Prostate Specific Antigen and Testosterone Levels in the Diagnosis of Prostate Tumour

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Abstract
Background: *Prostate tumour is the enlargement of the prostate gland which could be as a result of benign prostate hyperplasia (BPH) or prostate cancer (PCa) which causes urinary symptoms, erectile dysfunction and low back pain in most men from 40years and above.*
Aim*: This study was carried out to determine the level of prostate specific antigen (PSA) and Testosterone in the diagnosis of prostate tumor.*
Method: *Blood samples from 199 subjects consisting of 119 test subjects (40-90 years) from the Urology and General out Patient's Department (GOPD) of Irrua Specialist teaching Hospital, Irrua Nigeria, and 80 control subjects (40 years and above apparently healthy male adults) were assayed. The Method of assay used for both parameters was the Enzyme Linked Immunosorbent Assay (ELISA).*
Result: *The mean values of PSA for the control subjects (0.56 ± 0.45), test subjects (35.01±38.21), subjects with BPH (6.72±1.71), subjects with PCA (52.22±39.56). There was a statistical significant difference (P<0.05) between the control and the test subjects, BPH and Prostate cancer patients. The mean values of testosterone for the control subjects (2.45±2.30), Test subjects (2.43±1.58), subject with BPH (2.73±1.58), subjects with Prostate cancer (2.25±1.56).There was no statistical difference (P>0.05) between the control subjects, test subjects, BPH and Prostate cancer patients. However there was a significant statistical difference (P<0.05) in the testosterone and PSA levels between BPH and Prostate cancer patients. There was no correlation (r = 0.14) between PSA and Testosterone of the test subjects. Results of this study shows a significant elevation of PSA in the test subjects (BPH*

and Prostate Cancer patients) compared to the control subjects which makes it a potent marker for the diagnosis of prostate tumor.
Conclusion: *Testosterone assay when combined with PSA may further help differentiate between BPH and prostate cancer.*

Keywords: *Testosterone, Prostate, Antigen, Benign, Tumour, Diagnosis*

Introduction

Prostate tumor is the enlargement of the prostate gland which could be as a result of benign prostatic hyperplasia (BPH) or prostate cancer (PCa). Benign prostatic hyperplasia and prostate cancer are major sources of morbidity in older men. Benign prostatic hyperplasia (BPH) can lead to bothersome lower urinary symptoms (LUTS) and /to acute urinary retention (AUR) (Sausville *et al.*, 2010). However, Prostate cancer is a form of cancer that develops in the prostate, a gland in the male reproductive system. Most prostate cancers are slow growing (Lister, 2009). However, there are cases of aggressive prostate cancers (American Cancer Society, 2010). The cancer cells may metastasize from the prostate to other parts of the body, particularly the bones and lymph nodes. Prostate cancer may cause pain, difficulty in urinating, problems during sexual intercourse, erectile dysfunction, or death. Prostate cancer is the most common non-cutaneous cancer in American men (Struewing *et al*., 1997). Prostate cancer is least common among Asian men and most common among black men, with figures for white men in between (American cancer society, 2010). It has been declared a public health epidemic in black American men because of its high incidence (Pienta *et al*., 1995). Africa was reported in the past to have a low incidence of this disease (Ahluwalia *et al.*, 1981). However recent studies indicate a high and rising incidence in Nigerians (Osegbe, 1997; Ogunbiyi and Shittu, 1999). It is a malignancy with a broad range of biological potential; one challenge for physicians is to identify and cure aggressive cancers while not over-treating indolent tumors (www.bccancer.bc.ca). Other symptoms can potentially develop during later stages of the disease.

Rates of detection of prostate cancers vary widely across the world, with South and East Asia detecting less frequently than in Europe, and especially the United States (Moore and Dally, 1999). Prostate cancer tends to develop in men over the age of fifty. Globally it is the sixth leading cause of cancer-related death in men (Smith, 2007) (It is now the first in the UK and second in the United States). Prostate cancer is most common in the developed world with increasing rates in the developing world (Smith *et al*., 2007). However, many men with prostate cancer never have symptoms, undergo no therapy, and eventually die of other unrelated causes. Many factors, including genetics and diet, have been implicated in the development of prostate cancer. Recently the prevalence of light pollution has been implicated in the development of prostate cancer (Millar *et al*., 2003).

The presence of prostate cancer may be indicated by symptoms, physical examination, prostate-specific antigen (PSA), or biopsy. Early prostate cancer usually causes no symptoms. Sometimes, prostate cancer does cause symptoms, often similar to those of diseases such as benign prostatic hyperplasia. These include frequent urination, nocturia, difficulty starting and maintaining a steady stream of urine, haematuria, and dysuria. About a third of patients diagnosed with prostate cancer have one or more such symptoms, while two thirds have no symptoms (Jemal, 2011). Prostate Specific Antigen (PSA) test throughout the world is used as a screening tool for prostate cancer due to increased sensitivity produced by prostate cells. It is detected at low levels in men of all ages but increases when the prostate enlarges or

becomes inflamed. High PSA occurs not only in prostate cancer but in prostatitis or a benign enlargement of the prostate (BPH)

Testosterone is an androgen synthesized and secreted by the leydig cells of the testicles, adrenal and the ovaries. The dihydro derivative of the testosterone exerts a potent anabolic action responsible for the post pubescent growth rate and subsequent muscle and bone maintenance of the adult male. There has been diverse opinion on Testosterone - Prostate cancer link. There have been long standing prohibition of testosterone therapy in men with untreated or low risk prostate cancer. A testosterone level appears to have a major influence on PSA values and may warrant watching for patients at high risk of prostate cancer (Anaheim, 2007). Higher PSA values had a near- linear association with rising Testosterone levels (Barqawi, 2006). The strong correlation between PSA and Testosterone might have an impact on the PSA cut-off value for recommending prostate biopsy. PSA itself is not perfect. The correlation of PSA and Testosterone is very much indicative of a testosterone level that may actually improve our protection against prostate cancer. It may improve the meaning and interpretation of the PSA value (Barqawi, 2006). Testosterone could easily be including in a panel of laboratory test. Including testosterone in a panel should help hold down cost of testing (Crowford, 2006). Although he emphasized that the role of testosterone in prostate cancer screening remains unclear. Testosterone can help distinguish prostate cancer from BPH in at least some patients (Hiroyoshi, 2007). Low rather than high serum testosterone levels have been found to be associated with advanced or high-grade disease. Testosterone neither increases the risk of prostate cancer diagnosis nor affects the natural history of prostate cancer in men who have undergone definitive treatment without residual disease (Isbam *et al*., 2005).

There have been controversial reports on the influence of Testosterone on Prostate Cancer, and the relationship of PSA and Testosterone in the diagnosis of prostate tumor. However low rather than high serum testosterone is associated with advanced or high-grade prostate cancer (Isbam, 2009). In a surprising paradox, the male hormone testosterone generally thought to be a feeder of prostate cancer has been found to suppress some advanced prostate cancers and may reverse resistance. Low testosterone levels may indicate worsening of disease for men with prostate cancer (Ignacio *et al.*, 2014).
Provocative new research suggests that it is not high serum Testosterone that is problematic for Prostate Cancer, but to the contrary that it is low serum T that is associated with worrisome cancer features and outcomes *(*Khera *et al., 2014)* Testosterone can help distinguish prostate cancer from (Benigh prostate Hyperplasia) BPH in at least some patients (Hiroyoshi, 2007). Also higher PSA values had been found to have a near-linear association with rising testosterone levels (Barqawi, 2006).

This work was carried out to establish the relationship, if any between PSA and Testosterone in the diagnosis of prostate tumor as well as determining the levels of testosterone in prostate cancer.

Materials and Methods

Area of Study

The research was carried out at the Irrua Specialist Teaching Hospital, Irrua in Esan Central Local Government Area, Edo State. This is a hospital serving people from Edo central and Edo North senatorial district, part of Edo South senatorial district and the neighboring states.

Research Design

Sample Size:

119 patients of age 40years and above, visiting the Urology clinics of the hospital and 80 apparently healthy adults of 40 years and above as control was recruited for the study.

Subjects and Selection Criteria

Patient selection was done by sampling of individual presenting at the Urology clinic of Irrua Specialist Teaching Hospital with history of prostate enlargement, erectile dysfunction, frequent and painful urination, and difficulty starting and maintaining a steady stream of urine as well as blood in urine for a period of within one to two months. Also, patients with provisional diagnosis of either benign prostate hyperplasia or prostate cancer were involved in the study. For comparative purposes, a control group of 80 apparently healthy individuals of 40 years and above were enrolled.

Sample Collection

2.5ml of blood Samples was drawn once from the participants by me and other professionals trained in the act of collecting blood samples diligently adhering strictly to standard operating procedure of blood sample collection using sterile needles and syringes assuring participants of no risk but little pain from needle prick at the reception. The blood was centrifuged at 3000rev/min for 5mins and the serum was separated and stored frozen. The left over samples being clinical waste was disposed off appropriately. Consent of most of the participants was sought by making them sign a consent form. However consent was not sought on routine samples with request on provisional diagnosis of the disease conditions. Confidentiality was assured by the use of codes on sample labeling instead of names and such codes given to desiring participants.

Ethical Approval

Ethical approval was sought from the ethics and research committee of the Irrua Specialist Teaching Hospital, Irrua.

Analytical Techniques

Quantitative Determination of Prostate Specific Antigen (PSA)

PSA was determined using the Enzyme-Linked Immunosorbent Assay (ELISA) (Horton *et al.*, 1988).

Principle of the Test

Anti-PSA antibody directed against PSA for solid phase immobilization on the microtiter wells reacts with the test sample at room temperature, washed to remove any unbound antigen. A monoclonal anti-PSA-horseradish peroxidase conjugate is then added and allowed to react with the immobilized antigen at room temperature resulting in the PSA molecule being sandwiched between the solid phase and enzyme -linked antibodies. A solution of Tetramethylbenzidine (TMB) was added resulting in development of a blue color which was stopped by the addition of a stop solution to develop a yellow color. The concentration of PSA is directly proportional to the color intensity of the test sample and was measured spectrophotometrically at 450nm wavelength.

Quantitative Determination of Testosterone

Testosterone was determined using the Enzyme-Linked Immunosorbent Assay (ELISA) method (Klee and Heser, 2000).

Principle of the Test

It is a solid phase enzyme-linked immunosorbent assay based on the principle of competitive binding. The micro titer wells are coated with a monoclonal antibody directed towards a unique antigenic site on the Testosterone molecule. Endogenous Testosterone of a patient sample competes with a Testosterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of Testosterone in the sample. On addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of Testosterone in the patient sample.

Data Collation and Analysis

The data generated from the study was subjected to statistical analysis. The analysis of variance (ANOVA), student t-test and pearsman correlation co-efficient using computer software package SPSS was employed. Commercial quality control sera, internal control sera and standard/calibrators provided by kits manufacturer was used to monitor the performance of the procedures.

Results

Table 1: Mean values and standard deviation of PSA and testosterone of Patients (BPH and PCa) and Controls.

Sample	Control (n=80)	Test (n=119)	t-value	p-value	Remark
PSA (ng/ml)	0.56 ± 0.45	35.01±38.21	9.84	P < 0.05	S
Testosterone (ng/ml)	2.45 ± 2.30	2.43±1.58	-0.12	P > 0.05	NS

Key: BPH- Benign prostate hyperplasia, **PCa-** Prostate Carcinoma

Table 2: Mean values and standard deviations of PSA and Testosterone of Patients with BPH and Controls

Sample	Control (n=80)	Test (n=45)	t-value	p-value	Remark
PSA (ng/ml)	0.56 ± 0.45	6.72±1.71	24.12	P < 0.05	S
Testosterone (ng/ml)	2.45 ± 2.30	2.73± 1.58	1.21	P > 0.05	NS

NB: BPH (4-10ng/ml)

TABLE 3: Mean values and standard deviations of PSA and Testosterone of patients with PCa and controls

Sample	Control (n=80)	Test (n=74)	t-value	p-value	Remark
PSA(ng/ml)	0.56 ± 0.45	52.22±39.56	11.24	P < 0.05	S
Testosterone (ng/ml)	2.45 ± 2.30	2.25±1.56	-1.08	P > 0.05	NS

NB: PCa(>10ng/ml)

Table 4: Comparison of the mean values of PSA and Testosterone of Patients with BPH and those with PCa

Sample	BPH (n=45)	PCA (n=74)	t-value	p-value	Remark
PSA (ng/ml)	6.72±1.71	52.22±39.56	9.89	P < 0.05	S
Testosterone (ng/ml)	2.73± 1.58	2.25±1.56	-2.63	P < 0.05	S

Table 5: BPH, PCa and Control

Sample	Control (n=80)	BPH (n=45)	PCA (n=74)	f-value	P-value
PSA (ng/ml)	0.56±0.45[a]	6.72±1.71[a]	52.22±39.5[b]	98.260	0.000
Testosterone (ng/ml)	2.45 ± 2.30 [a]	2.73± 1.58 [a]	2.25±1.56 [a]	0.897	0.409

NB: values in a row with a different superscript is significantly different at P<0.05

Table 6: Correlation between PSA and Testosterone

PS n A (ng/ml) (=119)	Testosterone (ng/ml) (n=119)	R	P value	Remark
35.01±38.21	2.43±1.58	0.14	0.134	NS

Table 7: PSA and Testosterone against increasing age ranges

AGE	40 – 50 Years	51 – 70 Years	71 – 90 Years	T Value	P Value
PSA (ng/ml)	4.73 ±4.18[a]	35.43± 57.60[b]	42.91 ± 49.50[b]	0.781	0.463
Testosterone (ng/ml)	4.9±0.30[a]	4.10± 2.56[a]	3.52 ± 2.68[a]	0.547	0.583

NB: Values in a row with a same superscript is not significantly different at P<0.05

Discussion

The PSA **values of patients with** prostate tumor (benign prostate hyperplasia and prostate cancer) was significantly higher than that of the control subjects (P<0.05).This is in line with the works of Catalona *et al*., (1993) who noted that PSA is always elevated when a man has prostate tumor. Igwe *et al*., (2004.) in a separate study confirmed the relevance of PSA assay over ACP, PAP, ALP and HSAP in the diagnosis of prostate cancer patients. It highlights the need for the inclusion of PSA assay in hospitals for accurate diagnosis of prostatic carcinoma. There was no significant difference in the mean values of Testosterone for both patients with benign prostate hyperplasia, prostate cancer and the control group (P>0.05) even though testosterone values in the control subjects were higher than values in prostate cancer patients. This work did not agree with the work of Berqawi, (2006) who noted that higher PSA values had a near linear association with rising testosterone levels. It however agrees with the work of Isbam *et al*., (2009) who though asserted that broad androgen levels are not associated with prostate cancer risk noted that conversely at the time of prostate cancer diagnosis, low rather than high serum testosterone levels have been found to be associated with advanced or high grade disease. High testosterone levels increase the risk of prostate cancer' "This is a hypothesis based on a very simplistic understanding of testosterone metabolism and its effect on prostate cancer. It is simply wrong," Kristal, (2008) said. Unlike estrogen and breast cancer, where there is a very strong relationship, testosterone levels have no association with prostate cancer risk, he said. A study published in 2008 in the *Journal of the National Cancer Institute*, which combined data from 18 large studies, found no association between blood

testosterone concentration and prostate cancer risk, and more recent studies have confirmed this conclusion.

There was a significant difference between values of PSA and testosterone in Patients with BPH and prostate cancer, with testosterone levels lower in prostate cancer when compared with BPH. The increase in the mass of the prostate may be responsible for the increase in testosterone level in benign while the degeneration of the cells in prostate cancer may account for the noticeable reduction in testosterone levels. This may help differentiate between BPH and prostate cancer in a number of cases. This is in line with the work of Japanese investigators who contributed to a debate with data indicating that testosterone measurement may help distinguish prostate cancer from BPH in at least some patients.

Conclusion and Recommendation

Conclusively, although there was a lower level of testosterone in prostate cancer than in BPH and normal subjects, there was no correlation between PSA and Testosterone. Its inclusion along with PSA in trying to determine cut- off of prostate cancer will be of no value but an economic waste and unnecessary burden on patients.

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