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Research Article

Interleukin– 2 and 6 Levels in Nigerian Men with Prostate Cancer

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ABSTRACT

Prostate cancer is one of the leading causes of cancer mortality in Nigerian males. Multiple cytokines play a role in the aetiopathogenesis of prostate cancer. Interleukin – 2 (IL-2) and – 6 (IL-6) have been identified in the evolution of prostate cancer with possible therapeutic applications. The goal of this study was to investigate the serum levels of these cytokines in Nigerian males with prostate cancer. This was a longitudinal study of 40 serially recruited patients with prostate cancer and 40 age- and sex matched-controls. Blood sample was taken from participants after obtaining informed consent. The samples were processed and analysed for prostate specific antigen (PSA), free PSA, IL-2, and IL-6. Plasma levels of IL-2 and IL-6 were significantly elevated in individuals with prostate cancer and directly correlated with plasma PSA and free PSA. The PSA and free PSA levels were also significantly higher in the study group than the control group. The %free PSA was < 25% in all study individuals with malignant prostate cancer and most patients had advanced disease with a Gleason score of 7-9. The high plasma level of IL-2 in individuals with prostate cancer found in this study suggests active immune response; however, the failure to suppress malignancy may be due to activity modulation in the tumour microenvironment. Observed increase in IL-6 may contribute to disease progression by its direct androgenic action to prostate cancer cells. Hence, the prevalence of advanced disease in this study population may be the result of impaired IL-2 antitumour action and increased IL-6 activity.

Keywords: Interleukin-2, interleukin-6, prostate cancer, prostate specific antigen

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INTRODUCTION

Mortality from prostate cancer is significantly high among Nigerian males with most patients having advanced disease and responding inadequately to conventional therapies (Ikuerowo *et al*, 2013). Compared with people with prostate cancer in many other parts of the world, there is a disproportionately high prostate cancer-related mortality among Nigerian males; and key factors contributing to this include late presentation, late diagnosis, and limited management options for advanced disease (Ikuerowo *et al*, 2013). In Nigeria and many other low- and middle-income countries, the stages of the disease and tumour biology largely determine treatment options and overall prognosis (Adler *et al*, 1999). With this, there is an obvious need to develop efficacious therapeutic options that can reduce the morbidity and mortality burden of prostate cancer in these settings.

A number of immunotherapeutic approaches have been proposed based on the theory that tumour cells including prostate cancer cells escape immune surveillance via mutation of tumour antigens. These changes in antigens have been demonstrated in experimental animal models which show defective major histocompatibility complex (MHC) expression on cell surfaces (Kourtzelis *et al*, 2016). Naive cytotoxic T-cells (CTL) are functionally immature and hence incapable of eliminating altered self-cells or tumour cells (Rutkowski, 2010). The process of their maturation and activation is mediated by the action of interleukin-2 (IL-2) following antigen presentation by class 1 MHC (Coussens *et al*, 2013).

Inflammation has also been implicated in the initiation and progression of prostate malignancy with 80% of patient biopsies showing some degree of inflammation with cellular infiltrates (Natarajan *et al*, 2002). Inflammation modifies the tumour microenvironment by altering the balance of cytokines, chemokines, transcriptional factors, and reactive oxygen species via cellular and chemical mediators. Interleukin–6 (IL-6) has been recognized as an inflammatory chemical mediator of prostate cancer progression via multiple signalling pathways (Hanahan *et al*, 2000). It contributes to the initiation of prostate cancer, stimulates growth of the tumour cells, and transforms the tumour cells to more aggressive phenotypes. It also plays a role in the progression

of the malignancy to the castration resistant state (androgen-independent) as well as distant metastasis and resistance to chemotherapy. Serum IL–6 level is inversely related to tumour survival and response to therapy (Sfanos *et al*, 2012).

Considering the key roles that IL-2 and IL-6 play in the pathogenesis of prostate cancer; it becomes necessary to understand how the levels of these cytokines influence progression of the disease. This study compares serum levels of IL-2 and IL-6 in Nigerian patients with prostate cancer and in healthy controls.

MATERIALS AND METHODS

Study design: The study utilized a case-control study design involving 80 participants selected from the Lagos University Hospital (LUTH), Lagos State, Nigeria. The Health and Research Ethics Committee of the study hospital provided ethical approval for conducting the study. The ethical clearance was obtained on 26th March, 2019, with ethical approval number ADM/DCST/HREC/APP/2757. Written informed consent was obtained from all study participants and confidentiality of the data obtained was ensured in line with the principles of research ethics. Data collection was carried out between June 2019 and March 2020.

Participants: Forty consecutive males attending the urology clinic above the age of 18 years and with a histologic diagnosis of prostate cancer were recruited into the study. Healthy age-matched controls were also recruited into the study. Informed consent was obtained, and a validated interviewer administered questionnaire was used to obtain sociodemographic data, family and clinical history of the study participants. 5 mL of blood was collected in potassium EDTA tubes after venipuncture at the bleeding unit. The sample was transported to the central laboratory of the Department of Clinical Pathology, LUTH, for processing, separation, identification, storage, and subsequent laboratory analysis.

Biochemical analysis: Interleukin-2 (Cat.No E0094Hu, Lot Number 201910003) and IL-6 (Cat.No E0090Hu, Lot Number 201910003) was measured using Bioassay Technology Laboratory Human Interleukin 2 Enzyme-Linked Immunosorbent Assay (ELISA) kit based on the enzyme-linked immunosorbent assay method. The reagents and standard solutions were prepared and analysis was done in line with manufacturer's instructions. The optical density of each well was read in the Biotek Microplate reader at 450 nm wavelength. The concentration of IL-2 and IL-6 in the samples was calculated from the measured optical densities.

Total serum level of prostate-specific antigen (PSA; Cat No. PS235T, Lot No. PSS5541) and Free PSA (Cat No. PS233T, Lot No. PSS5811) was measured using Calbiotech prostate specific antigen ELISA kit. All reagents, controls and samples were brought to room temperature and laboratory analysis was done following manufacturers' recommendations and percentage free PSA (%fPSA) was calculated from both values.

Data analysis: Data obtained was analysed using IBM-SPSS software 20.0 version. Test for normality of continuous variables (age, IL-2, IL-6, fPSA, %fPSA, and total PSA) were done using Kolmogorov-Smirnov test. Mean/standard deviation or median/interquartile ranges were determined for these variables depending on the normality of the variable. Inferential tests were carried out using Mann Whitney-U test and unpaired t-test for non-normal and normal variables respectively. Nominal variables like Gleason's score, presence of prostate cancer symptoms, family history of prostate cancer, hypertension, diabetes mellitus and the presence of fever were presented in contingency tables and compared using Chi-square statistic and Fisher's exact test (where appropriate) to determine the level of significance. The relationships between the continuous variables were determined by correlation. P-value less than 0.05 was considered statistically significant.

Ethical Consideration: Ethics Approval was gotten from the Lagos University Hospital (LUTH), Lagos State, Nigeria. The Health and Research Ethics Committee of the study hospital provided ethical approval for conducting the study**.**

Table 1:
Study Participant Characteristics

Variable		Cases (n = 40)	Controls (n = 40)	statistic	df	p-value
Age *(mean ± SD; years)*		69.3 ± 5.4	69.7 ± 6.3	-0.285[a]	78	0.776
Symptoms of prostate cancer	Yes	13 (100.0%)	0 (0.0%)	15.522[b]	2	**0.000**[c]
	No	27 (40.3%)	40 (59.7%)			
Family history of prostate cancer	Yes	4 (100.0%)	0 (0.0%)	4.211[b]	2	0.116[c]
	No	36 (47.4%)	40 (52.6%)			
Recent history of febrile illness	Yes	6 (100.0%)	0 (0.0%)	6.486[b]	2	**0.026**[c]
	No	34 (45.9%)	40 (54.1%)			
History of diabetes mellitus	Yes	5 (100.0%)	0 (0.0%)	5.333[b]	2	0.055[c]
	No	35 (46.7%)	40 (53.3%)			
History of hypertension	Yes	12 (92.3%)	1 (7.7%)	11.114[b]	2	**0.001**[c]
	No	28 (41.8%)	39 (58.2%)			
Gleason's score	7	3 (7.5%)	NA	NA	NA	NA
	8	18 (45.0%)	NA			
	9	19 (47.5%)	NA			

*Bolded **p-values** are significant* [a] *T-test statistic* [b] *Chi-square statistic* [c] *Fisher's exact test*

RESULTS

All 80 study participants provided complete information and the mean ages of participants in the case and control groups were 69.3 (SD: 5.4) years and 69.7 (SD: 6.3) years respectively with no significant difference ($p > 0.05$). Approximately 33% of people with prostate cancer reported symptoms of advanced disease like weight loss, constitutional symptoms like fever and obstructive urinary symptoms with 30% having systemic hypertension. Only 10% reported a family history of prostate cancer. As much as 92.5% of cases were found to have advanced disease with Gleason scores 8 – 9 on confirmatory prostate biopsy (Table 1). Total PSA and free PSA was significantly raised in the cases and percentage free PSA was less than 25% in all individuals with prostate cancer suggesting malignant disease (Table 2). Cytokines IL-2 and IL-6 were significantly raised in the cases and also found to correlate significantly with each other and with PSA and Free PSA (Table 3).

Table 2:
Comparison of average (median, IQR) serum levels of IL-2, IL-6, TPSA, FPSA, and % FPSA

Variables	Cases (n = 40) Median (IQR)	Controls (n = 40) Median (IQR)	Statistic*	p-value
Interleukin-2 (ng/L)	700.0 (600.0)	600.0 (200.0)	596.5	**0.049**
Interleukin-6 (ng/L)	180.0 (98.8)	140.0 (57.5)	591.0	**0.043**
TPSA (ng/ml)	53.3 (32.0)	3.6 (1.1)	0.000	**<0.001**
FPSA (ng/ml)	4.2 (3.0)	1.4 (0.2)	276.0	**<0.001**
% FPSA	7.3 (3.2)	35.9 (9.5)	0.000	**<0.001**

Bolded p-values are statistically significant
**Mann Whitney-U test*

Table 3:
Spearman correlation analysis between plasma levels of IL-2, TPSA, fPSA, and % fPSA.

		Age	IL-6	TPSA	FPSA	%FPSA
IL-2	Correlation coefficient	-0.007	0.572	0.366	0.380	-0.139
	p-value	0.538	**<0.001**	**0.001**	**0.001**	0.219
	N	80	80	80	80	80

Bolded p-values are statistically significant

DISCUSSION

Previous studies have shown that prostate cancer incidence increases with advancing age and the diagnosis of most patients in Nigeria occurs in the seventh decade of life (Emiogun EF, 2019). The average age of study participants with prostate cancer in this study was 69.3 years which is similar to that obtained by Emiogun *et al* (median age of 70 years) (Emiogun EF, 2019). A third of the participants recruited into the study reported symptoms of malignancy such as weight loss and obstructive urinary symptoms. A significant proportion of respondents presented with a history of systemic hypertension possibly due to obstructive uropathy which is a relatively common complication of prostate cancer. This is similar to the report of Ikuerowo *et al* in their community-based screening in Lagos (Ikuerowo *et al*, 2013). There was no family history of prostate cancer in most of the study participants and this finding was in line with the report of Ikueruwo *et al* in their study where they evaluated the risk factors for prostate cancer (Ikuerowo *et al*, 2013)

The role of cytokines like IL-2 and IL-6 in the evolution and management of prostate cancer has been of interest in recent time (Culig *et al*., 2018). Interleukin–2 is a proinflammatory cytokine that regulates the activity of cytotoxic T-cells and contributes to the body's antitumour response (Culig *et al*., 2018). Elevated levels are desirable findings in individuals with prostate cancer; and in this present study, there was a significantly higher level of IL-2 in the cases compared to the controls. This is similar to a report by Tazaki *et al* (2011). A study in Kenya reported almost identical findings in individuals with prostate cancer across all Gleason's scores and the investigators therefore proposed IL-2 as a diagnostic and prognostic maker of disease in prostate malignancy (Culig *et al*, 2018). The levels of IL-2 among the cases in this study was also found to positively correlate with the measured plasma total PSA and free PSA, which is akin to the results reported in a Kenyan study where IL-2, IL-4 and IL-10 were correlated with the PSA in individuals with benign prostatic enlargement (Giri D, 2001) and those with prostate cancer (Dunn, 2002). Mechergui *et al* (2009) also found a significant correlation between IL-2 and PSA in their study to determine the profile of cytokines and its impact on plasma PSA levels among individuals with prostate cancer and those with benign prostatic hyperplasia (Mechergui *et al*., 2009). This elevated plasma level of IL-2 and its relationship with PSA found in individuals with prostate cancer suggests active immune response to the presence of the tumour. However, the failure to suppress malignancy as seen in this study with advanced disease may be due to modulation of the tumour microenvironment by TNF-β that impairs IL-2 activity (Tazaki *et al*., 2011; Quantan *et al*., 2006).

Interleukin-6 is a proinflammatory cytokine with androgenic properties (Culig *et al*, 2018). It has been implicated in the progression of prostate malignancy and elevated levels are said to contribute to the emergence of the castration-resistant class of prostate cancer. IL-6 is associated with multiple signaling pathways of cellular proliferative and apoptotic responses (Okamoto *et al*, 1997). In the current study, it was found that serum IL-6 level was elevated compared to that of the controls, and other researchers have linked elevated plasma levels of IL-6 with metastatic tumour spread. This finding is similar to the report of Shariat *et al* who investigated Plasma IL-6 in men with prostate cancer especially those with metastasis (Shariat SF, 2001). Nguyen *et al* also found increased serum levels of IL-6 in patients with untreated metastatic or castration-resistant prostate cancer compared with healthy patients or patients with organ confined disease (Nguyen *et al*, 2014). Another study by Okamoto *et al*. also reported elevated IL-6 levels in prostate

cancer patients and they went on in that study to demonstrate that IL-6 was endogenously secreted by castration-resistant prostate cancer cell lines implying autocrine action of IL-6 (Okamoto, 1997). This also corresponds to the findings of Adler *et al.* who posited that the increased plasma level of IL-6 in patients with hormone refractory prostate cancer Mechergui *et al*, 2009) contributed to angiogenesis and metastatic tumour spread with progressive elevation in circulating IL-6 in those with metastatic disease (Adler *et al*, 1999). This link between IL-6 and advanced prostate tumour characteristics may explain the advanced disease seen in this present study.

Additionally, this study found a significant positive correlation between the plasma levels of IL-6 and each of plasma total PSA and free PSA. This aligns with the finding of Adler *et al* who, on the basis of similar findings, proposed IL-6 use as a marker for progressive prostate cancer as it correlated with plasma PSA and advancing disease in their study. Similarly, Mechergui *et al* (2009) reported that prostate cancer patients with plasma PSA between 4 and 20 ng/mL showed high immune-expression of the profile of IL-6 with a positive correlation between both parameters Mechergui *et al* (2009). Shariat *et al* found that plasma IL-6 levels in patients with clinically localized prostate cancer correlated with PSA level and independently predicted biochemical progression after surgery using total PSA as a marker. (Shariat *et al*, 2001). Adler *et al* also found a significant positive correlation between the measured plasma IL-6 and the serum total PSA levels in individuals with prostate cancer (Adler *et al*, 1999). This relationship suggests a possible link between plasma IL-6 level and tumour burden as measured by circulating PSA concentration. This is however in contrast to what was found by Akimoto *et al* and Drachenberg *et al* who found no correlation between IL-6 and PSA, possibly because their study were exclusively for patients who had hormone-refractory prostate cancer. (Akimoto *et al* 1998) (Drachenberg DE, 1999)

The finding in this present study of elevated plasma levels of IL-6 in individuals with prostate cancer compared to controls and its direct correlation with plasma levels of total PSA and free PSA suggest that IL-6 may contribute to tumorigenesis in this cohort of patients by its direct androgenic action on prostate cancer cells. Therefore, the use of antibodies to IL-6 activity may be beneficial to counteract this androgenic effect of IL-6 in individuals with prostate cancer (Akimoto *et al*, 1998).

In conclusion, the incidence of prostate cancer is highest in the sixth decade of life and in Nigeria, a significant proportion of people with prostate cancer present late with symptoms of advanced disease. The plasma levels of both IL-2 and IL-6 are higher in individuals with prostate cancer and each correlates with plasma levels of total PSA and free PSA suggesting active immune response to the malignancy. However, the failure to suppress malignancy may be due to tumour microenvironment modulation that impairs IL-2 activity. The high levels of IL-6 could contribute to tumour progression by its direct androgenic action.

The prevalence of advanced disease in the study population is likely due to the impact of IL-2 activity inhibition since IL-2 was highly expressed in them yet tumour progression continued. The androgenic impact of IL-6 which promotes tumour progression and development of resistance to androgen deprivation therapy may also explain the advanced disease noted in the study population. Further research to define the immunologic contributors to disease progression would be beneficial in designing therapy for males with prostate cancer in Nigeria.

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