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Research Article

Immunohistochemical Study of PDL1 Expression in Urinary Bladder Carcinoma

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Abstract
Background: Urinary Bladder Carcinoma (UBC) is a worldwide health problem ranking as the ninth most common cancer globally and the second most common malignancy in Egypt. The effectiveness of PD-1/PDL-1 immune checkpoint inhibitors in metastatic disease has stimulated its evaluation as a treatment option. **Aim of the study:** Studying immunohistochemical expression of PDL-1 in UBC to evaluate the pattern of expression and its association with clinicopathological variables in addition to comparing the PDL-1 expression in the primary tumor and their corresponding metastasis. **Method**: The study included 50 formalin-fixed, paraffin-embedded tissue specimens of UBC. Tissue sections have been subjected to H&E staining and immunohistochemical study for PDL-1 expression. **Results:** PDL-1 expression was detected as positive membranous immunostaining ranging between -ve/ low and high expression. Thirty-four cases (68%) showed high expression while 16 (32%) showed -ve/ low expression. Significant association was found between PDL-1 expression and tumor type, tumor grade, stage, muscle invasion and LN status (p=0.012, 0.005, 0.005, 0.045 and <0.001 respectively). Significant associations was found between PDL-1 expression and LN spread (p. <0.001). Only 12 cases had lymph node metastasis, (4/12) (33.3%) had –ve/ low PDL-1 expression while the remaining eight cases (66.7%) had high PDL-1 expression. **Conclusion:** PDL-1 expression showed a significant association with tumor grade, stage, type, muscle invasion and lymph node metastasis.

Key words: Urinary bladder carcinoma, Programmed death ligand 1 (PDL-1), urothelial cancer, lymph node.

Introduction
Bladder cancer is considered the tenth most common cancer in our world, with an increasing occurrence yearly[1]. UBC is a common malignancy among Egyptian males which ranking the second most common malignancy, that accounts for 30% of whole malignant tumors [2].

The irritants that are filtered into the urine through kidneys are responsible for irritation of the urothelial lining of the urinary tract, especially the urinary bladder [3]. Expectedly, 90% of urinary bladder cancers arise from these urothelial cells, however, 10% of cases are squamous cell carcinomas [2]. The prognosis of urothelial carcinoma (UC) depends on the staging of the tumor. Localized forms have the best prognosis; however, the survival rate drops significantly with muscular invasion [2].

The recent research of immunotherapy in UBC led to approval of new treatments. With an initial studies and promising results of drugs used as anti-programmed cell death-1 (anti-PD-1) and anti-programmed cell death ligand-1 (anti-PDL-1) [4].

Programmed death ligand 1 (PDL-1) is an immunoglobulin-like type I transmembrane glycoprotein. PDL-1 is one of the B7 family, which includes seven ligands that are essential for the second signal of T-lymphocytes. The B7 family proteins are expressed mainly in immune cells B/T lymphocytes, monocytes, and dendritic cells [5]. We suggest making PDL-1

immunohistochemical study to evaluate the PDL-1 expression and prognostic advances among Egyptian UBC cases.

Materials and Methods

Case selection

Our study included 50 randomly selected UBC radical cystectomy cases obtained from Minia University Hospitals pathology laboratory (in the period between 2018 and 2021). The cases included; 30 urothelial carcinomas and 20 squamous cell carcinomas. The grades of differentiation ranged between low and high concerning TCC cases and grades I, II and III as regard SCC cases. Among the fifty selected cases, twelve cases of primary UBC and metastatic lymph nodes have been examined for PDL1 expression.

The clinicopathological data included age, gender, gross pattern, type, grade, stage, lymph node status, evidence of bilharziasis, presence of in situ element, tumor necrosis, lymph-vascular invasion and perineural invasion.

Five µm sections were made and stained with hematoxylin stain and eosin stain for revising the histological findings of all cases and examined according to WHO criteria [7] for confirming type of tumor, grade of tumor and stage.

Immunohistochemistry

Primary antibody

PDL-1

PDL-1 which is a rabbit anti-human mono-clonal AB for immunohistochemistry. The primary AB is used for qualitative recognition of AG in FFPE tissue specimens. The antibody produces cytoplasmic and/or membranous expression (Cat. No. 400100295). According to the manufacturer data sheet.

Scoring of immunostaining and cut off point:

PDL-1 expression was mainly membranous. Counting of 10 high power fields per section for each case was performed. In UBC PDL-1 expression is made with counting the number of positively stained tumor cells. Also positively stained immune cells (lymphocytes and macrophages) were divided by the whole number of non-necrotic malignant cellsx100. The PDL-1 expression was divided into 2 categories: high and low expressions. Negative and low expression cases were considered as one group. [8].

Statistical analysis

Analysis of statistics was conducted using (IBM SPSS software version 25). Data were compiled and used to determine the means ± standard deviations (SDs), range and median of various features. The Fischer's exact and Chi-square test was used to compare categorical features. Wilcoxon and McNamar tests were employed to compare PDL-1 expression in primary tumor and their corresponding lymph node metastasis. P-value was considered significant if <0.05**.**

Results

A positive significant association was found between PDL1 expression and tumor type, grade, stage, muscle invasion and lymph node metastasis **(*p*. = 0.005, 0.005, 0.001, 0.045, 0.045 respectively).** No significant relation was found between PDL1 expression and other variables.

Table 1: Clinicopathological data of Urinary bladder carcinoma (n= 50):

Clinicopathological Features	No. (%)	Clinicopathological Features	No. (%)
Age at surgery		**Lymph node metastasis**	
≤60 yrs.	18 (36%)	Negative	38 (76%)
>60 yrs.	32 (64%)	Positive	12 (24%)
Gender		**Lymph Node stage**	
Male	45 (90%)	N0	38 (76%)
Female	5 (10%)	N1	5 (10%)
		N2	7 (14%)
Growth pattern of tumour		**Evidence of bilharziasis**	
Fungating mass	15 (30%)	+ve	32 (64%)
Ulcer	25(50%)	-ve	18 (36%)
Infiltrative mass	10 (20%)		
Tumor Type		**Insitu Component**	
Urothelial Carcinoma (UC)	30 (60%)	+ve	12 (24%)
Squamous cell carcinoma (SCC)	20 (40%)	-ve	38 (76%)
Tumour Grade:		**Tumour Necrosis**	
UC: N=30		+ve	36 (72%)
High Grade UC	25 (83.3%)	-ve	14 (28%)
Low Grade UC	5 (16.7%)		
SCC: N=20			
SCC well differentiated	4 (20%)		
SCC moderately differentiated	12 (60%)		
SCC poorly differentiated	4 (20%)		
Stage of tumor		**Vascular Invasion**	
T1	7 (14%)	+ve	26 (52%)
T2a	5 (10%)	-ve	24 (48%)
T2b	16 (32%)		
T3	19 (38%)		
T4a	3 (6%)		
State of muscle invasion		**Perineural Invasion**	
NMIBC	14 (28 %)	+ve	7 (14%)
MIBC	36 (72 %)	-ve	43 (86%)
Lymphocytic infiltrate		**PDL-1 expression:**	
Intense	28 (56%)	High	34 (68%)
Non intense	22 (44%)	Low	16 (32%)

Table 2: Association between membranous expression of PDL-1 and different clinicopathological variables.

Clinicopathological Features	No. (%)	PDL-1 Expression		P value
		-ve/ low Expression N=16	High Expression N=34	
Age at surgery				0.581
≤60 yrs.	18 (36%)	1 (6.2%)	17 (50%)	
>60 yrs.	32 (64%)	15 (93.8%)	17 (50%)	
Gender				0.157
Male	45 (90%)	13(81.2%)	32(94.1%)	
Female	5 (10%)	3 (18.8%)	2 (5.9%)	
Growth pattern of tumor				0.130
Ulcer	25 (50%)	14 (41.2%)	1١ (68.8%)	
Fungating mass	15(30%)	2 (12.5%)	13 (38.2%)	
Infiltrative mass	10 (20%)	3 (18.8%)	7 (20.6%)	
Tumor Type				
Transitional cell carcinoma	30 (60%)	9(56.2%)	21 (61.8%)	**0.012***
Squamous cell carcinoma	20 (40%)	7 (43.8%)	13(38.2%)	
Tumor Grade:		N=9	N=21	
TCC: N=30				
High Grade TCC	25 (83.3%)	9(100%)	16(76.2%)	**0.005***
Low Grade TCC	5 (16.7%)	0 (0%)	5 (23.8%)	
SCC: N=20		N=7	N=13	
SCC well differentiated	4 (20%)	1 (14.2%)	3 (23.1%)	**0.005***
SCC moderately differentiated	12 (60%)	5 (71.4%)	7 (53.8%)	
SCC poorly differentiated	4 (20%)	1 (14.2%)	3 (23.1%)	
Stage of tumor				
T1	7 (14%)	3 (18.8%)	4 (11.8%)	**0.045***
T2a	5 (10%)	1 (6.2%)	4 (11.8%)	
T2b	16 (32%)	5 (31.2%)	11 (32.4%)	
T3	19 (38%)	6 (37.5%)	13(38.2%)	
T4	3 (6%)	1 (6.2%)	2 (5.9%)	
State of muscle invasion				**0.014***
Non muscle invasive bladder cancer	14 (28 %)	14 (87.5%)	0 (0%)	
Muscle invasive bladder cancer	36 (72 %)	2(12.5%)	34(100%)	
Lymph node metastasis				
Negative LN metastasis	38(76%)	11 (68.8%)	27 (79.4%)	
Positive LN metastasis	12 (24%)	5(31.2%)	7(20.6%)	**<0.001***
Lymph Node stage				
N0	38 (76%)	11 (68.8%)	27 (79.4%)	**0.369**
N1	5 (10%)	1 (6.2%)	4 (11.8%)	
N2	7 (14%)	4(25%)	3(8.8%)	
Evidence of bilharziasis				
Positive	32 (64%)	8 (50%)	24 (70.6%)	**0.330**
Negative	18 (36%)	8 (50%)	10 (29.4%)	
In situ Component				
Positive	12 (24%)	3 (18.8%)	9 (26.5 %)	**0.434**
Negative	38 (76%)	13 (81.2%)	25 (73.5%)	
Tumor Necrosis				
Positive	36 (72%)	11 (68.8%)	25 (73.5%)	**0.511**
Negative	14 (28%)	5 (31.2%)	9 (26.5%)	

Vascular Invasion				
Positive	26 (52%)	8(50%)	18 (52.9%)	**0.151**
Negative	24 (48%)	8 (50%)	16 (47.1%)	
Perineural Invasion				
Positive	7 (14%)	1 (6.2%)	6 (17.6%)	**0.978**
Negative	43 (86%)	15 (93.8%)	28 (82.4%)	
Lymphocytic infiltrate				
Intense	28 (56%)	8 (50%)	20 (58.8%)	**0.768**
Non intense	22 (44%)	8 (50%)	14(41.2%)	

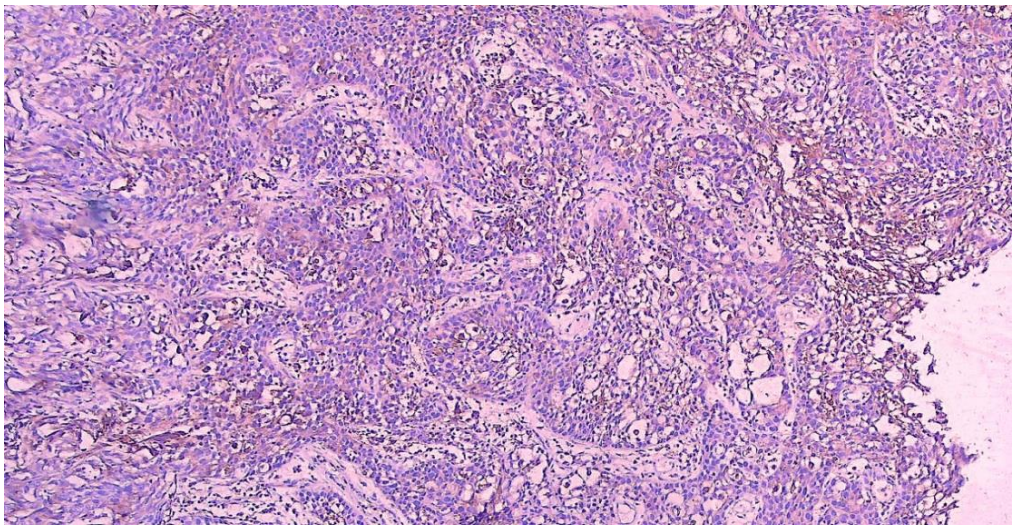

Fig. (1) Low expression with low grade UC (DAP and counterstain Hematoxylin x20).

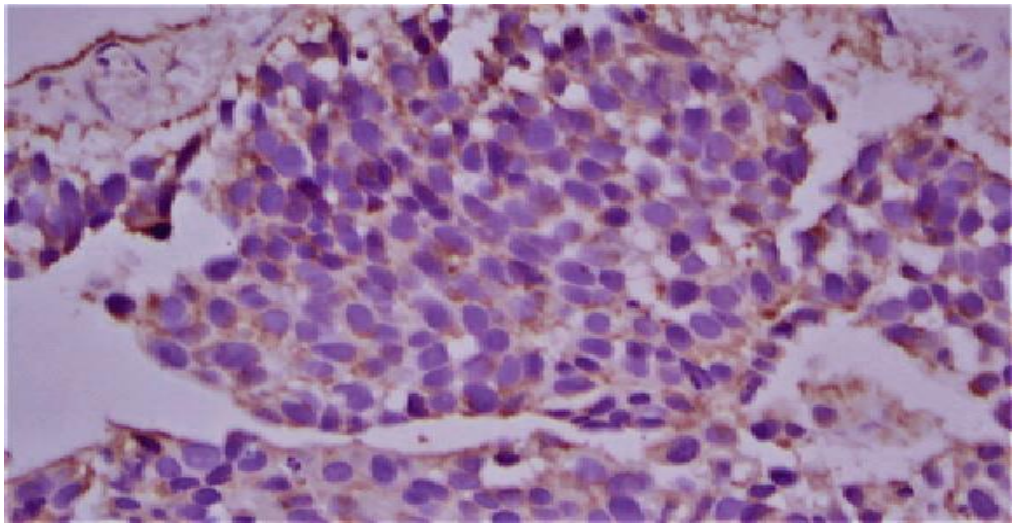

Fig. (2) High expression with High grade UC (H&E x40)

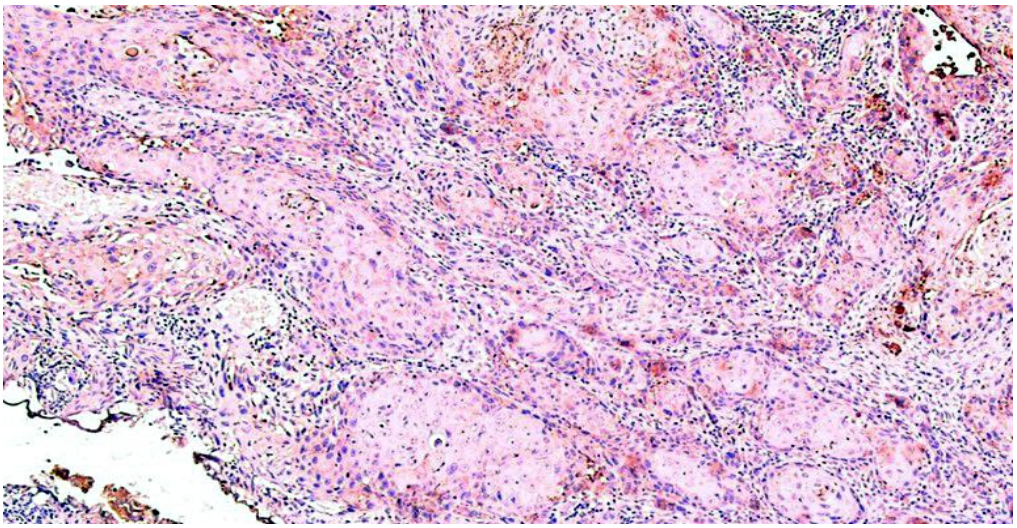

Fig. (3) Low expression with grade 1 SCC (DAP and counterstain Hematoxylin x20).

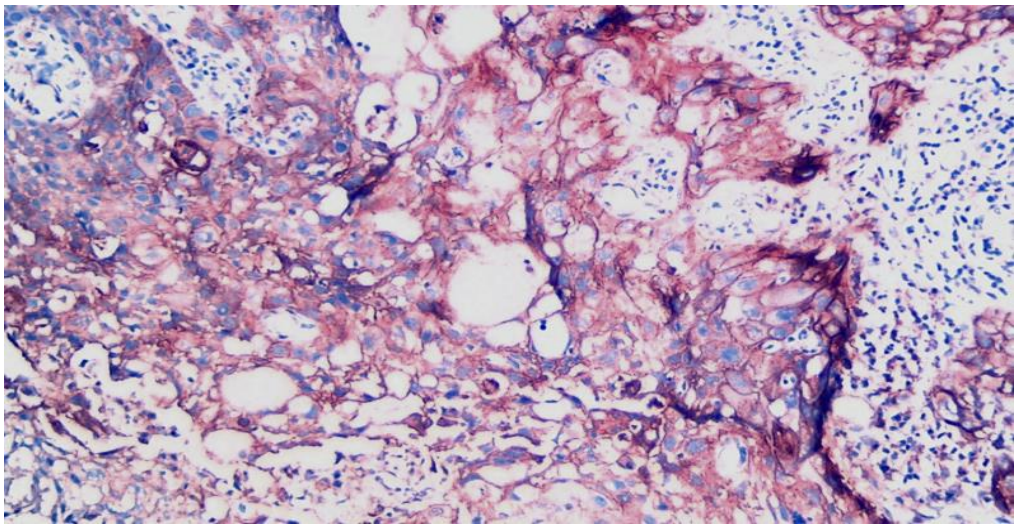

Fig. (4) High expression with high grade SCC (DAP and counterstain Hematoxylin x40).

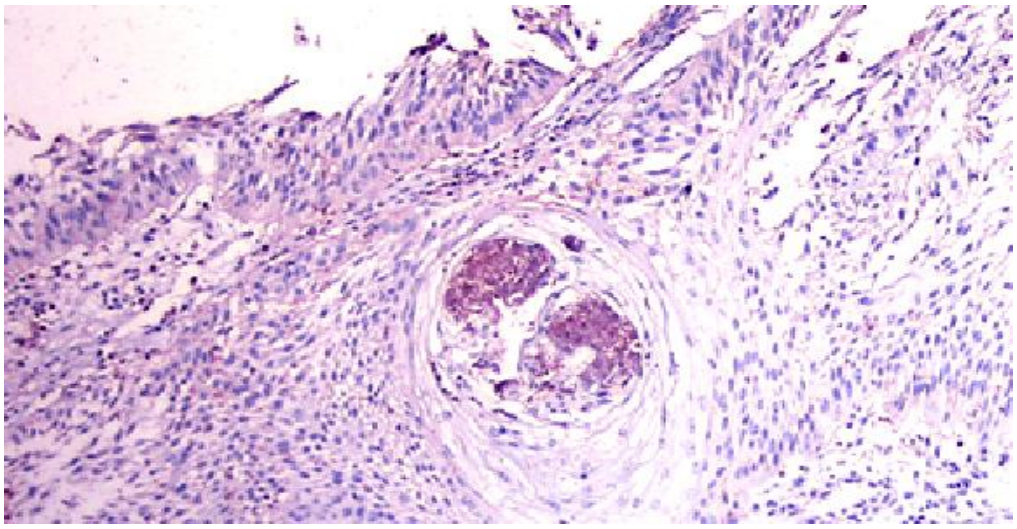

Fig. (5) High expression in vascular invasion of high-grade UC (DAP and counterstain Hematoxylin x40).

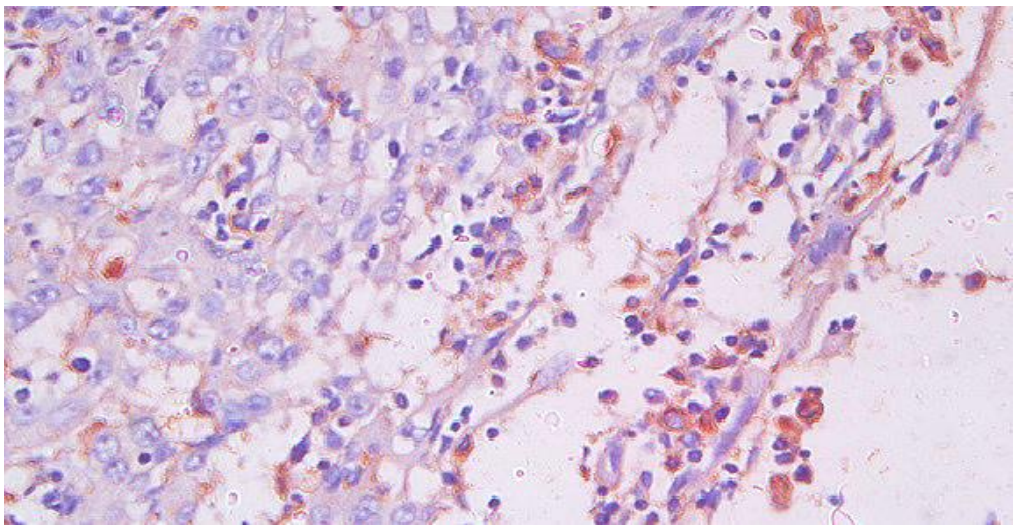

Fig. (6) High expression in immune cells (DAP and counterstain Hematoxylin x40).

Discussion

The study found that PDL-1 immunostaining is localized in the cytoplasm and membrane. As regards tumor type, in UC cases; 22 out of 30 (73.3%) showed high PDL-1expression but in SCC cases only 12 out of 20 (60%) showed significantly associated higher expression with in TCC cases (*p.* = 0.012). Like our results, Pichler et al., 2017 found that higher PDL-1 expression was seen in UC than patients with SCC (46.2% vs. 20.8%; p=0.002) [15].

Concerning grade, a significant association was found between PDL-1 expression and tumor grade. Twenty two out of 25 high grade UC cases (88%) showed high PDL-1expression, while UC cases of low grade showed –ve/low expression (*p value*= 0.005). As regards Squamous cell carcinoma, positive high PDL-1 expression was

detected in eight cases out of 12 (66.6%) in grade II SCC, while all grade I TCC cases showed –ve/low expression. All cases (100%) of grade III SCC showed high PDL-1expression (*p.* = 0.005). This was similar to the results of Nakanishi et al., 2007, who found that specimens from tumor of higher WHO grade or primary tumor classifications had significantly higher percentages of tumor-associated PDL-1expression [16].

Regarding muscle invasion; all cases of Non-muscle invasive bladder carcinoma cases showed –ve/low expression with no case showed high PDL-1 expression. In muscle invasive bladder carcinoma cases; 2 out of 36 cases (5.5%) showed low expression while the rest of cases (34/36 = 94.5%) had high expression, with significant association between high PDL-1expression and muscular invasion (p. = 0.001). These results go with the results of Gupta and his colleagues, 2022, who found that (92%) of muscle invasive bladder carcinoma showed high PDL-1 expression.[17].

Concerning tumor stage, we detect a significant association between stage of tumor and PDL-1 expression (*p.* = 0.045). In cases with stage T1 all of them (100%) showed –ve/low PDL-1expression as compared to cases with T2 stage where only 28.5% of them showed –ve/low expression while 71.5% of cases had high expression. However, in cases with stage T3; 3 out of 19 cases (15.8%) showed –ve to low expression, while 16 cases (74.2%) showed high expression. All cases of T4 stage showed high expression (100%) (*p.* = 0.045). This was matching with the results of Kim et al., 2020, who found that significant PDL-1 expression of tumor-infiltrating immune cells was correlated with higher stage of tumor [17]. While Zavalishina et al., 2018 who discovered that significant higher expression ($P < 0.04$) was detected among T2 stage (13/45 or 28.9%) and stage T≥ 3 (4/10 or 40%) disease compared with the proportion of cases with T1 stage (5/45 or 11.1%) [18].

Significant association was found between PDL-1 expression and nodal metastasis (*p.* <0.001). Only 12 cases had lymph node invasion, of them 4 cases (33.3%) had –ve low PDL-1 expression while the remaining eight cases (66.7%) had high expression. As regards the 38 cases without nodal metastasis, (12/38) accounting for 31.6% showed -ve/low PDL-1 expression whereas 26 cases (68.4%) showed high expression (*p.* <0.001). This was similar to Holah et al, 2022 who found that PD-L1 positivity in tumor cells was associated significantly with occurrence of nodal metastasis ($P = 0.028$) [16].

There were no significant associations were found between PDL-1 expression and other variables (age, gender, gross description, bilharzial infection, in-situ element, necrosis, lymph-vascular infiltration, perineural infiltration or lymph node metastasis (*p.* = 0.581, 0.157, 0.130, 0.330, 0.434, 0.511, 0.151, 0.978, 0.768 respectively). This was different from a study done by Holah, 2022, who found that PD-L1 immunohistochemical reactivity in tumor cells was significantly related with occurrence of lymph-vascular invasion ($P =$ 0.019). The different results may be explained by the differences of studied type of population[17].

Conclusion and recommendations

Our study pointed up the role of PDL1 in UBC and development of LN metastases. Among the clinicopathological variables investigated, we found a significant association with grade, stage, and LN metastasis. The results suggest that PDL1 expression can be helpful for recognition of UBC with poor prognosis. PDL1 also has a possible role in the development of LN infiltration. A high rate was detected between primary tumor and their LN metastasis regarding PDL1 expression explains the role of PDL1 and the metastatic ability of tumor cells.

A study of a large number of cases is recommended to confirm the previous outcomes and to approve the role of PDL1 in UBC.

Deeper studies are indicated to investigate the relationship between PDL1expression in different organ metastasis and their corresponding primary tumors. Such studies with follow up data would add help to follow up and survival analysis to identify aggressive tumors and to select cases for PDL1 targeted therapy.

More studies are needed to explore the role of PDL1 and its related family in UBC.

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