Original Article

Assessment of Immunohistochemical Expression of Programmed Cell Death Ligand-1 in Invasive Urothelial Carcinoma

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Abstract

Background: Infiltrating urothelial carcinoma is the most common histological variant (23.1%). This study was conducted to evaluate the immunohistochemical expression of PD-L1 in invasive urothelial carcinoma and to correlate PD-L1 expression with demographic data and the histological grade of urothelial carcinoma.

Methods: The study comprised a total of 65 cases of muscle-invasive urothelial carcinoma collected over a period of one year and evaluated for PD-L1 expression using the immunohistochemistry technique.

Results: Infiltrating urothelial carcinoma was the most common histological variant (23.1%), followed by poorly differentiated carcinoma (16.9%). PD-L1 positivity was observed in 47.7% of the total cases, while the remaining 52.3% showed no expression of PD-L1. Among the various histologic variants, poorly differentiated carcinoma (57.1%) was the largest group with weak expression of PD-L1; infiltrating urothelial carcinoma (26.7%) was the largest group with moderate expression of PD-L1, while the lymphoepithelioma-like variant (66.7%) was the largest group with strong expression of PD-L1. Our study was in concordance with other studies, showing that PD-L1 expression is not associated with clinicopathologic features like age, gender, and smoking status.

Conclusion: The present study shows a significant difference between the various groups in terms of the distribution of histologic variants and PD-L1 positivity according to the WHO classification (2016) as well as severity scoring by the IHC technique.

Keywords:

Urothelial carcinoma, PD-L1, Immunohistochemistry, Urinary bladder, Hematuria.

Introduction

Urothelial carcinoma (UC) is a malignant cancer that originates from the transitional epithelial cells of the urinary tract, accounting for about 95% of bladder cancers (BC). The remaining 5% comprises squamous cell carcinoma, adenocarcinoma, and small cell carcinoma [1]. Bladder cancer is the fourth most common cancer in men but is less common in women. UC recurs in more than half of cases, even with optimal treatment, and may progress to muscle-invasive UC in up to 20% of cases [2].

Smoking tobacco is the most important risk factor for bladder cancer, accounting for 50–65% of new cases each year due to the presence of known carcinogens such as beta-naphthylamine and polycyclic aromatic hydrocarbons. The older age of onset of

bladder cancer suggests a latency period of approximately 30 years from the initiation of smoking. However, cessation of smoking is related to a reduction of risk by approximately 40% within one to four years [3].

Occupational exposure to various carcinogens, including polycyclic aromatic hydrocarbons and chlorinated hydrocarbons, is correlated with 20% of cases, especially in industrial areas processing paint and dyes [4]. High levels of arsenic in drinking water are also associated with an increased risk of tumor progression [5]. The protozoan Schistosoma haematobium is known to be associated with bladder cancer due to inflammation, induction of endogenous synthesis of N-nitrosoamines, and DNA-damaging free oxygen radicals [3].

Furthermore, obesity, red meat, alcohol, long-term use of analgesics, heavy long-term exposure to cyclophosphamide, and irradiation for pelvic malignancies are the major risk factors contributing to UC [6]. Various genetic factors also contribute to the development of UC. Glutathione S-transferase (GST) encodes important enzymes in the process of carcinogen detoxification and plays a major role in its occurrence [7]. UC is relatively common in the elderly, as the median age of diagnosis is 72 years for men and 75 years for women. Male-to-female incidence ratios vary between 2:1 and 3:1 in different studies [8]. This discrepancy in the incidence of bladder cancer can be attributed to differential rates of tobacco smoking and industrial chemical exposure in males and females [3].

There are two distinct precursor lesions to invasive UC. The most common precursor lesions are the noninvasive papillary tumors, which originate from papillary urothelial hyperplasia. The other precursor lesion is carcinoma in situ (CIS). Once muscularis propria invasion occurs, there is a 30% five-year mortality rate [9]. Painless hematuria is the most common presenting symptom of UC. Frequency, urgency, and dysuria may accompany hematuria. Occasionally, obstruction of the ureteral orifice may lead to pyelonephritis or hydronephrosis [9].

The subsequent treatment and course of UC depend on tumor grade and stage, with the key variable being whether the tumor is muscle-invasive or not. Diagnostic transurethral resection is required for small and localized low-grade papillary tumors. CIS and papillary tumors that are large, high grade, multifocal, have a history of recurrence, or are associated with lamina propria invasion are treated with intravesical instillation of Bacillus Calmette–Guérin (BCG), which destroys the tumor by eliciting a local inflammatory reaction. Non-muscle-invasive urothelial neoplasms need lifelong surveillance and follow-up due to increased risk of recurrence and progression. Muscle-invasive UC is treated with radical cystectomy, cystoprostatectomy, or radiation therapy, with neoadjuvant and adjuvant chemotherapy [9].

Modern immunotherapy focuses on inhibitors of checkpoint proteins, molecules that impede immune function, thereby allowing tumor cells to grow and proliferate unregulated. Checkpoint inhibitors offer an effective alternative to patients who previously had very few options, including those ineligible for cisplatin-based regimens or at risk of significant toxicity. Ongoing research aims to further categorize responses, define ideal patient populations, and investigate combinations of checkpoint inhibitors to address multiple pathways in immune system functioning [10].

PD-L1 (Programmed Death Ligand 1) is the principal ligand of the programmed death receptor-1 (PD-1), a co-inhibitory receptor that can be constitutively expressed on lymphocytes, antigen-presenting cells, or induced on hematopoietic, endothelial, and epithelial cells. Under physiological conditions, the PD-1/PD-L1 interaction is essential in the development of immune tolerance, preventing excessive immune cell activity that can lead to tissue destruction and autoimmunity [11].

Most of the information regarding immunotherapy in UC is available from Western literature. There is limited data regarding the

role of PD-L1 in UC in the Indian population. This study was undertaken to determine the IHC expression of PD-L1 in invasive UC. We also aimed to understand the prevalence of PD-L1 in UC in our population, as well as its correlation with clinical parameters, histomorphological features, and demographic data.

Materials and Methods

The current study was conducted in the Department of Pathology, Pt B.D. Sharma PGIMS, Rohtak, over a period of one year. A total of 65 cases of muscle-invasive urothelial carcinoma constituted the study material.

Inclusion criteria: All cases of muscle-invasive urothelial carcinomas were included in the study.

Exclusion criteria: Non-invasive urothelial carcinomas, Cases for which blocks were not available, Urothelial carcinoma patients on chemotherapy.

All the specimens received fresh in the Department of Pathology were subjected to careful and detailed gross examination, fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. The sections were routinely stained with hematoxylin & eosin stain. The serial sections were prepared from paraffin-embedded blocks and incubated with primary antibodies. Immunohistochemistry stain was performed using the standard technique. Immunohistochemistry was assessed by subjecting one section from each representative block to PD-L1. Cases showing membranous staining for PD-L1 in ≥5% of tumor cells were considered positive. This cutoff is standard in other similar studies conducted by Tojyo et al. [12], Bellmunt et al. [13], and Feng et al. [14].

Immunohistochemical expression of PD-L1 in tumor cells: Negative: < 5%, Positive: ≥ 5%, Weak: 5–29%, Moderate: 30–59%, Strong: > 60%

Positive control: Tonsil and placenta were considered as positive controls.

Negative control: Negative control was obtained by substituting the primary antibody with an antibody of nonspecific relevance.

Statistical Analysis: All the data enlisted in the case proforma were collected and analyzed with the help of a software package (SPSS version 24.0). Frequency distribution and cross-tabulation were used to create summary tables and compare items within and across various categories. The Chi-square test was used for qualitative variables. Correlation was assessed using the Spearman test. Differences between groups were considered significant only when the p-value was <0.05.

Results

The overall observations and results from the study are as follows:

The range of patients varied from 34–88 years, with the majority of patients in the age group of 61–70 years. A higher incidence was observed in males, with a ratio of 12:1. Infiltrating urothelial carcinoma was the most common histological variant (23.1%), followed by poorly differentiated carcinoma (16.9%). There was a significant difference between the various groups in terms of the distribution of histologic variants.

Among the various histologic variants, the lymphoepithelioma-like (66.7%) histologic variant was the largest group with a strong expression of PD-L1. This can be attributed to the presence of mononuclear cell infiltration, a trait that predicts improved survival in metastatic patients. Weak expression of PD-L1 was seen in 57.1% of the total cases of poorly differentiated carcinoma, which can be attributed to the minimal presence of infiltrating immune cells. A histological variant can serve as a surrogate marker that

is helpful in identifying patients who are likely to benefit from immune checkpoint inhibitors.

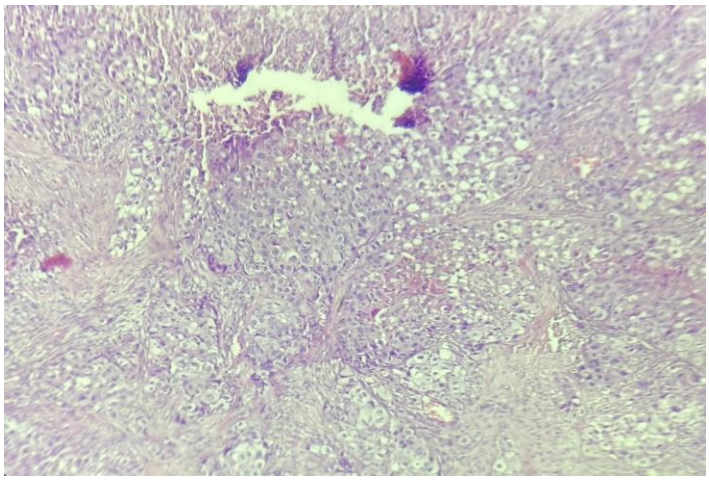

Figure 1: Photomicrograph showing muscle-invasive urothelial carcinoma, 100X (H&E).

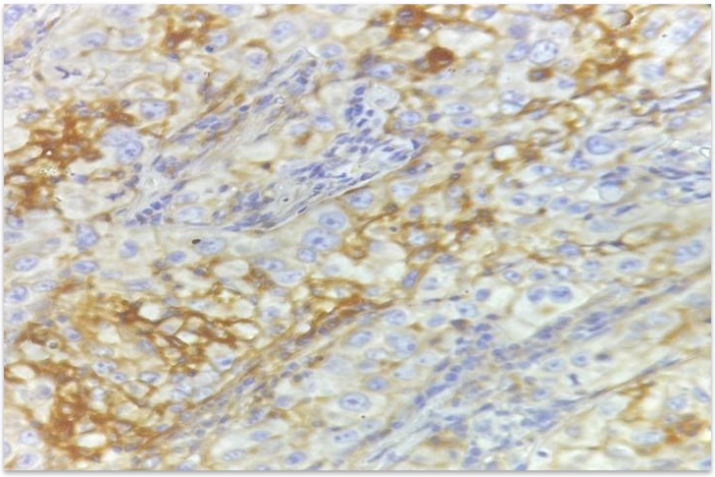

Figure 2: Photomicrograph showing membranous staining for PD-L1 positivity, 400X (IHC).

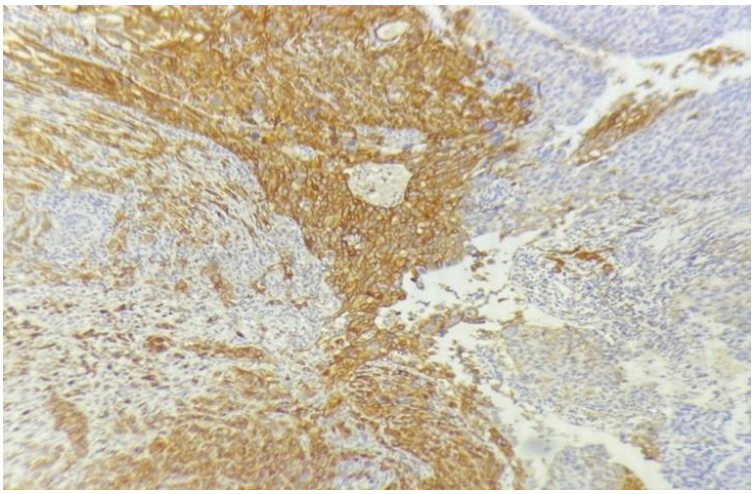

Figure 3: Photomicrograph showing muscle-invasive urothelial carcinoma with strong PD-L1 membranous positivity, 100X

(IHC).

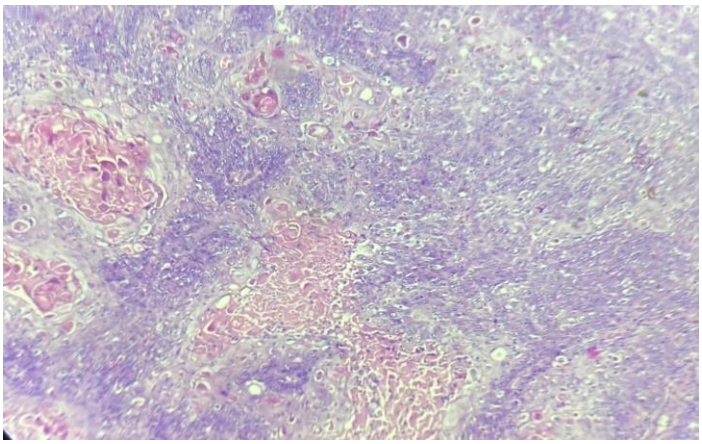

Figure 4: Photomicrograph showing urothelial carcinoma with squamous features (keratinizing cells consistent with squamous derivation), 100X (H&E).

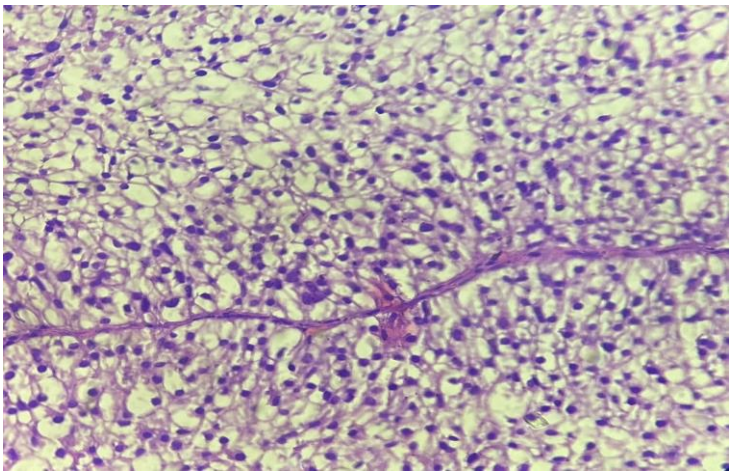

Figure 5: Photomicrograph showing clear cell variant in urothelial carcinoma, 400X (H&E).

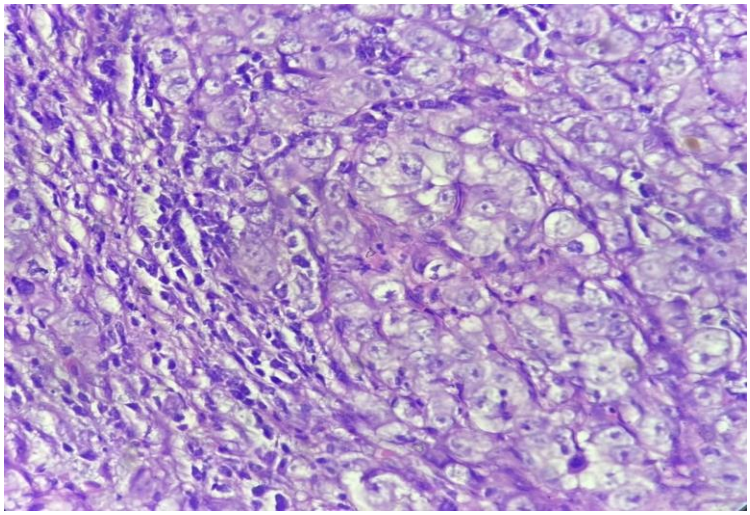

Figure 6: Photomicrograph showing lymphoepithelioma-like variant, 400X (H&E).

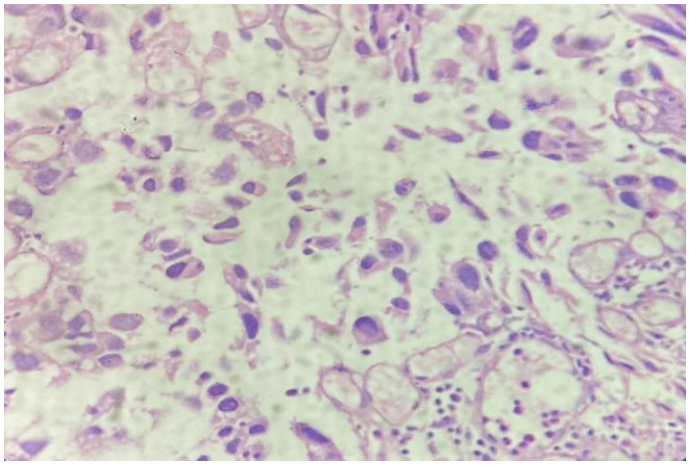

Figure 7: Photomicrograph showing plasmacytoid variant (single dyscohesive cells infiltrating within a myxoid background), 400X (H&E).

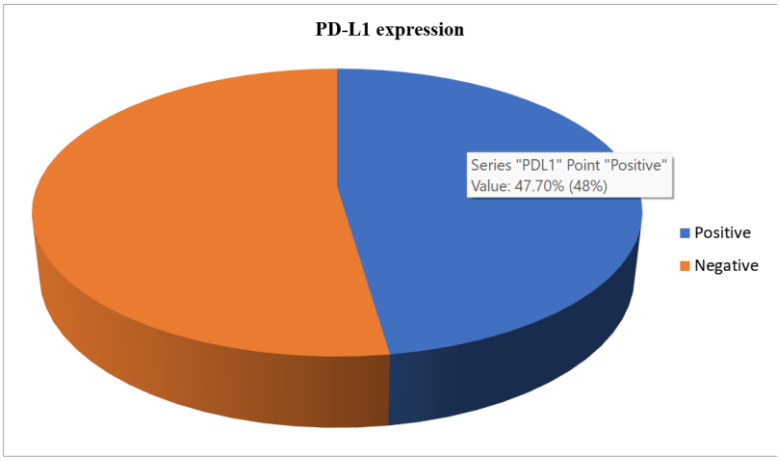

Figure 8: Case distribution as per PD-L1 expression.

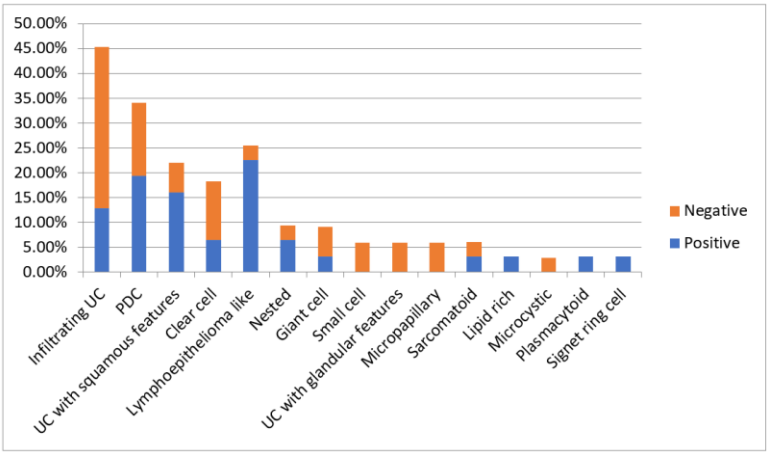

Figure 9: Association between PD-L1 and histologic variant.

Table 1: Age Distribution of Urothelial Carcinoma Cases

Age	Frequency	Percentage	95% CI
31-40 years	4	6.2%	2.0% - 15.8%
41-50 years	9	13.8%	6.9% - 25.2%
51-60 years	19	29.2%	18.9% - 42.0%
61-70 years	25	38.5%	26.9% - 51.4%
71-80 years	6	9.2%	3.8% - 19.7%
81-90 years	2	3.1%	0.5% - 11.6%

Table 2: Distribution of Urothelial Carcinoma Cases According to Gender

Gender	Frequency	Percentage	95% CI
Male	60	92.3%	82.2% - 97.1%
Female	5	7.7%	2.9% - 17.8%

Table 3: Distribution of Urothelial Carcinoma Cases According to Smoking

Smoking	Frequency	Percentage	95% CI
Present	36	55.4%	42.6% - 67.5%
Absent	29	44.6%	32.5% - 57.4%

Table 4: Distribution of Urothelial Carcinoma Cases According to Histologic Variants

Histologic variant	Frequency	Percentage	95% CI
Infiltrating urothelial carcinoma	15	23.1%	13.9% - 35.5%
Poorly differentiated carcinoma	11	16.9%	9.1% - 28.7%
UC with squamous features	7	10.8%	4.8% - 21.5%
Clear cell	6	9.2%	3.8% - 19.7%
Lymphoepithelioma like	8	12.3%	5.8% - 23.4%

Nested	3	4.6%	1.2% - 13.8%
Giant cell	3	4.6%	1.2% - 13.8%
Small cell	2	3.1%	0.5% - 11.6%
UC with glandular features	2	3.1%	0.5% - 11.6%
Micropapillary	2	3.1%	0.5% - 11.6%
Sarcomatoid	2	3.1%	0.5% - 11.6%
Lipid rich	1	1.5%	0.1% - 9.4%
Microcystic	1	1.5%	0.1% - 9.4%
Plasmacytoid	1	1.5%	0.1% - 9.4%
Signet ring cell	1	1.5%	0.1% - 9.4%

Discussion

Bladder cancer is usually suspected due to hematuria and then diagnosed by cystoscopy, telescopic endoscopy of the bladder, or computed tomography urography. As many as 7 out of every 10 cases of bladder cancer are detected in the early stages, thus allowing for resection and improved survival. In non-muscle-invasive bladder cancer, transurethral resection of the tumor is the mainstay treatment. In muscle-invasive bladder cancer, neoadjuvant or adjuvant chemotherapy is considered the standard to lower the risk of recurrence, and radical cystectomy is the mainstay surgical treatment. In metastatic disease, platinum chemotherapy remains the standard, though novel immunotherapies, namely checkpoint inhibitors, are gaining popularity as treatment options in the first line and beyond [3].

The expression of PD-L1, either in the tumor or tumor-infiltrating mononuclear cells, has been verified in a variety of tumors using immunohistochemistry techniques, suggesting a role for the PD-1/PD-L1 axis as a prognostic trait and therapeutic target across multiple histotypes. However, PD-L1 expression detection based on immunohistochemistry is constrained by pre-analytical and analytical variability, including heterogeneity in antibody clones, scoring methodology, and intrinsic biological variations. These variations in PD-L1 expression can be attributed to the type of specimen analyzed, including surgical resection vs. biopsy, primary tumor vs. metastasis, archival vs. frozen samples, and prior treatment status. The complex interplay between these factors plays a major role in the diffusion and clinical application of PD-L1 immunohistochemistry (IHC) assays as predictive biomarkers of response to PD-1/PD-L1 inhibitors [11].

The present study was conducted in the Department of Pathology, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences, Rohtak. Histopathologically confirmed 65 muscle-invasive urothelial carcinoma cases were included in the study group. IHC expression of PD-L1 in invasive urothelial carcinoma was evaluated and correlated with the demographic data and histological grade of urothelial carcinoma.

The age of patients ranged from 34 to 88 years, with a mean age of 60.14 ± 10.81 years. The age in the present study was in concordance with most studies in the literature conducted by Wang et al. (mean 67 years) [17], Jung et al. (mean 67 years) [18], Jones et al. (mean 69 years) [19], Goux et al. (>60 years) [20], and Stella et al. (mean 67 years) [21].

The majority of patients were male (92.3%). The gender distribution in the present study was consistent with the studies done by Wang et al. [17] and Hashmi et al. [22], who also observed male predominance. This gender discrepancy is most likely attributable to differential rates of tobacco smoking. Other hypothesized factors predisposing men to a higher risk for urothelial carcinoma include industrial chemical exposure, alcohol, and red meat consumption [3].

Smoking was present in 55.4% of the total number of urothelial carcinoma cases. Our findings were similar to the studies

conducted by Goux et al. (53.5%) [20] and Saginala et al. (50-65%) [3].

Gajjar et al. [23] studied the histomorphological spectrum of urothelial tumors according to the WHO/ISUP consensus classification. They found 14.3% as infiltrating urothelial carcinoma and 85.6% cases of non-invasive urothelial neoplasia. Among invasive UC, 72% of cases were infiltrating urothelial carcinoma, 20.9% had micropapillary differentiation, and 6.9% were diagnosed as sarcomatoid differentiation. In contrast, we found only 23.1% of cases as the infiltrating urothelial carcinoma subtype. The difference may be due to the small number of invasive UC cases in the above study, and only three histological subtypes were described, whereas we divided our cases into 15 different histological categories.

The expression of PD-L1, either in tumors or tumor-infiltrating mononuclear cells, has been verified predominantly by immunohistochemistry (IHC) in a variety of tumors, suggesting a role for the PD-L1/PD-1 axis as a prognostic trait and therapeutic target across multiple histotypes. In our study, 47.7% of cases were PD-L1 positive, while 52.3% were PD-L1 negative. Xylinas et al. [24] (25%) and Davis & Patel [25] (28.9%) reported lower expression of PD-L1, while Goux et al. (60.7%) [20] reported higher expression in their studies compared to our findings. The reasons for the wide variation in PD-L1 expression are the semi-quantitative IHC technique and high dependence on a range of variables, such as the choice of antibody, antibody concentration, fixation technique, variability in interpretation, and inconsistency in specimen handling and technical procedures.

In our study, PD-L1 expression was weak in 10.8% of the total cases, moderate in 23.1%, and strong in 13.8%. PD-L1 expression was negative in 52.3% of the total cases.

Gandini et al. [26], in a systematic review and meta-analysis on PD-L1 expression in cancer patients receiving anti-PD-1/PD-L1 therapy, found that PD-L1 expression in tumor tissues correlates with clinical response to antibodies targeting the PD-1/PD-L1 axis. PD-L1 expression is associated with a significantly better prognosis. In contrast, the study by Feng et al. [14] on the clinical significance of enhanced PD-L1 expression in cervical squamous cell carcinoma found that increased PD-L1 expression was significantly associated with high TNM stage, a reduced number of TILs, and worse prognosis in cervical carcinomas. No study in the literature focuses on bladder carcinoma based on the severity of PD-L1 expression and its role in prognosis.

Our study was in concordance with studies by Xylinas et al. [24], Owyong et al. [27], Wu et al. [28], Li et al. [29], Goux et al. [20], and Wang et al. [17], indicating that PD-L1 expression is not associated with clinicopathologic features.

Among the various histologic variants, poorly differentiated carcinoma (57.1%) was the largest group with weak expression of PD-L1. Infiltrating urothelial carcinoma (26.7%) was the largest group with moderate PD-L1 expression, while the lymphoepithelioma-like variant (66.7%) was the largest group with strong PD-L1 expression. The maximum number of cases of the infiltrating urothelial carcinoma (32.4%) histologic variant showed negative PD-L1 expression.

Takahara et al. [30], in a review article, stated that UC exhibits widely diverse histological variants, and recent studies have revealed that some histological variants may serve as markers of a very high risk for advanced cancers and poor prognosis. A histological variant acts as a surrogate marker, helping identify patients likely to benefit from immune checkpoint inhibitors, and its presence may caution against understaging by radiological imaging. In the present study, we found a significant difference between different histological subtypes and PD-L1 expression. However, more studies are required to support the role of PD-L1 in different histological subtypes for its usefulness in treatment and prognosis.

Limitation: The present study has a few limitations. First, PD-L1 expression was evaluated only in tumor cells. We did not study PD-L1 expression in inflammatory cells or using a semi-quantitative IHC technique. Secondly, correlation of staining with clinical

outcomes could not be attempted, as samples were not taken from patients treated with anti-PD-L1/PD-1 therapy. Thirdly, due to the small sample size and time-bound nature of our study, we were unable to carry out long-term follow-up of patients to determine the prognostic significance of our findings. A larger prospective study with long-term outcome analysis is needed for definitive conclusions.

Conclusion

The present study shows a significant difference between the various groups in terms of the distribution of histologic variants and PD-L1 positivity, as per the WHO classification of 2016, as well as severity scoring by the IHC technique. A histological variant acts as a surrogate marker, helping identify patients likely to benefit from immune checkpoint inhibitors, and its presence may caution against understaging by radiological imaging. Immune checkpoint inhibitors contribute to the treatment of patients with UC in combination with agents targeting other non-redundant immunosuppressive pathways, targeted therapy, and conventional chemotherapy. Additionally, the use of these agents earlier in the course of the disease can improve cure rates in patients with localized disease, demonstrating feasibility, safety, and promising efficacy. Our findings should be viewed with caution, and longer follow-up or a larger cohort of patients may be needed to reach significance for prognostic analysis.

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Competing Interests: There are no conflicts of interest in this study.

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