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Research Article

Fibroblast Growth Factor-23 may complement total PSA in prostate cancer and benign prostatic hyperplasia diagnosis.

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Abstract
The increase in the global incidence of prostate cancer (PCa) and benign prostate hyperplasia (BPH) can be adduced to increase in life expectancy, as both disorders are associated with aging. Prostate specific antigen (PSA) a pioneer biochemical marker for screening and diagnosis of prostate disorders has been found to be of limited specificity in differentiating PCa and BPH from prostatitis. We thus evaluated the role of fibroblast growth factor 23 (FGF-23) and estradiol (E2) in the development and progression of prostate disorders, their specificity and sensitivity in prostate disorders and the possible use of serum FGF-23 level as a diagnostic index for PCa and BPH. Thirty patients each with histological diagnosis of PCa or BPH were recruited from the Urology Unit of the University College Hospital Ibadan, and thirty aged-matched controls. Serum FGF-23, E2 and tPSA were assayed by ELISA technique and data generated, analyzed with IBM SPSS version 20.0 and Receiver Operating Characteristics (ROC) curve. The mean tPSA was found to be significantly higher (17.78 vs 5.25) and (17.78 vs 2.33) in PCa participants compared with BPH participants and controls respectively. FGF-23 was significantly higher in patients with PCa and BPH when compared with controls; (224.12 vs 207.33) and (222.88 vs 207.33) respectively. FGF-23 significantly differentiated participants with prostate disorders and controls, with greater sensitivity over PSA. The significantly raised serum FGF-23 in prostate cancer and BPH emphasizes its involvement in BPH and prostate carcinogenesis and can be used to exclude prostatitis in cases of raised PSA levels..

Key Words: Fibroblast growth factor 23 (FGF 23), Prostate cancer, Benign prostatic hyperplasia

INTRODUCTION

Prostate cancer is a major cause of cancer death among men (Siegel et al., 2017). Epidemiological surveys revealed Prostate cancer (PCa) as typically a disease of men over age 50 years and the incidence tends to increase with advancing age (Hsing et al., 2006), though incidence differs widely among races. Surveys have shown that PCa is most common among blacks; Africans and African-Americans, uncommon among Asians and the Americans and Europeans occupying the middle level in terms of prevalence (Ekman, 1999; Boyle et al., 2003). PCa is the most commonly diagnosed cancer among Nigerian men (Mohammad et al., 2008), constituting 11 % of all male cancers (Ogunbiyi et al., 1999). The incidence of prostate cancer in Nigeria remains on the rise and has become the number one cancer in men.

BPH is a noncancerous increase in size of the prostate due to proliferation of the epithelial and stromal cells of the transition zone. Enlargement occurs with age (Ojewola et al., 2017) and while all men experience prostate growth as they age, the growth rate differs among individuals (Wang et al., 2018). Cellular accumulation and gland enlargement has been attributed to a loss in glandular homeostasis; an altered balance between mitosis and apoptosis; (Torrealba et al., 2018). The hypertrophied gland may occlude the prostatic urethra leading to bladder outlet obstruction (BOO) with symptoms of impaired urine voiding, storage or both; known as Lower Urinary Tract Symptoms (LUTS) (Ojewola et al., 2017). Ojewola and colleagues reported a high burden of LUTS and BPH in Nigeria with a prevalence of 23.7% (Ojewola et al., 2017).

Prostate-specific antigen (PSA) is an enzyme protein of the kallikrein family serine proteases, produced by the prostate gland. Measurement of serum total PSA (tPSA) is the current method used in the screening, diagnosis and monitoring of therapy for prostate disorders, despite its limitation of non-specificity for prostate cancer. BPH and prostatitis can also induce a rise in serum PSA levels. This presents a pitfall in the use of serum PSA estimation in screening for PCa. The prostate has been shown to express receptors for fibroblast growth factor-23 (Fong et al., 2015) and estrogen (Kuiper et al., 1996). FGF-23 a family of 22-member fibroblast growth

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factors (Itoh et al., 2004) is an endocrine fibroblast growth factor of osteocytes essential in phosphate homeostasis (Yu et al., 2005). It is also expressed as autocrine growth factor in prostate, and present in increased levels in prostate cancer tissues (Fong et al., 2015), stimulating mitogenic and cell survival pathways by inhibition of vitamin D induced apoptosis and atrophy (Medici et al., 2008). Exogenous FGF-23 enhances proliferation, invasion and anchorage-dependent growth in vitro (Fong et al., 2015). Thus, reinforcing the possible role of FGF-23 as part of the multi-dynamics in the development of BPH and prostate cancer.

Estrogen, though a primary female sex hormone, has important physiological function in men (Lombardi et al., 2001). Estrogen is formed in the gonads of men from testosterone and dihydrotestosterone (DHT) by the action of enzyme aromatase. The presence of aromatase in the prostate tissue indicates intra-prostatic synthesis of estrogen, and is believed to play a role in the etiology of BPH and prostate cancer, perhaps by rendering prostate cells more susceptible to the action of testosterone and DHT (Wong et al., 2000) or act directly to initiate cellular proliferation. There has been a case report of the development of prostate cancer in a male to female transgender (MtFT) after years on estrogen therapy (Turo et al., 2013) Our previous report did not evaluate estrogen as a specific independent biomarker in the development of prostatic carcinoma (Adedapo et al., 2018a, b), hence its investigation alongside FGF-23 in this study.

The non-specificity of PSA informed this work to determine whether FGF-23 or estrogen could serve as independent biomarkers or complement PSA to improve the differential diagnosis of prostate disorders.

MATERIALS AND METHODS

Ethical clearance and approval for this study was obtained from UI/UCH joint ethical committee. Serum fibroblast growth factor 23 (FGF-23), estradiol (E2) and total prostate-specific antigen (tPSA) were determined in thirty (30) patients with histological diagnosis of PCa, thirty (30) patients with BPH from the Urology Unit of the University College Hospital Ibadan, and thirty (30) aged-matched controls. The sample size, 153, was calculated using this formula, $n = 2[(Z_\alpha + Z_\beta)^2 \pi (1 - \pi) / (P_1 - P_2)^2]$ (Bamgboye, 2008); n = required sample size, $\underline{Z_\alpha}$ = standard normal value corresponding to 95% confidence level set at 1.96, $\underline{Z_\beta}$ = standard normal value corresponding to a power of 80% set at 0.84, P_1 = proportion of patients within age ≥ 40 years with symptoms suggestive of BPH in Nigeria 25% (Ezeanyika *et al*., 2006); P_2 = prevalence of unexposed (with 15% increase = 40%); Π = average proportion, $(P_1 + P_2) / 2 = (0.25+0.40)/2 = 0.33$. However, for the purpose and duration of this study and the cost implication this preliminary study recruited and examined; a total of 90 participants (30 cases for PCa group, 30 cases for BPH group and 30 ages and sex matched control group). Participants for the test group were all willing patients who presented at the clinic and met the inclusion criteria; male above forty (40) years of age who had histopathological diagnosis for PCa or clinical diagnosis of BPH yet to commence therapy. The sample selection was done consecutively using every adult patient who registered to see the clinicians on each consulting day during the study period and who met the inclusion criteria and gave their consent. Participants for the control group had not been previously diagnosed or symptomatic for PCa or BPH, and were apparently healthy with PSA level less than 4ng/ml. Subjects for control were obtained randomly from within the city of Ibadan. Informed consent was obtained from all participants in the study. Semi structured questionnaire was used to obtain information on dietary pattern/lifestyle and other demographic indices from each participant, including the International Prostate System Score (IPSS) questionnaire.

Five milliliters (5 mL) venous blood samples was collected into lithium-heparin anticoagulant tube. All collected blood samples were centrifuged at 2000-3000 rpm. for 20 minutes and the plasma separated into plain tubes. Separated plasma were stored at -20^{o}C until adequate number was ready for laboratory analysis. Sample analysis was done at the research laboratory of the department of Chemical Pathology University of Ibadan. Serum FGF-23, estradiol (E2) and tPSA were determined using enzyme-linked immunosorbent assay (ELISA) kit for the quantitative determination of FGF-23, E2 and tPSA in serum (Wild, 1994). Blood pressure of the test groups were obtained from their clinical record while that of control group were determined using electronic blood pressure monitor. Data generated were analyzed using IBM SPSS statistics version 20.0. Analysis of Variance (ANOVA) and Post hoc tests were used for comparison of quantitative variables. Two-tailed independent t-test of significance at 95%confidence limit with $p < 0.05$ was considered significant for the variables. Chi-Square test (X^2) was used to determine the relationship between two qualitative variables. Receiver operating characteristics (ROC) curve used to determine the sensitivity and specificity of FGF-23, E2 and PSA.

RESULTS

Table 1 shows the comparison of mean anthropometric and biochemical variables in PCa, BPH and control participants. The mean ages for the PCa, BPH and control groups were 71.7 years, 70.2 years and 67.8 years respectively. There was a statistically significant difference between the mean PSA values of PCa, BPH and control participants (17.78ng/ml), (5.25ng/ml) and (2.33ng/ml) respectively; p=0.002. The mean values of FGF-23 in PCa group do not differ significantly from those from BPH; 224.12ng/ml vs 222.88ng/ml but both PCa and BPH groups were statistically significantly different from the control group; 207.33ng/ml (p=0.013). Both the mean systolic and diastolic blood pressure was significantly lower in PCa (126/82 mmHg) and BPH (137/81 mmHg) groups when compared to control group (140/90 mmHg).

Table 2 and Figure 1 show the receiver operating characteristics (ROC) and area under the curve (AUC) between PCa and control participants. The sensitivity and specificity of measured analytes were compared between prostate cancer patients and controls as shown in Table 2. FGF23 had a statistical significance AUC of 0.697 at 221.45ng/ml cutoff, with sensitivity of 66.7% and specificity of 83.3% (p=0.009). PSA and E2 had cutoffs of 1.22ng/ml and 40.19pg/ml respectively though not statistically significant. PSA had a sensitivity of 56.7% and specificity of 86.7%, while E2 had a much lower sensitivity and specificity of 50.0% and 46.7% respectively. Figure 1 shows the ROC curve of FGF23, E2 and PSA between PCa and control participants.

Table 1:
Comparison of mean anthropometric and biochemical variables in PCa, BPH and control participants.

Variables	Pca	BPH	Controls	F	P
Number (N)	30	30	30		
Age (years)	(71.70 ± 8.09)	(70.20 ± 10.41)	(67.77 ± 9.96)	1.299	0.278
SBP (mmHg)	126.33 ± 12.17[a,b]	137.20 ± 26.57	140.60 ± 22.10	3.722	0.028*
DBP (mmHg)	82.00 ± 8.34[a]	81.00 ± 12.42[a]	90.17 ± 11.22	6.504	0.002*
HEIGHT (meters)	1.67 ± 0.09[a]	1.68 ± 0.08	1.72 ± 0.08	3.109	0.050
WEIGHT (Kg)	62.23 ± 10.16	65.20 ± 9.85	68.10 ± 8.58	2.828	0.065
BMI (Kg/m^2)	22.42 ± 3.23	23.25 ± 3.31	23.17 ± 2.85	0.635	0.532
WC (cm)	87.00 ± 8.10	79.95 ± 14.55	84.45 ± 12.54	2.638	0.077
FGF-23 (ng/ml)	224.12 ± 24.47[a]	222.88 ± 25.74[a]	207.33 ± 21.40	4.581	0.013*
PSA (ng/ml)	17.78 ± 4.85[a,b]	5.25± 1.25	2.33 ± 1.38	6.964	0.002*
E2 (pg/ml)	41.85 ± 4.00	46.57 ± 1.89	41.96 ± 3.92	0.621	0.540

*Significant at p<0.05; [a]Significantly different from controls [b]significantly different from BPH

Table 2:
ROC AUC between PCa and Control participants.

VARIABLES	AUC	p	CUT-OFF	SENSITIVITY	SPECIFICITY
E2	0.513	0.859	40.19	50.0	46.7
FGF-23	0.697	0.009*	221.45	66.7	83.3
PSA	0.564	0.391	1.22	56.7	86.7

*Significant at p<0.05

Table 3:
ROC AUC between BPH and Controls

VARIABLES	AUC	p	CUT-OFF	SENSITIVITY	SPECIFICITY
E2	0.641	0.060	43.32	70.0	50.0
FGF-23	0.673	0.021*	224.52	60.0	83.3
PSA	0.467	0.657	1.016	40.0	86.7

*Significant at p<0.05

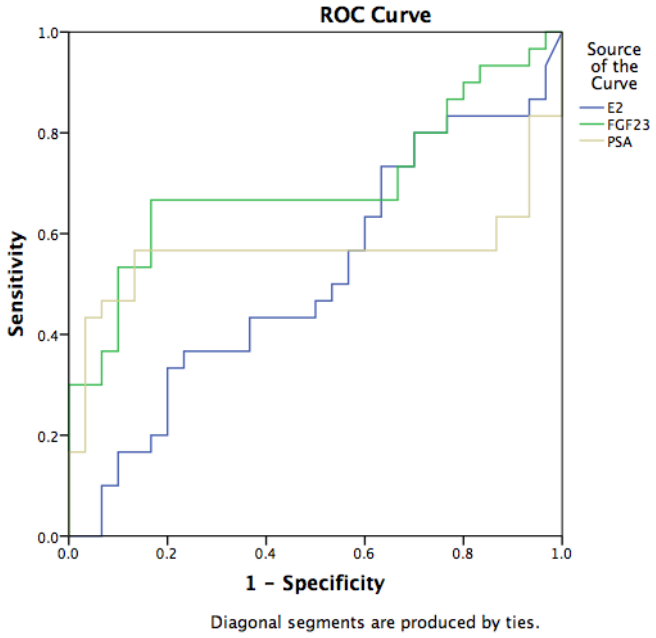

Figure 1:
ROC Curve for E2, FGF-23 and PSA between PCa and controls

Table 3 and Figure 2 show the ROC AUC between BPH and Controls. Comparing the sensitivity and specificity of FGF23, E2 and PSA between BPH and control participants as shown in Table 3; FGF23 had statistical significant AUC of 0.673 and a cutoff of 224.52ng/ml with sensitivity of 60.0% and specificity of 83.3% (p=0.021). E2 at 40.32pg/ml cutoff was 70% sensitive and 50% specific but not statistically significant with AUC 0.641. The ROC plot of FGF23, E2 and PSA between BPH and control groups is shown in Figure 2

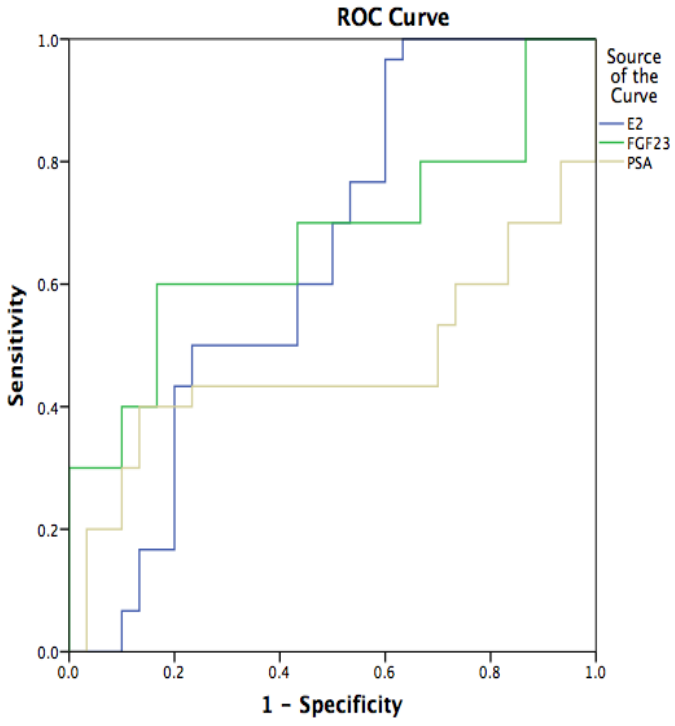

Figure 2:
ROC Curve for E2, FGF-23 and PSA between BPH and controls

ROC plot of the sensitivity and specificity of FGF23, E2 and PSA between PCa and BPH groups is shown in Table 4 and Figure 3.

Table 4:
ROC AUC between PCa and BPH

VARIABLES	AUC	p	CUT-OFF	SENSITIVITY	SPECIFICITY
E2	0.401	0.188	43.50	46.7	40.0
FGF-23	0.530	0.690	229.45	53.3	60.0
PSA	0.594	0.209	1.32	56.7	66.7

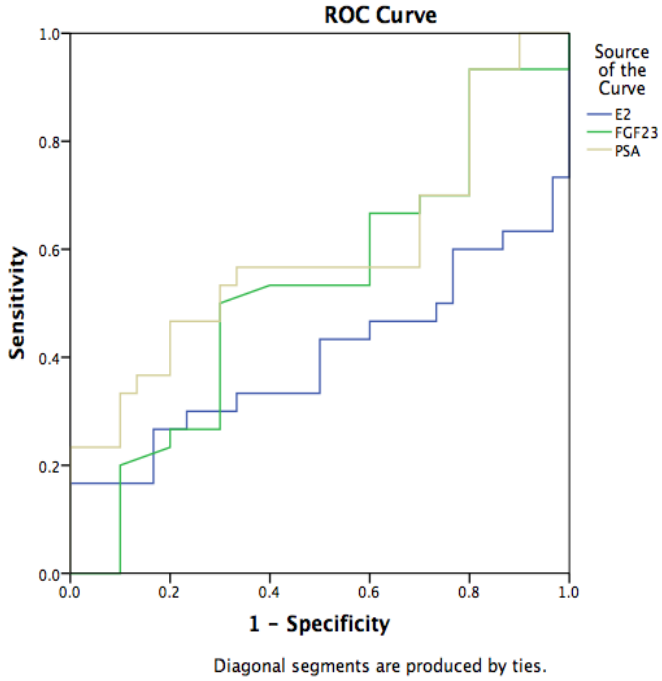

Figure 3:
ROC Curve for E2, FGF-23 and PSA between PCa and BPH

DISCUSSION

In this study, the mean total PSA level of PCa participants was significantly higher compared with BPH and control groups, this is consistent with the trend observed in other studies (Usoro *et al.,* 2015; Udeh *et al.,* 2016) which also obtained higher PSA levels in prostate cancer participants compared to control and BPH participants. However, in contrast to the same study by Usoro *et al.* (2015), the mean total PSA values for BPH was not significantly higher compared with controls in this study. This finding buttresses the non-specificity of PSA in differentiating benign prostate conditions; prostatitis or physical trauma which also cause a rise in PSA levels such as is obtainable in BPH.

Several in vitro studies have implicated the growth factor FGF-23 in the development and progression of prostate cancer and benign prostate hyperplasia. However, very few studies have measured serum levels of FGF-23 in prostate cancer or BPH patients. Volt *et al.,* (2017) reported that FGF-23 was not elevated in prostate cancer. As opposed to their finding, this study found the mean serum FGF-23 levels to be significantly higher in prostate cancer participants compared with control participants; and also higher in BPH participants compared to controls. A high serum FGF-23 level could indicate that FGF-23 acting via its receptors on the prostate induces glandular and stromal cell proliferation and consequent development of BPH or PCa on an already initiated prostate. Yu and colleagues reported FGF-23 promotes cell proliferation, growth, angiogenesis and consequent tumorigenesis; which is in tandem with the high serum levels found in both PCa and BPH patients in this study (Yu *et al.*, 2016). Notwithstanding, FGF-23 was unable to significantly differentiate between prostate cancer and benign prostate hyperplasia this had been earlier established in our previous work (Adedapo *et al.,* 2018b); however, FGF-23 had a significantly greater sensitivity over PSA for both prostate cancer and BPH. In addition, it markedly differentiated between prostate cancer and normal prostate at a serum concentration of 221.45ng/ml with 66.7% sensitivity and 83.3% specificity. Also at a concentration of 224.52ng/ml, FGF-23 also differentiated benign prostate hyperplasia from normal prostate with a sensitivity of 60.0% and specificity of 83.3%. FGF-23 could be a better maker than PSA in assessing the prostate. In combination with PSA, it can elucidate the apparent source of elevated serum PSA; a rise in PSA without a concomitant rise in FGF-23 predicts that the increased serum PSA could most likely be due to prostatitis rather than BPH or prostate cancer. The greater sensitivity of FGF-23 over PSA, moreover, affords for earlier detection of PCa and BPH, which is paramount to achieving a successful medical intervention.

In conclusion, FGF23 could be a promising biomarker to complement PSA, thus further elaborate studies should be carried out on a larger scale. The time frame, resources available and a minimal sample size form the limitations to this study.

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