ORIGINAL RESEARCH

Soluble Programmed Death-Ligand 1 (sPD-L1) is Elevated in Aggressive Prostate Cancer Disease Among African Men

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ABSTRACT

Introduction: Programmed death 1 (PD-1)/ programmed death-ligand 1 (PD-L1)-targeted immunotherapies have become a new mode of treatment for several tumours; however, there is limited evidence on the expression and prognostic value of PD-1/PD-L1 in prostate cancer, especially in African men.

Methods: Plasma concentrations of PD-L1/PD-1 were assessed using enzyme-linked immunosorbent assay in patients with prostate cancer and normal healthy controls at the Uganda Cancer Institute. The associations between plasma PD-L1/PD-1 concentration levels and serum prostate-specific antigen (PSA) levels, Gleason scores, age, and body mass index (BMI) were determined.

Results: We found significant differences in the median plasma concentrations of PD-L1 and PD-1 immune checkpoint molecules between prostate cancer cases and normal healthy controls of 0.285 vs 0.035 ($p = 0.001$) and 0.596 vs 0.355 ($p = 0.017$), respectively. We found no significant association between age, serum PSA levels, BMI and Gleason scores, and PD-1 among patients with prostate cancer and controls. However, elevated levels of PD-L1 were significantly associated with higher Gleason scores among patients with prostate cancer ($p = 0.014$).

Conclusions: Elevated PD-L1 levels were statistically significantly linked to high Gleason scores. These results may guide clinicians in assessing the prognosis of patients individually and selecting patients who will be suitable candidates for anti-PD-L1 immunotherapy.

Keywords: Immune checkpoint inhibitors; Immunotherapy; Prostate cancer; sPD-1; sPD-L1

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Key Summary Points

Prostate cancer is the second most frequently diagnosed cancer in men worldwide.

Men of African ancestry suffer disproportionately from prostate cancer at a rate almost twice as high as men of European origin.

Programmed death 1 (PD-1)/programmed death-ligand 1 (PD-L1)-targeted immunotherapies are promising treatment modalities for several tumours; however, there is limited evidence on the expression and prognostic value in prostate cancer, especially among African men.

We hypothesized that soluble programmed death 1 (sPD-1)/soluble programmed death-ligand 1 (sPD-L1) levels would be associated with prostate cancer disease aggression among men with prostate cancer in Uganda.

We found that elevated levels of sPD-L1 were significantly associated with higher Gleason scores among patients with prostate cancer ($p < 0.05$).

This study sheds more light on the role of sPD-L1 as a potential biomarker for prostate cancer patients, particularly African men.

INTRODUCTION

Prostate cancer is the second most frequently diagnosed cancer in men worldwide, accounting for approximately 15% of all new cancer diagnoses in men [1]. In 2020, the Global Cancer Observatory (GLOBOCAN) report noted an annual incidence of 1.4 million prostate cancer cases worldwide, with approximately 375,304 deaths [2]. Reports indicate that men of African ancestry suffer disproportionately from prostate cancer at a rate almost twice as high as men of European origin, irrespective of new treatment modalities [3–5].

The mainstay of treatment for advanced prostate cancer remains androgen deprivation therapy (ADT) [6]. Among patients with metastatic prostate cancer, various treatment options for metastatic castration-resistant prostate cancer (mCRPC), such as hormonal therapy, chemotherapy, and radiopharmaceuticals, have shown significantly increased overall survival (OS). Still, these patients ultimately develop resistance [7, 8].

Over the last decade, the introduction of immunotherapy, which works through the augmentation of the immune response against cancers, has yielded promising results and has significantly changed the treatment landscape owing to its efficacy and minimal side effects [9, 10]. Thus, immune checkpoint inhibitors have emerged as a complementary treatment arm in cancer clinical care and research [11, 12]. The co-inhibitory receptor programmed death 1 (PD-1) is expressed on activated T cells and B cells. Upon binding of PD-1 to its ligand, PD-L1, which is expressed on macrophages, dendritic cells and some tumour cells, T cell activation is downregulated via inhibition of CD28 signalling [13]. In addition to expression on cell membranes, several extracellular (soluble) forms of PD-L1 (sPD-L1), including spliced variants and proteolytic cleavage forms, have been reported in various types of cancer [14]. sPD-L1 can bind to PD-1 on T cells and suppress antitumour immunity, thus facilitating tumour growth. Blocking the PD-1/PD-L1 interaction using immune checkpoint inhibitors has been shown to reduce tumour growth and increase OS of patients [15].

Anti-PD-1 antibody immunotherapy (e.g. nivolumab) and anti-PD-L1 antibody (e.g. atezolizumab) have demonstrated improved response rates and OS among patients with melanoma and renal cell carcinoma, respectively [16, 17].

Ongoing clinical trials show that immunotherapy might provide a promising approach for the future treatment of prostate cancer [18]. Studies indicate that PD-L1 expression is upregulated in prostate cancer tissues

compared to paired benign prostate tissues. Similarly, high levels of PD-L1 expression have been shown to correlate positively with high Gleason scores and androgen receptor expression in patients with aggressive primary prostate cancer [19–21].

This study assessed the circulating levels of immune checkpoint molecules, PD-1 and PD-L1, among prostate cancer patients and normal healthy controls and their association with age, body mass index (BMI), serum prostate-specific antigen (PSA), and Gleason scores at the Uganda Cancer Institute.

METHODS

Study Population

This was a case–control study in which 86 men, including 57 prostate cancer patients on ADT and 29 normal healthy controls, were recruited from January 2020 to June 2020 at the Uganda Cancer Institute, Kampala, Uganda. All study participants were aged 40 years and older. Controls were men with no history of prostate cancer and with a PSA of less than 4 ng/ml. Cases were men with a histological diagnosis of prostate cancer who were receiving ADT.

Study Procedures

A predesigned questionnaire was used to obtain demographic data and the medical history of study participants. Six millilitres of blood was collected from each study participant in an ethylenediaminetetraacetate (EDTA) anticoagulation tube by a trained study nurse. Blood samples were transported within 1 h to the laboratory for processing. This study obtained ethical approval from the School of Biomedical Sciences Higher Degrees Research and Ethics Committee (REF: SBS-HDREC-779) and the Uganda National Council for Science and Technology (UNCST). All study participants provided written informed consent before enrolment in the study.

Plasma Collection and Immunoassays

Separation of Platelet-Poor Plasma

All laboratory procedures and tests were carried out in the Translational Research Laboratory at the Infectious Diseases Institute, Makerere University, Kampala, Uganda. Upon receipt in the laboratory, whole blood samples were immediately centrifuged at 6 °C at a speed of $1000 \times g$ for 10 min. The supernatant was collected in a sterile falcon tube and centrifuged again at $1600 \times g$ for 10 min at 6 °C. The resulting supernatant was then separated into two aliquots and stored at −80 °C for later use to perform the immunoassays.

Enzyme-Linked Immunosorbent Assay (ELISA)

Plasma levels of immune checkpoint regulators were measured in picograms per millilitre, and commercially procured control samples for each analyte were assayed in parallel to ensure good results. Stored plasma samples were retrieved and thawed at 4–8 °C. Immunoassays were performed in duplicate using human checkpoint marker PD-1 and PD-L1 Quantikine ELISA kits (R&D Systems), following the manufacturer's instructions.

Statistical Analysis

Categorical variables (demographic and clinical factors) were expressed as absolute numbers and proportions. Continuous variables (plasma concentration of cytokines) were expressed as medians and interquartile ranges. The Mann–Whitney U test was used to compare continuous variables, and Fisher's exact test for comparing categorical variables. The association of immune checkpoint molecules with different ages, Gleason scores, PSA, and BMI was explored using conditional linear regression. All analyses were performed using Stata 16 software.

RESULTS

Clinico-Demographic Factors in Prostate Cancer Patients and Controls

The median age was 70 years in cases and 59 years in the control group. The median BMI was 20.84 in the cases and 19.45 in the control group. Among the study participants, 49.43% of cases and 68.97% of controls had normal BMI in the range of 18.5–24.9. The median PSA was 33.01 ng/ml and 1.8 ng/ml among cases and controls, respectively. Among the cases, 31.58% had a PSA of > 100 ng/ml. Of the prostate cancer patients, 43.86% had advanced prostate cancer, with Gleason scores of 8–10. A full description of clinical and demographics characteristics is provided in Table 1.

Plasma Levels of Immune Checkpoint Molecules

The median concentrations of PD-1 and PD-L1 were generally higher in cases than in controls. We found significant differences in the plasma concentrations of PD-L1 and PD-1 immune checkpoint molecules between prostate cancer cases and normal healthy controls ($p = 0.0101$ and 0.017, respectively), as seen in Fig. 1. We found that high levels of PD-L1 were significantly associated with higher Gleason scores among patients with prostate cancer ($p = 0.014$) (Table 2).

However, the study found no significant association between age, serum PSA levels, BMI and Gleason scores, and PD-1 checkpoint molecules among patients with prostate cancer and controls.

DISCUSSION

This was the first study to assess the association between sPD-1/PD-L1 levels and prostate cancer clinical characteristics in African populations. The study evaluated the circulating concentrations of immune checkpoint molecules PD-1 and PD-L1 and their association with PSA levels, Gleason scores, age, and BMI among patients with prostate cancer and controls at the Uganda Cancer Institute. Consistent with previous studies, our results suggest a significant association between PD-L1 levels and high Gleason scores, predictive of aggressive tumour behaviour in prostate cancer [20, 22–24].

This finding further suggests that the PD-1/PD-L1 pathway activation supports the evasion of the antitumour immune response, driving tumour pathogenesis. The probability of antitumour immune response to anti-PD-1/anti-PD-L1 antibody therapy is associated with the expression of PD-L1 on the tumour cell surface [24–27].

Different mechanisms by which PD-L1-expressing cells evade T cell immunity have been hypothesized, namely: inducing (1) apoptosis, (2) T cell energy, or (3) functional exhaustion of T cells, (4) forming a molecular shield to keep lysis off tumour cells, (5) increasing production of the immunosuppressive cytokine IL-10, and (6) facilitating Treg cell-mediated suppression [28, 29]. Increased expression of PD-L1 on tumour cells has previously been described for several malignancies, including glioblastoma, pancreatic, ovarian, breast, renal, head and neck, oesophageal, and non-small cell lung cancer. PD-L1 [30] expression has also been associated with poor prognosis and adverse clinico-pathological characteristics [21, 31, 32]. A study by Gevensleben et al. (2016) provided early evidence of abundant expression of PD-L1 as a common occurrence in primary prostate cancer and as a negative predictor of biochemical recurrence (BCR)-free survival [23, 33]. Similarly, Heng Li et al. [34] looked at the expression of PD-1/PD-L1 in a retrospective cohort of men with prostate cancer who received adjuvant hormonal therapy (AHT) after radical prostatectomy (RP). They found moderate to high PD-L1 expression in 49.6% of primary prostate cancers after radical prostatectomy. In addition, PD-L1 expression was significantly associated with reduced BCR-free survival.

Topalian et al. [35] carried out an immunohistochemical assessment of PD-L1 in pretreatment cancer specimens from 42 patients. They reported that objective response to treatment was seen exclusively in (36%) PD-L1-positive

Table 1 Clinical characteristics of prostate cancer patients and controls

Clinical characteristics	Cases ($n = 57$)	Controls ($n = 29$)
Age (years)		
Median (IQR)	70 (44–89)	59 (44–82)
< 40	1 (1.75%)	0 (0.00%)
41–65	17 (29.82%)	18 (62.07%)
66–75	26 (45.61%)	6 (20.69%)
> 75	13 (22.81%)	5 (17.24%)
BMI (kg/m^2)		
Median (IQR)	20.84 (16.03–27.61)	19.45 (17.22–22.46)
< 18.5	8 (14.04%)	9 (31.03%)
18.5–24.9	43 (49.43%)	20 (68.97%)
25–29.9	6 (10.53%)	0 (0.00%)
30 and above	0 (0.00%)	0 (0.00%)
Gleason score		N/A
Median (IQR)	7 (6–10)	–
6 (3+3)	13 (22.8%)	–
7 (3+4) or (4+3)	19 (33.33%)	–
8 (4+4)	10 (17.54%)	–
9 (4+5)	9 (15.79%)	–
10 (5+5)	6 (10.53%)	–
Plasma PSA (ng/ml)		
Median (IQR)	33.01 (0.024–10,000)	1.8 (0.134–4.00)
≤ 4	16 (28.07%)	29 (100%)
5–20	9 (15.79%)	0 (0.00%)
20–100	14 (24.56%)	0 (0.00%)
> 100	18 (31.58%)	0 (0.00%)

tumours. Bishop et al. [22] recently reported that patients with CRPC resistant to enzalutamide showed elevated levels of PD-L1 in blood. The authors further concluded that PD-L1 expression on tumour cells might be a mechanism of non-androgen receptor (AR)-driven resistance to enzalutamide.

There is a need for predictive biomarkers that can identify patients who will benefit most from anti-PD-1/PD-L1 immunotherapy. Our study and many others provide evidence of sPD-L1 as a prognostic biomarker for prostate cancer patients [36, 37]. Based on our results, we provide credible evidence that prostate cancer patients with higher Gleason scores are more

PD-1

PD-L1
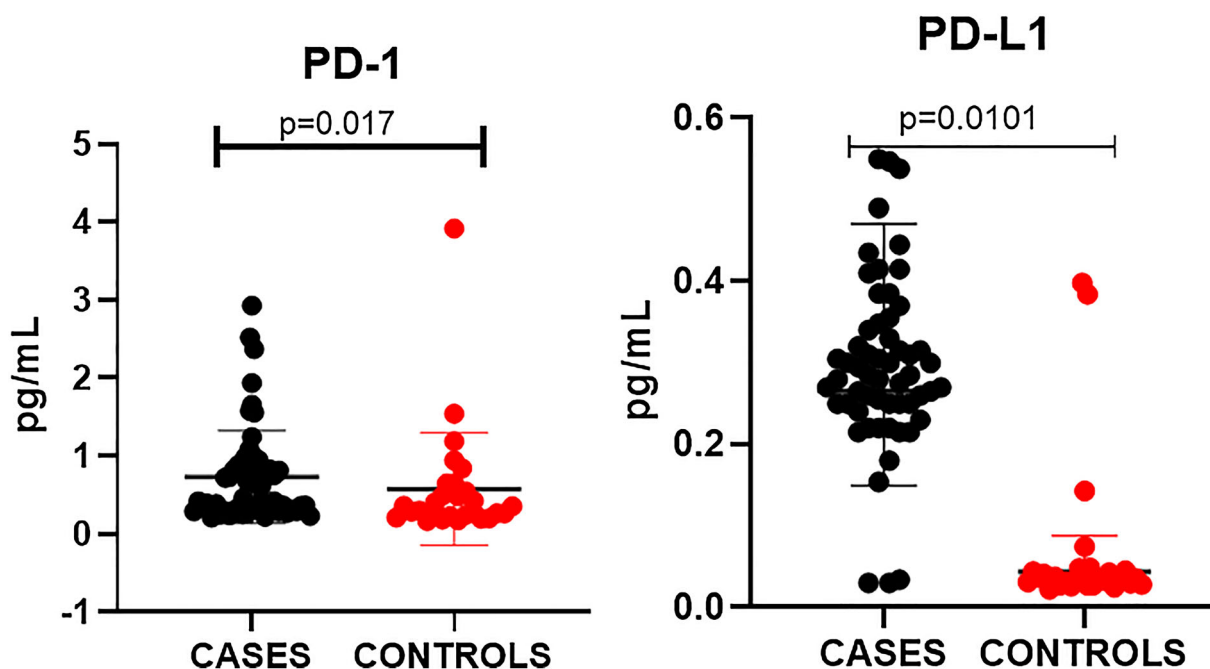
Fig. 1 Median concentrations of immune checkpoint molecules in prostate cancer patients and controls

Table 2 Association between immune checkpoint molecules and age, BMI, PSA, and Gleason score among prostate cancer patients and controls

Participants Cases ($n = 57$) Controls ($n = 29$)	Immune checkpoint molecules	Age p value correlation coefficient (r)	BMI p value correlation coefficient (r)	PSA p value correlation coefficient (r)	Gleason score p value correlation coefficient (r)
Cases	sPD-1	0.800 (-0.0222)	0.697 (0.0396)	0.758 (0.0180)	0.278 (0.1425)
	sPD-L1	0.492 (-0.0106)	0.247 (-0.1863)	0.485 (0.0657)	0.014 (0.3165)
Controls	sPD-1	0.397 (-0.0465)	0.087 (0.3003)	0.819 (-0.0538)	N/A
	sPD-L1	0.039 (-0.2776)	0.056 (0.2327)	0.606 stata (-0.0556)	N/A

likely to have higher levels of PD-L1 expression, with statistical significance. These patients are more likely to benefit from blocking the PD-1/PD-L1 pathway.

This study faced several challenges which could have affected the final results, including the relatively low median age of the controls and the fact that only the plasma levels of PD-L1/PD-1 were assessed and not tissue-based markers for comparison. The case–control study design was not able to determine the changes in checkpoint inhibitors over the course of treatments.

CONCLUSION

In this study we found that sPD-L1 was significantly associated with high Gleason scores. Our results shed more light on the possible role of

sPD-L1 as a biomarker in prostate cancer. We recommend extensive prospective cohort studies to further validate these findings.

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