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Serum Levels Of Interleukin-6 (Il-6), Tumour Necrosis Factor (TNF-A) and Prostate Specific Antigen (PSA) Following Administration of A Ph Modulator in Patients with Prostate Cancer

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Abstract
Background:*Although huge progress has been made towards thedesigning of effective therapeutic strategies against Prostate Cancer (PCa), its treatment remains a major cause for concern, as recurrence after treatment is very common, coupled with adverse effects anda limited survival window associated with many cases.Therefore, alternative cost-effective ways of managing prostatic diseases are needed. This study evaluated the effect of a pH modulator (pHmo) on serum levels of prostate specific antigen (PSA) and some pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) in patients with PCa.*
Materials and Methods:*This quasi-experimental study was carried out on thirty (30)men with PCa who presented at the Urology clinics of the Rivers StateUniversity Teaching Hospital (RSUTH) and the University of Port Harcourt Teaching Hospital (UPTH), Rivers State, Nigeria, from April to August, 2018. The product was administered for 10 days only according to the manufacturer's prescription and blood samples were taken before and after treatment to quantify serum levels of PSA, IL-6 and TNF-α.*
Results:*TNF-α serum levels were significantly reduced ($p<0.05$) following administration of pHmo. IL-6 levels were also reduced but not significantly. Conversely, PSA levels were increased, though not significantly.*
Conclusion:*The results of this preliminary study showed that administration of pHmo to men with Pca for 10 days only caused a slight improvement in cytokine levels.*
Key word: *Prostate cancer; Cytokine; Interleukin-6;Tumour necrosis factor-α; Prostate specific antigen; pH modulator*

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I. Introduction

Globally, prostate cancer (PCa) is the second most common cancer (after lung cancer) and sixth leading cause of cancer deaths among men, accounting for over 1.2 million cases and 350,000 deaths (3.8% of all deaths caused by cancer in men in 2018).[1] The incidence and mortality of PCa correlate with increasing age, with the average age at the time of diagnosis being 66 years.[1] Reports show that African men suffer disproportionately from PCa compared to men from other parts of the world.[2] In Nigeria, PCa is the most commonly diagnosed cancer in men, estimated at 1.046% or 1046 per 100,000 men of age ≥40, and is reported to be responsible for 11% of all male cancer deaths, making it second only to liver cancer.[3]

Current strategies for managing PCa include surgery, radiotherapy, hormonal therapy,cryotherapychemotherapy, and dendritic cells vaccine therapy,and these have been used either as monotherapy or in multimodal approach.[4,5]Despite the therapeutic efforts, PCa treatment remains a major cause of concern as recurrence of the condition once treated is common, with many cases having a limited survival window.[6]Furthermore, apart from being expensive, current conventional treatment regimens for PCa have produced adverse effects including causing urinary incontinence, erectile dysfunction and bowel problems.[7,8]In view of the above, alternative cost-effective ways of managing prostatic diseases are needed urgently especially in Sub-Saharan Africa, where cost of healthcare is increasing daily coupled with recent economic downturns.

Previous studies have shown that the immune system plays a critical role in recognizing and controlling tumour growth (a process termed elimination or surveillance), and it is now generally accepted that avoiding immune detection and elimination is a hallmark of cancer.[9] In contrast, deregulation of the immune system may promote tumour progression by supporting chronic inflammation, shaping tumour immunogenicity and

suppressing antitumour immunity. As part of the surveillance, several innate and adaptive immune effector cells and molecules work in concert to successfully eradicate developing tumours long before they become clinically apparent.[10] Cytokines, which are small soluble or membrane-bound glycoprotein molecules that regulate immunity, inflammation and haematopoiesis, also represent part of the complex pattern of the immune response that can assist in elimination of cancer as well as aid its development.[11] Cytokines such as Interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and interleukin 4 (IL-4) have been shown to have pro-tumorigenic effectsand their levels may be utilized as markers of immune system status and prognosis in cancer.[12,13]

It is well-recognized that the functional status of immune system has direct bearing on cancer.[14] As a result a lot of products have been marketed with the premise that they enhance the functions of immune cells and health of cancer patients. It remains unknown whethersome of the products indeed boost the immune system and scientific evidence backing up such claims appear to be lacking. This study sought to investigate the effect of a pH modulator (an immune booster approved by the National Agency for Food and Drugs Administration and Control, NAFDAC) on the serum levels of Prostate specific antigen (PSA) and some cytokines (TNF-α and IL-6) in men diagnosed with PCa

II. Materials and Methods

This Quasi-experimental studywas carried out on patients with Prostate cancer in the Urology units of the Department of Surgery of the Rivers State University Teaching Hospital (RSUTH) and University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State, Nigeria, from April to August, 2018. The study was approved by the Research Ethics Committee of UPTHand total of thirty (30) patients were utilized.

Sample Size Calculation: The sample size was estimated according to the formula for calculating sample size to determine a difference between 2 proportions as previously stated by Kirkwood and Sterne.[16]With a 10% attrition factor taken into account, a sample size of 30 was obtained.

Subjects and Selection: The study population consisted of men presenting with Prostate cancer at the Urology Clinic of the Department of Surgery at University of Port Harcourt Teaching Hospital. Individuals were consecutively selected by the purposive sampling technique

Inclusion Criteria:

1. Men with confirmed diagnosis of PCa who had not commenced treatment.
2. Men diagnosed with PCawho had no other comorbid conditions (diabetes, HIV/AIDS and other infections).

Exclusion Criteria:

1. Men with PCa who had commenced treatment.
2. Men without PCa.
3. Men diagnosed with PCawho had other comorbid conditions such as HIV/AIDS, diabetes and High blood pressure.

Procedure

Written informed consent was obtained from the participants, and thereafter, socio-demographic and clinical data were collected from the subjects' hospital records using a PROFORMA data collection sheet. A pH buffer solution made of sodium and phosphate(pH 11.0- 13.3) manufactured by AllgoneInc, USA, and marketed in Nigeria under the trade name of CleanShield, with NAFDAC registration number A1-8184, was administered according to the manufacturer's prescription for duration of 10 days only.

Five millilitres (5 mls) of venous blood were collected from the study subjects prior to the administration of the supplement and on the 11th day after the administration of the supplement into plain sample bottles and appropriately labelled. Sera were separated from the blood samples immediately by centrifugation and stored at -20^{o}C until PSA, TNF-α and IL-6estimations were carried out. Serum levels of PSA and cytokines were quantified using capture Enzyme Linked Immunosorbent Assay (ELISA) kits, according to the manufacturer's instructions (Elabscience, USA). The concentrations of the factors 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 ng/ml protein.

Statistical Analysis of Data

Data were analysed using SPSS version 25 (SPSS, Chicago, IL). The data were presented as means, frequency and percentages. Serum cytokine and PSA levels of the different groups were compared with Analysis of Variance (ANOVA). Correlation of serum cytokine and PSA levels of the different groups was also done. All analysis was considered significant at a p-value of less than 0.05.

III. Results

The age range of the study subjects was 50-83 years, with a mean of 68.03±7.74 years.

Table 1.1 and Figure 1.1 show the effect of the pH Modulator (pHmo) on serum levels of PSA, IL-6 and TNF-α in PCa subjects.The mean PSA level before treatment was 21.27±18.39ng/ml and 23.60±23.65pg/ml after treatment. The PSA level was higher following administration of the pHmo but the difference was not significant. The mean IL-6 level was 301.16±352.22pg/ml before treatment and 94.81±84.05pg/ml after treatment. Although IL-6 level was lower following the administration of the pHmo, there was also no significant difference between these values. The mean TNF-α levelswere 21.06±6.36pg/ml and 12.47±2.00pg/ml before and after treatment respectively, showing that TNF-α levels were significantly ($p<0.05$) reduced in PCa subjects following administration of the pHmo.

Table no 1: Effect of pH Modulator (pHmo) on serum levels of PSA, IL-6 and TNF-α

Variable	Serum level					
Intervention	PSA (ng/mL)		IL-6 (pg/mL)		TNF-α(pg/mL)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
pH Modulator among PCa group	21.27±18.39	23.60±23.65	301.16±352.22	94.81±84.05	21.06±6.36	12.47±2.00
Paired t-test	$t_{(9)} = -0.64$; $p = 0.54$		$t_{(9)} = 1.91$; $p = 0.09$		$t_{(9)} = 3.91$; $p = 0.001$	

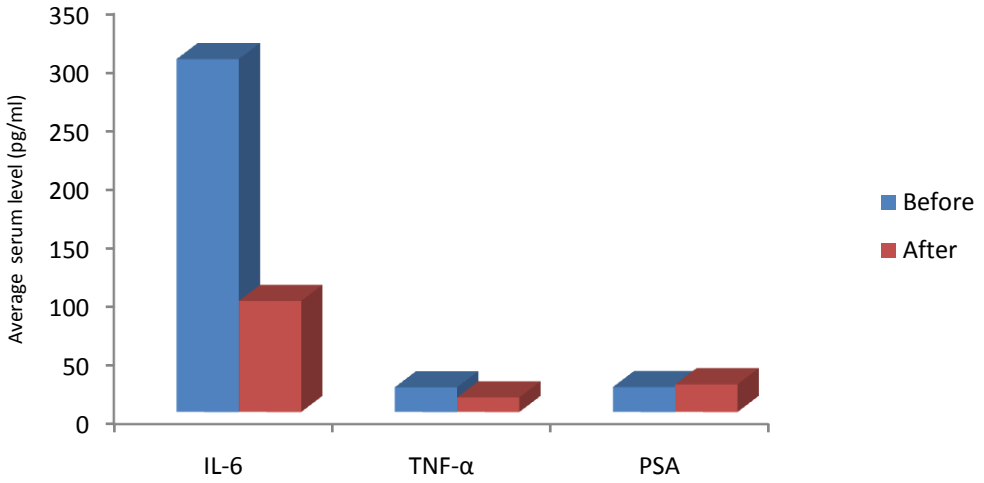

Fig no 1: Effect of pH Modulator (pHmo) on serum levels of PSA, IL-6 and TNF-α

IV. Discussion

The sequalae of Prostate Cancer (PCa) on the quality of life of patients have necessitated the urgency for search for low-cost and effective alternatives for the treatment and management of the condition. Spontaneous immune responses in cancer patients have been shown to determine disease course and impact positively on prognosis.[16] Immune cells such as cytotoxic T cells ($CD8^+$ T cells) and natural killer (NK) cells play a crucial role in immune mediated tumour rejection. They act by detecting antigenic determinants of tumour proteins, which are quantitatively or qualitatively altered in their expression because of neoplastic transformation,thereby destroying the tumour.[16]Although neglected for decades, immunotherapy has now emerged as a crucial strategy to improve survival in patients with most types of cancer. This renewed interest in immunotherapy is connected to the discovery of 'immune checkpoints' and the development of therapeutic tools to annul the inhibiting effects of these regulators on tumour immunity such as the cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed cell death protein-1 (PD-1) inhibitors.[17,18]

Parallel to traditional medicine, alternative medical products have been marketedfor a range of diseases, including cancer, with some making claims of healing diseases and significantly improving health. This study evaluated the effect of a pH modulator (a marketed immune booster registered with NAFDAC) on the serum levels of PSA and some cytokines (TNF-α and IL-6) in men diagnosed with Pca. The manufacturer of

the product of interest claims that the product 'boosts the body's pH levels and neutralizes the cascading avalanche of internal acidity in key areas, thereby boosting the immune system'.[19]To the best of our knowledge, no previous studies investigating the effects of an immune booster in the management of PCa have been published in Nigeria.

TNF-α and IL-6 are multifunctional cytokines which play key roles in tumour growth and metastasis and their serum levels have been shown to correlate directly with the extent of disease in patients with PCa.[20] In this study, the levels of TNF-α were found to be significantly reduced in PCa subjects after administration of the pHmo. Similarly, IL-6 levels were reduced after pHmo treatment, although the reduction was not significant. It remains unknown whether or not the levels of IL-6 would have reduced significantly if the treatment duration was extended.

Prostate specific antigen (PSA) is a glycoprotein primarily produced by the epithelial cells of the prostate gland and is the most commonly used serum marker for prostate cancer.[21] PSA levels can inform doctors of the possible presence of malignancy, while it is still small, low grade and localized. Men with PCa may have high serum PSA levels because of enhanced production of PSA and micro-architectural disruption in the prostatic gland, thereby allowing PSA greater access to the circulation.[22] PSA is also increased in other prostatic diseases such as benign prostatic hyperplasia (BPH) and prostatitis.[23] The results of this study showed that there was an increase, though not significant, in serum PSA levels following the administration of the pH modulator (pHmo) regimen for 10 days. Traditionally, the normal reference range of serum PSA is from 0.1 to 4ng/mL.[22] However, there have been reports of cases where patients with PCa have PSA less than 4ng/ml. [24,25] The reason for the observed increased in our study is not clear and again, it remains unknown whether or not the PSA levels would have reduced if the treatment duration was extended.

V. Conclusion

This study showed that the administration of the supplement (pHmo) for only 10 days caused a slight improvement in serum cytokine levels but not on PSA levels. Further studies are needed to assess the effects of administration of pHmofor a longer duration of time on the serum levels of these factors in PCa subjects. Probably, the utilization of a larger sample size may also help.

Declaration of interests: None

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