

## RESEARCH ARTICLE

## The value of PD-L1 immunohistochemical expression in Egyptian urinary bladder carcinoma patients

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### Abstract

**Objective:** To evaluate programmed death-ligand 1 immunohistochemical expression in the available variants of urinary bladder carcinoma, and to correlate its expression with the available clinicopathological features.

**Method:** The retrospective study was conducted at the Faculty of Medicine, Kafrelsheikh University, Egypt, from February 2020 to April 2021, and comprised formalin-fixed and paraffin-embedded specimens of urinary bladder carcinoma belonging to patients who had no history of radiotherapy or chemotherapy. Immunohistochemical staining of all cases was done using anti-programmed death-ligand 1 antibody. Data was analysed using SPSS 20.

**Results:** Of the 70 specimens, 58(82.86%) had been obtained through transurethral resection of bladder tumours and 12(17.14%) through radical cystectomy. Also, 53(75.7%) specimens belonged to males and 27(24.3%) to females. The age of the cases ranged 34-83 years, and 59(84.3%) were aged  $\geq 45$  years. There were 27(38.6%) noninvasive bladder tumours and 43(61.4%) were infiltrating bladder carcinomas. Positive programmed death-ligand 1 expression was detected in 42(60%) cases. Age, gender and histopathological type were not significantly associated with the expression of programmed death-ligand 1.

**Conclusions:** Programmed death-ligand 1 could be considered a predictive marker for aggressive bladder carcinoma and its immunohistochemical expression may aid in identifying selective patients for targeted immunotherapy.

**Keywords:** Carcinoma, Cell, Urinary bladder, Neoplasms, Antigen, Receptor, Immunotherapy, Muscles.

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### Introduction

Nearly 430,000 new cases of urinary bladder carcinoma (UBC), one of the most malignant neoplasms in the world, are reported each year.<sup>1</sup> UBC is the 5th most prevalent cancer globally, with a significant illness and death rate among those affected.<sup>2</sup> In Egypt, bladder cancer ranks third among all malignancies, with a prevalence of 90.71% among tumours of the urinary system.<sup>3</sup> The most frequent type of urinary tract cancer, UBC is classified as either muscle-invasive (MI) or non-muscle-invasive (NMI), and it has a varying spreading potential.<sup>4</sup>

The pathological stages pTa and pT1, where the tumours are contained to the urothelium and the lamina propria, respectively, are regarded as NMI, whereas pT2, pT3 and pT4, which invade the muscle, are regarded as MI in the context of UBC. Tumour staging in this context refers to the extent of bladder wall invasion. Decisions about the best course of action are influenced by the prognostic, predictive and therapeutic implications of various molecular subtypes. However, the majority of patients first experience the less severe NMI, which makes up about 70%

of UBCs, having 5-year recurrence-free overall survival (OS) of about 40% for minimal risk, and 30% for intermediate risk, but as severe as 20% for high-risk MI progression.<sup>5</sup>

About 30% of the patients have MI disease when they first arrive, which indicates a worse prognosis because of its potential for metastatic spread. While 75% of newly-diagnosed MIs do not metastasise, if they do, they become clinically aggressive and have a significant death rate. In contrast, OS rate for all stages at five years for UBC is still around 80%.<sup>6</sup>

First-line therapy for metastatic UBCs has historically relied on cisplatin-based combinations. Unfortunately, despite the initial response linked with cisplatin-based combinations, almost all patients eventually advance and pass away from bladder cancer. Immune checkpoint inhibitors are a common treatment choice for many solid cancers nowadays.<sup>7</sup> Recurrences happen frequently despite the best treatment. The absence of well-characterised representative preclinical model systems poses presents significant obstacles to the advancement of targeted therapies as well as critical limits in the understanding of bladder cancer progression. Complex tumour heterogeneity has been revealed by genetic profiling, and this heterogeneity likely causes variation in the therapy response.<sup>8</sup>

A key immune checkpoint molecule, programmed death-ligand 1 (PD-L1), also known as B7 homolog 1 (B7-H1) or

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cluster of differentiation 274 (CD274), is expressed on the surface of many carcinoma cells, including lung, colon, melanoma and leukaemic cells.<sup>9</sup> In UBC, advanced and aggressive malignancies with poor prognoses for survival have been linked with high levels of PD-L1 overexpression. In numerous cancer types, inhibiting the interaction between programmed cell death protein 1 (PD-1) and PD-L1 produced positive therapeutic outcomes. The identification of patients who gain from PD-1/PD-L1-targeted immunotherapy is still a crucial clinical issue.<sup>10</sup>

The current study was planned to evaluate PD-L1 immunohistochemical (IHC) expression in the available UBC variants, and to correlate its expression with the available clinicopathological features.

## Materials and Methods

The retrospective study was conducted at the Faculty of Medicine, Kafrelsheikh University, Egypt, from February 2020 to April 2021, and comprised formalin-fixed and paraffin-embedded (FFPE) specimens of UBC belonging to patients who had no history of radiotherapy or chemotherapy. The specimens had been obtained by through transurethral resection of bladder tumours (TURBT) or through radical cystectomy. Informed consent had been obtained at the time from all the patients. The specimens were retrieved from the database of the Pathology Department and some private laboratories after approval from institutional ethics review committee. Specimens of patients who had previously received chemotherapy or radiotherapy were excluded and so were those with insufficient tissue for immunostaining or poor quality of the blocks. Collection of clinic-pathological data of the patients, like age, gender and clinical presentation, was done from patient files containing surgery and oncology reports.

Histopathological examination of each case under the light microscope was done for the confirmation of the diagnosis and classification of histopathological types according to the World Health Organisation (WHO) 2016 classification of urinary tract tumours.<sup>11</sup> Other histological characteristics, like necrosis, mitosis and lymph-vascular invasion (LVI) were assessed. NMI UBCs of pTa and pT1 subtypes (primary tumour size of pathologic stage) were classified as low-grade (LG) and high-grade (HG) tumours, while all MI UBCs were classified as HG tumours. Staging of tumour was done in line with the American Joint Committee on Cancer (AJCC) system.<sup>12</sup>

Sections 3 micron-thick of the relevant block, cut from the typical haematoxylin and eosin (H&E)-stained section for each case, were then placed on positively-charged coated slides. As indicated by an earlier study, streptavidin-biotin IHC staining was carried out.<sup>13</sup> Endogenous peroxidase

activity was blocked with 0.3% hydrogen peroxidase after tissue sections were deparaffinized in xylene and rehydrated in an alcohol gradient. Slides were then incubated with the primary antibody (PD-L1[ZR3, the catalogue code number of the clone, which is used in research, named by manufacturing company] rabbit monoclonal antibody [438R-28], 7.0 ml pre-diluted; Cell Marque, Sigma Aldrich Co., USA) for an overnight period. at 8°C. Primary antibodies were removed the following morning using phosphate-buffered saline (PBS). PD-L1 IHC positivity was considered by the presence of cytoplasmic and/or membranous immunostaining with a cut-off value  $\geq 5\%$  of tumour cells.<sup>14</sup> Semi-quantitative scoring was applied, depending on the proportion of tumour cells stained favourably, and independent of staining intensity.<sup>15,16</sup> Tumour proportion score (TPS) represented the estimated fraction of positively stained tumour cells, thus: +1 = 5-25%; +2 = 26-50%; and +3 = >50% of positively stained tumour cells.

Data was analysed using SPSS 20. Frequencies and percentages were used to describe qualitative data. Quantitative data was expressed as mean and standard deviation or median and interquartile range (IQR), as appropriate. Fisher's exact or Monte Carlo adjustment for chi-square was used when >20% of the cells had an anticipated count of <5 Chi-square test was used to compare categorical variables across the groups.  $P < 0.05$  was considered statistically significant.

## Results

Of the 70 specimens, 58(82.86%) had been obtained through TURBT and 12(17.14%) through radical cystectomy. Also, 53(75.7%) specimens belonged to males and 27(24.3%) to females. The age of the cases ranged 34-83 years, and 59(84.3%) were aged  $\geq 45$  years.

According to the histopathological examination of H&E-stained slides, there were 27(38.6%) NMI UBCs and 43(61.4%) had MI tumours. Among the NMI cases, there were 2(2.8%) cases of papillary urothelial neoplasm of low malignant potential (PUNLMP), 17(24.3%) cases of LG papillary (LGP) urothelial carcinomas (UCs), and 8(11.4%) cases of HG papillary (HGP) UCs. Among the MI UBCs, the predominant histological type was conventional UCs 20(28.6%) cases, followed by UC with divergent differentiation 9(12.9%) cases, including UCs with squamous differentiation (UCSDs) 6(8.5%), UCs with glandular differentiation (UCGDs) 2(2.8%) and 1(1.4%) case of UC with mucinous differentiation (UCMD). Other MI variants accounted for 8(11.4%) cases, while 4(5.7%) cases were diagnosed as pure squamous cell carcinomas (SCCs), and 2(2.8%) as pure glandular adenocarcinomas (ADCs).

Regarding the grading of the studied UBCs, 30(42.9%) cases had LG carcinoma, while 40(57.1%) had HG carcinoma. NMI cases were 33(47.2%), while 37(52.8%) were MI. There were 12(%) cystectomy specimens; 2(16.6%) pT2a, 4(33.3%) pT2b, 2(16.6%) (pT3a), 3(24.9%) pT3b, and 1(8.3%) pT4a. None of the studied cases showed nodal metastases. Only 7(10%) cases showed perineural invasion and 8(11.4%) showed LVI.

PD-L1 expression showed positivity in 42(60%) cases; 10(14%) showed +1 expression, 18(26%) +2, and 14(20%) cases showed +3 expression (Figures 1-2). The mean age of

PD-L1-positive cases was 59.40±12.50 years (range: 34-83 years). Among them, 30(71.4%) were males and 12(28.6%) were females. There was no significant relationship of age ( $p > 0.05$ ) and gender ( $p > 0.05$ ) with PD-L1 expression.

Regarding the histopathological types of the cases, PD-L1 overexpression was detected in conventional MI UCs (14/20; 70%), UCSD) (5/6; 83%), and other uncommon variants, like micropapillary, undifferentiated, and UC with signet ring cells (7/8; 87.5%). However, the difference in PD-L1 expression in different histopathological types was statistically non-significant ( $p > 0.05$ ).

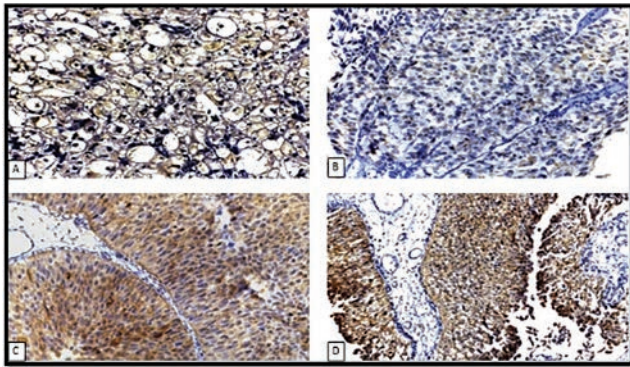
**Table:** Relation between PD-L1 expression and clinicopathological characteristics (n = 70).

The clinicopathological data of the studied cases (n = 70)	PD-L1 immunohistochemical score				$\chi^2$	MCp=
	Negative (n = 28) n (%)	+1 (n = 10) n (%)	+2 (n = 18) n (%)	+3 (n = 14) n (%)		
<b>Histopathological types</b>						
<b>Non-invasive urothelial tumours:</b>						
PUNLMPs	1 (3.6)	0 (0.0)	1 (5.6)	0 (0.0)		
LGP UCs	11 (39.3)	1 (10.0)	3 (16.7)	2 (14.3)		
HGP UCs3	3 (10.7)	0 (0.0)	2 (11.1)	3 (21.4)		
<b>Infiltrating urothelial carcinomas:</b>						
Conventional UCs	6 (21.4)	5 (50.0)	6 (33.3)	3 (21.4)		
Infiltrating urothelial carcinoma with divergent differentiation including squamous and glandular, mucinous differentiation	3 (10.7)	2 (20.0)	3 (16.7)	1 (7.1)	18.391	0.327
■ UCSDs	1 (3.6)	2 (20.0)	2 (11.1)	1 (7.1)		
■ UCGDs	1 (3.6)	0 (0.0)	1 (5.6)	0 (0.0)		
■ UCs with mucinous differentiation	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)		
Other variants of infiltrating urothelial carcinoma	1 (3.6)	1 (10.0)	1 (5.6)	5 (35.7)		
Miscellaneous histopathological types including pure SCC and adenocarcinoma	3 (10.7)	1 (10.0)	2 (11.1)	0 (0.0)		
■ ADCs	1 (3.6)	1 (10.0)	0 (0.0)	0 (0.0)		
■ SCCs	2 (7.1)	0 (0.0)	2 (11.1)	0 (0.0)		
<b>Histopathological grade</b>						
LG	16 (57.1)	4 (40.0)	6 (33.3)	4 (28.6)	4.200	0.0241*
HG	12 (42.9)	6 (60.0)	12 (66.7)	10 (71.4)		
<b>Degree of muscle invasion</b>						
Non-muscle invasive (Ta, T1)	18 (64.3)	4 (40.0)	8 (44.4)	7 (50.0)	2.688	0.442
Muscle invasive (T2, T3, T4)	10 (35.7)	6 (60.0)	10 (55.6)	7 (50.0)		
<b>Perineural invasion</b>						
With perineural invasion	3 (10.7)	1 (10)	1 (5.6)	2 (14.3)	2.814	0.572
Without perineural invasion	25 (89.3)	9 (90)	17 (94.4)	2 (85.7)		
<b>Lymph vascular invasion</b>						
with lymph vascular invasion	1 (3.6)	0 (0)	5 (27.8)	2 (14.3)	2.965	0.0432*
Without lymph vascular invasion	27 (96.4)	10 (100)	13 (72.8)	12 (85.7)		

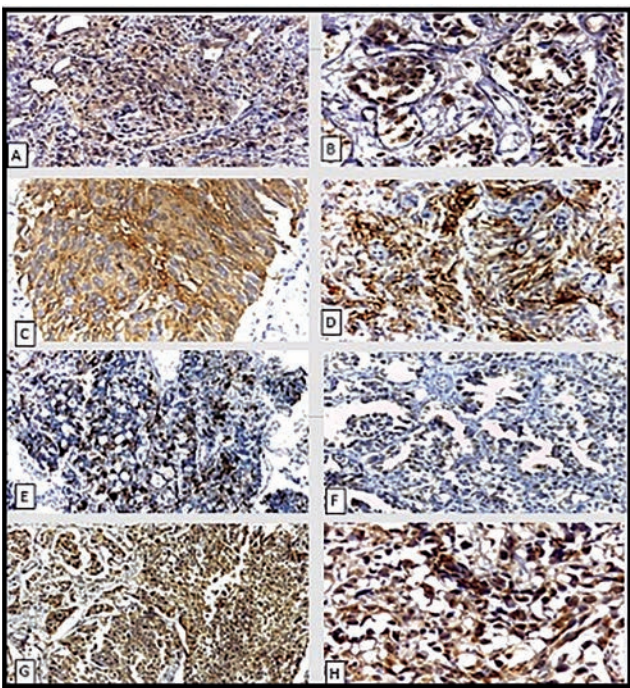
**PD-L1:** Programmed death-ligand 1, PUNLMP: Papillary urothelial neoplasm of low malignant potential, LGP: Low-grade papillary, HGP: High-grade papillary, US: Urothelial carcinoma, UCSD: Urothelial carcinoma with squamous differentiation, UCGD: Urothelial carcinoma with glandular differentiation, SCC: Squamous cell carcinomas, ADC: Adenocarcinoma

$\chi^2$ : Chi-square test, MC: Monte Carlo, p: p for association between different categories.





**Figure 1:** A: Positive control in which programmed death-ligand 1 (PD-L1) expressed cytoplasmic and membranous staining in neoplastic glandular cells of non-small lung adenocarcinoma (x200); B: Low-grade non-invasive papillary urothelial carcinoma showing positive PD-L1 expression, score +1 (x400); C: Low-grade non-invasive papillary urothelial carcinoma showing positive PD-L1 expression, score +3 (x200); D: high-grade non-invasive papillary urothelial carcinoma, showing positive PD-L1 expression, score +3 (x100), Immunoperoxidase.



**Figure 2:** High-grade infiltrating urothelial carcinoma (UC). A: UC conventional type showing positive programmed death-ligand 1 (PD-L1) expression, score +2 (x200); B: UC conventional type showing positive PD-L1 expression, score +3 (x400); C: UC conventional type showing positive PD-L1 expression, score +3 (x400); D: UC with squamous differentiation showing positive PD-L1 expression score +2 (x200); E: UC with glandular differentiation showing positive PD-L1 expression, score +2 (x100); F: UC micropapillary variant showing positive PD-L1 expression score +2 (x200); G: Undifferentiated carcinoma showing positive PD-L1 expression, score +3 (x100); H: UC with signet ring cells showing positive PD-L1 expression, score +3 (x400), Immunoperoxidase.

PD-L1 expression was more frequent in HG UBCs compared to LG cases ( $p < 0.05$ ), as HG carcinoma showed positivity in 28(70%) of the 40 such cases, while only 14(46.7%) of the 30 LG cases showed PD-L1 positivity.

PD-L1 expression showed higher expression in MI carcinomas 23(69.7%) than in 19(51.3%) NMI cases

( $p > 0.05$ ). Positive immune-expression of PD-L1 was detected in 7(58.3%) cystectomy specimens; 4(33.3%) were pT2b, 2(16.6%) were pT3a, and 1(8.3%) was pT4a stage. The pathological stage of the tumour and PD-L1 expression were not significantly correlated ( $p > 0.05$ ).

Among the 7(10%) cases with perineural invasion, 4(57.1%) showed PD-L1 positivity, while 3(42.8%) cases were negative ( $p > 0.05$ ). Tumours with LVI showed positive PD-L1 expression in 7(87.5%) of the 8 cases ( $p < 0.05$ ) (Table 1).

## Discussion

One of the biosimilar diagnostic antibodies utilised in clinical practice is the PD-L1 ZR3 clone.<sup>17</sup> PD-L1 expression showed positivity in 60% of the studied 70 cases. Nearly similar results were reported by Morsch et al. in 2020.<sup>16</sup> In contrast, Hodgson et al. in 2018<sup>15</sup> reported 14% positivity. These discrepancies may be due to differences in the evaluation algorithms.<sup>18</sup> Sanguedolce et al. in 2021<sup>19</sup> mentioned that many PD-L1 clones showed reactivity to genito-urinary tumours, and each clone needed to be assessed using the specific scoring formula and/or cut-off criterion.

The current results showed a more prevalent immune expression of PD-L1 in infiltrating HG bladder carcinomas compared to LG bladder carcinomas ( $p < 0.001$ ), which is a finding in line with earlier studies.<sup>20,21</sup> Also, the studied cases with LVI in the current study showed a significant relation with positive PD-L1 expression. Tsai, et al. in 2021<sup>22</sup> reported contrasting findings. Regarding the histopathological types of studied cases, the present study found that PD-L1 overexpression was in certain histopathological types of MI UBCs, including conventional type, UCSD, and other uncommon variants, including micropapillary, undifferentiated and UC with signet ring cells, but the difference in PD-L1 expression was not significant in different histopathological types ( $p > 0.05$ ). Kawahara et al. in 2018<sup>21</sup> reported that a sizeable fraction of PD-L1 was expressed in UCSDs (52%). Additionally, Morsch et al.<sup>16</sup> showed a substantial expression of PD-L1 in UCSDs comparable to pure UCs, whose disease management has been successfully improved by checkpoint inhibitors. These findings may be significant for using PD-1/PD-L1 blockades in this type of bladder carcinoma.

PD-L1 immune expression was higher in MI tumours in the current study than in NMI tumours, but the difference was not significant. PD-L1 expression in tumour cells was linked to MI UBS and a shorter OS, according to Ding et al. in 2019.<sup>23</sup> However, Zibelman et al. in 2016<sup>7</sup> discovered that there was no distinction in PD-L1 expression between NMIs and MIs. In the present study, the TNM-determined tumour stage decreased with increased PD-L1 expression in the

examined cystectomy specimens, but the connection was not significant. These outcomes run counter to the findings of Eckstein et al. who in 2019<sup>20</sup> reported that there was a concurrent rise in tumour stage and PD-L1 expression without any discernible relationship. Tsai et al.<sup>22</sup> reported that PD-L1 positivity was significantly correlated ( $p=0.049$ ) with a higher tumour stage. The small number of cystectomy specimens in the current study may be the cause of these conflicting outcomes.

There was no significant correlation between perineural invasion and PD-L1 immune expression in the current study. This was in line with earlier findings.<sup>24</sup> Additionally, neither age nor gender was significantly associated with PD-L1 expression in the current study, and the finding is supported by literature.<sup>25</sup>

Numerous variables, such as various antibody clones, scoring methods and cut-off criteria for positivity, tumour heterogeneity, sample type, variations in the number of studied cases, inter-observer variability in interpretation, and other pre-analytical variables, can be blamed for the differences in PD-L1 IHC expressions and the positivity rate among various studies. The current study has limitations in the shape of a small sample size.

## Conclusions

High PD-L1 expression was found to be a reliable biomarker for aggressive UBC. Compared to chemotherapy and cystectomy, checkpoint inhibitors considerably outperformed the treatment for patients, and their expression may be influenced by the histological type, the degree of muscle invasion, and other factors.

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**Conflict of Interest:** None.

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