

# IL-6 and IL-8 cytokines are associated with elevated prostate-specific antigen levels among patients with adenocarcinoma of the prostate at the Uganda Cancer Institute

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**Background:** The possible clinical application of specific cytokines and chemokines contributing to tumorigenesis and the clinical outcome of several cancers has been reported. However, less invasive and easily applicable biomarkers in prostate cancer diagnosis and prognostication are still lacking. This study assessed the levels of plasma cytokines in prostate cancer patients as potential biomarkers for noninvasive early diagnosis. **Methods:** The plasma levels of nine cytokines, IL-6, IL-8, IL-10, IL-1 $\beta$ , IL-17A, IL-2, M-CSF, IL-12 and IFN- $\alpha$ , were detected by Luminex<sup>®</sup> liquid array-based multiplexed immunoassays in 56 prostate cancer patients on androgen deprivation therapy and radiotherapy and 27 normal healthy controls. **Results:** Levels of plasma proinflammatory cytokines IL-6 and IL-8 were markedly increased in prostate cancer patients compared with controls. There was, however, no significant difference in the concentrations of all cytokines in prostate cancer patients compared with controls. Increasing levels of IL-6 and IL-8 were significantly associated with high levels of plasma prostate-specific antigen ( $p < 0.05$ ). **Conclusion:** Proinflammatory cytokines IL-6 and IL-8 are potential biomarkers for prostate cancer pathogenesis and could serve as markers of disease progression.

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**Keywords:** androgen deprivation therapy • cytokines • inflammation • prostate cancer

Prostate cancer is the second most common cancer in men worldwide. The Global Cancer Observatory reported 1,414,259 new prostate cancer cases and approximately 375,304 deaths in 2020 alone [1]. In 2020, Uganda recorded over 2375 new prostate cancer cases and 1329 related deaths. The age-standardized prostate cancer incidence in eastern, western, northern, central and southern parts of Africa is 23.9, 31.9, 13.2, 35.9 and 64.1, respectively, per 100,000 men [2]. The age-standardized incidence rate of prostate cancer among Ugandan men is 65 per 100,000 [3].

The mainstay of treatment for prostate cancer is androgen deprivation therapy, which is often augmented with other treatment options, such as chemotherapy, radiotherapy and surgery [4,5]. Prostate cancer treatment options depend on several tumor and patient factors, such as prostate-specific antigen (PSA); tumor, node, metastasis staging; digital rectal exam; and Gleason score [6,7]. However, these pretreatment factors cannot adequately be used to predict response to chemotherapy and acute toxicity to radiotherapy [8]. Cytokines are water-soluble, low-molecular-weight proteins that transport signals between cells [9]. Several studies have demonstrated the role of cytokines in the development of many types of cancer; however, data on prostate cancer remain limited [10,11]. The

development and progression of prostate cancer is a multistep process involving several growth factors, hormones and cytokines [12–14].

Culig *et al.* reported elevated levels of IL-6 in prostate tumors and tumor microenvironment, which was associated with poor disease outcome and tumor differentiation [15]. Similarly, Maynard *et al.* identified IL-8 as a cytokine with heightened expression in primary and metastatic prostate cancer patients compared with normal healthy controls [16]. There are, however, limited data on the possible role of cytokines in prostate cancer in African men. The authors' study examined the plasma concentrations of cytokines in prostate cancer patients on androgen deprivation therapy and normal healthy controls at the Uganda Cancer Institute.

## Methods

### Study population

This was a case–control study in which 86 men, 57 prostate cancer patients on androgen deprivation therapy and 29 normal healthy controls, were recruited from January 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 a PSA of less than 4 ng/ml. Patients were men with a histological diagnosis of prostate cancer who were on androgen deprivation therapy.

### Study procedures

A predesigned questionnaire was used to obtain demographic data and medical history of study participants. A total of 6 ml of blood in an EDTA anticoagulant tube was collected by a trained study nurse from each study participant. Blood samples were transported within 1 h to the laboratory for processing. The authors obtained ethical approval from the School of Biomedical Sciences Higher Degrees Research and Ethics Committee (SBS-HDREC-779) and the Uganda National Council for Science and Technology. All study participants provided written informed consent before enrollment in the study.

### Plasma collection & 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. On receipt by the laboratory, whole blood samples were immediately centrifuged at 6°C at a speed of 1000 × g for 10 min. The supernatant was collected in a sterile Falcon tube and centrifuged again at 1600 × g for 10 min at 6°C. The resulting supernatant was then separated into two aliquots and stored at -80°C for later use in the immunoassays.

#### *Luminex immunoassays*

Stored plasma samples were retrieved and thawed at 4–8°C. Immunoassays were performed in duplicates using a nine-plex Magnetic Luminex<sup>®</sup> performance assay (Human Angiogenesis Premixed KitA; R&D Systems, a biotechnique brand, MI, USA) and following the manufacturer's instructions. The nine-plex cytokine assay kit contained IL-6, IL-8, IL-10, IL-1β, IL-17A, IL-2, M-CSF, IL-12 and IFN-α. Plasma concentrations of each cytokine were measured in pg/ml, and commercially procured control samples for each analyte were assayed in parallel to ensure good results. After all reagents were prepared, the authors added 50 ul of standards to each well (after adding 50 ul of diluted microparticle cocktail to each well) and then incubated for 2 h at room temperature on a shaker at 800 rpm. A total of 100 ul of wash buffer was added to each well, and the liquid was then removed; this process was repeated three times. Next, 50 ul of diluted biotin antibody cocktail was added to each well followed by incubation for 1 h at room temperature on a shaker at 800 rpm. The authors then added wash buffer and washed three times. A total of 50 ul of diluted streptavidin–phycoerythrin was added to each well followed by incubation for 30 min at room temperature on a shaker at 800 rpm. Finally, 100 ul of wash buffer was added to each well followed by incubation for 2 min at room temperature on a shaker at 800 rpm. The plate was later inserted into a MAGPIX (Luminex Corporation, TX, USA) and the results read within 90 min.

### Statistical analysis

Categorical variables (demographic and clinical factors) were summarized as absolute numbers and proportions. Continuous variables (plasma concentrations of cytokines) were summarized as means and interquartile ranges. Mann–Whitney U test was used to compare continuous variables and Fisher's exact test was used to compare

**Table 1. Clinical and demographic characteristics of prostate cancer patients and controls.**

Characteristic	Case (n = 57)	Control (n = 29)
<b>Age, years</b>		
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)
<b>BMI, kg/m<sup>2</sup></b>		
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	0 (0.00)	0 (0.00)
<b>Gleason score</b>		
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)	–
<b>Plasma PSA, ng/ml</b>		
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)

IQR: Interquartile range; PSA: Prostate-specific antigen.

categorical variables. The association of cytokine concentrations with variables such as age, Gleason score, PSA and BMI was explored using conditional linear regression. All analyses were performed in Prism 8 (GraphPad Software, CA, USA).

## Results

### Clinicodemographic factors in prostate cancer patients & controls

The authors collected 86 plasma samples – 57 from prostate cancer patients on androgen deprivation therapy and 29 from normal healthy controls. The median age of patients was 70 years and the median age of the control group was 59 years. The median BMI of patients was 20.84 and the median BMI of the control group was 19.45. The majority of both patients and controls (49.43 and 68.97%, respectively) were in the normal BMI range of 18.5–24.9. The median PSA was 33.01 ng/ml, with the majority of patients (31.58%) having a PSA > 100 ng/ml. A total of 43.86% of the prostate cancer patients had Gleason scores of 8–10. A full description of the clinical and demographic characteristics is presented in Table 1.

### Plasma cytokine concentrations in prostate cancer patients & controls

The authors found no significant differences in the plasma concentrations of IL-6, IL-8, IL-10, IL-1 $\beta$ , IL-4, IL-17A, IL-2, IL-12, M-CSF and IFN- $\alpha$  cytokines between prostate cancer patients and normal healthy controls (Figure 1 & Supplementary Material). However, the authors found a significant association between plasma PSA levels and concentrations of cytokines IL-6, IL-8 and M-CSF ( $p < 0.05$ ; Figures 2 & 3). High BMI was found to be significantly associated with increased levels of cytokine IL-8. Age and Gleason score were not associated with plasma cytokine concentrations. On multilinear regression, a combination of IL-6 and IL-8 was found to be significantly associated with rising plasma PSA levels ( $p < 0.05$ ).

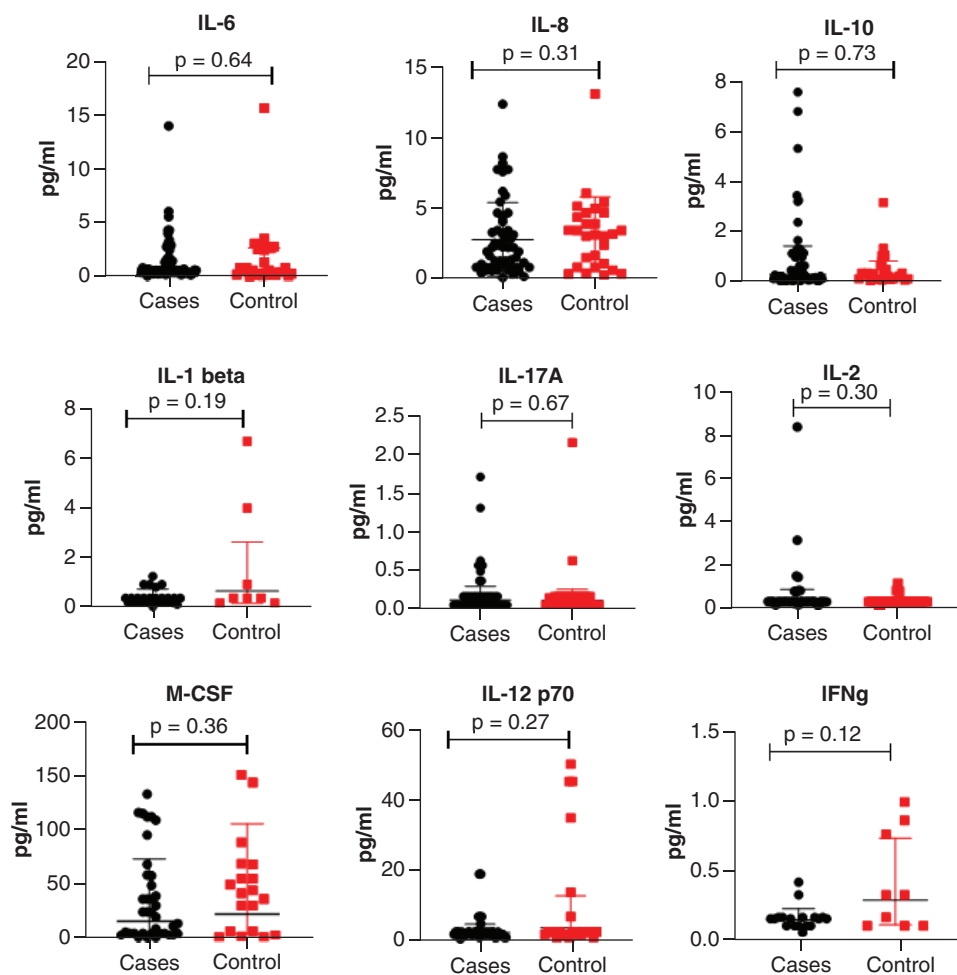


Figure 1. Concentrations of plasma cytokines among prostate cancer patients and controls.

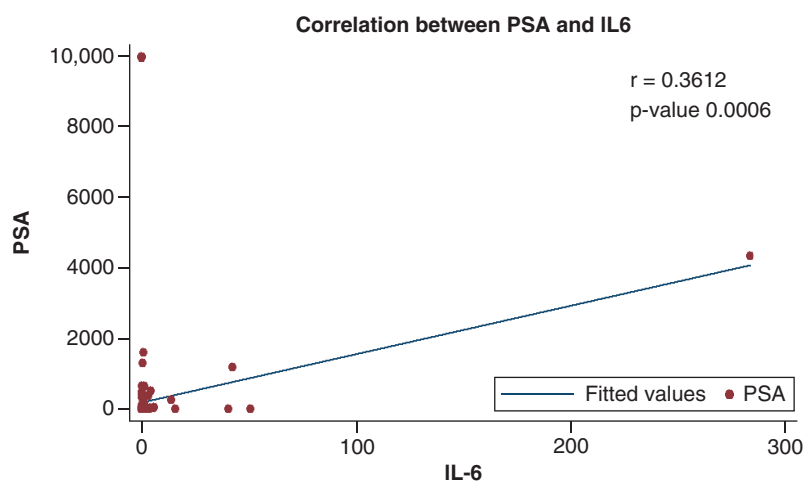
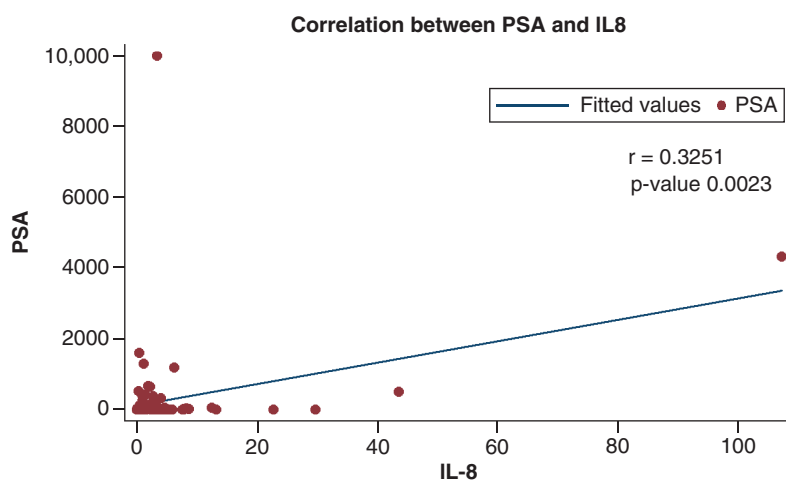


Figure 2. Correlation line graph of prostate-specific antigen and IL-6. Outliers were found not to affect the significance of the results. PSA: Prostate specific antigen.



**Figure 3. Correlation line graph of prostate-specific antigen and IL-8.** Outliers were found not to affect the significance of the results.

PSA: Prostate specific antigen.

## Discussion

Prostate cancer tumor heterogeneity plays a critical role in shaping tumor immune responses, contributing to tumorigenesis [17]. Elevated plasma levels of specific cytokines have been previously described in prostate cancer patients [18,19]. This study aimed to evaluate the plasma concentrations of nine cytokines (IL-6, IL-8, IL-10, IL-1 $\beta$ , IL-17A, IL-2, M-CSF, IL-12 and IFN- $\alpha$ ) in prostate cancer patients receiving androgen deprivation therapy and normal healthy controls. In this study, there was no significant difference in the concentrations of these nine cytokines between prostate cancer patients and controls. However, the authors found markedly elevated concentrations of IL-6, IL-8 and M-CSF in prostate cancer patients compared with controls. Concentrations of IL-6 and IL-8 were significantly associated with rising PSA levels ( $p < 0.05$ ). The authors did not observe any significant association between other cytokines and PSA, age, BMI or Gleason score. A study by Liu *et al.* showed that IL-6 induced prostate neoplasm in mouse models [20]. Similarly, Wu *et al.* reported IL-6 to be a clinical predictor of biochemical failure-free survival in prostate cancer patients on radiotherapy [21]. Ma *et al.* reported elevated serum levels of the cytokines IL-6 and TNF- $\alpha$  in patients with prostate cancer compared with controls, and this was associated with disease stage, PSA level and the presence of metastatic disease [22]. IL-6 is a key driver of tissue proliferation and also induces the metastatic potential of cancer cells [23]. This cytokine has been characterized as a prostate exocrine gene that interacts with its receptors in prostate cells, regulating proliferation and differentiation. Maynard *et al.* identified IL-8 as a cytokine with heightened expression in primary and metastatic prostate cancer patients compared with normal healthy controls [24]. The study theorizes a role for IL-8 in prostate cancer progression, likely via promotion of AR-independent mitogenic pathways.

The authors acknowledge that most of the studies referenced contain data from Caucasian and Asian populations. This clearly illustrates the gap in understanding the role immune cytokines play in prostate cancer disease pathogenesis in African men and hence the need for more work [8]. There is also a gap in understanding the key switches that control IL-6 and IL-8 signaling in cancer. A better understanding of how these cytokines contribute to the pathophysiology of prostate cancer would provide new targets for therapeutic intervention. However, this study is limited by a small sample size, which potentially affects its power.

## Conclusion

Viewed in the context of similar observations made for other cancers, these data further support a relationship between elevated IL-6 and IL-8 levels and prostate cancer disease prognosis. IL-6 and IL-8 correlate with rising PSA levels and extent of disease, and the authors recommend plasma monitoring in conjunction with other disease indicators. Future studies are needed to assess the precise relationship between IL-8 and IL-6 and AR and whether these cytokines or receptors may be therapeutic targets in managing advanced forms of prostate cancer. In addition, a longitudinal study to measure changes in IL-6 and IL-8 levels before initiation of androgen deprivation therapy and 6 months or 1 year after treatment may provide more insightful information.

## Future perspective

The role of non invasive biomarkers in cancer diagnosis and prognostication is key in ensuring sustainable cancer early detection and provision of personalized therapies.

### Summary points

- Prostate cancer is the second most common cancer in men worldwide.
- In Uganda, the age-standardized incidence rate of prostate cancer is 65 per 100,000 men.
- The mainstay of treatment for prostate cancer is androgen deprivation therapy, which is often augmented with other treatment options.
- Plasma cytokines have proved to be possible biomarkers for the prediction of clinical prognosis in cancer patients.
- Studies have demonstrated the role of proinflammatory cytokines in prostate cancer disease pathogenesis and response to radiotherapy and androgen deprivation therapy.
- There is limited information on the association and role of proinflammatory cytokines in prostate cancer disease pathogenesis in African men.
- This study assessed the levels of plasma cytokines in prostate cancer patients receiving care at the Uganda Cancer Institute.
- The plasma levels of nine cytokines, IL-6, IL-8, IL-10, IL-1 $\beta$ , IL-17A, IL-2, M-CSF, IL-12 and IFN- $\alpha$ , were detected by a Luminex assay.
- IL-6 and IL-8 cytokines were markedly increased in prostate cancer patients compared with controls.
- Increasing levels of IL-6 and IL-8 were significantly associated with high levels of plasma prostate-specific antigen ( $p < 0.05$ ).

### Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: [www.futuremedicine.com/doi/suppl/10.2217/fon-2021-0683](http://www.futuremedicine.com/doi/suppl/10.2217/fon-2021-0683)

### Author contributions

P Katongole, OJ Sande and N Niyonzima contributed to the conceptualization of the manuscript. S Nabweyambo and M Atuheirwe contributed to the running of the laboratory assays. P Katongole collected and analyzed the data, ran the laboratory assays and drafted the manuscript. OJ Sande, M Joloba, H Kajumbula, S Kalungi, K Ssebambulidde, J Orem, SJ Reynolds and N Niyonzima reviewed, read and approved the final manuscript.

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No writing assistance was utilized in the production of this manuscript.

### Ethical conduct of research

The authors obtained ethical approval from the School of Biomedical Sciences Higher Degrees Research and Ethics Committee (SBS-HDREC-779) and the Uganda National Council for Science and Technology and followed the principles outlined in the Declaration of Helsinki. All study participants provided written informed consent before enrollment in the study.

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