International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Expression of PDL-1 IN Urothelial Carcinoma of the Bladder and Correlation with Clinicopathological Parameters in a Tertiary Care Centre in South India

PDL-1 Expression in Urothelial Carcinoma

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Abstract: ***Background**: Bladder Cancer is the ninth most common cancer worldwide. In India it ranks seventeenth among all malignancies. Urothelial carcinoma accounts for more than 90% of all bladder cancer cases. Introduction of immune checkpoint inhibitors targeting PD-1/PD-L1 has transformed the management of advanced cases of urothelial carcinoma. PD-L1 immunohistochemistry has emerged as a key biomarker to select patients and predict response to immune therapy **Aim**: To evaluate immunohistochemical expression of PD-L1 in Urothelial carcinoma of the bladder and assess its correlation with clinicopathological parameters. **Materials and Methods**: A hospital-based observational study was conducted on 55 confirmed Urothelial carcinoma cases. PDL-1 immunohistochemistry was performed on formalin-fixed paraffin-embedded sections. Associations were analyzed using Chi-square test. PDL-1 scoring was done by CPS (Combined Positive Score). CPS <10 is taken as Negative and CPS ≥10 is taken as Positive. **Results**: PD-L1 expression was observed in 38.2% of cases. Significant associations were found between PD-L1 expression and high tumour grade (p=0.015), and muscle invasion (p=0.026). **Conclusion**: PD-L1 positivity correlates with adverse histopathological features in Urothelial carcinoma. It may serve as a prognostic biomarker reflecting tumor microenvironment dynamics in addition to being a therapeutic tool.*

Keywords: PD-L1, urothelial carcinoma, immunohistochemistry, muscle-invasive, tumour grade

1. Introduction

Bladder cancer is the ninth most common cancer worldwide and accounts for about three per cent of all malignancies, with marked male predominance. [1,2,3] In India, it contributes nearly 32,000 new cases annually and constitutes about three to four per cent of male cancers. [4,5] Urothelial carcinoma represents more than 90 per cent of bladder cancers and typically presents in the sixth to seventh decades of life. Established risk factors include tobacco smoking, occupational exposure to aromatic amines and benzidine-based dyes, certain drugs such as cyclophosphamide and radiation. [6,7]

PD-1/PD-L1 (Programmed death-1/ Programmed cell death ligand 1) receptor ligand pathway plays a crucial role in maintaining immune tolerance within the tumour microenvironment.[8] Higher PD-L1 expression has been correlated with adverse clinicopathological parameters such as high grade and muscle-invasive stage, suggesting a link between immune checkpoint activation and aggressive tumour biology.[9]

Recent advances in tumour immunology have highlighted the pivotal role of the PD-1/PD-L1 immune checkpoint pathway in enabling tumour immune escape. PD-L1, expressed on tumour cells and antigen-presenting cells, binds PD-1 on T cells, leading to inhibition of anti-tumour immune responses and promotion of tumour progression. Immune checkpoint inhibitors targeting PD-1/PD-L1 have significantly improved outcomes in advanced urothelial carcinoma and in cisplatin ineligible cases. PD-L1 immunohistochemistry has emerged as an important biomarker for patient selection.

However, data on PD-L1 expression in urothelial carcinoma from Indian cohorts remains limited. Present study was undertaken to evaluate immunohistochemical expression of PD-L1 in urothelial carcinoma of the urinary bladder in a tertiary care centre in South India and to assess its correlation with clinicopathological parameters including age, sex, histological grade, muscle invasion and histological variants.

2. Materials and Methods

Study design and setting

This was a cross-sectional study conducted over a period of two years in the Upgraded Department of Pathology, Osmania General Hospital, Afzalgunj, Hyderabad, Telangana, India. Institutional ethics committee approval was obtained and written informed consent was taken from all participants as per departmental protocol.

Study population and inclusion criteria

A total of 55 consecutive cases of histologically confirmed urothelial carcinoma of the urinary bladder were included. Specimens comprised 52 transurethral resection of bladder

tumour (TURBT) samples and 3 radical cystectomy specimens received in the department during the study period

Inclusion criteria were: (i) cases diagnosed as urothelial carcinoma on routine histopathology, and (ii) availability of adequate formalin-fixed paraffin-embedded tumour tissue and patient consent.

Exclusion criteria were: (i) prior chemotherapy or radiotherapy before sampling, (ii) metastatic carcinoma, (iii) insufficient biopsy material and (iv) lack of consent.

Clinical and histopathological evaluation:
Demographic and clinical details including age, sex, presenting complaints and cystoscopy findings were recorded from case sheets and requisition forms. Routine haematoxylin and eosin (H&E) staining was performed on 5 µm sections from paraffin blocks using standard protocols. Tumours were classified and graded as low-grade or high-grade urothelial carcinoma according to WHO/ISUP (World Health Organization/ International society of Urologic Pathology) criteria and assessed for muscle invasion and histological variants.

Immunohistochemistry for PD-L1:
Representative tumour areas were selected and additional sections were cut at 5 µm on poly-L-lysine coated slides for PD-L1 immunohistochemistry. Placenta tissue was used as positive control for PD-L1 expression. Immunostaining was performed using a Ventana Benchmark Ultra automated system with a rabbit monoclonal primary antibody against PD-L1 (clone 411), following the manufacturer's instructions. After deparaffinization and rehydration, heat-induced antigen retrieval was carried out in Tris-EDTA buffer (pH 9) in a pressure cooker. Endogenous peroxidase activity was blocked using 3 per cent hydrogen peroxide, followed by incubation with blocking reagent to prevent non-specific protein binding. Sections were then incubated with the PD-L1 primary antibody, followed by polymer-based horseradish peroxidase-labelled secondary reagent and visualization with 3,3′-diaminobenzidine (DAB) chromogen, and counterstained with Harris haematoxylin

Scoring of PD-L1 expression:
Membranous staining of any intensity in tumour cells was considered specific. PD-L1 expression was evaluated using the combined positive score (CPS), defined as the number of PD-L1 positive cells (tumour cells, lymphocytes and macrophages) divided by the total number of viable tumour cells, multiplied by 100. A CPS <10 was considered negative and CPS ≥10 was taken as positive for PD-L1 expression. Immunostaining was independently assessed by a senior professor and a pathology resident and discordant cases were reviewed jointly to reach consensus.

Statistical analysis
Data were compiled in a master chart and analyzed using IBM SPSS version 25 statistical software. Associations between PD-L1 expression (positive vs negative) and clinicopathological variables (age, sex, histological grade, muscle invasion and histological variants) were assessed by Chi-square test for categorical variables and comparison of means for continuous variables. A p-value of <0.05 was considered statistically significant.

3. Results

Clinicopathological profile:
Of the 55 urothelial carcinoma cases, 40 (72.7%) were males and 15 (27.3%) females, with a male to female ratio of 2.6:1. Patients age ranged from 22 to 88 years, with mean age 61 years and median 63 years; males had a mean age of 62.1 years and females 58 years. Most cases occurred in the sixth and seventh decades of life. Hematuria was the most common presenting symptom, with some patients also reporting urinary frequency and dysuria. Cystoscopy most frequently revealed papillary lesions.

High-grade urothelial carcinoma accounted for 39 (71%) cases, (Figure I, H&E) while 16 (29%) were low-grade tumours. Muscle invasion was identified in 31 (56.4%) cases, (Figure II, H&E) whereas 24 (43.6%) were non-muscle invasive. Pure urothelial carcinoma was seen in 49 (89.1%) cases; urothelial carcinoma (UC) with squamous differentiation in 4 (7.3%) (Figure III, H&E) and with sarcomatoid differentiation in 2 (3.6%) cases. (Figure IV, H&E) The clinicopathological profile of urothelial carcinoma (UC) cases is summarized in Table I.

PD-L1 expression profile:
Overall, PD-L1 positivity (CPS ≥10) was observed in 21 of 55 cases, with an expression of 38.2 per cent. PD-L1 expression according to gender showed positivity in 15/40 (37.5%) males and 6/15 (40%) females; the association between PD-L1 status and sex was not statistically significant (p=1.0). Mean age did not differ significantly between PD-L1 positive (60.2 ±14.1 yr) and negative (61.4 ±14.9 yr) groups (p=0.729).

A clear association was observed between PD-L1 expression and histological grade. PD-L1 positivity was present in 2/16 (12.5%) low-grade tumours compared to 19/39 (48.7%) high-grade tumours (Figure V, IHC), and this difference was statistically significant (p=0.015) (Figure VIII). Similarly, PD-L1 positivity was more frequent in muscle-invasive urothelial carcinoma: 16/31 (51.6%) muscle-invasive (Figure VI, IHC) versus 5/24 (20.8%) non-muscle-invasive cases (p=0.026).

Regarding histological variants, PD-L1 positivity was noted in 17/49 (34.7%) pure urothelial carcinomas, 3/4 (75%) tumours with squamous differentiation and 1/2 (50%) tumours with sarcomatoid differentiation. Although variant histology showed a higher proportion of PD-L1 positive cases compared to pure urothelial carcinoma, this trend did not reach statistical significance (p=0.140), likely due to the small number of variant cases. PDL-1 expression and its association with clinicopathological parameters is summarized in Table II.

4. Discussion

In this study of 55 cases of urothelial carcinoma of the urinary bladder from a tertiary care centre in South India, PD-L1 expression (CPS ≥10) was observed in 38.2 per cent of

tumours and showed significant association with high histological grade and muscle-invasive disease. The age distribution (mean 61 yr, median 63 yr) and male predominance (M:F 2.6:1) in our cohort are comparable to previous studies and global epidemiological data on urothelial carcinoma. Similar age ranges and male predominance have been reported by kumar et al., [10]Anand et al. [11] and Al Nabhani et al.,[12] reflecting the consistent demographic profile of bladder cancer across different populations.

The overall PD-L1 positivity rate in our study is in line with several published series, which have reported expression rates between about 28 and 44 per cent in urothelial carcinoma using various antibodies and scoring systems. Singh et al. [13] and Kumar et al. have documented PD-L1 positivity rates of 30 and 32.7 per cent, respectively, while Kim et al. [14] reported 28 per cent positivity, all comparable with our findings. Differences in reported prevalence likely reflect heterogeneity in patient cohorts, pre-analytical variables, antibody clones, scoring methods and cut-offs used for positivity.

Our observation of a statistically significant association between PD-L1 expression and high tumour grade corroborates multiple earlier studies that linked higher PD-L1 levels to adverse pathological features. Kumar et al. and others [12,14,15] have reported similar association in their respective studies. In the present cohort, high-grade tumours showed nearly four-fold higher PD-L1 positivity than low-grade tumours (48.7% vs 12.5%), supporting the concept that aggressive urothelial carcinomas are more likely to exploit the PD-1/PD-L1 axis for immune evasion.

We also found a significant association between PD-L1 expression and muscle-invasive disease, with more than half of muscle-invasive tumours being PD-L1 positive compared to about one-fifth of non-muscle-invasive cases. This is consistent with studies by Kim et al., [14] Singh et al., [13] and others [10,16] indicating that PD-L1 positivity is more common in muscle-invasive tumours and is associated with advanced stage. Clinically, this is relevant, as muscle-invasive bladder cancer carries a substantially worse prognosis.

In contrast, PD-L1 expression did not show significant association with age or sex in our study, which is also in agreement with several prior reports by Holland et al. [17] Male preponderance in bladder cancer incidence has been attributed largely to environmental and occupational exposures.

Variant histologies (squamous and sarcomatoid differentiation) exhibited a numerically higher frequency of PD-L1 positivity compared with pure urothelial carcinoma. However, the small number of variant cases in our cohort precluded demonstration of statistical significance, underscoring the need for larger studies focusing on these subtypes.

The strengths of this study include the use of a standardized CPS-based scoring system, correlation with detailed clinicopathological data and focus on a well-defined single-centre cohort. Limitations include the relatively small sample size, particularly for variant histologies, and the lack of follow-up data to correlate PD-L1 expression with treatment response and survival. Additionally, this is a single-centre study and findings may not be generalizable to all populations, highlighting the need for multi-centre studies with larger sample sizes and longitudinal follow-up.

5. Conclusion

In this two-year cross-sectional study of 55 cases of urothelial carcinoma of the urinary bladder, PD-L1 expression (CPS ≥10) was observed in 38.2 per cent of tumours and was significantly associated with high histological grade and muscle-invasive disease, but not with age or sex. Variant histologies showed a trend towards higher PD-L1 positivity, although statistical significance could not be established due to small numbers. These findings, concordant with international literature, underscore the role of PD-L1 as a marker of aggressive tumour biology and support its utility as a prognostic and potential predictive biomarker in urothelial carcinoma in our setting.

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Acknowledgements

The authors thank the laboratory technical staff for assistance with immunohistochemistry procedures. No external funding was received. The authors declare no conflicts of interest.

Table I: Clinicopathological profile of urothelial carcinoma cases

Variable	Category	n (%)
Sex	Male	40 (72.7)
	Female	15 (27.3)
Age (yr)	Mean ± SD	61.0 ± 14.5
Grade	Low-grade	16 (29.1)
	High-grade	39 (70.9)
Muscle invasion	Non-muscle-invasive	24 (43.6)
	Muscle-invasive	31 (56.4)
Histology	Pure urothelial carcinoma	49 (89.1)
	Squamous differentiation	4 (7.3)
	Sarcomatoid differentiation	2 (3.6)

n= No. of cases, SD – Standard deviation

Table II: PDL-1 expression and its association with clinicopathological variables

Variable	Category	PD-L1 positive n (%)	PD-L1 negative n (%)	p value
Sex	Male	15 (37.5)	25 (62.5)	1.000
	Female	6 (40.0)	9 (60.0)	
Grade	Low-grade	2 (12.5)	14 (87.5)	0.015
	High-grade	19 (48.7)	20 (51.3)	
Muscle invasion	Non-muscle-invasive	5 (20.8)	19 (79.2)	0.026
	Muscle-invasive	16 (51.6)	15 (48.4)	
Histology	Pure urothelial	17 (34.7)	32 (65.3)	0.140
	Squamous differentiation	3 (75.0)	1 (25.0)	
	Sarcomatoid differentiation	1 (50.0)	1 (50.0)	

n= Number of cases

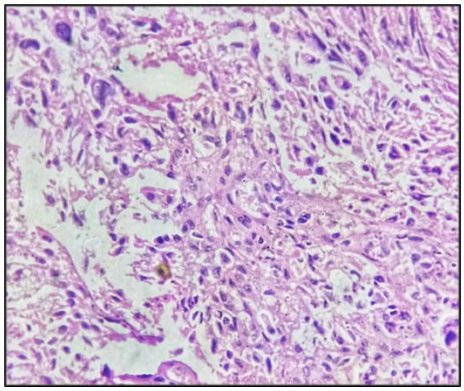

Figure I: Hematoxylin and Eosin (H&E) stained section of high- grade urothelial carcinoma (UC), showing marked nuclear pleomorphism and atypia x 400

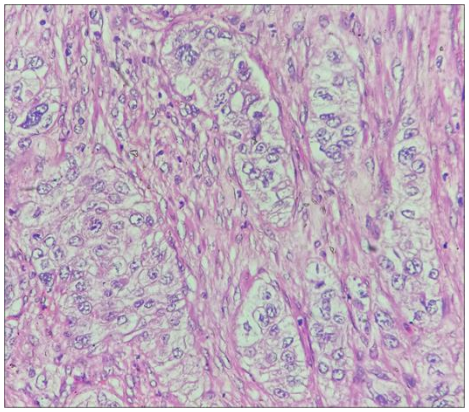

Figure II: Invasive urothelial carcinoma (H&E, x400)

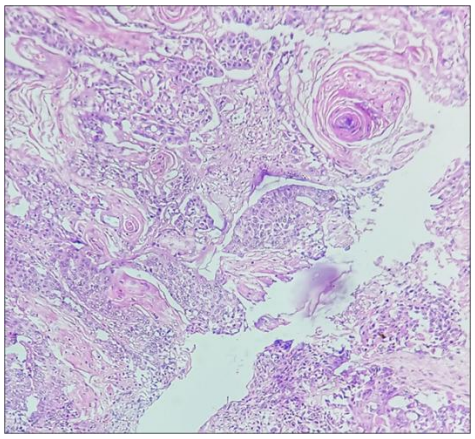

Figure III: Urothelial carcinoma with Squamous differentiation (H&E, x100)

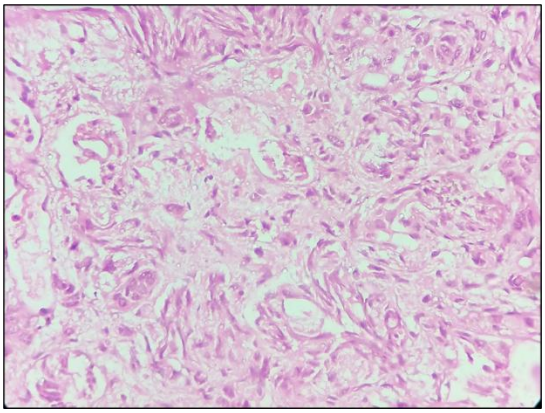

Figure IV: Urothelial carcinoma with Sarcomatoid differentiation (H&E, x400)

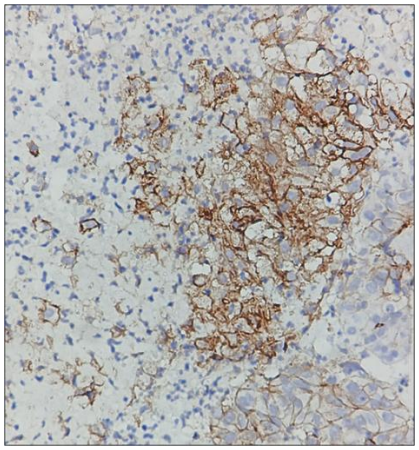

Figure V: Immunohistochemical (IHC) staining for PDL-1 in High grade urothelial carcinoma (UC), x400

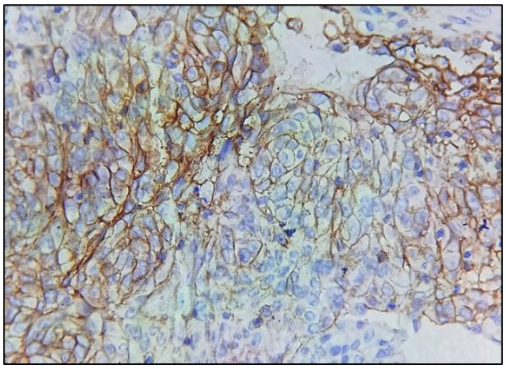

Figure VI: Immunohistochemical (IHC) staining for PDL-1 in Invasive UC, x400

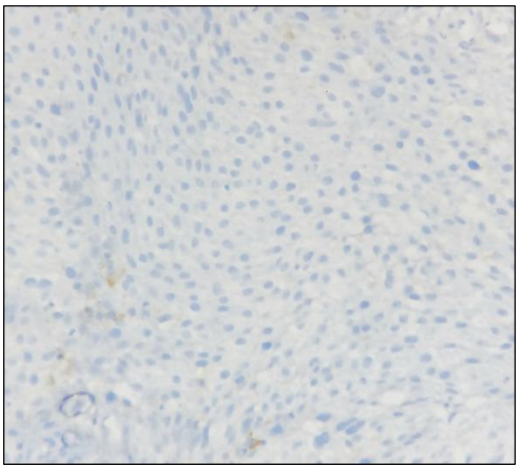

Figure VII: IHC staining for PDL-1 in low grade Urothelial carcinoma, x400

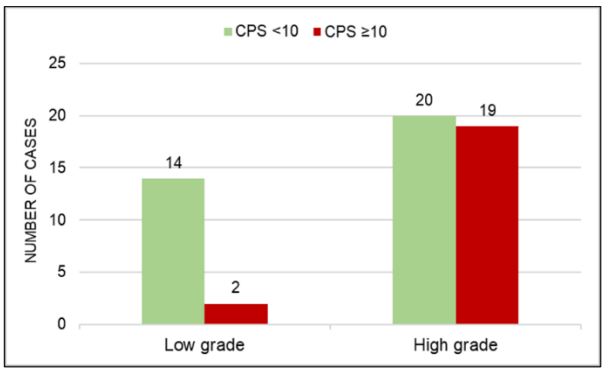

Figure VIII: Chart depicts PDL-1 expression according to histological grade