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Original Article

Oncology Section

Expression of PDL-1 Receptors in Prostate Cancer Patients and its Association with Tumour Aggressiveness: A Cross-sectional Study

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ABSTRACT

Introduction: Prostate cancers are infiltrated with Programmed Death-1 (PD-1) expressing Cluster of Differentiation (CD)8+ T-cells which interact with Programmed cell Death Ligand-1 (PDL-1) receptors on Tumour Cells (TC). However, in many studies, male with prostate cancer did not respond to monotherapy (PDL blockade). This unresponsiveness could be due to the fact that prostate cancer usually does not express PDL-1. The PDL-1 expression has demonstrated a significant correlation with increased risk of disease progression in various tumours but data regarding its role in prostate cancer is conflicting.

Aim: To study the occurrence rate of PDL-1 expression and its association with tumour aggressiveness in prostate cancer.

Materials and Methods: This cross-sectional observational study was conducted at ABVIMS and Dr. Ram Manohar Hospital, New Delhi, India, from October 1st, 2018 to April 30th, 2020. A total of 120 males with prostate cancer who had their diagnosis established by a prostate biopsy were included. Histopathology reports were analysed and PDL-1 immunohistochemical staining was carried out with PDL-1 monoclonal antibodies. PDL-1 expression on TCs was defined by the percentage of PDL-1 positive TCs (<1%=0 or negative, 1 to 5%=+1, ≥5%=+2). The relationship between PDL-1 expression in prostate cancer cells and clinicopathological factors like Gleason grade, lymph node positivity, perineural invasion, lymphovascular invasion, distant metastasis and Prostate Specific Antigen (PSA) level was investigated using univariate tests and multivariate logistic regression analyses.

Results: Overall, high PDL-1 expression was observed in 21.7% of patients. PDL-1 positivity 1+ and 2+ was found among 11.67% and 10% cases, respectively. Significantly higher expression (p-value <0.05) of PDL-1 was noted in cases with higher preoperative PSA levels (>40), high Gleason score (≥7), distant metastasis and cases with lymphovascular invasion.

Conclusion: Present study suggests that PDL-1 is associated with the tumour aggressiveness in prostate cancer patients and can be used for the identification of more aggressive diseases.

Keywords: Biomarkers, Immune checkpoint inhibitors, Prognostic marker, Programmed cell death ligand-1

INTRODUCTION

Programmed cell Death Ligand 1 (PDL-1) is a cell surface glycoprotein that functions as an inhibitor of the immune response through promoting T-cell apoptosis by binding to programmed cell death-1 (PD-1) receptor on the surface of T lymphocytes [1]. Although prostate tumours generally have low PDL-1 expression, PDL-1 expression is clearly not a universal marker of response as demonstrated by the experience in renal cell carcinoma and urothelial carcinoma [2]. Efforts to enhance the immunogenicity of prostate cancer by combining chemotherapy, androgen deprivation, radiation, Deoxyribonucleic Acid (DNA) damaging agents, and vaccines with PD-1/PDL-1 inhibitors are all in clinical development [3].

Previous research has discovered wide variability in PDL-1 expression in prostate cancer, ranging from 14-92% [4-7]. PDL-1-positive expression has demonstrated a significant correlation with increased risk of disease progression and cancer death in various tumours like hepatocellular carcinoma, melanoma, colorectal carcinoma, etc., [8,9], but data regarding the prognostic role of PDL-1 expression in prostate cancer are conflicting. These findings prompted researchers to investigate the relevance of PDL-1 as a prognostic marker in prostate cancer. PDL-1 could be a potential biomarker for prostate cancer risk stratification. In the future, providing additional meaningful predictive information to the existing clinical characteristics. Future research are required to investigate the response of PDL-1 therapy in such patients (PDL-1 and high-risk patients). Hence, present study aimed to assess the PDL-1 expression in prostate cancer and its relation with clinicopathological factors of cancer.

MATERIALS AND METHODS

This was a cross-sectional observational study conducted from 1st October 2018 to 30th April 2020 in ABVIMS and Dr. Ram Manohar Hospital, New Delhi after obtaining Ethical Committee approval (No. TP(MD/MS) (09/2018) /IEC/PGIMER/RMLH 3004/18) and informed written consent.

Inclusion criteria: Patients who were diagnosed with prostate cancer on Transrectal Ultrasound (TRUS) guided prostate biopsy or who underwent radical prostatectomy were included in the present study.

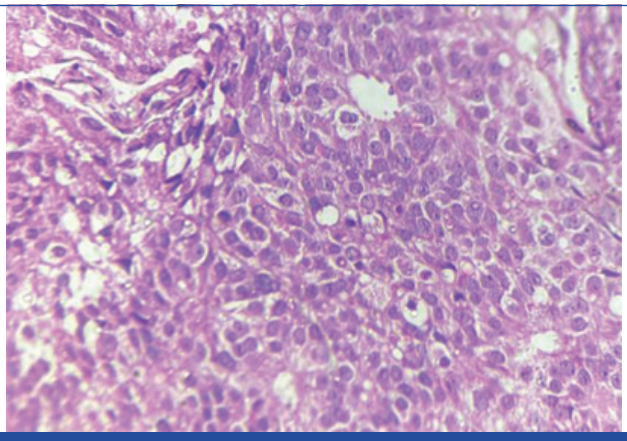
Exclusion criteria: Those who had previously received immune-modulator or checkpoint inhibitor therapy were excluded.

A total of 120 patients were subjected to standard tests, for e.g., routine blood and urine tests, serum PSA, Multiparametric-Magnetic Resonance Imaging (mpMRI) for local staging. Radiographic staging with Computed Tomography (CT) scan whole abdomen, chest plus spine screening or Prostate-Specific Membrane Antigen-Positron Emission Tomography (PSMA-PET) scan was done for patients with suspected locally advanced disease, Gleason score of 8 or greater (as per World Health Organisation (WHO) 2016 modified Gleason scoring system) [10], or PSA level greater than 20 ng/mL, as per 2018 clinically localised prostate cancer: American Urological Association/American Society for Radiation Oncology/Society of Urologic Oncology (AUA/ASTRO/ SUO) guideline [11].

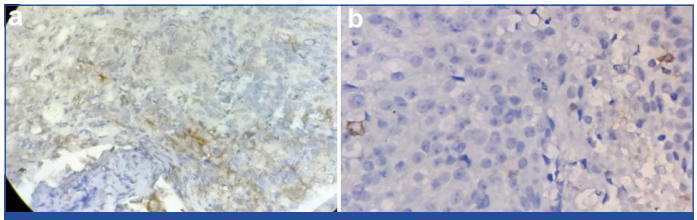
Histopathological Examination

The patients underwent either TRUS biopsy or radical prostatectomy. Out of 120 cases, 96 patients underwent TRUS biopsy, while 24 patients underwent radical prostatectomy with pelvic lymph node dissection for clinically organ confined prostate cancer at present Institution. The limits of pelvic lymph node dissection included all

lymphatic tissue along the external iliac vein from the lymph node of cloquet distal to the bifurcation of the common iliac vein proximal, including all lymphatic tissue in the obturator fossa. The tissue samples with adjacent normal tissue samples were fixed in 10% phosphate buffered formalin and embedded in paraffin. About 5 mm thick sections were stained by Haematoxylin and Eosin (H&E) [Table/Fig-1] for routine histopathological analysis/Gleason grading (as per WHO 2016 modified Gleason scoring system) [10]. PDL-1 immunohistochemical staining was carried out with the following antibodies: PDL-1 (Rabbit) ZR3 (438R-25) monoclonal antibodies [Table/Fig-2]. Other histopathological markers like lymph nodes positivity, perineural and lymphovascular invasion were also analysed. (TNM staging-T (primary tumour), N (lymph nodes) and M (metastasis) as per American Joint Committee on Cancer (AJCC) 8th Edition, 2016) [12].



[Table/Fig-1]: Histopathological slide of a carcinoma prostate (H&E, 400x).



[Table/Fig-2]: Immunohistopathology slides of carcinoma prostate showing staining for PDL-1 (H&E, 200x).

Immunohistochemical (IHC) Expression Analysis of PDL-1 in Tumour Samples

PDL-1 expression was evaluated based on immunostaining in the membrane of TCs in carcinoma prostate according to the intensity and extent on a semiquantitative scale as follows: PDL-1 expression on TCs was defined by the percentage of PDL-1 positive TCs [6]: (<1%=0 or negative, ≥1% but <5%=+1, ≥5%=+2).

STATISTICAL ANALYSIS

Data so collected were tabulated in an excel sheet, under the guidance of a statistician. The means and standard deviations of the measurements per group were used for statistical analysis (Statistical Package for the Social Sciences (SPSS) 22.0 for windows; SPSS inc, Chicago, USA). Normality of data was tested by Kolmogorov-Smirnov test. Student's t-test, Mann-Whitney U test, Pearson's Chi-square test, Fisher's-Exact test and the Receiver Operating Characteristic (ROC) curve test were used to analyse the data and the level of significance was set at $p<0.05$. Moreover, multivariate logistic regression analysis was also conducted to determine the relationship of PDL-1 expression in prostate cancer with different clinicopathological factors of the malignancy.

RESULTS

The mean age of the study group was 59.6 years. There was no significant difference among subjects with diabetes, hypertension and Body Mass Index (BMI) as compared to their counterparts in terms of PDL-1 positivity.

The association of various clinicopathological factors of enrolled patients with PDL-1 expression is shown in [Table/Fig-3]. There were a total of 120 cases out of which 96 and 24 subjects underwent TRUS Biopsy and radical prostatectomy respectively. A total of 26 (21.7%) samples of tumour tested positive for PDL-1 [Table/Fig-4].

Characteristics	N=120	PDL-1 (0)	PDL-1 (1+)	PDL-1 (2+)	Total PDL-1 (+)	p-value
Prostate size						
<45	56	46	7	3	10	0.19
>45	64	48	7	9	16	
PSA (ng/mL)						
0-4	5	4	1	0	1	0.04*
4-10	24	21	2	1	3	
10-40	56	48	4	4	8	
>40	35	21	7	7	14	
Gleason score						
≤6	32	29	3	0	3	0.04*
=7	49	38	7	4	11	
≥8	39	27	4	8	12	
T-staging						
T1	96	79	11	6	17	0.03*
T2	16	12	2	2	4	
T3/T4	8	3	1	4	5	
N stage (Total 24 cases of RP+Extended pelvic lymph node dissection (ePLND)						
pN0	20	7	9	4	13	0.07
pN1	4	0	1	3	4	
Distant metastasis						
M0/Mx	97	85	9	3	12	0.02*
M1	23	9	5	9	14	
Perineural invasion						
No	89	74	10	5	15	0.04*
Yes	31	20	4	7	11	
Lymphovascular invasion						
No	17	15	2	0	2	0.04*
Yes	7	1	1	5	6	
Total	**120**	**94**	**14**	**12**	**26**	

[Table/Fig-3]: Baseline clinicopathological characteristics of all enrolled carcinoma prostate patients.
*Statistically significant; Radical Prostatectomy+extended pelvic lymph node dissection; Student's t-test, Mann-Whitney U-test, Pearson's Chi-square test, Fisher-exact test

PDL-1 positivity 1+ and 2+ was found among 14 (11.67%) and 12 (10%) cases respectively. Significantly higher expression (p-value <0.05) of PDL-1 (+1 or +2 in comparison to 0) was noted in cases with higher T stage, distant metastasis, higher preoperative PSA levels, high Gleason score (≥8), perineural invasion, and cases with lymphovascular invasion. No significant difference was found between PDL-1 expression with prostate size, and lymph node staging [Table/Fig-3]. PDL-1 (+2) was found to have significantly higher expression than PDL-1 (+1) in cases with distant metastasis (M1) and lymphovascular invasion, but no such significant difference was found between PDL-1 (1+) and (2+) expression in cases with high Gleason score (8), T stage (T4), N stage (pN1), and perineural invasion [Table/Fig-4].

Likewise, multivariate analysis confirmed that higher preoperative PSA levels (>40), high Gleason score (7 or more), distant metastasis, and cases with lymphovascular invasion had significantly higher expression (p-value <0.05) of PDL-1. Conversely, no significant associations were found between the expressions of PDL-1 in TCs with other factors as depicted in [Table/Fig-5].

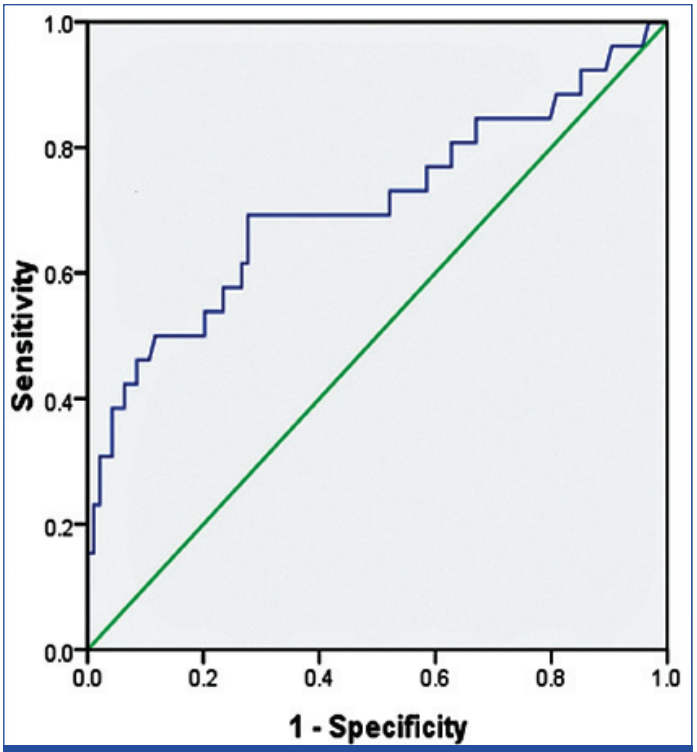
Characteristics	PDL-1 (1+)	PDL-1 (2+)	p-value
Prostate size			
<45	7	3	0.19
>45	7	9	
PSA (ng/mL)			
0-4	1	0	0.91
4-10	2	1	
10-40	4	4	
>40	7	7	
Gleason score			
≤6	3	0	0.21
=7	7	4	
≥ 8	4	8	
T-staging			
T1	11	6	0.20
T2	2	2	
T3/T4	1	4	
N stage (Total 24 cases of RP+ePLND)			
pN0	9	4	0.11
pN1	1	3	
Distant metastasis			
M0/Mx	9	3	0.04*
M1	5	9	
Perineural invasion			
No	10	5	0.13
Yes	4	7	
Lymphovascular invasion			
No	2	0	0.035*
Yes	1	5	
Total	**14**	**12**	

[Table/Fig-4]: Clinicopathological characteristics of all enrolled carcinoma prostate patients according to PDL-1 positivity.
*Statistically significant; Student's t-test, Mann-Whitney U-test, Pearson's Chi-square test, Fisher-exact test

Variables	Multivariate analysis		
	Odds ratio	p-value	95% Confidence intervals (Lower-upper bound)
Prostate size >45	1.78	0.23	0.72-2.86
PSA[2] >40	2.87	0.04*	1.31-4.98
PSA, 10-40	2.47	0.11	1.18-4.23
PSA, 4-10	1.09	0.67	0.79-1.46
Gleason score ≥8	2.91	0.037*	1.32-5.07
Gleason score=7	2.73	0.044*	1.24-4.81
T3/T4 stage	2.11	0.13	1.04-3.93
T2 stage	1.49	0.38	0.96-2.04
pN1 (nodal metastasis)	1.93	0.17	1.09-3.59
M1 (distant metastasis)	3.24	0.008*	1.24-12.38
Perineural Invasion	1.82	0.28	1.01-3.72
Lymphovascular invasion	2.99	0.042*	1.35-5.24

[Table/Fig-5]: Multivariate analysis of various parameters.
[2]Prostate specific antigen; Mann-Whitney U test, Pearson's Chi-square test, Fisher's-Exact test; *Statistically significant

The median PSA level was significantly higher among PDL-1 positive as compared to PDL-1 negative patients {41.7 (15.1-53) vs. 17.3 (9.8-37.5) ng/mL; p<0.001}. An optimal PSA cut-off of ≥30.75 ng/mL had a sensitivity of 69.2%, specificity of 72.3%, accuracy of 71.7%, positive predictive value of 40.9% and negative predictive value of 89.5% in detecting PDL-1 positivity. It has an area under the ROC curve of 0.708 (95% CI: 0.579-0.838); p<0.001 as shown in [Table/Fig-6].



[Table/Fig-6]: Receiver Operating Characteristic (ROC) curve analysis of PSA levels with PDL-1 positivity.

DISCUSSION

Prostate cancer has been shown to have little response to immune checkpoint inhibitors. In order to reveal the underlying mechanisms of resistance, many investigators have examined the expression of PDL-1 in primary prostate tumours. Martin AM et al., speculated that the likely reason for the failure of anti-PD-1 monotherapies in prostate cancer is due to paucity of PDL-1 expression in the prostate tumour microenvironment, as only three out of 20 prostate tumours in their study showed positive PDL-1 staining [4]. In present study, 21.7% of cases were tested positive for PDL-1. However, previous studies found huge variations in terms of PDL-1 expression. PDL-1 positive rates vary from 15%, 14%, 35% to 52.2%, and even 92% [4-6].

Baas W et al., found no significant association between expression of PDL-1 and patient or disease characteristics [13] while some published studies [7,14-16] reported that PDL-1 expression was a negative predictor for prognosis. Sharma M et al., found that PDL-1 expression was not very helpful in predicting tumour recurrence in prostate cancer patients who underwent radical prostatectomy [14]. Because of these conflicting results in different studies, one might be able to question the role of PDL-1 in local immune suppression in prostate cancer.

Li Y et al., in their meta-analysis revealed that the prevalence of PDL-1 in prostate cancers was 35% [15]. They also revealed that PDL-1 tends to have high expression levels in high Gleason score cases. However, PDL-1 had a relatively weak correlation with age, pathologic stage, lymph node metastasis, and preoperative PSA level. Xian P et al., in their study found that PDL-1 positive tumours were found in patients with advanced tumour stage, lymph node metastasis, and high Gleason score, which was similar to present study [17]. However, they did not find a significant association with PSA levels. Petitprez F et al., found that expression of PDL-1 by TCs was associated with a higher risk of clinical progression in men with node-positive prostate cancer [18]. He J et al., reported that the PDL-1 expression in TCs or lymphocytes was associated with Gleason score, but not related to age, preoperative PSA level, clinical T-stage, lymph node metastasis and grade of risk factors [19].

Present study also explored the relationship of PDL-1 expression with clinicopathological features in prostate cancer. The results

present study revealed that high PDL-1 expression was more likely to be observed in patients with higher preoperative PSA levels (>40), high Gleason score (7 or more), distant metastasis, and cases with lymphovascular invasion. Although nodal involvement was not found significant, this must have happened because of a low number of cases (24 cases) who underwent pelvic lymph node dissection during radical prostatectomy. These findings indicated that patients with PDL-1 overexpression showed more aggressive and advanced disease than those without PDL-1 expression and might obtain a survival benefit from anti-PDL-1 immunotherapy. In the future, PDL-1 may be a promising biomarker for risk stratification of prostate cancer and might offer additional relevant prognostic information to the implemented clinical parameters. The response of PDL-1 therapy in such patients (PDL-1 positivity 1+ and high-risk patients) need to be assessed in future studies. The cut-off values distinguishing negative and positive PDL-1 expression varied in different studies. The different antibodies used in the previous studies might also affect the precision of the positive rate of PDL-1 expression and might therefore affect the estimation of the prognostic and clinicopathologic value of PDL-1 expression [14-16].

Limitation(s)

The main limitation of present study was its observational nature, single-Institution data with a limited number of cases. Secondly, the present study did not evaluate the response of anti-PDL-1 therapy in these patients. Thus, a large multicentric study employing the same antibody and cut-off value is expected to provide more precise and reliable results.

CONCLUSION(S)

The present research reveals that PDL-1 expression is linked to high-risk prostate cancer patients. PDL-1 expression by prostate cancer cells could be utilised in the future to identify more aggressive diseases and hence, guide the use of anti-PDL-1 therapy. The current investigation was carried out in a single facility with a modest number of patients. To corroborate the findings of the present study, more research with a bigger sample size and multicentre population is required.

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