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Immunohistochemical Expression of Programmed Death-Ligand 1 (Pd-L1) in Urothelial Cancer in Urinary Bladder

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ABSTRACT

Objective: To determine the frequency of Programmed death-ligand 1 (PD-L1) immunohistochemistry expression in urothelial carcinoma in our population

Study Design: Cross-sectional study

Place and Duration of Study: Histopathology Department, Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan, from Jan 2021 to Jun 2022.

Methodology: The study comprised of 80 patients diagnosed with urothelial cancer. The frequency of different subtypes of urothelial carcinoma (papillary and non-papillary) was recorded. Immunohistochemical staining with PD-L1 antibody (Clone 28-8) was performed.Patients with no expression for PD-L1 were considered negative, whereas immunohistochemical expression of ≥1% was considered positive. PD-L1 statuses of all urothelial cancer tumour cells was determined.

Results: The study comprised 80 instances of urothelial carcinoma. The age range at presentation was 32 to 95 years. Among the patients, 71(88.8%) were men and 9(11.12%) were women. Papillary urothelial carcinoma was the most prevalent histological type, found in 63 cases (78.8%). Urothelial carcinoma without papillary features was detected in 17 instances (21.2%). Of the former, 23 were found to be low-grade papillary urothelial carcinoma and 40 cases turned out to be high-grade papillary urothelial carcinoma. Urothelial carcinomas other than papillary were all high grade, and each patient had a different histology. PD-L1 immunohistochemistry expression was seen in 15(18.75%) instances of urothelial carcinoma.

Conclusion: The prevalence of urothelial cancer was higher in men than women. PD-L1 expression in urothelial carcinoma was not commonly observed.

Keywords: Immunohistochemical marker, PD-L1, Papillary Urothelial Carcinoma, Urinary Bladder Carcinoma

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INTRODUCTION

Carcinoma of the urinary bladder is the fifth commonest malignant growth in the US and the ninth-most prevalent globally.[1] Pakistan has the highest documented prevalence of urinary bladder cancer in South Asia.[2] According to statistics from the Tumour Registry at the Armed Forces Institute of Pathology in Rawalpindi, urinary bladder cancer is the second most common malignancy, comprising about 7.4% of all tumors among both males and females.[3] Urinary bladder cancer is a complex pathology with a metastatic potential and significantly high mortality and morbidity rate. According to World Health Organization data, mortality due to bladder cancer is approximately 0.19% of total deaths and with an overall rank of 82 worldwide.[4] Females appear to have a poorer prognosis.[5] Patients with advanced cancer have a more dismal prognosis, with a five-year relative-survival rate ranging from 4% to 50%.[6] Risk factors include genetics, cigarette-smoking and occupational exposure to chemical carcinogens like aromatic amines including benzidene and β-naphthylamine.[7]

Programmed Cell Death Ligand 1 (PD-L1) is a glycoprotein that belongs to the B7/CD28 co-stimulatory factor family.[8] Cancer cells can circumvent immunological checkpoints by up-regulating PD-L1, and the immune inhibitory/exhaustion signaling caused by the PD-1/PD-L1 signaling axis may cause activated T-cells, drastically altering the anti-tumor immune activity.[9]

Due to the potential predictive role of PD-L1 expression onto immune cells from patients receiving checkpoint-inhibitors as treatment therapy for advanced urothelial carcinoma, more focus is being placed on the therapeutic implications of PD-L1 expression on immune cells.[10] We aimed to study the

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immunohistochemical expression of PD-L1 in urothelial carcinoma for selection of receptor targeted immunomodulator therapies.

METHODOLOGY

This cross-sectional study was conducted at the Department of Histopathology at the Armed Forces Institute of Pathology Rawalpindi, Pakistan, from January 2021 to June 2022.

Inclusion Criteria: Patients of all age groups and either gender diagnosed with urothelial carcinoma, and registered with the Tumour Registry at Armed Forces Institute of Pathology, Rawalpindi were included.

Exclusion Criteria: Patients with other active cancers, or those who had previously had any other cancer were excluded.

Considering the frequency of PD-L1 expression of 15.5% in prior studies, a sample size of 80 was determined to be sufficient using the WHO sample-size calculator, with a confidence-interval of 95% and a margin of error of 5%.[11] The sampling method being used was non-probability consecutive sampling, and data was collected after obtaining informed consent.

Formalin-fixed, paraffin-embedded tissues were selected. 3-5µm tissue sections were cut with rotary semi-automated microtome. Tissues were sectioned, mounted onto Dako FLEX IHC® microscope slides and then placed in the oven at 58°C for an hour. De-paraffinization, rehydration and target retrieval was done by heat-induced epitope-retrieval method. 3,3N-Diaminobenzidine Tertrahydrochloride (DAB) and substrate-chromogen solution were used. Leica Bond® Autostainer was used for staining, preprogrammed with Dako Link® software. PD-L1 28-8 pharmDx clone is used for immunohistochemiscal expression.

Cases with ≥100 viable tumor cells were included for scoring. Positive tumour cells were defined as those with entire circumferential or partial linear plasma membrane staining at any intensity. Immunohistochemical expression of PD-L1 in immune cells, normal cells, necrotic cells, and in-situ lesion sites was not included.

Quantitative variables, including age, were presented by calculating mean and standard deviation while qualitative variables, including gender and expression of PD-L1 marker, were presented using frequency and percentages. Utilizing Statistical Package for the Social Sciences 20.

RESULTS

The study comprised of a total of 80 cases of urothelial carcinoma. The median age at presentation was 64.58 ±12.45 years, ranging from 32 to 95 years. As shown in Figure-1, the male to female ratio was 7.8:1, 71(88.8%) patients being men and 9(11.12%), women.

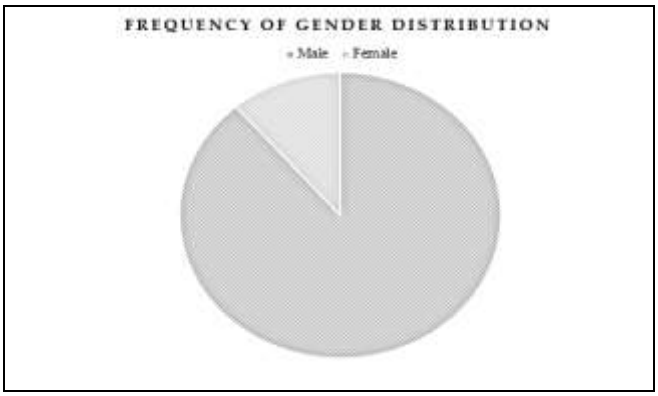

Figure-1: Frequency of Gender Distribution (n=80)

Papillary urothelial carcinoma was the most prevalent histological type, found in 63 cases (78.8%). "Non-papillary" urothelial carcinoma was found in 17 instances (21.2%). Forty instances with high-grade papillary urothelial cancer and 23 instances of low-grade papillary urothelial carcinoma were reported. Non-papillary urothelial carcinomas were all high grade, and each patient had a different histology. As illustrated in Figure-2, the variations comprised nested, glandular, and micro-papillary variants in 1 case each. Squamous differentiation was observed in 9 cases (11.3%), plasmacytoid morphology in 3 and sarcomatoid differentiation in 2 patents.

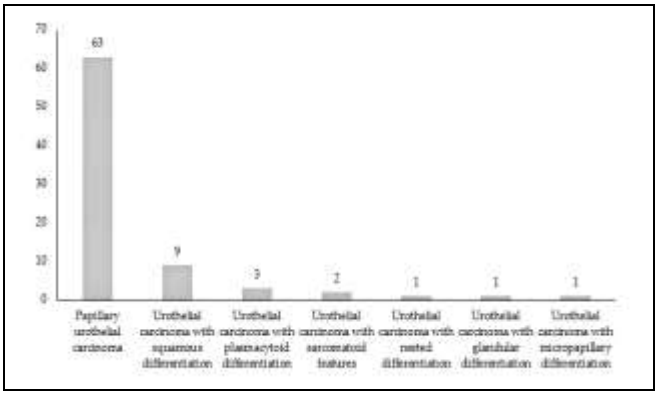

Figure-2: Frequency of Histological Subtypes of Urothelial Carcinoma (n=80)

PD-L1 immunohistochemical expression was seen in 15(18.75%) cases of urothelial carcinoma as

shown in Figure-3. Out of 15(18.75%) positive cases for PDL-1, 13(86.67%) were papillary lesions and 2(13.33%) were non-papillary lesions. And out of papillary lesions, 9(69.23%) were high grade and 4(30.76%) were low grade. Similarly, 10(66.67%) were high grade and 10(66.67%) were low grade stained for PDL-1.

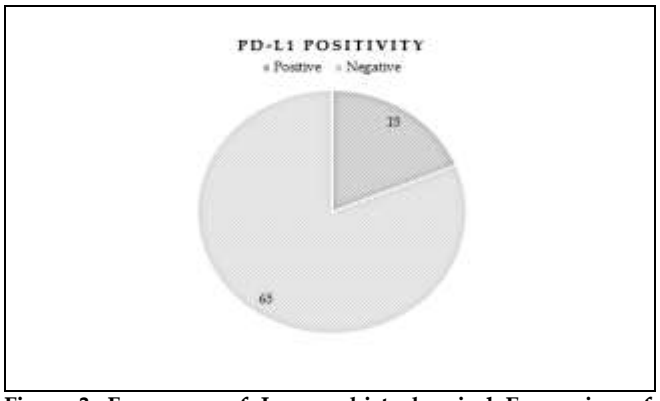

Figure-3: Frequency of Immunohistochemical Expression of PD-L1 in Urothelial Carcinoma (n=80)

DISCUSSION

In several malignancies, PD-L1, a part of the B7 family, is expressed on cancerous cells. It has ability to control T lymphocyte activities by interacting with PD-1. There is no regular assessment for PD-L1 found in urothelial cancer in Pakistan. Urothelial carcinomas make up the bulk of epithelial tumours that grow in the urinary bladder.[12] Even though US-FDA has authorized five immunotherapeutic drugs (PD-1/PD-L1 inhibitors) for treatment in bladder cancers, to-date it has been debatable what the best test for patient selection is. Immunohistochemistry is the most widely used test for PD-L1 and PD-1. However, the standards for "positivity" and which clones to utilize are matters still unclear. A new CAP-ACP recommendation permits the use of laboratory-developed tests for the particular purpose for which they have been approved if they have been fully verified clinically in the laboratory.[13]

In our research, papillary urothelial carcinoma was the most frequent histological subtype, and urothelial carcinoma was more prevalent in men. In patients with urothelial carcinoma, 18.8% of patients had PD-L1 immunoexpression. Studies show that favorable prognosis and improved treatment responsiveness are linked to increased PD-L1 expression.[14-16] There is no statistically-significant correlation between gender and PD-L1 expression, as was also seen in research done in Illinois, USA. Most cases of positive PD-L1 range between 53 to 66 years of age. However no significant differences are found in immunoexpression.[17]

One regional study showed that 32.7% of people had PD-L1 expression (38 out of 116 cases). Forty-three percent (33 out of 77) of high-grade instances and 49% (21 out of 43) of muscle-invasive patients displayed the expression. When 1% was utilised as the cut-off value for positive, PD-L1 expression was found in tumour cells in 62% of instances, and in 51% when a cut-off value of 5% was used. Parallel to this, PD-L1 expression was detected in tumor-infiltrating lymphocytes in 79% of instances where a cut-off mark of 1% was utilised as positive and in 68% of instances when a cut-off of 5% was used as positivity.[18]

In an Oman-based research, PD-L1 testing was performed on 63 individuals. Contrary to our results, using a cut-off value of 5%, PD-L1 was positive in 44% of instances, but dropped to 30% of instances when a cut-off value of 25% was used.[19] In one single-arm clinical trial, 270 participants were randomly assigned at 63 sites in 11 countries to receive the drug CA209275, and the therapeutic value of PD-L was assessed. Using CA209275, the prevalence of PD-L1 expression in tumours found in specimens of urothelial carcinoma was determined to be 1%, with 29 out of 122 participants reaching the primary efficacy threshold and 23 out of 81 patients exhibiting the PD-L1 expression of under 5%.[20]

The FDA recently authorised the use of Pembrolizumab and Atezolizumab for use as an initial line of treatment in PD-L1 positive urothelial carcninoma.[21] These results allow us to recommend PD-L1 immunohistochemistry testing for urothelial carcinoma patients who are not candidates for platinum-based treatments. This supports our efforts to expand the use of immunomodulatory drugs in areas with low economic standing.

LIMITATIONS OF STUDY

Being a single-centre study, with a relatively small sample size, our findings may not be generalizable to the general populace. Also, our study did not follow patients to see outcomes at different intervals during and after treatment.

CONCLUSION

The prevalence of urothelial cancer was higher in men than women. PD-L1 expression in urothelial carcinoma was not commonly observed.

Conflict of Interest: None.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MAK & NZ: Data acquisition, data analysis, critical review, approval of the final version to be published.

HT & WAK: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SA & MOQ: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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