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RESEARCH ARTICLE

Current Evidence for the Involvement of Hypogonadism and Modulation of Steroid Receptors in Prostate Carcinoma

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ABSTRACT
Prostate cancer (PCa) is the most prevalent cancer in the Nigerian male population, similar to other black populations. It is postulated that exposure to endogenous or environmental steroids prompts prostatic mediated changes via steroid receptors as well as a decrease in the androgen/estrogen ratio and aging. Thereby contributing to prostatic carcinogenesis and disease progression. This study is aimed to determine the plasma levels of testosterone, 17β-estradiol as well as the pattern of expression of steroid receptors in subjects with prostate cancer, benign prostatic hyperplasia, and controls. Study participants are made up of a total of 195 consented volunteers consisting of 65 Prostate cancer (PCa) and 65 benign prostatic hyperplasia (BPH) treatment naïve participants and 65 apparently healthy subjects as controls. Anthropometric data were measured using standard methods and biochemical parameters determined by enzyme-linked immunosorbent assay (ELISA). The gene expression is quantified by Real-Time PCR with PerfeCTa SYBR Green SuperMix on CFX96 Bio-Rad, USA. The results of this study showed increased levels of 17β-estradiol, total androgen receptor (AR) and estrogen receptor-beta (Erβ) in prostate cancer participants compared with controls ($P<0.05$). A Significant reduction in plasma levels of testosterone in prostate cancer subjects compared with BPH and controls was observed ($P<0.001$). The plasma levels of androgen receptors were significantly increased in PCa and BPH participants ($p<0.05$). We observed a positive correlation between ERβ levels and PSA levels in the PCa group ($r=0.32$, $p=0.02$). There was a differential expression of AR, ESR1 and ESR2 in the studied group. This study shows hypogonadism and an increased 17β-estradiol, ERβ, and AR levels in subjects with prostate cancer. This data suggests that modulation of these hormones and their receptors may be associated with initiation and progression of prostate cancer and could be valuable in the interpretation of PSA kinetics and stratification of cases after screening.

Keywords: Prostate Cancer, Benign Prostatic Hyperplasia, Androgen Receptor, Hypogonadism 17β-estradiol, Oestrogen Receptor-β.

INTRODUCTION

Prostate cancer is the world's second most frequent malignancy among males. It affects people of African descent disproportionately, regardless of where they live, and is less frequent in White and Asian populations. A complex interaction of hormonal, environmental, and genetic variables is thought to be responsible for racial disparities in prostate cancer incidence and prognosis[1]. Early research on the hormonal basis of prostate cancer concentrated on androgens, however 17β-estradiol has lately been identified as a possible factor in the pathogenesis and progression of prostate cancer[2]. In the early era of prostate cancer research, the role of 17β-estradiol was primarily seen as an indirect anti-androgen action mediated through feedback inhibition of hypothalamic luteinizing hormone (LHRH) and pituitary luteinizing hormone (LH) release, resulting in decreased testicular androgen synthesis and release [3].

Low levels of testosterone had been associated with reduced risk of developing prostate cancer. However, men with very low levels of testosterone who did get prostate cancer were reported to be more likely to develop an aggressive form of the disease[4]. However, the actions of testosterone is mediated via the androgen receptor (AR), a ligand-dependent nuclear transcription factor and member of the steroid hormone nuclear receptor family. Although androgen receptor (AR) signaling is the primary molecular tool for controlling prostate gland development and function, estrogen receptor (ER) is involved in prostatic epithelial cell differentiation as well as multiple antiproliferative activities on prostate cancer cells. ER splice variants, on the other hand, have been linked to prostate cancer initiation and progression pathways. ER seems to be effective as an anticancer treatment and in the prevention of prostate cancer[5].

Also estrogen receptors mediate the activities of direct estrogens. The nuclear receptor superfamily includes estrogen receptors (ER1) and (ER2). ER2 has been considered as a novel therapeutic target for prostate cancer due to its growth inhibitory effect in prostate tissue[5]. Estrogen signaling pathways are selectively stimulated or inhibited depending on a balance between the activities of estrogen receptors; ER-α or ER-β in target organs. These receptors are members of the nuclear receptor superfamily which act as transcription factors after binding to estrogen. The gene expression regulation by estrogen receptors is to modulate biological activities, such as reproductive organ development, bone modeling, cardiovascular system functioning, metabolism, and behavior in both females and males [6]. A report suggested that estrogenic action via ER-β can result in an inhibited cellular proliferation within the prostate and prostate cancer [7]. It is noteworthy that 17β-estradiol has been classified as a carcinogen by the International Agency for Research on Cancer, primarily based on its association with endometrial and breast carcinoma in women [6]. It is also now recognized that estrogens and their metabolites play a role in the normal growth of the prostate as well as in prostate cancer [8].

There is multiple consistent evidence suggesting that 17β-estradiol and its receptors especially ER-β are critical players in human prostate cancer. Recently, their role in prostate cancer is becoming of interest. As estrogenic stimulation and phenotypic changes to estrogen-receptors may contribute to prostatic carcinogenesis [2], the molecular networks of estrogenic and anti-estrogenic signaling in prostate cancer have not been fully deciphered.

METHODOLOGY

Study site and design

This is a case-control study. The study was conducted at the Lagos University Teaching Hospital, Idi-Araba, Lagos State. The samples were obtained from consented participants who have been diagnosed to have prostate cancer visiting Urology Clinic, Lagos University Teaching Hospital, Idi-Araba, Lagos State.

Study population

The study populations are older men (45-85 years), divided into three groups Viz; Prostate cancer, benign prostatic hyperplasia and apparently healthy controls. All participants were screened using digital rectal examination (DRE) and plasma prostate specific antigen concentration. Participants with enlarged prostate as detected via digital rectal examination and /or those with PSA greater than 4ng/ml were recommended for histopathology analysis of the prostate biopsy for confirmation of diagnosis as either prostate cancer (having cancerous tissues) or benign prostatic hyperplasia (absence of cancerous cells). Control group are individually screened using DRE, plasma PSA concentration and free of any prostatic disease and have no family history of prostate cancer or any other cancer and who have not had their prostate surgically removed. A total of 195 participants including 65 PCa and 65 BPH and 65 apparently healthy aged, matched controls.

Ethical consideration

Research protocol was submitted to the Health Research Ethics Committee, College of Medicine, University of Lagos and approval was obtained; (CMUL/HREC/04/19/512) prior to the commencement of the study. Inform consent was sought from each of the participants of the study.

Biochemical analysis

Total and free Prostate specific antigen, testosterone, DHT, 17β-estradiol, total androgen receptor, and estrogen receptor activity were determined using ELISA kits from Merlin Biotechnologies (Ontario, Canada) following the manufacturers protocol.

RNA extraction

Circulating RNA was extracted from plasma using QIAamp Circulating Nucleic Acid Kit following the manufacturers procedure[9].

Gene expression analysis

Extracted RNA was reverse transcribed to cDNA using ABM 5x all-in-one rt mastermix cDNA kits following the manufacturers instruction and an Eppendorf mastercycler PCR machine. Expression of AR, ESR1 and ESR2 were determined using quantitative PCR[10]. Real-Time PCR was done with PerfeCTa SYBR Green SuperMix on CFX96 Bio-Rad, USA. was synthesized and produced by RT-primers limited (USA). The annealing temperature for all primer was 60. The qPCR was performed for 45 cycles at the following temperature: 95°C for 3minutes, 95°C for 15 seconds, 60°C for 45 seconds [10]. Gene expression was determined by normalizing with GADPH house-keeping gene as reference.

RESULTS

The results show significant increase in weight, waist circumference, hip circumference and waist-hip ratio between group (*$p<0.05$). While the weight and waist-hip ratio increased in PCa (78.00±1.66 and 1.02±0.03, respectively), the waist and hip circumference increased in BPH (43.94±2.32 and 45.47±2.31, respectively), Table 1. Obesity defined by waist hip ratio was a significant risk factor in PCa (3.27) and PBH (2.87), $p<0.05$. The odd ratio (OR) estimates shows that Obesity as defined by waist-hip ratio greater that 0.90 is a better predictor of both BPH (OR=2.87,p=0.033) and Prostate cancer (OR=3.27,p=0.018) (Table 2). Table 3 shows the plasma levels of hormones, steroid receptors activity, and prostate specific antigen and (testosterone, 17β-Estradiol, Total PSA, free PSA, TARA, ERβ). ANOVA shows significant difference in total PSA, free PSA, TARA, Erβ ($p<0.05$), Post hoc comparisons using the Tukey test show statistically significant difference was in Testosterone (2.85±3.31 **Vs** 25.83±9.40), 17β-estradiol (173.97±13.74 **Vs** 129.1±6.09), TARA (557.24±85.16 **Vs** 366.57±16.45), ERβ (716.61±105.37 **Vs** 413.55±95.73) between PCa subjects and controls (†= $p<0.01$). Testosterone, TARA and ERβ had statistically significant difference between BPH and controls (¶= $p<0.05$). There is a significant negative correlation between testosterone and PSA levels(p=**-** 0.31, r=0.014*), ($p<0.05$) while a positive correlation exists between PSA and ERβ (p=0.32, r=0.02*) ($p<0.05$) as presented in Table 4.

Table 1 Comparative analysis of anthropometric variables in the study participants

Variable	Control Mean±SEM	BPH Mean±SEM	PCa Mean±SEM	F value	P value
Age	62.80±1.74	64.13±1.29	66.48±0.82	1.04	0.06
Weight (Kg)	63.70±2.17	68.69±2.31†	78.00±1.66¶†	11.267	<0.001*
Height (m)	1.60±0.02	1.66±0.02	1.69±0.01	6.190	0.08
BMI (Kg/m2)	25.33±1.18	25.10±0.89	27.26±0.66	1.744	0.181
Waist Circumference	36.04±0.80	43.94±2.32¶†	38.50±0.94†	5.819	0.004*
Hip Circumference	36.65±0.70	45.47±2.31¶†	37.79±0.76†	8.922	<0.001*
Waist-Hip Ratio	0.98±0.01	0.97±0.01†	1.02±0.03†¶	3.645	0.031*

Values are presented as mean±SEM ; $P<0.05$ is denoted as * One way ANOVA followed by Tukey's post-hoc test to determine significance ¶$p<0.05$ between cases versus the control group and †$p<0.05$ between (PCa and BPH).

Table 2 : Chi-square of anthropometric indices in studied groups

Factor	Odd ratio	95% CI	P-value
Obesity-BMI (PCa)	1.57	0.62 – 4.06	0.062
Obesity -BMI (BPH)	1.78	0.69 – 4.59	0.230
Waist-Hip ratio (Pca)	3.27	1.22 – 8.74	0.018*
Wasit-Hip ratio (BPH)	2.87	1.09 - 7.59	0.033*

Risk stratified using chi-square at 95% CI; Obesity is considered BMI >25 or Waist-Hip Ratio >0.90 (WHO, 2011).

Table 3: Comparative analysis of TPSA, Hormones, and their Receptors in Cases and Controls

Variables	Control (n=65) Mean±SEM	BPH (n=65) Mean±SEM	PCa (n=65) Mean±SEM	F-value	p-value
TPSA (ng/ml)	1.38±1.04	66.56±12.17	87.45±19.48	13.15	0.00016*
% Free PSA (ng/ml)	27.45±0.64	13.12±12.17	7.83±1.37	12.26	0.0083*
Testosterone (ng/ml	25.83±9.40	12.89±1.08	2.85±3.31	10.18	0.00022*
17β-Estradiol(pg/ml)	129.1±6.09	109.63±4.44	173.97±13.74	4.72	0.014*
TARA	366.57±16.45	409.83±25.06¶	557.24±85.16†	3.86	0.025*
Erβ	413.55±95.73	516.52±205.64¶	716.61±105.37†	2.16	0.007*

Values are presented as Mean±SEM; $p<0.05$ is denoted as * using One way ANOVA followed by Tukey's post-hoc test to determine the level significance ¶$p<0.05$, †$p<0.01$ versus the control group.

Table 4: Correlation between hormones/receptors and PSA in cases and controls

Variable	PCa	BPH	Control
ERβ	0.32, 0.02*	0.04, 0.28	0.09, 0.48
Testosterone	**-** 0.31, 0.014*	0.13, 0.34	0.15, 0.11
17β-Estradiol	0.18, 0.07	0.12, 0.46	0.07, 0.66
AR	-0.16, 0.209	0.16,0.29	0.05,0.72

Pearson's correlation expressed as (r, p-value) * Significant when $p<0.05$.

Figure 1 shows an early upsurge in the levels of 17β-estradiol in prostate cancer group and a sustained increase from 45 year. The pattern is similar in the BPH and control groups but the plasma levels were reduced. There was a significant upregulation of AR gene in prostate cancer and benign prostatic hyperplasia group compared to control ($p<0.01$), similarly the ESR2 gene was significantly upregulated in prostate cancer and BPH ($p<0.01$). However, a downregulation of ESR1 gene was observed in prostate cancer group versus BPH group and controls ($p<0.05$) (Figure 2) showing a significant difference in the expression of genes between PCa and BPH.

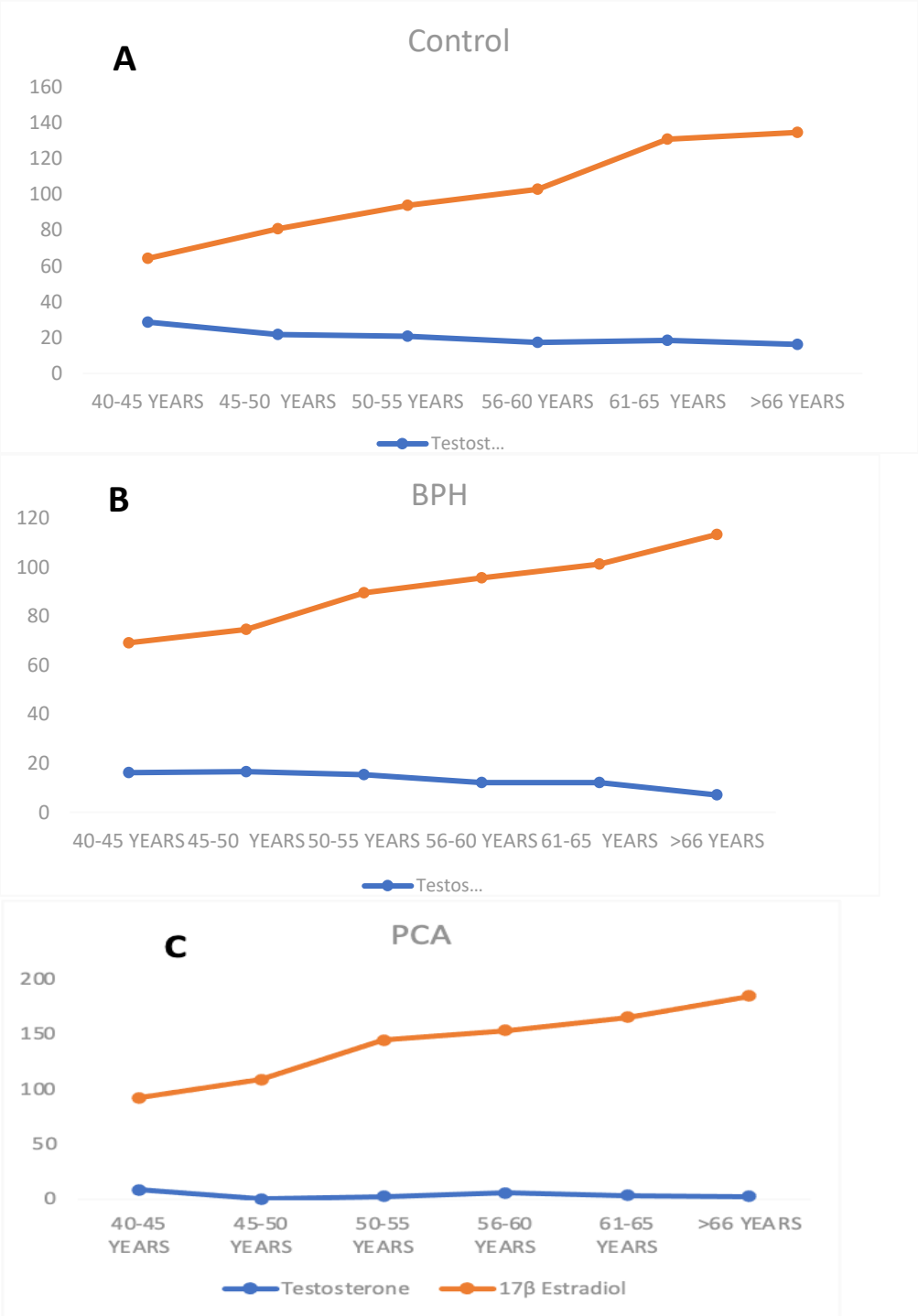

Figure 1. Age-related changes in testosterone and 17β-estradiol levels in (A) controls, (B) benign prostatic hyperplasia and (C) prostate cancer group

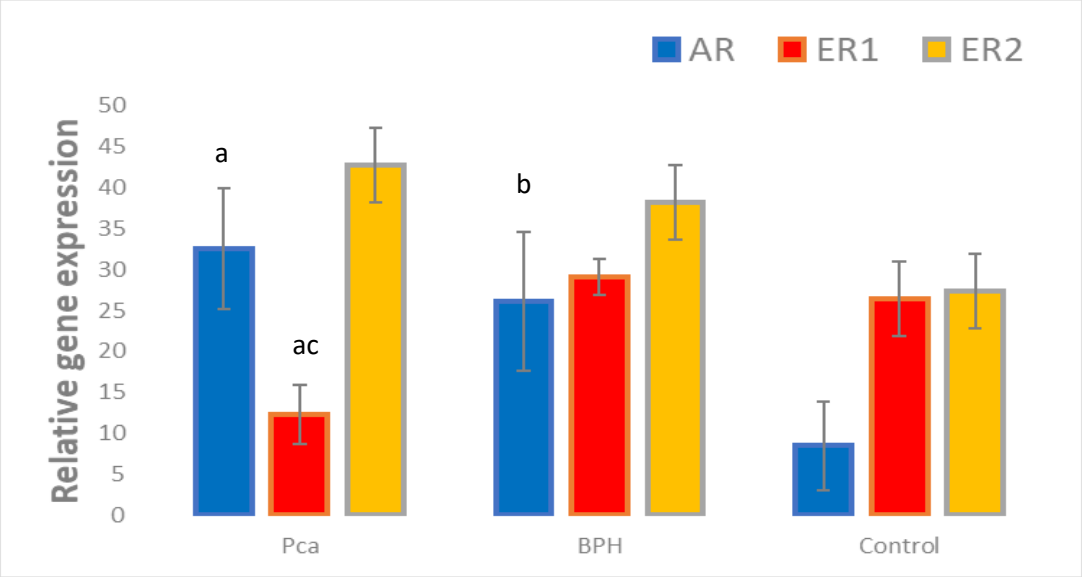

Figure 2: Relative gene expression in PCa, BPH and Controls; a =p<0.05 between PCa and controls, and b =p<0.05 between BPH and controls. c =p<0.05

DISCUSSION

Prostate cancer growth believed to be driven by androgens and androgen receptor modulation strategy is important in management of prostate cancer[11]. The serum levels of testosterone in patients with prostate cancer have largely been a source of controversy (Rove *et al.*, 2014)[12]. Although other risk factors like obesity and family history may also be an important factor in prostate cancer development. Obesity particularly is gradually becoming a global health epidemic, crucial to the effect of obesity is its association with hormonal changes especially the androgens [13]. There are several conflicting reports on the association of obesity and the risk of prostate cancer. The findings from this study shows that increase in weight is associated with prostate cancer and BPH while waist circumference in BPH only. Interestingly, the BMI show no difference in the study groups, this may be associated with the limitation of BMI which include inability to distinguish between muscle and fat accumulation and its inability to reflect fat distribution [14]. Measures of central adiposity by waist circumference (WC) and waist-to-hip ratio (WHR) have been shown to better reflect abdominal adiposity than BMI and have been documented to have stronger associations with cardiometabolic risk factors and outcomes [15]. Obesity or abnormal distribution of fat have been documented to affect steroid hormone receptor expression pattern in hormone dependent cancers [16]. Simultaneous expression of ERβ2 and ERβ5 are prognostic markers of biochemical relapse, post-operative metastasis and general poor prognosis in PCa [17]. We observed reduced testosterone level in prostate cancer and BPH participants. This implies that hypogonadism in older men is a common phenomenon in prostate cancer [18]. Usoro *et al* (2015)[19] observed no significant difference in the serum levels of testosterone between subject with prostate cancer and BPH. The group however reported a significant difference between testosterone levels between PCa group and controls, supporting an important association between testosterone and development of prostate cancer[17]. The study of Ferro *et al* (2021)[20] documented an association between low levels of serum testosterone and unfavorable prognosis in prostate cancer patients, a similar report has corroborated the evidence that hypogonadism increases the rate of morbidity in cancer patients [19, 21]. Watts et al (2014)[4] reported that men with the lowest levels of testosterone had a 23% reduced risk of developing prostate cancer compared to all the other men. In addition they reported that men with very low levels of testosterone who did get prostate cancer were more likely to develop an aggressive form of the disease. However, discrepancy documented in studies of testosterone and prostate cancer can be attributed to differences in environment and ethnicity of the study groups, which may affects the steroid physiology resulting in different outcome in cohorts studied[22].

Reduced plasma level of testosterone could in turns leads to over expression of androgen receptor (AR) and androgen biosynthesis in benign prostate cells [23]. The human androgen receptor saturation is reached at relatively low levels of testosterone as described by the androgen saturation hypothesis [24]. The overexpression of AR

can alter androgen-receptor binding sites and transcriptional programs [25] . The increase androgen synthesis induced by chronic low testosterone exposure, coupled with increased AR, may also drive chromosomal rearrangements [26]. Therefore, in the presence of low systemic testosterone level, the cell of the prostate may not respond as they would in the presence of normal testosterone levels. It is possible that AR binds to and regulates a distinct set of genes following chronic exposure to low testosterone which may result to selection of preneoplastic cell that are less dependent on survival and growth signals from stromal and basal cells. These could initiate AR malignancy switch that causes AR-driven proliferation of luminal cells, common in prostate cancer[27]. Data from this study show a positive correlation between ER2 and PSA, a negative correlation of testosterone and PSA in the prostate cancer group. This association further proves that elevation of this receptor may drive proliferation in this group of participants.

Elevated circulating estrogens have been found in some high-risk populations, such as African American men, suggesting estrogen involvement in prostate cancer. This effect is postulated to involve genotoxic activity of estrogens, as well as receptor-mediated changes in prostatic sex steroid metabolism and receptors [27]. We observed significant increase in 17β-estradiol levels in the PCa and BPH participants in our study. This increased serum levels of 17β-estradiol in patients with prostate cancer could be due to increased activities of the enzyme, aromatase, which converts testosterone to 17β-estradiol in the prostatic epithelia [19]. A previous study has implicated estrogen action in the progression of prostate cancer[2] which may be via proposed mechanisms like epigenetic genotoxicity, hyper-prolactinaemia, immunotoxic or inflammatory changes and prostatic estrogen receptor-mediated changes. The elevation of estrogen observed in our study is in agreement with the study of Bosland (2006)[28] and Dobbs *et al*.(2019) [29] which supports the critical role of increase levels of estrogen in prostate cancer and mediated effects by the expression of estrogen receptors in the prostate. Furthermore, aging as demonstrated in our study contributes to increased 17β-estradiol and reduced testosterone levels as a probable factor in prostate carcinogenesis. This is because as men age, their testosterone levels drop and their 17β-estradiol levels increase due to increased aromatization[30]. Estrogen's primary hormonal function is mediated through estrogen-specific receptors, of which there are several types with opposing functions, but a significant amount of evidence has accumulated demonstrating that direct estrogen signaling pathways within prostate cells play an important role in the development of the prostate gland and possibly in the development of cancer especially the nuclear steroid receptors like ER-β[31]. The result from this study showed a significant increase in the plasma levels of oestrogen-receptor β in the prostate cancer group. This result add credence to the study by Lazari and colleagues (2009)[31], which revealed that the prostatic epithelial ER-β function in pro-differentiation, anti-proliferation, anti-inflammation and as an inducer of anti-oxidant genes may contribute to the progression of prostate cancer. Although, its exact role has not clearly been established but there are report of ER-β involvement in colorectal cancer (CRC)[32] and may contribute to initiation and progression of chemical carcinogen-induced neoplastic transformation in breast epithelia cells [33]. Estrogen receptor can be found both in the nucleus and the cytoplasm of cells including the prostate cells. Its presence in the nucleus supports its ability to activate tumor promoting genes and modulation of tumor suppressor genes on one hand and interaction with tumor growth factors that enhance cell survival and proliferation of prostate cells on the other hand. This study supports the hypothesis of Hua and colleagues that describe the action of ERβ as an oncogene depending on the isoform involved[34]. Interestingly, we reported a significant positive correlation between PSA and 17β-estradiol in prostate cancer patients. This finding indicates that the level of 17β-estradiol increases with the concentration of PSA hence relating the activities of aromatase enzyme with prostate cellular proliferation. The action of 17β-estradiol in prostatic hormonal carcinogenesis is thought to be mediated by the estrogen receptor, this finding is consistent with the report of Usoro *et al* (2015)[19]. The significant increase in 17β-estradiol and significant increase in ER-β however indicates that there may be an increase in activity at a cellular level and an exaggerated increase in 17β-estradiol functions thereby promoting prostatic metastasis. However, we found no correlation between 17β-estradiol and PSA in prostate cancer, this is consistent with previous studies by Yao *et al*. (2011) [35] and Roddam *et al*. (2008) [36] that stipulate no relationship between serum 17β-estradiol and the risk of prostate cancer. We demonstrated an interesting pattern of testosterone and 17β-estradiol in different age groups. The pattern looks similar other that a significant increase from the age of 56 and above with consistent hypogonasm is associated with upsurge of 17-βestradiol in PCa.

This indicate an increased activity of 5α-reductase activity at this age, which may predispose this group to risk of developing prostate cancer [17] and probably metastasis of prostate tumor.

Furthermore, estrogen is dynamically integrated into the signaling mechanism that support prostate cancer development and co-ordinate growth and differentiation of prostate cells. However, there are scanty information on the possible role of different isoform of estrogen receptors in the initiation and progression of prostate cancer[37]. Previous data have shown that an imbalance in the levels of the hormones androgen and estrogen may encourage the growth of prostatic stromal cells and alter the morphological properties of the prostatic tissues. It is possible that ESRα has a positive activating role in the proliferation of prostate cells because it had exceptionally high proliferative effects on normal prostate tissue and caused intraepithelial neoplasia in prostate tissue. Additionally, it was reported in numerous studies that the location of ESRα expression shifts following the development of cancer, primarily in the form of high levels of ESRα expression found in epithelial cells, which were primarily expressed in prostate stromal cells. It is however unclear if both ESR1 and ESRβ upregulation in our treatment naïve cohort is activated simultaneously as recently postulated by Tong (2022) [38].

Previous studies in LNCaP cells (an estrogen-versus-androgen-dependent prostate cancer cell line), have reported that ERβ induced the cell line into S-phase, stimulate cell proliferation, and secrete epidermal growth factor [38, 39]. Upregulation of ESRα in a ESRβ loss prostate cancer results in therapeutic resistance and development of endothelial mesenchymal transmission (EMT) and activation of ESRα using agonist can bring about reversal of EMT. The upregulation of both ESRα and ESRβ and the downregulation of ESRα in PCa reported in this study may be due to the stages of metastasis and suggest that this cohort may be responsive to androgen ablation therapy while the upregulation of ESRβ may be due to their tumor promoting[38 39].

CONCLUSION

This study provides evidence that plasma levels of 17β-estradiol and estrogen receptor-β are significantly higher in prostate cancer subjects thus emphasizing the role of aromatization in prostate carcinogenesis and probably metastatic progression. We reported a significantly reduced level of testosterone in both BPH and PCa group, this may be associated with upregulation of androgen receptor. This might be a pointer to a possible crosstalk between androgen receptor, the aromatized estrogen, and prostate proliferative activities. This also implies that clinical symptom of hypogonadism such as low libido, erectile dysfunction, decrease energy, may be an important concern in PCa. The associated hypogonadism may increase the expression of androgen receptor, a common factor in the progression of prostate cancer thus, worsen the prognosis of the disease. Therefore, monitoring of testosterone level and steroid receptor may be useful in the management of prostate cancer, especially in interpreting PSA kinetics in stratifying patients that will require biopsy and management strategy, especially those that will benefit from androgen depriviation therapy.

Conflict of Interest

The authors declare no conflict of interest.

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