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Programmed Death Ligand 1 expression in Urothelial Carcinoma.A retrospective study in Duhok City-Iraq.

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Abstract

Background and objective: Programmed death ligand-1 protein expression has been linked to the severity of Urothelial cancer. The objectives of this study were to evaluate the expression of programmed death-ligand 1 and determine the association with tumour grade, stage, type (Papillary and non papillary), muscle invasion, age, and gender.

Methods: This retrospective study was carried out from 2015 to 2022 on fifty-eight blocks belonging to patients with urothelial carcinoma, which were obtained from Duhok central lab and private laboratory. From each block, two sections were made; one of which was stained with Hematoxylin and Eosin for histological re-assessment and the other one for immunostaining for programmed death-ligand-1, which was calculated in tumour cells and inflammatory cells regardless of staining intensity and then the combined positive score was determined and considered positive when is more than 10.

Result: This study included 58 cases of Urothelial carcinoma, Females represent 8 (13.79%) cases and males 50 (86.21%) cases, with a female-to-male ratio of 1/6.25. No statistically significant association existed between the histological subtypes and gender. Combined positive score was positive in (32.76%) of cases and negative (67.24%) cases. The association was significant with the stage of the tumor ($p=0.03$), muscle invasion ($p=0.02$) and highly significant with grade ($p<0.001$).

Conclusion: patients with urothelial carcinoma who have higher tumour grade, advanced stage and those who with muscle invasion can benefit from immune check point inhibitors.

Keywords: Combined positive score; PDL-1, Urothelial cancer

Introduction

Programmed death ligand (PD-L1) is a 33-kDa type 1 transmembrane glycoprotein.[1] The gene PDCDL1, that codes for PD-L1, is located on human chromosome 9.[2] Programmed death ligand-1 is a predominantly expressed on dendritic cells, macrophages, activated T and B cells and

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tumour cells.[3] Programmed death ligand-1 is connected with an immunological milieu abundant in CD8 T cells.[4] Interferon-γ activates protein kinase D isoform 2 (PKD2), which is required for PD-L1 regulation. Inhibiting PKD2 activity reduces PD-L1 expression and generates a robust antitumor immune response. Natural killer cells release IFN-γ through the Janus kinase (JAK) 1, JAK2, and (STAT) 1 pathways, boosting PD-L1 expression on tumour cell surfaces. [5] Numerous tumour cells can express PD-L1 to elude the immunological response of the body.[6] The binding of PD-L1 to PD-1 exhausts effector T cells and allows tumour cells to escape the immune system, resulting in a poor prognosis.[7] It is possible for PD-L1 to be overexpressed during carcinogenesis as a consequence of oncogenic driver events.[8] In a number of tumour tissues, high PD-L1 expression has been found to be a poor predictor sign, and an abundance of evidence indicates a correlation between PD-L1 expression and the metastatic potential of cancer cells.[9] Bladder tumours have been identified as one of the tumour types whose immune responses are reasonably well retained. In bladder cancer, a large percentage of tumor-infiltrating lymphocytes (TIL) are present. Thus, bladder cancer has been proven to be immunotherapy sensitive.[10,11] Expression of PDL-1 in urothelial carcinoma has inconsistent result in trials evaluating its potential prognostic or predictive usefulness.[6] Programmed death ligand -1 had been linked to a higher recurrence frequency and a shorter survival rate.[10]

The objectives of this study were to evaluate the expression of programmed death-ligand 1 and determine the association with tumour grade, stage, type (Papillary and non papillary), muscle invasion, age, and gender.

Material and methods

This retrospective cross sectional study was carried out on fifty eight formalin-fixed paraffin-embedded (FFPE) tumour tissues from transurethral resection of urothelial carcinoma and cystectomy specimens, PD-L1 expression was evaluated in the tumor cells and the infiltrating inflammatory cells and the combined positive score was calculated. Cases were gathered from the Duhok central lab and private laboratory for a period of seven years, from 2015 to 2022. From each block, two sections were obtained; one of which was stained with Hematoxylin and Eosin for histological examination and the other one for immunohistochemical staining with PDL-1. The association of PDL-1/CPS with age, gender, type, muscle invasion, grade, and stage was determined by Chi-square test and Fisher's exact test. The urothelial carcinoma cases were microscopically classified and graded using the 2016 World Health Organization classification system.[12] and the pathological staging was done using the ninth edition of the American Joint Committee on Cancer (AJCC).[13] Programmed death ligand-1 protein expression was detected on FFPE slides. Antibody used 22C3 (DAKO), For 28-8 / 22C3, automated pre-treatment was carried out at pH 6. At room temperature, primary monoclonal antibodies were incubated for 30 minutes and visualized using the Morsch et al. appropriate DAB-based detection kits and hematoxylin counterstains. In contrast to positive controls used in immunohistochemical studies, negative controls were carried out by removing the main antibody for both pH conditions. PD-L1 expression was determined for tumor cells (CPS, combined positivity score) regardless of the staining intensity as follows: combined positivity score (CPS) given by summing the number of PDL1–stained cells (tumor cells, lymphocytes, macrophages) and subtraction of the outcome from the total viable tumor cells, multiplied by 100.[14] Scores with staining artifacts or damage were excluded. The statistical analysis of data was

performed by SPSS (IBM Corporation, New York, NY, USA) (version 28.0) software. Chi-square test and Fisher's exact test were applied to figure out a proposed statistical association. $p \leq 0.05$ was considered statistically significant.

The Kurdistan Higher Council of Medical Specialties Ethics Committee granted its ethical approval for this study.

Results

This cross sectional retrospective study included 58 cases of UC, 54 (93.1%) are TURBT specimens, and 4 (6.9%) are cystectomy specimens. Females represented 8 (13.79) and males 50 (86.21%), with a female-to-male ratio of 1/6.25 (Figure 1).

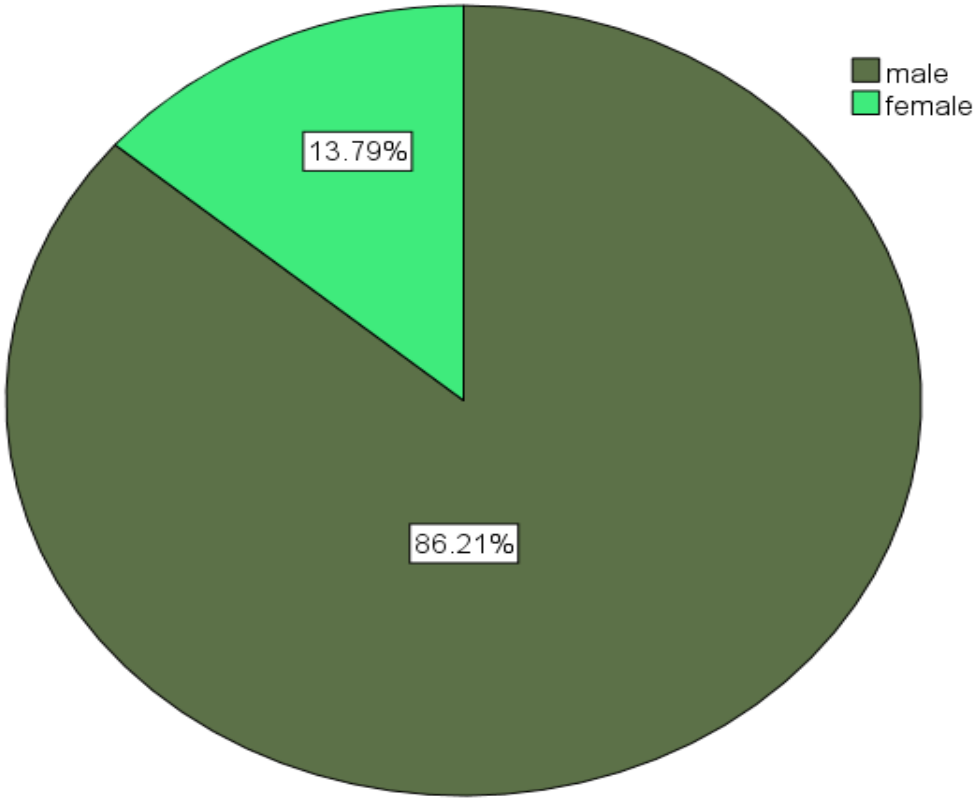

Figure (1): The gender distribution of the patients.

The youngest was 47 years old male, and the oldest was a 91-year-old male. The peak incidence was in the 8th decade of life (Figure 2).

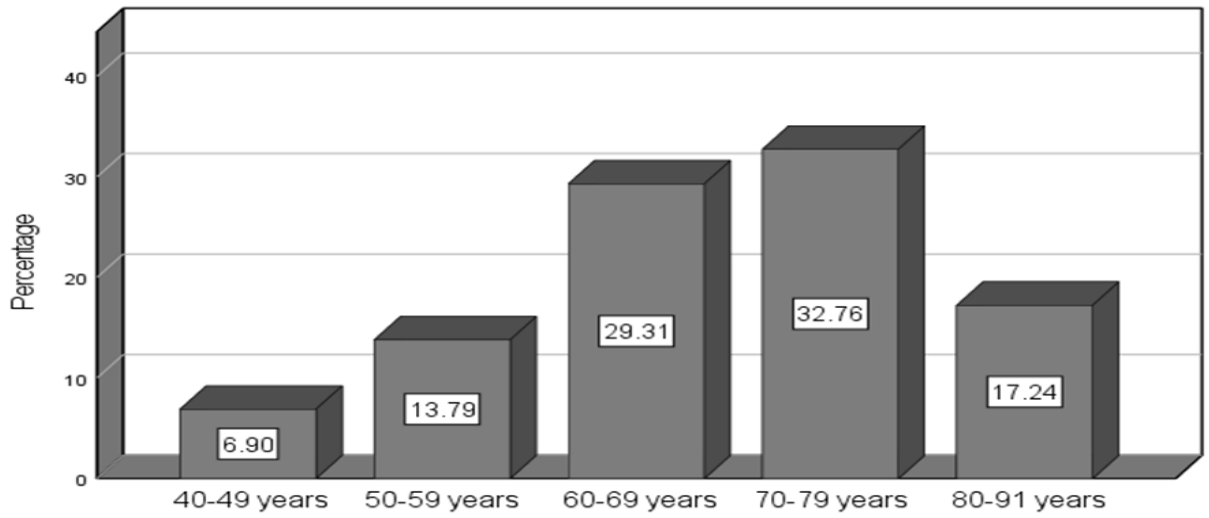

Figure (2): The results indicated that 6.9%, 13.79%, 29.31%, 32.76%, and 17.24% of patients belonged to the 40-49, 50-59, 60-69, 70-79, 80-91 years, respectively.

Twenty-three cases (39.66%) were in stage T2a, and (figure 3) shows the stage of all the included specimens.

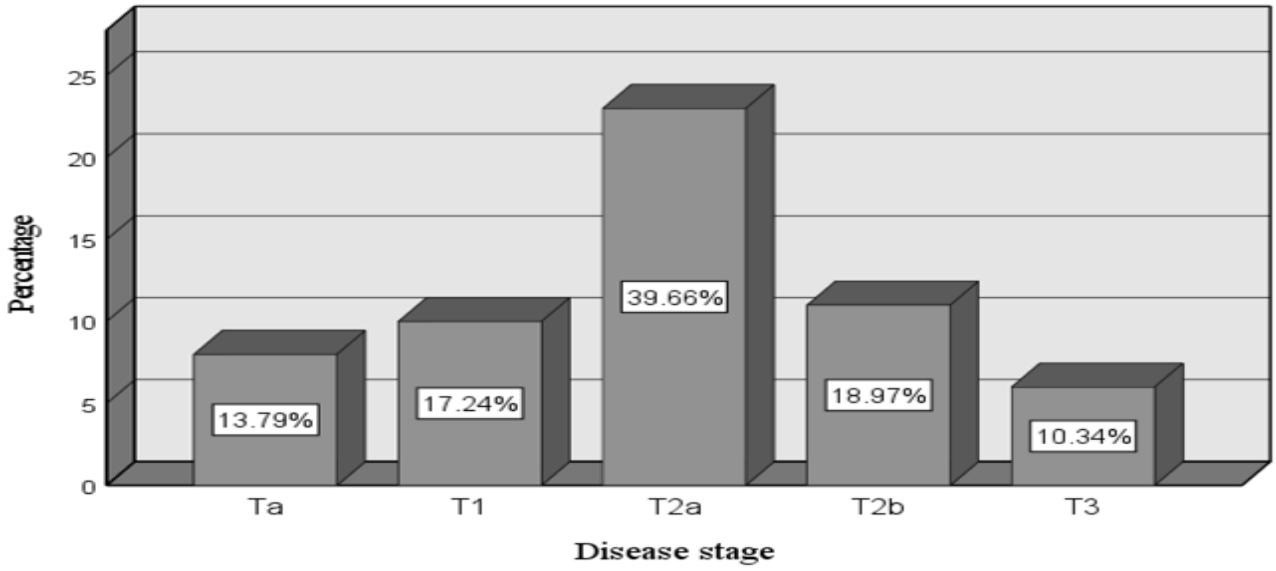

Figure (3) : The stages of urothelial carcinomas in all the enrolled cases.

The mean age (67.6±11.15). Of the included specimens, 33 (56.9%) were papillary urothelial carcinomas and 25 (43.1%) non-papillary. Histologically, 19 (32.76%) cases were low grade Urothelial cancer, and 39 (67.24%) cases were high grade. Muscle invasion was detected in 41(70.6%) cases. (Figure 4).

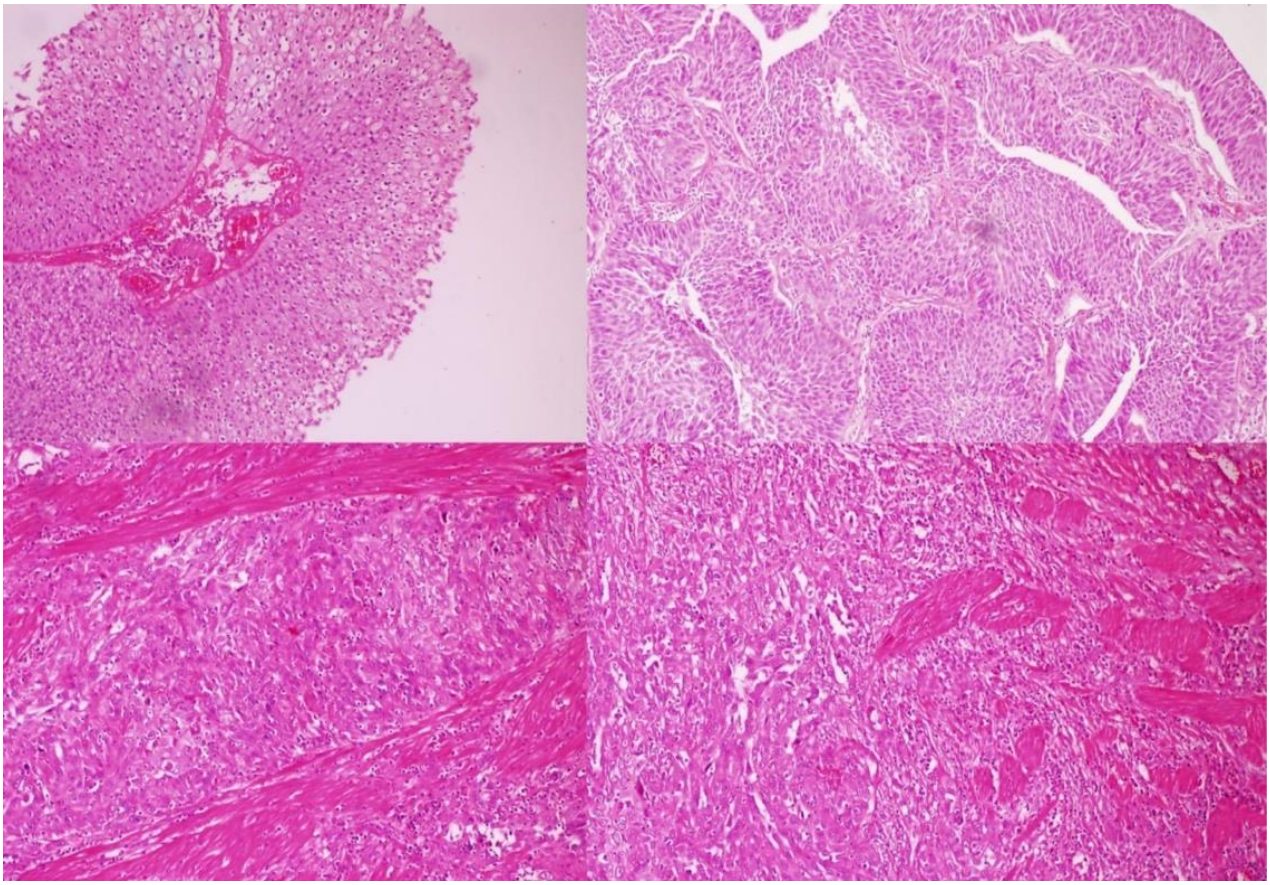

Figure (4): Low grade papillary urothelial carcinoma with fibrovascular core. B, high grade papillary urothelial carcinoma with multilayering. C and D, high grade of urothelail carcinoma with invasion to muscle fibers (H&E 10 X HPF).

Programmed death ligand-1/CPS was positive in 19 (32.76 %) cases and negative in 39 (67.24%) cases. (Figure 5)

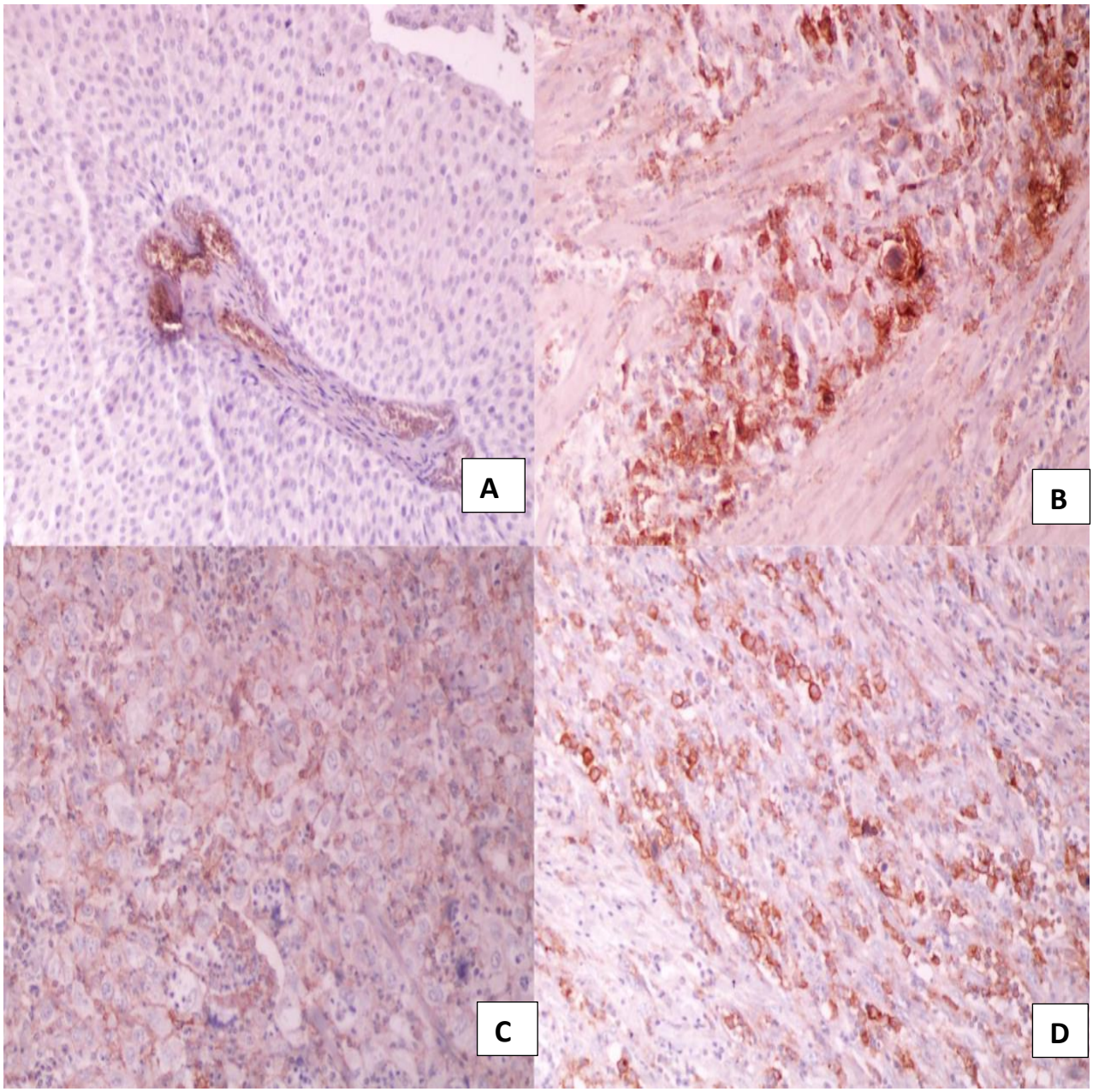

Figure (5): A, Low grade papillary urothelial cancer negative membrane staining for PDL-1. B, membranous staining of high grade tumor cells invading muscle. C&D, PDL-1 membrane and cytoplasmic staining of inflammatory cells infilterating tumor cell and membranous staining of high grade tumor cells (20X HPF).

Statistically speaking, PDL-1/CPS is not correlated with gender (p= 0.41), age (p=0.7), and histological subtypes (p=0.31), while the association was significant with the stage (p=0.03), muscle invasion (p=0.028) and highly significant with grade (p<0.001). The correlation between the PDL-1 score and various parameters is presented in table 1.

Table (1) shows that there was no significant association when gender and PDL-1lCPS test categories were investigated (p=0.41) while significant associations obtained when the PDL-1/CPS expression versus grade, muscle invasion, and stage were considered with p values of <0.001, 0.028, 0.03, respectively.

Table (1): The association of PDL-1/CPS expression with gender and other clincopathological parameters.

		PDL-1/CPS Negative	PDL-1/CPS positive	Total	p value
Gender	Male	35	15	50	0.41
	Female	4	4	8	
Grade	High	20	19	39	<0.001***
	Low	19	0	19	
Muscle invasion	Negative	15	2	17	0.028*
	Positive	24	17	41	
Disease stage	Ta	7	1	8	0.03*
	T1	8	2	10	
	T2a	17	6	23	
	T2b	6	5	11	
	T3	1	5	6	

Discussion

PD-L1 regulates T cell activities by interacting with PD-1 and is expressed on dendritic cells (immature, mature, and follicular) as well as many types of cancer cells.[15] Agents that inhibit either PD-1 or PD-L1 are expected to boost the T cell-mediated immune response to tumor cells. It has recently been proposed that PD-L1 expression on tumour cells and tumor-infiltrating cells has distinct consequences for tumor response to anti-PD-1/PD-L1 therapy.[16] A recent study appears to link PD-L1 expression to increased survival as well as lymphocyte infiltration into the tumor microenvironment.[17] In this study of 58 FFPE tissue samples, PD-L1 membranous expression

was discovered in 19 cases (32.76 %) of transitional cell carcinoma this result is consistent with study performed by Al Nabhani S et al who found that 19/63(30.2%)cases were PDL-1 postive.[18] In this studt, the majority of patients with Urothelial carcinoma were male (86.21%), with the mean age of the patients being (67.6±11.15) and there was no association between PDL-1 with age and gender. These results are similar to what was reported by Nechifor-Boilă IA et al who failed to demonstrate an association between PDL-1expression and the age and gender[19] and in contrast to the finding of Faraj SF et al who found that younger patients with urothelial carcinomas were more likely to express PD-L1.[20] There was no association between PDL-1 expression and the histological subtype of urothelial carcinoma wheather papillay or non-papillary (p:0.31) and to the best of our knwolegde, no previous study was done to determine such correlation. At the time of diagnosis, 23 (39.66%) cases were in stage T2a, while 39 (67.24%) cases were high-grade. There was a highly significant association between grade and PDL-1 expression, similarly Al Nabhani S et al , and Kawahara T et al found postive correlation between PDL-1 and grade[18,21]. There was a significant correlation between PD-L1 expression on tumor cells and inflammatory cells with a positive CPS and increasing pathological T stage. Some authors reported results consistant with the results of this study[18, 21], while others did not find any correlation between PDL-1 and stage[19, 22]. The statistical correlation between PDL-1 and muscle invasion was significant (p:0.02), in consistence with the result of Al Nabhani S et al and Ding X et al who determined highly significant association between PDL-1 and muscle invasion.[18, 22] The presence of PD-L1 on tumour cells can serve as a marker for tumour aggressiveness, which may help determine the efficacy of immunotherapy.[23] These findings suggest that PD-L1 expression on tumour cells (TCs) may be helpful for assessing tumour aggressiveness and that PD-L1 status may be utilised to identify patients who will respond better to anti-PD-1/PD-L1 therapy. Furthermore, it is challenging to compare the results of this study with those of other researchers due to the variety of PD-L1 antibodies available on the market, each of which has various staining platforms, scoring criteria, and positivity definitions.[24] Despite the fact that the samples analysed in this study were limited and retrospective data collection may add bias, the findings of this study revealed that PD-L1 levels might be used as a predictor factor for Urothelial carcinoma cases aggresivness and treatment protocol. Other studies with larger sample sizes are required to investigate the link between PD-L1 expression (using both IHC and RT-PCR) and outcomes in patients who do not undergo or receiving immunotherapy.

Conclusions

There was a strong link between PD-L1 expression and tumor grade, stage, and muscle invasion. No significant association was found between PDL-1 expression with age, gender, and histological subtype of Urothelial carcinoma.

The results indicate that anti-PD-L1 immunotherapy may be beneficial for patients with higher-grade and advanced-stage tumors.

Conflict of interest:

The authors recorded no conflict of interest.

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