5: DOI: 10.15761/JTS.1000324.

17. Joaquin Bellmunt, Thomas Powles, Nicholas J. Vogelzang, A review on the evolution of PD1/PD-L1 immunotherapy for bladder cancer: The future is now,Cancer Treatment Reviews, Volume 54,2017, Pages 58-67, ISSN 0305-7372,https://doi.org/10.1016/j.ctrv.2017.01.007.

18. Eckstein, M., Cimadamore, A., Hartmann, A., Lopez-Beltran, A., Cheng, L., Scarpelli, M., Montironi, R. and Gevaert, T., 2019. PD-L1 assessment in urothelial carcinoma: a practical approach. Annals of Translational Medicine, 7(22), pp.690-690.

19. Ribas A, Hu-Lieskovan S. What does PD-L1 positive or negative mean?. J Exp Med. 2016;213(13):2835-2840.

20. J. Bellmunt, S.A. Mullane, L. Werner, F.S. Hodi, G.J. Freeman, S. Signoretti, et al. Association of PD-L1 expression on tumor-infiltrating mononuclear cells and overall survival in patients with urothelial carcinoma. European Society for Medical Oncology. Published by Elsevier Inc., April 2015. Volume 26. Issue 4, pages 613-825. https://doi.org/10.1093/annonc/mdv009.