The Immunohistochemical Expression of PDL-1 in Prostate Carcinoma and Benign Prostatic Hyperplasia/ Clinico-Pathological Study

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ABSTRACT:

BACKGROUND:

Programmed death ligand 1 is tumor marker which is anti apoptotic factor (PD-L1, B7-H1, and CD274) and is a member of the B7 family of cell surface ligands that regulate T cell activation and immune responses.

THE AIMS OF STUDY :

To investigate the expression of Programmed death ligand 1 (PD-L1, B7-H1, CD274) in prostate cancer, benign prostatic hyperplasia, and normal prostatic tissue using anti-PD-L1 antibody; and to correlate the result of study with some clinico-pathological parameters such as the age of the patients and grade of tumor.

PATIENTS AND METHODS:

A retrospective case control study, included 90 cases was divided into three groups include 30 cases of Prostate cancer; 30 cases of benign prostate hyperplasia and 30 cases of normal prostatic tissue.

RESULTS:

Programmed death ligand 1was expressed in 10% of Prostate cancer cases and there is no expression in benign Prostatic hyperplasia and normal prostatic tissue. In Programmed death ligand1positive cases there is a significant correlation with age of patients, high Gleason score.

CONCLUSION:

Immunohistochemically, it is concluded that the expression of Programmed death ligand 1 increases as the age of the patient increases, high Gleason score and there is no expression of Programmed death ligand 1in benign prostate hyperplasia and normal control tissue.

KEYWORDS: PC: prostate cancer, PD-L1: programmed cell death-1ligand.

INTRODUCTION:

Prostate cancer (PC) is the second most frequently diagnosed cancer in men, accounting for approximately 15% of the newly diagnosed male cancers worldwide. In men, it is the fifth most common cause of death from cancer, with 307.000 estimated deaths (6.6% of all estimated deaths) in 2012 [(1).]

Benign prostate hyperplasia (BPH) is one of the most common diseases in elderly men [(2)]. Histological enlargement of the prostate gland begins at age 30 years and at age 50 years the prostate continues to enlarge gradually in some men, while in others the size is scarcely changes [(3)].

Programmed Death Ligand 1 (PD-L1) was discovered following a search for novel B7 protein homolog's and was later implicated to be expressed by antigen presenting cells, activated T cells, and tissues including placenta, lung and heart [(4,5,6)]. Moreover, PD-L1 expression has been associated with adverse clinic-pathological characteristics and poor prognosis [(7)].

PD-L1 expression seems to be an important biomarker as it has been associated with increase recurrence rate in PC (8). PD-L1 was not expressed in localized Prostate cancer or benign prostate hyperplasia, and was showed only in a minority of castration-resistant prostate cancer tumors and infiltrating immune cells [(9)].

PATIENTS AND METHODS:

A retrospective case control study, involved 90 cases was divided into three groups including 30 cases of PC, 30 cases of BPH and 30 cases of normal prostatic tissue. The samples of PC and BPH were taken by TURP (Transurethral Resection of Prostate) from the patients and collected from the archives of teaching lab of Al-Yarmook Teaching Hospital and private lab in Baghdad for a period from October 2018 to May 2019.

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Thirty cases of control group were collected from Institute of Forensic Medicine in Medical city of Baghdad after consents were taken from their relatives; those cases were collected from young age group (below25year) to avoid inflammation especially after puberty and to avoid BPH changes. Each biopsy block was sliced into 2 slides (1 for H&E stain and the other for immunohistochemical stain) and all cases were reviewed for the diagnosis by consultant pathologists.

Clinico-pathological data including age of patients, Gleason score in PC, age of patients, prostatitis in BPH and the age in normal control group were collected from forensic medicine department.

Quality control

1- Positive control: normal placental tissue from abortion specimens were considered as a positive control for PDL_1 stain.
2- Negative control: normal prostate tissue was taken from young patients for PDL_1 stain.

Microscopical study

A digital light microscope (Micros Austria) was used in the examination of slides, then the images were captured in high definition (HD) using the same device built in camera that displays the image on the LCD screen.

Scoring of PD-L1

The cells were scored as positive for anti PD-1when these cells displayed a distinct brown color of cytoplasmic membrane staining. All samples were assessed for anti PD-L1antibody regardless whether they were from a cancer case or a control, and of the age of the patient. According to the de-facto consensus, a malignant cell was regarded positive for PD-L1 if the cell membrane was partially or completely stained. Cytoplasm PD-L1 staining in the carcinoma was disregarded and considered negative. Cancer cells were counted by evaluating the ratio of stained and unstained cells (Number of PD-L1 positive carcinoma cells/ number of all carcinoma cells). Necrotic area was excluded from scoring, and minimum number of 100 carcinoma cells was used easy to fulfill in all cases (10, 11, 12, 13, and 14).

- Negative 0-1% of cancerous cells were stained
- Low/weak 1-5% of cancerous cells were stained
- Medium 5-10% of cancerous cells were stained
- High/strong ≥10% of cancerous cells were stained

As a percentage of positively stained cells in each five zones, denoted intensity of PD-L1 expression as

- Zero (no staining)
- 1+ (weak)
- 2+ (moderate)
- 3+ (strong)

The H score was calculated by multiplying the intensity by proportion of total cells staining (10, 11, 12, 13, and 14).

Statistical analysis

The experiment was created with a purely random design. The statistical program for the social science (SPSS) version 24 was used to examine the association between the pattern of PD-L1 expression and the clinicopathological factors. If the probability (p value was 0.05 and very significant at 0.01 and greater), the results were statistically significant [15].

RESULTS:

The present study is a retrospective case control study involved 90 cases subdivided into 30 cases of PC, 30 cases of BPH and 30 cases of normal control prostatic tissue. These samples were studied concerning the pattern of expression of PD-L1 and its relation to certain clinico-pathological parameters. 10% of PC cases were positive for PD-L1 and there is no expression of PD-L1 in BPH. In prostate cancer the patient's age ranged from 50 years upwards, most of them are over 70 years (36.6%).

As illustrated in table 1, the age group divided each 10 year, 6.7% out of 10% PD-L1 were positive in patients at age ranged between 80-89 years and 3.3% out of 10% were between 70-79 years. The correlation of PD-L1 expression showed significant differences with ages of the patients, at a p value 0.05.

The Gleason scores ranged from 7 to 10, (46%) of them 4+3=7 as shown in table 2. Most of cases with positive PD-L1 expression (6.7%) out of 10% were associated with Gleason score 10 and 3.3% out of 10% associated with Gleason score of 8-9. There was a significant correlation between Gleason score and PD-L1 expression with p value 0.008.

The age of patients in BPH ranged from 50 up to 79 years divided each ten, the majority was between 60-79 years (66.6%), most of the patients had prostatitis (96.6%) showing a significant correlation between prostatitis and BPH with p value 0.01 as shown in table 4.

There is no expression of PD-L1 in BPH (as illustrated in table 3). In table 5, all of the cases were below 25 years, most of them ranged between 10-20 years (66.6%) and they showed neither prostatic inflammation nor prostatic tumor and there was no expression of PD-L1 in all these cases.

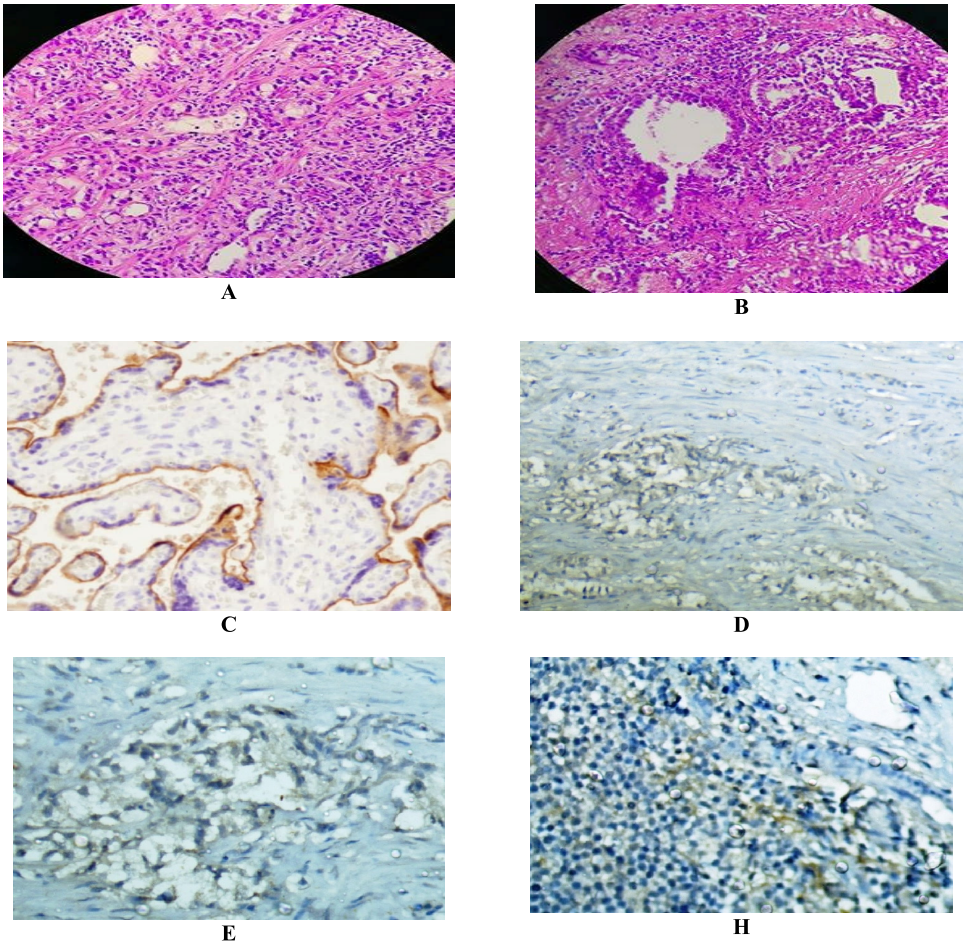

Figure 1: A: H&E of prostate cancer at x400 magnification. B: prostatitis in Benign prostatic hyperplasia at x400 magnification. C: PD-L1 expression in positive control placenta tissue at x400 magnification. D, E: PD-L1expression in prostate cancer weakly stained taken at x100 magnification (D), and at x400 magnification (E),(H): PD-L1expression in prostate cancer moderately stained taken at x400 magnification.

Table 1: The relationship between age and PD-L1expression in Prostate cancer in 30 studied case.

Age (year)	No. of cases	%	PD-L1 +ve	Total(%) ofPD-L1 +ve
50-59	5	16.6	0 (0%)	10
60-69	8	26.6	0 (0%)	10
70-79	11	36.6	1(3.3%) **	10
80-89	6	20	2 (6.7%) **	10

** Correlation is significant at the 0.05 level (p value 0.05).

Table 2: The relationship between Gleason score and PD-L1 expression in Prostate cancer in 30 studied cases.

Gleason score	No.	%	PD-L1 +ve	Total % of PD-L1+ve
7	14	46.6	0 (0%)	10%
8	5	16.6	0 (0%)	10%
9	9	30	1(3.3%) **	10%
10	2	6.6	2 (6.7%) **	10%

** Correlation is significant at the probability $p \leq 0.01$ level (p value 0.008).

Table 3: The relationship between age and PD-L1expression in Benign prostate hyperplasia in 30 studied cases.

Ages	No.	%	PD-L1 +ve
50-59	3	10	0
60-69	10	33.3	0
70-79	10	33.3	0
80-89	7	23.3	0

- There is no Correlation between BPH& PD-L1 expression.

Table 4: The relationship between prostatitis and PD-L1expression in Benign prostate hyperplasia in 30 studied cases.

Prostatitis	No.	%	PD-L1
+ve	29	96.6	0
-ve	1	3.3	0

- There is no Correlation between BPH& PD-L1 expression.

Table 5: The relationship between age and PD-L1expression in normal prostate tissue in 30 studied patients.

Age	No. of cases	%	PD-L1+ve
<10	2	6.6	0
10-20	20	66.6	0
20-25	8	26.6	0

- There is no Correlation between normal prostate tissue &PD-L1expression.

DISCUSSION:

In this study, we found most of the patients were between 70-79 years (table 1) as shown in previous studies which stated that Prostate cancer incidence strongly increases with age. Based on US Surveillance, Epidemiology and End Results Program statistics from 2000–2008, the incidence rate of prostate cancer is 9.2/100,000 for men aged 40–44 years. The rate increases sharply to 984.8/100,000 in men aged 70–74 years, after which it slightly decreases [16].

In this study, the PD-L1 expression assessed on 30 cases of PC, 30 cases of BPH and 30 cases of normal prostatic tissue and then assessing the IHC expression of PD-L1 on normal prostatic tissue, PC and BPH result indicated that there was no PD-L1 expression in BPH and normal prostatic tissue as have been stated previous report, with limited expression in PC [17].

It has been found that PD-L1 expression in PC is (10%), which is relatively comparable with the result of previous study which used two different antibodies (3.7%), (6%) respectively on 82 case of PC [17]. This slight variation in the percentage of expression may be related to small size of the sample and some factors like tissue fixation and antigen preservation, because prolonged tissue fixation (more than 24 hr) may cause masking of the antigenic epitope and results in strong non-specific background staining. Also the differences between our results and the previous report may be interpreted that PD-L1 IHC was conducted using only one antibody, which is certainly different from the using more than one antibody in previous studies, since the type of antibodies and cut offs were used to detect PD-L1 expression giving contrast results. Moreover, this variation in PD-L1 expression may reflect differences in subjective evaluation of PD-L1expression status.

Results of the present study also show that most cases had Gleason score 7. This could be due to small size of the sample taken and the PD-L1 expression increases with increasing Gleason score. About 67% of PD-L1 positive occurs in Gleason score of 10 with p value 0.008, like previous study of PD-L1 expression in prostate carcinoma showing an increase in Gleason score [9].

In this study, it was observed that 66.6% of BPH cases are between 60-80 years old, the most contributing factors to BPH are aging and androgen factor may lead to lower urinary tract symptoms like those reported by previous study which showed that BPH age related tissue remodeling [19].

It also noticed that 96% of cases with BPH had prostatitis. This association between BPH and prostatitis disagrees with previous study in inflammation and BPH in which showed only 19% of cases had prostatitis. Little attention was given to the relationship between BPH and prostatitis although the high spread of both

at aged men [19]. Differences could be due to variability in age group and to some extent, to the environmental factors causing a decrease in prevalence of inflammation in developed countries.

Almost all cases of normal prostate tissue not expressed the PD-L1 and there was no significant statistical correlation between them, these tissues were taken from young men most of them were between 10-20 year old and they had no history of prostatic inflammation or tumors.

CONCLUSION:

From the results of the current study, it is concluded that:

1. PD-L1 expression increases as the ages of the patients increase and high Gleason score.
2. There is no expression of PD-L1in BPH and normal control tissue.

Recommendations

1. Increase the sample size from different sectors that must be examined
2. More clinic-pathological parameters are suggested to be used such as (stage and type of sample TURP or RP, other histological types of prostate cancer).
3. Molecular methods such as (FISH, CISH, and PCR) are recommended to evaluate the specificity and sensitivity of PD-L1 expression in PC.

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