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Tumour Necrotic Factor Alpha, Interleukin 4 AndInterleukin 6 Levels In Patients With Prostatic Enlargements in A Nigerian Tertiary Hospital.

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Abstract
Objectives*: To determine the levels of TNF-α, IL-4 and IL-6 in the plasma of patients with benign and malignant prostate enlargements in Port Harcourt.*
Subjects and Methods: *Plasma of 30 patients with benign prostate enlargement, 30 patients with malignant prostate enlargement and 20 healthy controls at the University of Port Harcourt Teaching Hospital (UPTH) were analysed with the ELISA method to determine the levels of TNF-α, IL-4 and IL-6. The ages, PSA levels and volume of the prostate gland were retrieved from the patients' case notes and analyzed along with the levels of the cytokine using the SPSS version 21.*
Results: *The mean age of the patients with benign lesion (65.5±1.8)years was lower than those with malignant enlargement (67.5±1.5)years, but was not statistically significant. The average PSA level was also significantly higher (p < 0.05) in malignant enlargements (76.3±6.9ng/ml) when compared to benign prostate enlargement (3.3±0.3ng/ml). The mean plasma concentrations of TNF-α, IL-4 and IL-6 in patients with benign lesions were 73.3±7.4 (±SEM), 47.9±15.2 (±SEM) and 15.4 ± 2.1 (±SEM) respectively while that for malignant lesions were 38.1± 1.7 (±SEM), 4.4± 0.5 (±SEM) and 1.9± 0.2 (± SEM) respectively. The values were statistically significantly higher in patients with benign enlargement than those with malignant enlargement.*
Conclusion: *The plasma concentrations of TNF-α, IL-4 and IL-6 were found to be higher in patients with benign prostate enlargement than those with malignant enlargement in our environment.*
Keywords:*TNF-α, IL-4, IL-6, Prostate enlargements.*
Abbreviations: *Tumour Necrotic Factor-alpha (TNF-α), Interleukin 6(IL-6), Interleukin 4(IL -4), Benign prostatic hyperplasia (BPH), Enzyme linked immunosorbent assay (ELISA).*

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I. Introduction

Tumor necrosis factors were first found in tumor cells inducing lysis. TNF-α is the most prominent member of the TNF superfamily, consisting of at least 19 different peptides with diverse biological functions. The cytokine is chiefly produced by activated macrophagesand its primary role is regulation of immune cells. It has several pro-inflammatory properties and exhibits considerable pleiotropic characteristics. It has also been linked with the development and progression of prostate cancer [1]. TNF-α has been proposed to be a tumoral promoter andseveral studies have shown that endogenous TNF-α production is related to tumour invasion and development of metastasis [2]. In addition, elevated levels of TNF-α have been observed in the serum of individuals with prostate cancer[1, 3].

Interleukin-4 (IL-4)is believed to be secreted mainly by basophils and has functions related to IL-13 [4]. Observed biological roles of IL-4 include stimulating activated B-cell, T-lymphocyte proliferation and differentiation of B-cells to plasma cells. It has also been shown that IL-4 has a stimulatory effect on the proliferation of prostatic tumour cell lines [5]. Furthermore, Goldstein*et al* [6] reported elevated levels of IL-4 in patients with benign prostate hyperplasia and prostate cancer. On the other hand, high concentrations of IL-4 have been shown to inhibit the growths of both breast and colorectal cell lines [7]. However, work conducted using IL-4 knock-out model showed contradictory results on the specific role of this cytokine on prostate cancer[8].

Interleukin -6 (IL-6)has up and down regulatory inflammatory properties. It is mainly secreted by macrophages and T lymphocytes, and stimulates immune responses in the course of infections and trauma. The anti-inflammatory role of IL-6 is characteristically mediated via its inhibitory effects on TNF-α and IL-1 which are primarily pro-inflammatory cytokines. It has been reported that IL-6 activates the development of several neoplasms such as melanoma, other forms of carcinoma in renal and ovarian cells[9]as well as in prostate cancer[10].Several studies from different parts of the world have reported the expression of cytokines in patients with prostate enlargement (some of which were mentioned above). However, there is dearth of data on the expression of cytokines in this part of the world, especially among black Africans. This study was therefore designed to assay the levels of some cytokines (TNF-α, IL-4 and IL-6) in patients with benign and malignant prostate enlargements in University of Port Teaching Hospital (UPTH), Port Harcourt, Nigeria

II. Materials And Methods

This was a cross-sectional prospective study conducted among patients with benign and malignant prostate enlargements in the Urology Division of the UPTH. The study was approved by the Research Ethics Committee of the hospital (Appendix A.1).Sixty (60) consecutive presenting patients thirty (30) with benign enlargement and thirty (30) with malignant enlargement were recruited into the study. Those excluded were those who were already on therapy and those that declined to be part of the study. Also recruited as controls were twenty (20) healthy clinical dental students with no signs of prostate diseases. Information collected from the case notes of patients that met the inclusion criteria are ageand the PSA levels. Five (5) mls of venous blood was collected from each into a specimen bottle and immediately spurn with a centrifuge for 15-20mins. The supernatant plasma was transferred into another bottle and stored at -20^{o}C until analyzed. Cytokine concentrations were assayed using capture Enzyme Linked Immunosorbent Assay (ELISA) kits, according to the manufacturer's instructions (Avivabio systems, San Diego, CA, USA). Concentrations for each sample were extrapolated from the standard curve and expressed as mg/ml and were ultimately normalized to total protein in the sample and expressed as pg/mg protein. The data were transferred into a computer and analyzed using Prism software package (Graphpad Software Version 6.0). Summary and descriptive statistics were generated. The levels of the cytokines were compared between the two groups using the student T test and Chi square. Anova was used to compare the means of three groups (control, malignant and benign). An observation was considered significant if the P value was ≤ 0.05.

III. Results

The age range of patients with malignant prostate enlargements was 57-87 years, with a mean of 67.5±1.5 years, while the range for those with benign prostate enlargements was 40-93 years, with a mean of 65.5± 1.8 years. There was no significant difference in the mean ages between the two groups. The PSA levels in patients with malignant prostate enlargements ranged from 20-154 ng/ml with a mean of 76.3±6.9 ng/ml, while the range for benign prostate enlargements was 0.5-4.0ng/ml with a mean of 3.3±0.3 ng/ml. The mean PSA level was significantly higher in patients with malignant prostate enlargements ($P<0.05$). [Table A.1].

The mean concentration of TNF-α in patient with malignant enlargement was 38.1 ± 1.7 (±SEM) pg/ml, while the values for patients with benign enlargement and controls were 73.3 ± 7.4 (±SEM) pg/ml and 77.0 ± 10.5 (±SEM) pg/ml respectively. There were statistically significant differences in the values among the three groups ($p<0.05$). The mean TNF-α was significantly higher in patients with benign enlargement when compared with the malignant group ($p<0.05$). It was also higher in the controls compared with the malignant group ($p<0.05$). There was no statistically significant difference in the mean values between the benign group and the controls ($p>0.05$). This is shown in table A.2

The mean concentration of IL-4 in patients with malignant enlargement was 4.4 ± 0.5 (±SEM) pg/ml while for patients with benign enlargement; the mean was 47.9 ± 15.2 (±SEM) pg/ml. The control group had a mean of 12.7 ± 2.2 (±SEM) pg/ml. There were also statistically significant differences in the values among the three groups ($p<0.05$). There were significantly higher IL-4 levels in patients with benign prostate enlargement compared with the malignant group ($p<0.05$) and the controls ($p<0.05$). There was however no statistically significant difference in the mean level of the malignant group and the controls ($p>0.05$).

The mean concentration of IL-6 in patients with malignant enlargement was 1.9 ± 0.2 (±SEM) pg/ml, while the mean for patients with benign enlargement and controls were 15.4 ± 2.1 (±SEM) pg/ml and 14.0 ± 2.7 (±SEM) pg/ml respectively. There were statistically significant differences in the values among the three groups ($p<0.05$). The values were significantly higher in patients with benign prostate enlargement when compared with the malignant ($p<0.05$) and the controls ($p<0.05$) groups. There was no statistically significant difference in the mean values between the benign group and the controls ($p>0.05$).

IV. Discussion

Prostate neoplasm including benign prostate hyperplasia and prostate carcinoma are age- related diseases and are common in the elderly. De Nunzio et al estimated that benign prostate hyperplasia affects approximately 25% of men in their 50s, 33% of men in their 60s and 50% of those in their 80s, thus, aging is regarded as one of the significant risk factors for the development of benign prostate hyperplasia [11]. Also, Goossens et al had identified age as an important risk factor in the development of prostate cancer [12]. In this study, the mean age of patients with benign prostate hyperplasia was 65.5±1.8years while that of patients with prostate cancer was 67.5±1.5years. The mean age observed for patients with prostate cancer in this study is in agreement with previous reports from both Port Harcourt [13] and other parts of Nigeria [14].

However, in this study, the mean PSA level for patients with benign prostate enlargement was 3.3±0.3ng/ml, which is similar to mean value of 3.6ng/ml reported in a similar study [15]. Although, Brawer and Lange [16] had reported a low PSA mean of 2.1ng/ml, some other studies had reported higher values ranging from 4-9.8ng/ml in patients with benign prostate enlargement [16]. Also, the mean PSA level in patients with malignant prostate enlargement was significantly higher than those with benign enlargement in this study. Generally, patients with malignant prostate enlargement tend to have higher PSA levels compared to those with benign enlargement. However, there have been reports of instances when patients with malignant lesions have low PSA levels as observed by Ekeke et al [13] in which 20% of 294 patients with prostate cancer had PSA levels of less than 4ng/ml. The normal reference range of PSA as established in previous studies [17] was 0 – 4ng/ml. A serum PSA level above the range of 4 – 10ng/ml increases the probability of prostate cancer [17].

The mean plasma concentration of TNF-α in this study was significantly higher in patients with benign prostate enlargement compared to those with malignant enlargement, but there was no significant difference in the mean concentrations between the controls and patients with benign enlargement. This observation is contrary to the observations of many researchers who had reported higher serum concentrations in patients with malignant enlargement than those with benign enlargement and controls [3]. The reason for this apparent difference is not clear. Michalaki*et al* [3], had attributed high serum levels of TNF-α in prostate cancer to advanced disease, poor prognosis and resistance to therapy. However, it had also been previously reported that chronic synthesis of low amounts of TNF-α within a tumour microenvironment promotes tumour growth, thereby favouring angiogenesis, while high levels induce necrosis of tumour cells, stimulation of anti-tumour immunity, and triggering of vascular collapse. Thus, the high levels of this cytokine observed in patients with benign enlargement in this study may indicate a protective role.

The role of IL-4 in the pathogenesis and progression of prostate cancer and other cancers has remained controversial. As observed by Toi*et al* [5], a high concentration of this cytokine inhibits the growth of breast and colorectal cell lines, but Prokopchuk*et al* [7] showed that IL-4 has a stimulatory effect on the proliferation of prostatic cell line. Recently, Goldstein *et al* [8] studied the role of this cytokine in both benign and malignant prostate enlargements and their finding was similar to the finding in this study, in which the mean concentration of IL-4 was significantly higher in patients with benign enlargement than both the controls and patients with malignant enlargement. As found in this study, they also observed that the level of this cytokine was lower in patients with organ-confined radically treatable disease, and that the serum level was raised in castrate resistant (advanced) prostate cancer. Thus the findings in this study and those of other workers suggest that the production of high levels of IL-4 within the tumour microenvironment promotes rejection of the tumour. In contrast, however, lower levels of IL-4 produced by infiltrating leukocytes; make pro-tumour effects dominant and the disease progresses.

The mean plasma concentration of IL-6 in patients with benign prostate enlargement was higher than that of the controls in this study. This finding is similar to the observation of Pace *et al* [18]. However, while Pace *et al* [18] and El-far *et al* [19] reported higher mean serum concentrations of IL-6 in patients with prostate cancer than those with benign enlargement, the reverse was the case in the present study. This finding may be related to the associated chronic inflammation usually seen in patients with benign prostate enlargement in this environment, due to their late presentation. Also, it should be noted that Mandic*et al* [20] had reported genetic variation in the gene that controls the production of IL-6, as they observed that IL-6 higher producer genotypes were more frequent in patients with benign prostate hyperplasia than those with prostate cancer. This underscores the need for further studies on the genetic composition of patients with prostate neoplasms in this environment, in order to determine their role on the production of IL-6 and other cytokines.

V. Conclusion

The mean age and PSA level were higher in patients with malignant prostate enlargement compared to those with benign enlargement. The mean plasma concentrations of the cytokines TNF-α, IL-4 and IL-6 were higher in patients with benign prostate enlargement than those with malignant enlargement. Also, the concentrations in patients with benign enlargement were higher than in the control group except for TNF-α where that of the control was slightly higher.

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Table A. 1: Distribution of age and PSA level of Patients

Parameters	Malignant (M)	Benign (B)	T-Test (p-value)
Age (years)	67.5±1.5	65.5±1.8	0.3791
PSA (ng/ml)	76.3±6.9	3.3±0.3	<0.0001*

* Difference of the parameters between both groups is statistically significant.

Table A.2: Mean Concentrations of Cytokine in Control, Malignant and BPH

Parameters	**Control (C)**	**Malignant (M)**	**Benign (B)**	**ANOVA (*p*-value)**	**Multiple Comparisons (*p*-value)**		
					C v M	**C v B**	**M v B**
IL-4 (pg/ml)	12.7±2.2	4.4±0.5	47.9±15.2	<0.0042*	0.8419	0.0522	0.0044*
IL-6 (pg/ml)	14.0±2.7	1.9±0.2	15.4±2.1	<0.0001*	<0.0001*	0.8579	<0.0001*
TNF-α (pg/ml)	77.0±10.5	38.1±1.7	73.3±7.4	<0.0001*	0.0005*	0.9257	0.0003*

Values are expressed in Mean ± standard error of mean

*Difference between cytokine concentrations of the different groups is statistically significant