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The Association Between Immune-Histochemical Expressions of Program Death Ligand with some Aggressive Features of Prostate Cancer

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Abstract

Prostate cancer (PC), accounts for more than one-fourth of all cancer diagnoses, and the most frequently diagnosed cancer among men in 2022. The immunoglobulin (IG) Program death ligand-1(PD-1) cell surface receptor is predominantly expressed on the surface of many cells. The purpose of this study was to demonstrate the relationship between Program death ligand expression and some aggressive features of prostate cancer including perineural invasion, vascular invasion and necrosis. Thirty cases of prostate cancer with age range from 60 to 80 year old and 30 cases of normal prostate tissue with age under 25 year old were separated into two groups in a retrospective case-control research that encompassed 60 cases. All malignant cases were examined by consultant pathologists for the diagnosis of prostate carcinoma, and each block of tissue was divided into two slides, one for hematoxylin and eosin (H&E) staining and the other for immunoglobulin (IHC) staining of PDL-1. The expression pattern of Program Death Ligand was investigated in these samples and its relationship to particular clinic-pathological characteristics. Despite it was not expressed in healthy prostatic tissue, program death ligand demonstrated to be positive in prostate cancer with vascular invasion, perineural invasion, and necrosis, while it was negative in healthy prostatic tissue. High expression of Program death ligand was correlated with poor differentiation, neural invasion, and vascular invasion; these criteria indicate that the expression of Program death ligand is associated with high grade and aggressive tumors. The current study confirms that perineural invasion, vascular invasion, and necrosis are all accompanied by a rise in Program death ligand expression regardless their grade and stage.

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1. Introduction

Prostate cancer (PC), which has a high incidence, is prevalent in the USA and worldwide [1].The tumor stroma is also significant in addition to the epithelial cell in the development of prostate cancer cells, as evidenced by growing study into the functions of the neuronal components in this context [2]. Meanwhile, a mechanism known as perineural invasion (PNI) allows cancer cells to move along nerves.This is a crucial strategy for metastasis and invasion used by several malignancies, especially pancreatic and prostate cancer, as well as head and neck, colon, and rectum cancer [3,4]. The presence of tumor cells in an endothelium-lined area is known as lymphovascular invasion, or LVI. The International Society of Urological Pathology (ISUP) advises that LVI to be included in the routine evaluation of radical prostatectomy specimens [5]. It was shown that cribriform architecture was linked to genomic instability and hypoxia and that the occurrence of comedonecrosis would suggest rapid growth or metabolic aberration being associated with more aggressive biological activity of cribriform

and solid carcinoma [6]. The immunoglobulin super family's PD-1 (CD279) cell surface receptor is predominantly expressed on the surface of activated T lymphocytes [7-9]. The primary PD-1 ligand, PD-L1, also known as "programmed-death ligand 1" (CD274), is a transmembrane protein that is expressed on a range of cell types, primarily dendritic cells and macrophages that transmit antigens [7]. Non-lymphoid tissues like the heart, lung, and other organs also constitutively express PD-L1 (10). In the peripheral effector phase of T-cell activation, binding of PD-L1 reduces the activity of activated T cells, which is a crucial mechanism for negative feedback control of inflammation and autoimmune disease [7]. PD-L1 expression seems to be an important biomarker because it has been associated with biochemical recurrence in PC [8]. Since the disease harvests millions of people yearly, thus the current research aimed to study the association between Pdl-1 and some aggressive features of prostate cancer include perineural invasion, vascular invasion and necrosis using anti Pdl-1 immune histochemicalstain.

2. Materials and Methods

2.1. Patients and Methods

A retrospective case control study with 60 cases was split into two groups, 30 cases were prostate cancer cases and 30 of which were cases of normal prostate tissue. The samples were gathered by Transurethral resection of the prostate (TURP) from a private hospital which sends the biopsy to private lab preserved in 10% formaline between January 2023 and August 2023. After receiving permission from the patients' families, the Institute of Forensic Medicine in Medical City of Baghdad collected 30 cases for the control group; these instances were taken from patients who were under 25 years old to prevent inflammation, particularly after puberty, and to prevent BPH changes. All cases were examined by consultant pathologists for a diagnosis of prostate carcinoma, and each block was divided into two slides—one for H&E staining and the other for IHC staining, PolyExcel two steps detection systems is non biotin, micro polymer based on an HRP labeled polymer, which was conjugated with secondary antibodies was used. For IHC staining, rabbit primary antibody was added to the tissue which bound to tissue specific antigens in the specimen, then any excess antibody was removed by washing, then secondary antibody or poly excel HRP labeled polymer added and reacted with primary antibody. Again any excess secondary antibody was removed by washing; the end brown color which formed as 3-3 diaminobenzidine HCL (DAB) which in turn oxidized by denoting electrons to activate HRP/H_2O_2 reaction.

2.2. Quality control

1. Normal placental tissue from abortion specimens was used as a positive control.
2. Young patient prostate tissue was employed as a negative control.

3. Experimental Work

3.1. Microscopically study

A digital light microscope (Micros Austria) was utilized to inspect the slides. The photographs were taken in high definition (HD) using the same device's built-in camera that displays the image on the LCD screen from five randomly chosen zones on the slide (corners and center).

3.2. Scoring of PD-L1

The cells were classified as anti-PD-1 positive when the cytoplasmic membrane staining had a distinct brown tint. Neither matter the patient's age nor they were cancer patients or healthy individuals, all samples were examined for anti-PD-L1 antibodies. According to the de-facto agreement, a malignant cell was deemed positive for PD-L1 if the cell membrane was stained partially or entirely. The cytoplasmic PD-L1 staining of the tumor was disregarded and labeled negative. The number of cancer cells was calculated by dividing the total number of carcinoma cells by the number of PD-L1 positive carcinoma cells. In every instance, necrotic portions were cut off before scoring, and it was simple to use at least 100 carcinoma cells.

i. A negative staining test revealed 0-1% malignant cells.
ii. Low/weak There was 1-5% of labeled malignant cells.
iii. Medium Staining revealed 5–10% of malignant cells.
iv. More than 10% of the cells were highly/strongly stained.

In each of the five zones, the proportion of positively stained cells was used to gauge the level of PD-L1 expression. Null (no stains).

- 1+ (weak)
- 2+ (moderate)
- 3+ (strong)

By multiplying the intensity by the percentage of all stained cells, the H score was determined [9, 11, 14].

3.3. Statistical Analysis

The design of the experiment was entirely random. The relationship between the clinicopathological variables and the pattern of PD-L1 expression using

the statistical program for social science (SPSS) version 24. The results were statistically significant if the probability was 0.05 and very significant at 0.01 and more [13].

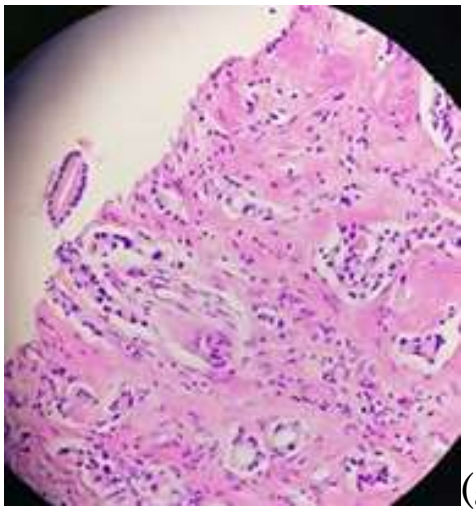
4. Results and Discussion

The current study used 60 patients divided into 30 PC cases and 30 cases with normal control prostatic tissue in a retrospective case control design. The pattern of PD-L1 expression in these samples and its correlation with specific clinic-pathological traits was studied. Although it is not expressed in healthy prostatic tissue, PD-L1 demonstrated to be positive in PC with vascular invasion, perineural invasion, and necrosis. Table 1 shows that the percentages of cases with perineural invasion were 20 and 10% expressing Pdl-1. Eighty percent exhibited no perineural invasion and negative for pdl-1 expression as shown in figure 1. A statistically significant link between the existence of peri-neural invasion and PD-L1 expression ($p \leq 0.01$) and that all tumors with peri-neural invasion were positive (10% out of 10%). While negative cases for perineural invasion were 80% and there was no Pdl-1 expression.

Table 1. The relationship between peri-neural invasion and PD-L1 expression in Prostate cancer in 30 studied cases.

Perineural invasion	Number of cases	%	PD-L1+ve	Total % of PD-L1 +ve
+ve	6	20	3(10%) **	10%
-ve	24	80	0 (0%)	10%

** Correlation is significant at the 0.01 level (p value 0).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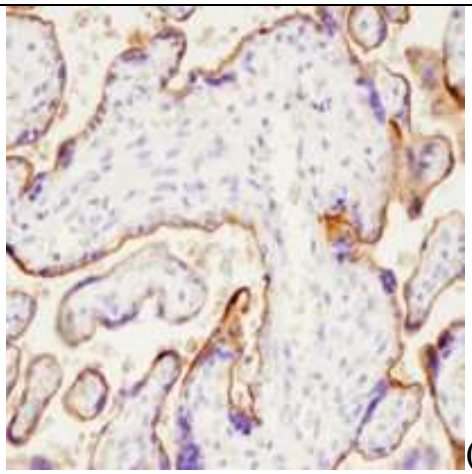
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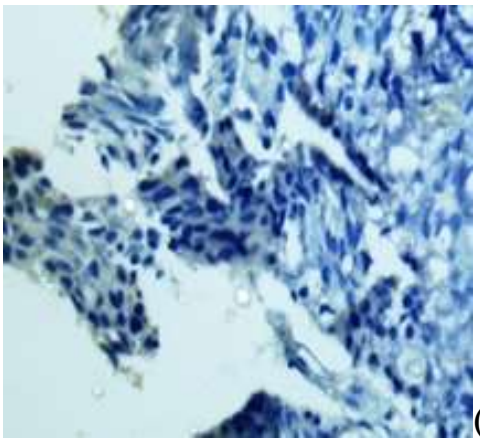
(c)

Figure 1. Prostate cancer with grading score 7 and perineural invasion, A: perineuralinvasion in Prostate cancer at 400X magnification, B: PD-L1expression in positive control placenta tissue at 400X magnification positive, C:Prostate cancer with Pdl-1 expression at 400Xmagnification.

Only 10% of cases exhibited vascular invasion (table 2) since 100% of those instances exhibited PD-L1 expression and 90% were negative to vascular invasion and not exhibited Pdl-1 expression.

Table 2. The relationship between vascular invasion and PD-L1 expression in Prostate cancer in 30 studied cases.

Vascular invasion	No. of cases	%	PD-L1 +ve	Total% of PD-L1+ve
+ve	3	10	3(10%) **	10%
-ve	27	90	0 (0%)	10%

** Correlation is significant at 0.01 levels (p value 0.008).

Table 3 displays that 10% of the cases had necrosis and 90% without necrosis. Perineural invasion,

vascular invasion, and the presence of necrosis all showed a statistically significant correlation with PD-L1 expression ($p \leq 0.01$).

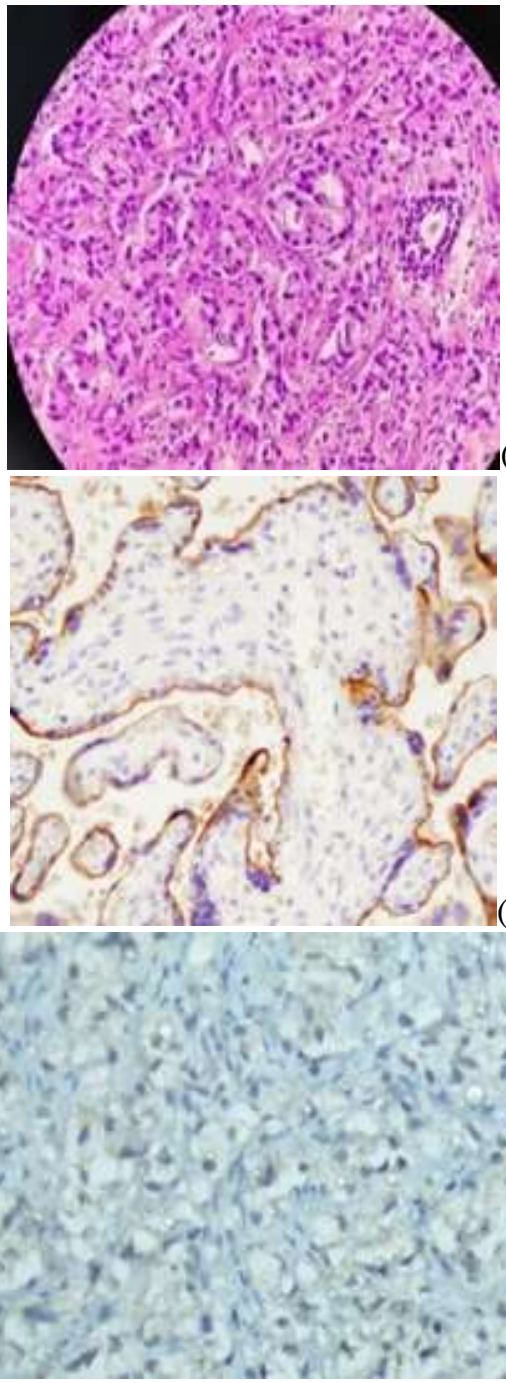

Figure 2. Prostate cancer with grading score 7 and vascular invasion, A: vascular invasion in Prostate cancer at 400X magnification, B: PD-L1expression in positive control placenta tissue at x400 magnification, C: PD-L1 expression of moderately stained prostate cancer taken at 400X magnification.

Table 3. The relationship between necrosis and PD-L1expression in Prostate cancer in 30 studied cases.

Necrosis	No.of cases	%	PD-L1 +ve	Total %of PD-L1+ve
+ve	3	10	3(10%) **	10%
-ve	27	90	0 (0%)	10%

** Correlation is significant at the 0.01 level (p value 0).

In this study, the presence of peri-neural and vascular invasion as well as necrosis in the tumor elevated the expression of PD-L1. These factors indicate that PD-L1 expression is linked to aggressive and high-grade cancers. The current study indicated that 80, 90, and 90% of the cases respectively, lacked necrosis, peri-neural invasion, and vascular invasion. This can be the result of the incidental finding of prostate cancer in patients with benign prostate hyperplasia undergo prostatectomy or early detection of cancer in such cases. According to a previous study on pancreatic carcinoma, which found that high expression of PD-L1 was associated with poor differentiation between neural and vascular invasion, vascular and peri-neural invasion in the tumor are correlated with increased PD-L1 expression(15). Another study on PD-L1 expression in small lung carcinoma found no significant correlation between PD-L1 expressions in tumor cells and/or those of tumor infiltrating cells, and that PD-L1 expression in tumor cells was significantly related to the absence of nodal metastasis accompanied by the presence of vascular invasion. The current p value is 0.0, whereas the vascular invasion and PD-L1 expression p values were 0.022, in previous study (16). This variation could result from different antibody usage or from different case counts.

Necrosis in the mass also led to increased PD-L1 expressionin the cases exhibiting PD-L1 positive which were associated with necrosis. This correlation was also shown in a prior study on pulmonary pleomorphic carcinoma that high PD-L1 expression is correlated with a poor prognosis and can provide a rationale for using specific immunotherapy in the subtype with high grade carcinoma. It is suggested that the aggressive behavior of pleomorphic carcinoma may be partially related to PD-L1 mediated immune escape and intra-tumoral hypoxia (17).

5. Conclusion

When there is peri-neural invasion, vascular invasion, and necrosis, PD-L1 expression rises. So Pdl-1 is associated with poor prognostic features in prostate cancer.

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Ethics Declaration: The project was permitted by the ethical community in the Kindy College of Medicine, University of Baghdad.

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