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The Role of pD_L1 expression in bladder cancer

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Abstract:

The aim of this study is to determine the expression of the PD_L1protein in human bladder cancer, and significant correlations between these parameters and clinico pathologic variables like (grade and stage), and also by using this marker can classify bladder cancer in to basal and luminal type. the bladder cancer is one of the most common cancers worldwide. the incidence and mortality of this disease increased during last decades alarmingly in Iraq. The current study is designed to detect the role of pD_L1 expression in bladder carcinoma as a possible marker for detecting the biological behavior of malignancy and its correlation with grade and muscle invasiveness for both diagnostic and prognostic purposes. The study focuses on a technique of immunohistochemistry for detection pD_L1 expression in bladder cancer. The samples are collected randomly in southern Iraq, in AL-Nasiriya city, from AL Hussein teaching hospital. Number of samples is 100 ,70 bladder cancer tissue and 30 controls benign tissue. Results of this study reveal that pD_L1 expression is positive in 45 of 70 sample. The study demonstrated pD_L1 expression is increased in high grade bladder cancer represent (44.19%), while in low grade (33.33%), pD_L1 expression high in T2 stage (41.03%) , and in Ta(26.3%) T1(33.3%) T3 (50%) T4(100%). expression pD_L1 excessive in muscle invasive (42.86%), whilst the low expression for pD_L1 in the non-muscle invasive type (35.71)

So pD_L1 can be used as a marker for assessment of bladder cancer aggressiveness. This study represents an important step because there are few of studies about this topic in Iraq; we are needing more studies to prove the function of this pD_L1 in biological behavior of bladder cancer.

Key words: Bladder cancer, PD_L1, Immunohistochemical.

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INTRODUCTION

Bladder cancer has been the most Popular pathological conditions among tumors of the Urinary tract that require a mixture of some techniques and immunological examinations to reach the kind of tumor, and despite the advance of pioneering techniques in the field of medication, the exact identification of the case had not been prepared without depend on taking a biopsy from bladder tissue. Depended on their deep of invasion within bladder wall, they have been distributed into either non-muscle-invasive bladder carcinoma (NMIBC) or muscle-invasive bladder carcinoma (MIBC). NMIBC Representfor around over70% of wholly newly identified BCs , counting non-invasive papillary urothelial carcinomas (stage pTa), Carcinoma In Situ (CIS) and invasive carcinomas exclusive to the lamina propria (stage pT1) [1,2].

Kinds of bladder cancer contain Transitional cell carcinoma (TCC)has been the most communal kind about (97%), followed by Squamous Cell Carcinoma (SCC) around (2%) and the lowest frequency has been adenocarcinoma as (1%) [3]. Bladder tumor has been the fourth most communal tumor in men, with approximately 60,000 new-fangled diagnosis each year ranking as the eighth principal reason of cancer associated deaths in the United States, with about 12,000 deaths yearly, Specifically, in 2017, there have been 79, 030 cases of bladder tumor and 16,870 associated deaths in the United States [4]. In Iraq, bladder malignancy has been the fifth of ten tumors, the third tumor in men and the eighth in women. 866 men had been diagnosed with bladder tumor, Comparison to 297 women, agreeing to the Iraqi Cancer Registry (2011) [5]. As for the year 2021, it would have ranked fifth among other most communal tumors, with an infection rate of 1,769, as the number of male infections has been 1,360, while females have been 409, and this designates that male infections have been greater than females [6]. Whereas the current suggested management for advanced bladder tumor has been systemic cisplatin-based chemotherapy, immunotherapy has been developing as a viable salvage management where first-line chemotherapy failed to control the illness [6].

Immunotherapy usages monoclonal antibodies aiming Programmed Death - 1 (PD-1) or Programmed Death-Ligand 1 (PD-L1) to block the PD-1/PD-L1 path, release T cells to perform their immunological roles [7]. PD-L 1 has been a trans membrane protein whose chief function has been to prevent immune cells, chiefly stimulated T cells. PD-L 1 has been expressed in a width range of human tissues counting heart, pancreas, spleen, placenta, lymph node, and thymus, whereas it has been few in the brain and renals [8]. There has been a specified receptor that could be ligated by PD-L1 called programmed death receptor 1 (PD-1) which has been expressed on CD4+ and CD8+ T cells, monocytes, natural killer T cells, B cells, and Stem Cells [7].

PD-L1 has been locate on the superficial of tumor cells and antigen-presenting cells. In common tissues, PD-1 signing in T cells regulates immune reactions to reduction injury to adjacent tissue and plays a major role in the development of autoimmunity by inducing tolerance to self-antigens. Binding of PD-L1 to PD-1 leads to the preventing of T cell stimulation through generation of programmed cell death, decrease of propagation, and preventing of cytokine excretion. The mechanism of expression of PDL-1 on tumor cells has been associated to the cancer immune-editing procedure which had three stages: removal, balance, and escape. Throughout the first phase (removal), cancer cells have been documented and damaged through immune cells before they become detectable clinically. Tumor cells that survive the removal phase come in the second phase(balance) were Adaptation immunity modifications cancer cell immunity, suppressing the outgrowth of the cancer cells. In rare cases, alternative cancer cells capable of dodging the immune system might rise and enter the third phase (escape). They progress to suppress more immune reactions by adjusting the superficial antigens and creating immunosuppressive particles and cytokines, causing in clinically evident tumor [9].

The blockage of the PD-1/PD-L1 relations led to good clinical reactions in some tumor

kinds. Yet, defining which patients gain benefit from PD-1/PD-L1–immediately immunotherapy remnants a significant clinical question. Data propose that patients whose cancers overexpress PD-L1 through IHC have improved reactions with anti-PD-1–immediately therapy, but the strong reactions in various patients with little expression of these markers create this procedure controversy [10]. Raised levels of PDL1 expression on immunity cells had been related with augmented reaction to therapy [11,12]. PDL-1 expression as immediately through Immune Histo-Chemistry (IHC) appears to be the best presently available biomarker and might be indicative of a dose-response association between PD-L1 expression and drug effectiveness [13]. through IHC has been hard at times for the reason that in histological investigation the staining could be either focal or diffused. Hence, choosing an optimum location for biopsy to measure PD-L1 expression station remnants challenging [7]. Overexpressed PD-L1 in bladder cancer cells had been related to advanced clinical stage tumor and decreased illness-free survival rates, and a positive association had been shown between PD-L1 overexpression and tumors increasing Lymph node malignancy and loco-regional failure [14].

One of the most important factors in the incidence of bladder cancer is smoking, as there is a direct relationship between the increase, the number of cigarettes, years of smoking, and the incidence of bladder cancer, as the absorption of nicotine, which is a toxic substance, leads to a lack of blood absorption of oxygen that the body needs and is replaced by carbon dioxide that is rapidly absorbed by the blood, which leads to hemoglobin saturation [15].

The aim of this study was to evaluate the expression of PD-L1 in bladder cancer cases by using IHC, and to investigate a possible association between the level of PD-L1 expression and tumor grade, stage. We also sought to bring out the clinic pathological features of bladder cancer among Iraq's patients.

MATERIALS AND METHODS

Collection of samples

This project included100 bladder tissue samples divided into two groups: 70 samples (patients' group) of bladder cancer tissues and 30 samples (control group) of benign bladder lesion. The age range was between 20-80 years old. All samples in study were collected from Al-Hussein Hospital and a Private Laboratory in Thi-Qar province during the period between January 2022and February 2023 All samples kept at 10% formaldehyde and room

temperature for histopathological and immunohistochemical analysis.

Immuno Histo Chemistry assays (IHC)

In this study the immunohistochemical technique was been performed as designated earlier [16]. First step has been the deparrafination, accomplished by soaking samples in xylene and then rehydrated through a chain of graded alcohol. After that, Antigen retrieval by placing the slides in Retrieval Solution in high temperature Then left in room temperature for 20 min to cooled, subsequently adding of peroxidase to eliminate internal peroxidase action of tissue for 10 min and slides had been again washed 3 times for 10 min in phosphate buffered saline. Then let sections overnight for incubated with primary antibodies PD-L1, (PD-L1, - Rabbit Monoclonal antibody) (PD-L1,1:100). In the next day, the slides imperiled to washing 3 times with (PBS) for 10 min then dropping of anti- Rabbit labeled polymer HRP primary anti body and incubated then at room temperature with gentle vibrator for 30 min. Then, the slides washed three times with PBS buffer for 5 min each. After that drops DAP Chromogen additional to the slides and incubated for 10 min at room temperature agreeing protocol Dako. Next step, slides washed with D.W (distilled water) for 10 min. Then, drops of the counter stain (Haematoxylin) has been utilized onto the slides and let for incubation at room temperature for 2 min, to stain the nucleus of cells, then, slides washed or cleaned by the running tap water for 2 min and distilled water for 1 min. After that, the slides dehydrated by soaked in ascending concentrations of alcohol (70, 95, and 100%) for 1 min each one. After dehydration slides soaked in xylene 2 times for 2 min each. Then drops of DPX practical onto slides and protected with cover slides (22 × 22 mm) and let them to dry.

Statistical analysis

The data has been analyzed by the statistical package for available from SPSS., percentages have been utilized to display the data. The Pearson Chi-square test has been utilized to determine the significance of the difference between significant qualities (qualitative data). Whenever the P-value has been less than 0.05, statistical significance has been considered.

RESULTS

The study shows that 31 case out of 70 bladder cancer show over expression of PD-L1 (40.0%) and 2 out of 30 controls are positive for PD-L1. high expression of PD-L1 in stage T2 (16) case (p-0.001). As for the (7) cases of stage Ta, (3) cases of T1, and (1) cases of T3 stage and (1) case ofT4 stage as in (Table 1). The results of this study shows that (9) cases of (28) positive PD-L1expression of bladder cancer are of low-grade urothelial carcinoma and high expression PD-L1 in high grade of bladder cancer (19) (P - 0.110) as in (Table 2). This study shows over expression of PD-L1 in
muscle invasive of bladder cancer (18) case out of (28) (0.311) while low expression of PD-L1 in non-muscle invasive bladder cancer (10) case out of (28) as in (Table 3).

A Total Score (TS) is the sum of PS plus IS (ranging from 0, 2–8). A positive result for PD-L1 is defined as TS ≥ 3, which was validated in numerous large clinical studies.

Tab. 1. Relationship between PD-L1 and T staging of bladder cancer

Stage	Positive PD-L1 No.	%	Negative PD_L1 No.	%	Total No.	%
Ta	7	36.84	12	63.16	19	27.14
T1	3	33.33	6	66.67	9	12.86
T2	16	41.03	23	58.97	39	55.71
T3	1	50	1	50	2	2.86
T4	1	100	0	0	1	1.43
Total	28	40	42	60	70	100

CalX2= 116.1 TabX2= 9.49 DF= 4 *p*-value < 0.001high-sig

Tab. 2. Represent PD-L1expression and grades tumor

Grade Tumor	Positive PD-L1 No.	%	Negative PD_L1 No.	%	Total No.	%
Low grade	9	33.33	18	66.67	27	38.57
High grade	19	44.19	24	55.81	43	61.43
Total	28	40	42	60	70	100

CalX2= 2.555 TabX2= 3.84 DF= 1 p. value 0.110non-sig OD high/low grad 1.595 (0.898 – 2.833).

Tab 3. correlation PD-L1expression and muscle invasive of bladder cancer.

Type of Tumor	Positive PD-L1 No.	%	Negative PD_L1 No.	%	Total No.	%
Muscle invasive	18	42.86	24	57.14	42	60
Non-muscle invasive	10	35.71	18	64.29	28	40
Total	28	40	42	60	70	100

CalX2= 1.025 TabX2= 3.84 DF= 1 p. value 0.311non-sig
OD invasive/non-invasive 1.341 (0.759 – 2.368)

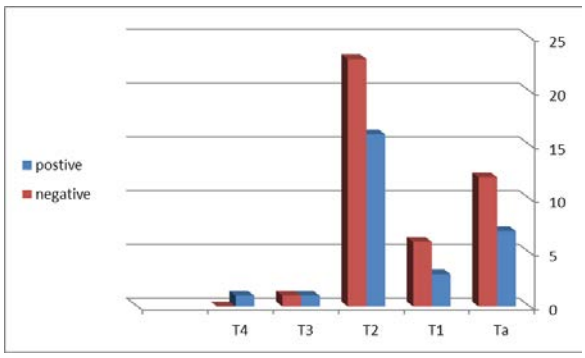

Fig 1. Represent PD-L1 expression, T stages of tumor

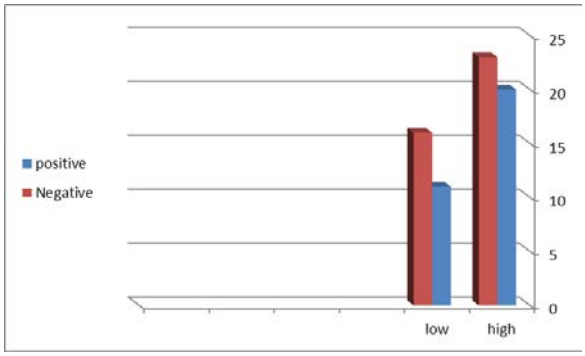

Fig. 2. Represent PD-L1 expression and tumor grades

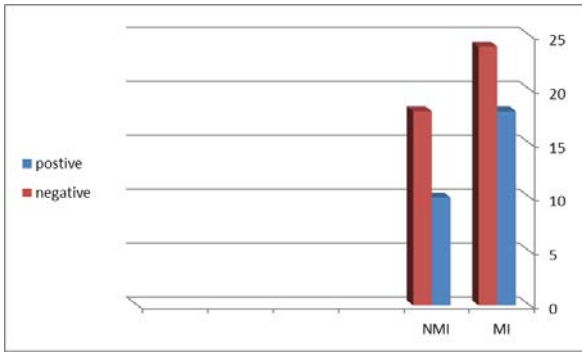

Fig. 3. Correlation PD-L1 expression and muscle invasive of bladder cancer

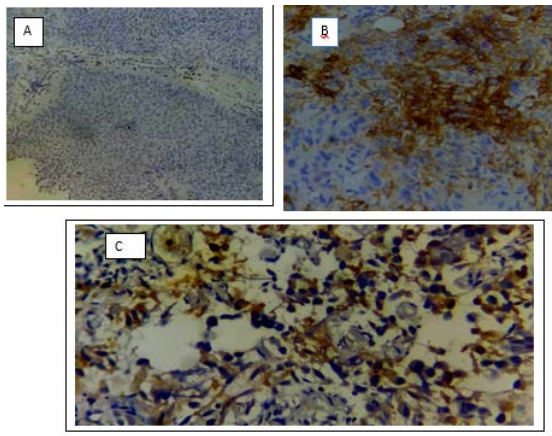

Fig. 4. (A) show no expression of PD-L1 (10X). (B) high- grade urothelial carcinoma, show high expression of PD-L1 (20X) (c) low grade papillary urothelial carcinoma, no expression PD-L1 (40X).

DISCUSSION

In the current study the results showed high expression of PD-L1 in stage T2 (41.03%) cases, The expression of PD-L1 is low in the T stages of tumor (7) cases of stage Ta, (7) T1, (3) T3, (1) cases of T4. the reason PD-L1 higher expression for the reason that PD-L1 marker of stronger difference in tumor cells, and it indicates a bad prediction for the patient, with cancer growth and malignant, sometimes in elevation levels of PD-L1 have been usage as recommendation therapy of patients. This result agreement with Safia et al [17]. In which the researcher showed that the high expression of PD-L1 in T2 stage had 40.02%. The effects of the study showed that PD-L1 expression remained high with a high grade of bladder tumor (44.19%), whereas a lessening was observed in PD-L1 expression with a low grade (33.33%). The cause for the in-elevation PD-L1 expression with a high grade has been because of the statement that the expression of PD-L1 protein rises with the advancement of the grade of bladder tumor, for the reason that high-grade cancer cells excrete more quantities of PD-L1 associated to other tumor We also found a important relationship between PD-L1 expression and cancer grade, as utmost tumors that expressed PD-L1 remained of high grade, similar to the results Safia et al [17].

The relations between expression of PD-L1 and cancer pathological T stage, had also examined. However positive relations remained detected between the expression of PD-L1 on cancer cells and in elevation pathological T stage [18]. T lymphocytes remove cancer cells through immunity investigation usage T cell receptor and main histocompatibility compound relations. The interface of PD-1 expressed in T lymphocyte with PD-L1 expressed in cancer cell clues to Barriers in immunity regulation designated over. PD-1/PD-L1 path has been usage by cancer cells as a process of immune evasion. But, effect PD-L1 expression on scientific diagnosis has been poorly definite in UC. Several studies have proposed that PD-L1 overexpression remained associated with poor prediction in bladder tumor, in agreement with Kawahara et al [19] .In our study, valuation of all PD-L1 scoring algorithms (ICs, TPS, CPS) providing important relations with cancer stage (pT2) and grade (HG), in agreement with Kawahara et al [19]. A study that assessed PD-L1 expression in cancer cells in the bladder in addition to metastatic location showed homogenous positivity, signifying that PDL1 might be verified usage samples from either location due to their like natural behavior.15 In adding, PD-L1 expression infiltrating immunity cells warrants depth examination for the reason that the obtainable data proposes that PD-L1 expression in infiltrating immunity cells has been an analyst for better overall endurance even without PD-L1 inhibitor therapy, in agreement with Ding et al.

as the study presented the expression of PD-L1 protein rises with high grade signifies (19) cases. The results of the present study revealing that PD-L1 has been above expressed in muscle-invasive bladder tumor (42.86%), whereas PD-L1 expression lessened in non-muscle-invasive bladder tumor (35.71%). also found that non-invasive papillary urothelial carcinoma remained meaningfully lesser in PD-L1 expression than invasive UC, chiefly in the squamous and sarcomatoid histologies, change to the other variants [20]. Quantified that the basal/squamous subtype has been much subtle to anti-PD-L1/PD-1 comparison with papillary luminal cancers [21]. In our study, positive PD-L1 expression remained meaningfully related with high grade and muscle-invasive cases. Our results agreement with Kawahara et al [19]. this has been specified that PD-L1 expression on bladder carcinoma cancer cells was related to high tumor grade, muscle-invasive disease, increased resistance to Bacillus Calmette- Guerin (BCG) treatment and worse overall perseverance [18]. Davick et al found no important relative between PD-L1 expression on bladder carcinoma cancer cells and advanced tumor grade, lymph node and Distant metastasis, but it remained related with muscle-invasion, signifying that positive PD-L1 expression might be a potential predictive indicator for patients with bladder tumor [22]. Charlton et al found that in elevation PD-L1, remained meaningfully related with higher tumor stage, distant metastasis and poor whole survival, but not with gender, cancer grade, lymph node status, and multimodality [23].

CONCLUSION

PD-L1 expression is significantly associated with two important prognostic factors; stage and grade. While PD-L1 expression was significantly correlated with the tumour grade and degree of invasion suggesting the suitability of PD-L1 as prognostic marker of urothelial carcinoma. invasive urothelial carcinoma patients with multiple tumor characteristic which has high PD-L1 immunoexpression more than patients no-invasive muscle.

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