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Programmed Death-ligand 1 (PD-L1) Expression in Bladder Cancer and its Correlation with Tumor Grade, Stage, and Outcome

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ABSTRACT

Objectives: To evaluate the expression of programmed death-ligand 1 (PD-L1) in bladder cancer cases in Oman using immunohistochemistry, and to determine whether the level of PD-L1 expression is associated with tumor grade, stage, or outcome. An additional objective was to identify the clinicopathological features of bladder cancer among Omanis. ***Methods***: This was a retrospective cohort study of patients where we subjected archived tissue samples to prospective analysis. All patients diagnosed and treated for bladder cancer in Sultan Qaboos University Hospital from January 2006 to December 2017 and followed up for at least one year were included. Clinical and demographical information of the patients was obtained from their 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 conducted independently by two pathologists. Positivity was defined using two different cut-off values (≥ 5% and ≥ 25%) of tumor cells showing membrane or cytoplasmic staining. The outcome was divided into two categories either no recurrence at the last follow-up, or recurrence/disease progression/death. ***Results:*** There were 68 cases of bladder cancer; 72.1% were male; the age range was 35–89 years (mean = 65.3 and median = 66). The largest number of patients were diagnosed with stage II cancer (38.8%) followed by stage I cancer (32.8%). Hematuria was the most common presentation (58.7%). High-grade tumors were seen in 83.8% (57/68) of patients. Invasive urothelial carcinoma appeared in 79.4% (54/68). PD-L1 tests were performed on 63 cases where tissue blocks were available. PD-L1 was positive in 44.4% of cases using a cut-off value of 5%; however, it dropped to 30.2% at a cut-off value of 25%. At the cut-off value of 5%, PD-L1 was significantly associated with tumor grade (p = 0.033), but the significance was lost when the cut-off value of 25% was applied (p = 0.250). No significant association was found between PD-L1 expression and outcome using both cut-off values and stage at diagnosis (p = 0.798 and p = 0.102, respectively). ***Conclusions:*** This study showed that at a cut-off value of ≥ 5%, 44.4% of cases of bladder cancer were PD-L1 positive. There was a significant association between PD-L1 expression in bladder cancer and tumor grade. No statistically significant association was found between tumor stage and outcome. The results indicated the potential benefit of anti-PD-L1 immunotherapy for patients with high tumor grades.

Bladder cancer is a complex disease associated with high morbidity and mortality if not treated optimally.[1,2] There are about 380 000 new cases and 150 000 deaths per year worldwide.[1,3] As of 2011, the yearly incidence of bladder cancer in Oman stood at 2.2 per 100 000 for males and 0.8 for females.[4] While the current recommended treatment for advanced bladder cancer is systemic cisplatin-based chemotherapy, immunotherapy is emerging as a viable salvage treatment where first-line chemotherapy failed to control the disease. Immunotherapy uses monoclonal antibodies targeting programmed death 1 (PD-1) or programmed death-ligand 1 (PD-L1) to block the PD-1/PD-L1 pathway, freeing T cells to perform their immunological functions.[5]

PD-L1 is a transmembrane protein whose main 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,

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lymph node, and thymus, while it is absent in the brain and kidneys.[6] There is a specific receptor that can be ligated by PD-L1 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 plays 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 the cancer immune-editing process which has three phases: elimination, equilibrium, and escape. During the first phase (elimination), cancer cells are recognized and destroyed by immune cells before they become detectable clinically. Tumor cells that survive the elimination phase enter the second phase (equilibrium) where adaptive immunity edits tumor cell immunogenicity, suppressing the outgrowth of the tumor cells. In rare cases, variant tumor cells capable of evading the immune system may arise and enter the third phase (escape). They proceed to suppress more immune responses by modifying the surface antigens and producing immunosuppressive molecules and cytokines, resulting in clinically evident cancer.[8]

PD-1/PD-L1 pathway targeting monoclonal antibodies have been used in the treatment of different types of malignancies including melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, colorectal cancer, and gastric cancer. The overall response rates achieved were in the range of 16%–100%.[7]

Atezolizumab, a PD-L1 inhibitor, was approved in 2016 by the US Food and Drug Administration as a second-line therapy for several late-stage cancers including bladder cancer.[9] Increased levels of PD-L1 expression on immune cells have been associated with increased response to treatment.[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] Detecting PD-L1 by IHC is difficult at times because in histological examination the staining can be either focal or diffuse. Hence, selecting an optimum site for biopsy to assess PD-L1 expression status remains challenging.[5] Overexpressed PD-L1 in bladder tumor cells has been linked to higher clinical stage cancer and reduced disease-free survival rates, and a positive correlation has been shown between PD-L1 overexpression and cancers developing lymph node metastasis and loco-regional failure.[13] Testing for PD-L1 using IHC helps identify patients who are most likely to benefit from immunotherapy and avoid introducing unnecessary toxicity in others.[5]

The aim of this study was to evaluate the expression of PD-L1 in bladder cancer cases by using IHC, and to investigate a possible association between the level of PD-L1 expression and tumor grade, stage, or outcome. We also sought to bring out the clinicopathological features of bladder cancer among Omani patients.

METHODS

In this retrospective cohort study of bladder cancer patients, we used archived tissue samples for prospective analysis. The study included all patients diagnosed and treated for bladder cancer in Sultan Qaboos University Hospital during the period January 2006 – December 2017 with at least one-year follow-up. Cases without sufficient follow-up or sufficient tissue block were excluded from PD-L1 testing. The patients' list was retrieved from the hospital information system using the keywords 'urothelial carcinoma' and 'bladder cancer.' A data collection sheet was used to record patient data such as age, sex, risk factors, stage at diagnosis, and outcome. To protect privacy, each case was given a unique serial number instead of the hospital file number. The cases were divided into two categories based on the outcome: (a) no recurrence at last follow-up and (b) recurrence/disease progression/death.

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

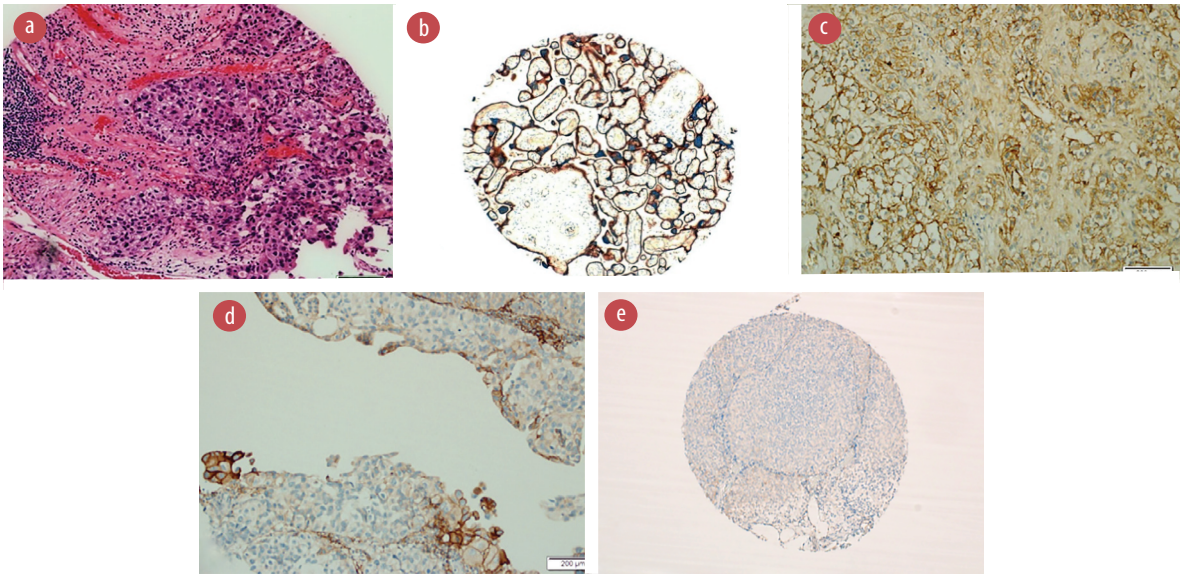

Figure 1: (a) Tissue microarray section of urothelial carcinoma, **(b)** programmed death-ligand 1 (PD-L1) control (placental tissue), **(c)** PD-L1 expression in tumor cells showing cytoplasmic/membranous positivity in 100% of tumor cells, **(d)** 20% PD-L1 expression, and **(e)** complete negativity for PD-L1 in tumor cells.

The PD-L1 expression of tumor cells was scored independently by two pathologists. For cases with discrepancies, the consensus was reached using a double-head microscope discussion. Positivity was defined using two different cut-off values (≥ 5% and ≥ 25%) for tumor cells showing membrane or cytoplasmic staining. SPSS statistics software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used for statistical analysis. Chi-squared test was used to assess correlation.

Ethical approval was obtained from the medical research ethics committee of the College of Medicine and Health Sciences, Sultan Qaboos University (Ref. No. SQU-EC/107/17 MREC#1502).

RESULTS

A total of 68 cases were included, 72.1% were male and 27.9% were female, with an age range of 35–89 years (mean = 65.3 and median = 66). Hematuria was the most common presentation (58.7%). Other forms of presentations were incidental (22.2%), lower urinary tract symptoms other than hematuria (15.9%), and flank/back pain (3.2%). Regarding risk factors for bladder cancer, the only available data was that 10.3% of the patients were smokers.

At the time of diagnosis, 38.8% of the patients were at cancer stage 2 (pT2), followed by 32.8% at pT1, 10.4% at pT3, and 4.5% at pT4 stages. The remaining patients (13.4%) were diagnosed at pTa stage. High-grade tumors were present in 57/68 (83.8%) of patients, while 54/68 (79.4%) had invasive urothelial carcinoma. Cases of non-invasive urothelial carcinoma (13.2%), squamous cell carcinoma (5.90%), and carcinosarcoma (1.50%) were identified. Among those with invasive urothelial carcinoma, three patients had coexisting prostatic adenocarcinoma, one had hereditary nonpolyposis colorectal cancer, and one had cervical cancer.

PD-L1 testing was performed on 63/68 (92.6%) cases; five were excluded due to non-availability of tissue blocks. PD-L1 was positive in 28/63 (44.4%) of cases, using a cut-off value of 5%; however, PD-L1 positivity dropped to 19/63 (30.2%) when the cut-off value was raised to 25% [Figure 1]. PD-L1 expression was significantly associated with the tumor grade of ≥ 5% ($p = 0.033$) and insignificantly associated with tumor grade at ≥ 25% ($p = 0.250$)

Table 1: PD-L1 positive cases among patients with high grade bladder cancer tumors (n = 53).

PD-L1 expression using different cut-off values	PD-L1 expression ≥ 5%	PD-L1 expression ≥ 25%
Positive	27 (50.9)	18 (34.0)
Negative	26 (49.1)	35 (66.0)
Total	53 (100)	53 (100)

PD-L1: programmed death-ligand 1.

[Table1]. No significant association was found between PD-L1 expression and outcome using both cut off values (p = 1.000 for both) and stages at diagnosis (p = 0.798 and p = 0.102, respectively).

DISCUSSION

In this study, the majority of bladder cancer patients were male (72.1%). The most common clinical presentation was hematuria. These results were comparable to those from studies conducted elsewhere, such as in Yemen and Pakistan.[12,13] At the time of diagnosis, 38.8% of the patients were at pT2, 32.8% at pT1, 10.4% at pT3, and 4.5% at pT4 cancer stages. We also found a significant association between PD-L1 expression and tumor grade, as most tumors that expressed PD-L1 were of high grade, similar to the results of other studies.[14–16] The associations between expression of PD-L1 and tumor pathological T stage, as well as outcome, were also investigated. Though positive associations were observed between the expression of PD-L1 on tumor cells and high pathological T stage and worse outcomes, these were not significant. This is contrary to several studies which reported significant associations between higher expression of PD-L1 and worse outcomes.[11,15,16] A meta-analysis that included 27 studies and 4032 bladder cancer patients found PD-L1 expression in bladder cancer tumor cells to be associated with muscle invasion and shorter overall survival.[15] In other words, PD-L1 expression on tumor cells can serve as an indicator of tumor aggressiveness and hence may predict the usefulness of immunotherapy.[16] It is likely that the small sample size in our study contributed to the lack of significance in this respect.

Knowing the status of PD-L1 can help direct the use of PD-L1 inhibitors because tumors with increased expression are more likely to respond to anti-PD-L1 therapy.[14–16] The first PD-L1 inhibitor approved by Food and Drug Administration in 2016 was atezolizumab, effective against both locally advanced and metastatic urothelial carcinoma.[11,15,16] In 2017, more PD-L1 inhibitors such as pembrolizumab, nivolumab, and durvalumab were approved.[16] A study that evaluated PD-L1 expression in tumor cells in the bladder as well as metastatic site showed homogenous positivity, suggesting that PD-L1 could be tested using samples from either site due to their similar biological behavior.[15]

Our study has several limitations. The number of cases was limited. Some of the patients had also been treated outside Oman for whom proper documentation and material were not available. Partial data in some cases made it difficult to draw reliable statistical conclusions. Moreover, there are different PD-L1 antibodies in the market with variable staining platforms, scoring guidelines, and positivity definitions, making it harder to compare our results with other studies.[17] 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.[18] In spite of the limitations, this study has the status of being the first one to investigate the PD-L1 expression in bladder cancer among Omanis.

Future studies need to include bladder cancer patients from other institutions within Oman, as well as from ethnically similar populations in the neighboring countries. In addition, PD-L1 expression infiltrating immune cells warrants deeper investigation because the available data suggests that PD-L1 expression in infiltrating immune cells is a predictor for better overall survival even without PD-L1 inhibitor treatment.[15,19] Standardizations of testing and result scoring are also warranted to render the results more understandable and practical for clinicians.

CONCLUSION

This study showed that 44.4% of bladder cancer cases studied were PD-L1 positive. There was a significant association between PD-L1 expression and tumor grade. No significant association was found between tumor stage and outcome. The results indicate that anti-PD-L1 immunotherapy may be beneficial for patients with higher-grade tumors. In addition, this study brings out the clinicopathological features of bladder cancer among Omani patients.

Disclosure
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