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Research Article

Relationship Between Serum Vitamin D and Adrenal-derived Androgens Upon Castrate-Resistant Prostate Cancer Diagnosis

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Abstract

Introduction: Following primary androgen deprivation therapy (PADT) for prostate cancer (PCa) treatment, most patients develop castration-resistant PCa (CRPC). Intra-prostatic biosynthesis of androgens from adrenal-derived precursor androgens promotes CRPC evolution after PADT and vitamin D (VitD) have been shown to inhibit this intra-prostatic biosynthesis. However, the relationship between VitD and these adrenally-derived androgens in PCa patients who later develop CRPC following PADT is unknown, hence, this study. *Methods:* This prospective longitudinal study was conducted among locally advanced PCa patients in the Department of Chemical Pathology at the Rivers State University Teaching Hospital, Southern Nigeria. Patients were followed up for 36 months (January 2021 to December 2023) from when they had surgical PADT until they developed CRPC. Relevant data were obtained at 4-time points during the studied period: at PCa diagnosis before PADT, at PADT commencement, at the attainment of castrate status following PADT, and at CRPC evolution/diagnosis to evaluate the influence of VitD on adrenal-derived androgens [dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEAS), androstenedione (A-dione), 5-androstenediol (5-adiol), and 11-keto-testosterone (11-KT)] in CRPC. Descriptive/inferential parameters were used to evaluate data at an alpha value of <0.05. *Results*: At baseline before PADT, the majority of the study cohorts (n=220) had VitD deficiency (n=121; 55.0%) compared to those with VitD insufficiency (n=72; 32.7%) and VitD sufficiency (n=27; 12.3%). At the attainment of castrate status following PADT, the VitD deficient cohorts had a longer duration to attain castrate status, a shorter time to attain TPSA nadir, and higher serum TT levels ($p<0.05$, respectively). At the attainment of CRPC status, 47 subjects developed CRPC with the majority (n=24; 51.0%) having VitD deficiency compared to the VitD insufficient (n=13; 27.7%) and VitD sufficient cohorts (n=10; 21.3%). The VitD-deficient cohorts also had a shorter time to CRPC onset following PADT and higher serum total prostate-specific antigen (TPSA), total testosterone (TT), and adrenal-derived androgens (DHEA, DHEAS, A-dione, 5-adiol, and 11-KT) levels compared to the VitD insufficient/sufficient cohorts ($p<0.05$, respectively). Moreover, the CRPC cohorts had higher serum levels of adrenocorticotropic hormone (ACTH), TT, TPSA, free testosterone, bio-available testosterone, and adrenal-derived androgens but lower VitD than the non-CRPC cohorts ($p<0.05$, respectively). An inverse relationship was observed between VitD and the adrenal-derived androgens among all CRPC cohorts which were more amplified among the VitD-deficient CRPC cohorts ($p<0.05$). *Conclusion:* Current findings indicate the role of VitD in CRPC through its influence on the adrenal-derived androgens. However, further studies are recommended to verify these findings and their clinical implications.

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Keywords

Prostate Cancer, CRPC, Vitamin D, Adrenal Androgens

1. Introduction

Primary androgen deprivation therapy (PADT) remains the cornerstone of treatment for locally advanced and metastatic prostate cancer (PCa) in Nigeria [1]. However, these common treatment protocols fail to sustain over time resulting in castration-resistant prostate cancer (CRPC) variants within months to years after PADT with more devastating and catastrophic consequences to the male patients [2-4].

This unfavorable outcome following PADT has been attributed to the enhanced intra-prostatic utility of adrenal-derived androgen precursors and downstream metabolites for the continued testosterone (TT) and dihydrotestosterone (DHT) biosynthesis within the PCa cell lines which continue to favor and promote PCa growth and metastasis despite achieving castrate levels of serum TT level status by PADT [5-7]. Some of these adrenal-derived androgens include dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione (A-dione), 5-androstenediol (5-adiol), 11-keto-testosterone (11-KT) among others [5-7].

Recent experimental studies, using some PCa cell lines, have shown that the intra-prostatic androgen biosynthesis from the adrenally-derived androgens in CRPC can be down-regulated by sufficient vitamin D (VitD) [8-10]. These previous studies highlighted in great detail how vitamin D analogs, acting through the VitD Receptors (VDR), within these PCa cell lines tend to counter the intracrine conversion of the adrenal-derived androgens to other androgen metabolites that favor PCa growth and CRPC evolution [8-12].

However, the relationship between serum VitD status and the adrenal-derived androgens among CRPC patients following successful PADT for locally advanced PCa is unknown, hence, the purpose of this study.

2. Materials and Methods

2.1. Study Design, and Site

This was a prospective longitudinal study conducted in the Department of Chemical Pathology of the Rivers State University Teaching Hospital (RSUTH)] within the southern region of Nigeria. RSUTH is a tertiary healthcare hub with specialized clinical departments/units including the Urology Unit, Department of Histopathology, and Department of Radiology that are adequately staffed and required for core clinical services, medical education/training, and research purposes. The Department of Chemical Pathology has a well-equipped laboratory with diverse biochemical analyzers, a metabolic clinic/ward, and experienced laboratory scientists and physicians. Patients from diverse clinics/units are usually referred to the Department of Chemical Pathology from within and outside of RSUTH for diverse biochemical tests including cancer biomarkers such as the total prostate-specific antigen (TPSA) or free prostate-specific antigen (FPSA) tests.

2.2. Ethical Considerations

The Health Research Ethical Committee of RSUTH reviewed and approved the study procedures. All study populations agreed to participate and provided written and signed informed consent. The study strictly adhered to the RSUTH-recommended guidelines and the principles embodied and laid down in the Helsinki Declarations of 1964, revised in 2013.

2.3. Study Population

The study populations were PCa patients who were referred to the Department of Chemical Pathology from the Urology Unit of RSUTH for diverse biochemical investigations including the confirmation of prostate gland pathology using PSA.

2.4. Sample Size Determination

The calculated minimum sample size required for this study is approximately 220. The sample size was determined using a sample size mathematical formula for cross-sectional studies for characteristics in an infinite population >10,000 using a 5% margin of error, 95% confidence interval, and a reported 15.7% prevalence of PCa previously documented in the study region [13, 14]. Though the result from the calculation was approximately 200, 220 participants were enrolled to make up for an anticipated 10% attrition rate.

2.5. Eligibility Criteria

The criteria for inclusion included men with incident, treatment-naïve, biopsy-confirmed with Gleason grade score ≥6, and locally advanced stage PCa who had successfully undergone surgical PADT during the study period. The criteria for exclusion were those with localized, non-advanced, or

metastatic PCa, those with acute/chronic hepatic or renal diseases, those who have undergone prostatectomy, those with other tumors, those on any hormonal therapies, and those with any endocrinopathies including hypothalamic/pituitary gland disorders, diabetes mellitus, thyroid disorders, and adrenal gland disorders such as Cushing's syndrome, Addison's disease, or congenital adrenal hyperplasia.

Those on medications used in the treatment of PCa reported to influence adrenal androgen pathways such as ketoconazole, glucocorticoids (prednisolone, dexamethasone), abiraterone acetate, and enzalutamide were also excluded.

Eligible PCa patients who did not develop CRPC at the end of the follow-up period were recruited as controls.

2.6. Sampling Method

A convenience sampling technique was employed to recruit participants until the target sample size was reached.

2.7. Data Collection

Stage one (at PCa screening/diagnosis before PADT): Following referral to the Department of Chemical Pathology for a serum PSA test on clinical suspicion of PCa from the Urologic Unit, patients were educated and counseled to grant consent to participate.

If consented, participants were given a basic information questionnaire to acquire socio-demographic, clinical examination (systolic/diastolic blood pressure, symptoms, signs, etc.), and anthropometric (weight, height, waist circumference, etc.) data and to determine eligibility status.

If the serum PSA results aligned with the clinical suspicion of PCa by the Urologists, the patients were followed up in the Urology Unit to obtain digital rectal examination (DRE) findings and PCa clinical staging, to the Department of Histopathology for histology reports on PCa grade and its Gleason scores (GS), and to the Department of Radiology for trans-rectal ultrasound (TRUS) scan findings of the prostate gland.

If PCa diagnosis and eligibility status were confirmed, a baseline whole blood specimen for laboratory parameters was taken. The participants were then monitored regularly via phone calls, text messages, and/or email messages until the day of androgen deprivation therapy (PADT) commencement.

The baseline laboratory parameters were serum adrenocorticotropic hormone (ACTH), serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17β-estradiol (E2), total testosterone (TT), sex-hormone binding globulin (SHBG), albumin, TPSA, and Vitamin D (VitD) including the adrenally-derived androgens including dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione (A-dione), 5-androstenediol (5-adiol), and 11-keto-testosterone (11-KT).

Stage two (at PADT commencement): The commencement date, the mode of PADT, and the therapeutic modalities were noted and documented on the day of PADT initiation.

Stage three (at the attainment of castration status after PADT): Following PADT commencement, participants were monitored using serum TT and TPSA to detect/define castration status and TPSA nadir, respectively.

Stage four (at CRPC evolution/diagnosis): At CRPC diagnosis, specimens were obtained finally for serum ACTH, LH, FSH, E2, TT, albumin, TPSA, SHBG, and VitD levels including the adrenal-derived androgens such as DHEA, DHEAS, A-dione, 5-adiol, and 11-KT.

2.8. Specimen Collection, Processing, and Laboratory Analysis

In each stage of the 4 stages of data acquisition, blood specimens were aseptically acquired, processed, and analyzed using standardized protocols in the RSUTH Department of Chemical Pathology. Five milliliters (5 ml) of venous whole blood were acquired by 8-10 am after an overnight fast for at least 8-12 hours and transferred into a well-labeled plain non-anticoagulated tube. The acquired specimens in plain tubes were allowed to clot undisturbed at room temperature (22-28°C) for at least one hour, later centrifuged at 1,500 revolutions/minute for 10 minutes, the serum supernatant separated then transferred immediately with a Pasteur's pipette into plain tubes, and finally stored frozen at -80 °C until batched analyses. Analyses for ACTH, LH, FSH, E2, TT, DHEAS, SHBG, TPSA, and VitD levels were done on an automated immunoassay analyzer (Architect i1000, Abbott Laboratories, IL, USA). Serum albumin analysis was done on an automated chemistry analyzer (BS200, Mindray, China).

The enzyme-linked immunoassay (ELISA) method was used for the analysis of serum DHEA (Elabscience ELISA reagent kits, Texas, USA), A-dione (Abcam ELISA reagent kits, Cambridge, UK), 5-adiol (Innovative Research Inc. ELISA reagent kits, MI, USA), and 11-KT (Thermo-Fisher Scientific ELISA reagent kits, MA, USA) using standardized laboratory reagent kits.

2.9. Quality Assurance

The utilized study questionnaire was explored for reliability and validity using appropriate methodologies. Two levels of control sera were used to monitor intra-assay and inter-assay analytic precision, and the intra- and inter-assay coefficients of variation were always below 5% and 10%, respectively.

2.10. Variable Definitions / Stratifications

2.10.1. PCa Diagnosis / Characteristics

This was based on DRE findings (hard, surface irregularity, and nodular prostate gland ± prostatic enlargement, loss of the median groove, and asymmetry of the prostatic lobes), TPSA greater than or equal to the upper limit of the age-specific reference values within the study zone, positive TRUS find-

ings of PCa features (hypoechoic lesion in the peripheral zone, asymmetry, protrusion into the prostatic capsule, and asymmetrical bulging of the prostate's contour), and positive prostate biopsy diagnosis with a Gleason score of ≥6. PCa tissues were graded from 1-5 based on the International Society of Urological Pathology (ISUP) recommendations [15]. The staging was done clinically (stages 1 to 4) based on the recommendations of the American Joint Committee on Cancer (AJCC) [16]. Locally advanced PCa was defined as: PCa with spread outside of the prostate capsule (clinical stage T3a) and/or PCa involvement of seminal vesicles (clinical stage cT3b) and/or PCa involvement of adjacent organs (clinical stage cT4) and/or PCa with regional lymph node involvement without distant metastasis (clinical stage T3-4 N±M_0).

2.10.2. Surgical *PADT*

For the current study, surgical PADT was defined as initial bilateral orchiectomy plus an antiandrogen (flutamide given orally at 125mg thrice a day or bicalutamide administered 80mg once a day) therapy.

2.10.3. Post-PADT Castrate Status

Post-PADT castrate status was defined as serum TT concentration <50 ng/dL (<1.7 nmol/L) following surgical PADT, as previously described [17].

2.10.4. Biochemical Definition of CRPC

The biochemical definition of CRPC was based on meeting the following European Association of Urology Guidelines criteria: serum TT level <50 ng/dL (<1.7 nmol/L) following PADT plus 1) successive increases in PSA level during three consecutive measurements obtained ≥1 week apart, and an increase of ≥25% on two PSA readings, and a PSA level ≥2.0 ng/mL [18].

2.10.5. VitD Status

VitD status was defined using total serum 25(OH)D status. It was further categorized as deficient (total serum 25(OH)D <30 nmol/L), insufficient (total serum 25(OH)D 30-50 nmol/L), and sufficient (total serum 25(OH)D >50 nmol/L) based on the recommendations of the National Academy of Medicine [19].

2.10.6. Calculated Free Testosterone (FT) Bioavailable Testosterone (BT)

FT and BT were calculated from the measurement of serum TT, SHBG, and albumin, according to the mass action law using the Vermeulen formula [20].

2.10.7. Data Management

Data management and analyses were done using statistical product and service solutions software for Windows version 25. The continuous data were initially evaluated for conformity to a normal distribution pattern using the Shapiro-Wilk tests. Those continuous data violating the normal distribution patterns were log-transformed before analysis, expressed using means ± standard deviations, and compared by independent student t-test or analysis of variance (ANOVA), as appropriate. The categorical data were reported as counts/percentages and compared with the Chi-square or Fisher's exact tests, as appropriate. Pearson's correlation coefficient was used to evaluate the relationships between continuous data. An alpha value of <0.05 was deemed statistically significant.

3. Results

Table 1 depicts the study cohort demographic, clinical, anthropometric, and laboratory characteristics, including VitD status categories. As shown, the majority of the study cohorts had VitD deficiency (n=121; 55.0%) compared to those with VitD insufficiency (n=72; 32.7%) and VitD sufficiency (n=27; 12.3%) (Table 1).

Table 1. *Basic Characteristics of Study Subjects (n = 220) at Initial PCa Diagnosis.*

Nonadrenal-derived Parameters	Mean ± SD/n (%) n = 220	Range (Min. – Max.) n = 220	p-value
Age, mean, years	62.45 ± 7.14	52 – 72	NA
BM1, kg/m^2	30.17 ± 3.55	26 – 33	NA
WC, cm	96.22 ± 6.92	94 – 109	NA
HC, cm	95.15 ± 7.08	95 – 107	NA
Waist-hip ratio	0.95 ± 0.70	0.93 - 1.05	NA
SBP, mmHg	135.10 ± 8.60	120 – 140	NA
DBP, mmHg	82.33 ± 5.33	70 – 100	NA

Nonadrenal-derived Parameters	Mean ± SD/n (%) n = 220	Range (Min. – Max.) n = 220	p-value
Serum ACTH, pmol/L	7.44 ± 1.76	1.9 – 15.4	NA
Serum LH, IU/L	3.63 ± 1.12	2.2 – 7.5	NA
Serum FSH, IU/L	4.20 ± 1.87	2.4 – 9.8	NA
Serum E2, pmol/L	67.51 ± 10.12	38.8 – 110.9	NA
Serum TT, nmol/L	13.40 ± 2.07	9.7 – 19.86	NA
Serum SHBG, nmol/L	35.72 ± 5.84	19.60 – 49.51	NA
Serum TPSA, µg/L	73.44 ± 4.88	38.80 – 107.47	NA
Serum total VitD, nmol/L	27.86 ± 4.17	22.75 – 54.87	NA
Serum albumin, g/L	38.7 ± 3.22	36.73 – 49.8	NA
FT, pmol/L (calculated)	184.65 ± 12.33	168.36 – 289.88	NA
BT, nmol/L (calculated)	15.77 ± 2.71	14.73 – 31.10	NA
Adrenal-derived Parameters			
Serum DHEA, nmol/L	4.96 ± 1.86	2.6 – 11.67	NA
Serum DHEAS, µmol/L	3.87 ± 1.67	1.66 - 5.70	NA
Serum A-dione, nmol/L	2.45 ± 1.17	1.16 – 3.44	NA
Serum 5-adiol, nmol/L	2.38 ± 1.08	1.65 – 5.66	NA
Serum 11-KT, nmol/L	1.66 ± 0.90	0.10 – 2.67	NA
PCa Clinical Stage			0.103
CT3a	70 (31.8%)	NA	
CT3b	73 (33.2%)	NA	
CT4 (NoMo)	77 (35.0%)	NA	
PCa ISUP Grade			0.264
1 (GS = 3 + 3)	43 (19.5%)	NA	
2 (GS = 3 + 4)	42 (19.1%)	NA	
3 (GS = 4 + 3)	43 (19.5%)	NA	
4 (GS = 4+ 4 or 5 + 3 or 3+ 5)	45 (20.5%)	NA	
5 (GS = 4 + 5 or 5 + 4 or 5 + 5)	47 (21.4%)	NA	
Serum VitD [25(OH)D] Status			<0.001*
Sufficient	27 (12.3%)	NA	
Insufficient	72 (32.7%)	NA	
Deficient	121 (55.0%)	NA	

*Statistically significant; NA: not applicable; BMI: body mass index; WC: waist circumference; HC: Hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; ACTH: adrenocorticotropic hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: 17β-estradiol; TT: total testosterone; FT: free testosterone; BT: bioavailable testosterone; SHBG: sex hormone binding globulin; TPSA: total prostate-specific antigen; VitD: vitamin D; DHEAS: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone sulphate;; A-dione: androstenedione; 5-adiol: 5-anhydrostenediol; 11-KT: 11-keto-testosterone; PCa: prostate cancer; GS: Gleason's score; ISUP: International Society of Urological Pathology

Table 2 shows the follow-up characteristics of PCa subjects by VitD status at attainment of castrate status. As shown in Table 2, the VitD deficient cohorts had a longer duration to attain castrate status from PADT commencement, a shorter time to attain TPSA nadir from PADT commencement, and higher serum TT levels at castrate status attainment compared to the VitD insufficient and sufficient cohorts (Table 2) ($p<0.05$, respectively).

Table 2. *Follow-up Characteristics of PCa Subjects by VitD Status at Attainment of Castrate Status (n=220).*

Variables	Overall, n=220 Mean ± SD, Range	Sufficient VitD, n=27 Mean ± SD/n	Insufficient VitD, n=72 Mean ± SD/n	Deficient VitD, n=121 Mean ± SD/n	p-value
Observation period from PADT, hours	16.54 ± 3.44	16.01 ± 3.61	15.78 ± 3.06	16.31 ± 3.33	0.166
Duration of castrate status from PADT, hours	10.11 ± 2.79	8.13 ± 1.50	11.07 ± 2.84	13.44 ± 2.96	<0.001*
Time to TPSA nadir from PADT, weeks	4.77 ± 1.34	5.97 ± 1.88	5.01 ± 1.38	4.27 ± 1.09	<0.001*
Serum TPSA nadir at castrate status, µg/L	0.19 ± 0.06	0.15 ± 0.07	0.22 ± 0.08	0.29 ± 0.09	<0.001*
Serum TT at castrate status, nmol/L	1.30 ± 0.76	1.27 ± 0.85	1.43 ± 0.69	1.59 ± 0.58	<0.001*

*Statistically significant; **VitD sufficient versus VitD insufficient versus VitD deficient; VitD: vitamin D; SD: standard deviation; PADT: primary androgen deprivation therapy; TPSA: total prostate-specific antigen; CRPC: castration resistant prostate cancer; VitD: vitamin D

Table 3 depicts the characteristics and distribution of the adrenal-derived VitD Status of PCa Subjects (n = 47) at attainment of CRPC Status. By the end of the 3-year follow-up, 47 subjects developed CRPC with the majority (n=24; 51.0%) having VitD deficiency compared to the VitD insufficient (n=13; 27.7%) and VitD sufficient cohorts (n=10; 21.3%) (Table 3). The VitD deficient cohorts also had a shorter time course to CRPC onset following PADT and higher serum levels of TPSA, TT, DHEA, DHEAS, A-dione, 5-adiol, and 11-KT at attainment of CRPC status compared to the VitD insufficient and VitD sufficient cohorts ($p<0.05$, respectively) (Table 3).

Table 3. *Characteristics and Adrenal-derived Hormone Distribution by VitD Status of PCa Subjects (n = 47) at Attainment of CRPC Status.*

Variables	Overall CRPC Subjects Mean ± SD	Sufficient VitD Mean ± SD	Insufficient VitD Mean ± SD	Deficient VitD Mean ± SD	p-value**
n (%)	47 (100)	10 (21.3)	13 (27.7)	24 (51.0)	<0.001*
CRPC onset timeline following castration status, months	26.55 ± 5.09	31.70 ± 4.91	28.41 ± 3.54	24.80 ± 3.08	<0.001*
Serum TPSA at CRPC diagnosis, µg/L	2.65 ± 0.76	2.56 ± 0.41	2.88 ± 0.67	3.22 ± 0.90	<0.001*
Serum TT at CRPC diagnosis, nmol/L	1.32 ± 0.59	1.29 ± 0.88	1.46 ± 0.74	1.63 ± 0.63	<0.001*
Serum DHEA, nmol/L	5.46 ± 1.22	1.77 ± 1.16	2.39 ± 1.25	7.74 ± 1.84	<0.001*
Serum DHEAS, µmol/L	4.77 ± 1.89	1.14 ± 0.65	2.53 ± 1.18	6.88 ± 1.75	<0.001*
Serum A-dione, nmol/L	3.95 ± 1.28	0.88 ± 0.10	1.94 ± 1.01	5.74 ± 1.75	<0.001*
Serum 5-adiol, nmol/L	3.35 ± 1.30	1.39 ± 0.92	2.17 ± 1.02	4.31 ± 1.62	<0.001*
Serum 11-KT, nmol/L	2.06 ± 0.95	0.09 ± 0.06	1.14 ± 0.57	3.78 ± 0.61	<0.001*

*Statistically significant; SD: standard deviation; **VitD sufficient versus VitD insufficient versus VitD deficient; VitD: vitamin D; DHEAS: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone sulphate; A-dione: androstenedione; 5-adiol: 5-anhydrostenediol; 11-KT: 11-keto-testosterone

Table 4 depicts the biochemical characteristics of study subjects at attainment of CRPC status compared to those with non-CRPC status. From Table 4, the CRPC cohorts had higher serum levels of ACTH, TT, TPSA, FT, BT, DHEA, DHEAS, A-dione, 5-adiol, and 11-KT but lower serum VitD status compared to the non-CRPC cohorts ($p<0.05$, respectively) (Table 4).

Table 4. *Biochemical Characteristics of Study Subjects at CRPC Diagnosis (n=47) Versus Non-CRPC Subjects (n=173).*

Nonadrenal-derived Parameters	Non-CRPC, n=173 Mean ± SD	CRPC, n=47 Mean ± SD	p-value***
Serum ACTH, pmol/L	5.08 ± 1.31	8.85 ± 1.87	<0.001*
Serum LH, IU/L	16.90 ± 2.67	17.64 ± 2.74	0.204
Serum FSH, IU/L	18.74 ± 3.86	19.07 ± 3.93	0.367
Serum E2, pmol/L	1.44 ± 1.31	1.27 ± 1.07	0.068
Serum TT, nmol/L	1.20 ± 0.57	1.55 ± 0.86	0.014*
Serum SHBG, nmol/L	75.47 ± 6.08	74.24 ± 5.76	0.117
Serum TPSA, µg/L	0.13 ± 0.07	2.12 ± 1.06	<0.001*
Serum Total VitD, nmol/L	29.17 ± 4.23	25.45 ± 4.05	<0.001*
Serum albumin, g/L	37.44 ± 3.12	38.01 ± 3.20	0.224
FT, pmol/L (calculated)	12.12 ± 1.65	14.29 ± 1.73	<0.001*
BT, nmol/L (calculated)	1.34 ± 0.67	1.56 ± 0.74	0.021*
Adrenal-derived Parameters			
Serum DHEA, nmol/L	3.34 ± 1.01	5.46 ± 1.22	<0.001*
Serum DHEAS, µmol/L	3.42 ± 1.55	4.77 ± 1.89	<0.001*
Serum A-dione, nmol/L	2.61 ± 1.09	3.95 ± 1.28	<0.001*
Serum 5-adiol, nmol/L	2.07 ± 0.91	3.35 ± 1.30	<0.001*
Serum 11-KT, nmol/L	1.53 ± 0.86	2.06 ± 0.95	0.017*

*Statistically significant; SD: standard deviation; ***Non-CRPC versus CRPC; CRPC: castration resistant prostate cancer; ACTH: adrenocorticotropic hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: 17β-estradiol; TT: total testosterone; FT: free testosterone; BT: bioavailable testosterone; SHBG: sex hormone binding globulin; TPSA: total prostate-specific antigen; VitD: vitamin D; DHEAS: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone sulphate; A-dione: androstenedione; 5-adiol: 5-anhydrostenediol; 11-KT: 11-keto-testosterone

Table 5 shows the relationship between VitD and the adrenal-derived hormones (DHEA, DHEAS, A-dione, 5-adiol, and 11-KT) among the CRPC cohorts (n = 47) at attainment of CRPC Status. As shown in Table 5, an inverse relationship was observed between VitD and all the five adrenal-derived hormones among the overall CRPC cohorts (n=47) which were more amplified among the VitD deficient CRPC cohorts ($p<0.05$, respectively) (Table 5).

Table 5. *Relationships between VitD and the Adrenal-derived Hormones among the PCa Subjects (n = 47) at Attainment of CRPC Status.*

	Serum Vitamin D Status, nmol/L			
	Overall, n=47	Sufficient, n=10	Insufficient, n=13	Deficient, n=24
Adrenal-derived hormones	r; p-value	r; p-value	r; p-value	r; p-value

	Serum Vitamin D Status, nmol/L			
	Overall, n=47	**Sufficient, n=10**	**Insufficient, n=13**	**Deficient, n=24**
Serum DHEA, nmol/L	-0.443; <0.001*	-0.294; 0.067	-0.267; 0.176	-0.638; <0.001*
Serum DHEAS, µmol/L	-0.378; <0.001*	-0.246; 0.118	-0.239; 0.088	-0.573; <0.001*
Serum A-dione, nmol/L	-0.407; <0.001*	-0.189; 0.144	-0.210; 0.220	-0.688; <0.001*
Serum 5-adiol, nmol/L	-0.460; <0.001*	-0.251; 0.089	-0.198; 0.109	-0.556; <0.001*
Serum 11-KT, nmol/L	-0.388; <0.001*	-0.189; 0.106	-0.201; 0.121	-0.670; <0.001*

*Statistically significant; DHEAS: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone sulphate; A-dione: androstenedione; 5-adiol: 5-anhydrostenediol; 11-KT: 11-keto-testosterone

4. Discussion

4.1. Major Findings

Following PADT for locally advanced or metastatic PCa, most patients develop the lethal CRPC. It is now recognized that the intra-prostatic (intracrine) biosynthesis of T and DHT from adrenal-derived androgens promotes CRPC evolution after PADT. Consequently, recent in vitro studies have provided molecular evidence that VitD analogs counteract the impact of these adrenally-derived androgens on CRPC evolution. However, the relationship between VitD and these adrenally-derived androgens in PCa patients who later develop CRPC following PADT is unknown. Hence, this study evaluated this vital relationship among Nigerian PCa patients with several significant findings. At baseline before PADT, most of the study cohorts had VitD deficiency compared to those with VitD insufficiency and VitD sufficiency. At the attainment of castrate status following PADT, the VitD deficient cohorts had a longer duration to attain castrate status, a shorter time to attain TPSA nadir and higher serum TT levels. At attainment of CRPC status, 47 subjects developed CRPC with the majority having VitD deficiency compared to the VitD insufficient and VitD sufficient cohorts. The VitD deficient cohorts also had a shorter time course to CRPC onset following PADT and higher serum levels of TPSA, TT, and adrenal-derived androgens (DHEA, DHEAS, A-dione, 5-adiol, and 11-KT) compared to the VitD insufficient and VitD sufficient cohorts. The CRPC cohorts also had higher serum levels of ACTH, TT, TPSA, FT, BT, and adrenal-derived androgens but lower VitD status than the non-CRPC cohorts. An inverse relationship was observed between VitD and all the adrenal-derived androgens among the overall CRPC cohorts which were more amplified among the VitD-deficient CRPC cohorts.

4.2. Relationship with Previous Studies

Research data from human-based observational studies on the relationship between VitD and adrenal-derived androgens regarding CRPC evolution following PADT is scarce within the existing literature. However, few experimental studies have historically highlighted the clinical relevance of VitD in the intracrine regulation of adrenal-derived androgens in CRPC evolution [8-10]. Intracrine pathways that utilize these adrenal-derived steroids are thought to generate relevant levels of growth-stimulating androgens such as T and DHT in the emergence of CRPC [8]. Smith and colleagues explored the capacity of the active VitD hormone to interact and elicit changes upon prostatic intracrine pathway at a metabolic level using androgen-dependent LNCaP cells cultured under steroid-depleted conditions and assessed the impact of VitD-based compounds upon intracrine pathways that convert exogenously added DHEA to relevant metabolites, through Mass Spectrometry [8]. The authors confirmed that exposure to VitD-based compounds, within LNCaP cells, elicited a measurable and significant reduction in the intracrine conversion of DHEA to T, DHT, and other intermediate metabolites within the androgenic pathway within the prostatic cancer cell line. These data confirmed how a vitamin D-based regime may be used to counter intracrine mechanisms contributing to the emergence of castrate-resistant tumors [8]. Similar findings have previously been described by Doherty and colleagues in 2014 and Maguire and colleagues in 2012 [9, 10].

4.3. Mechanistic Considerations

The exact mechanisms underlying the suppressed intracrine conversion of the adrenal-derived androgens in PCa tissues remain ill-defined within the existing literature. However, VitD, acting through its receptors (VitD receptors) in PCa cell lines can influence vital genes (including CYP3A4 and CYP3A5) necessary for the increased level of metabolic inactivation of T and DHEA within LNCaP and LAPC-4 prostate cancer cells [9, 10]. The enzymes of the CYP3A enzymes have been found to cause hydroxylation of steroidal compounds that include T, DHEA, A-dione, and DHT [21-23].

This mechanism is corroborated by some clinical studies

that indicate patients with higher levels of CYP3A4 expression in prostate tissue have a successful chance of cancer-specific survival and lower levels of biochemical relapse assessed using PSA [24].

4.4. Relevance to Clinical Practice and Future Studies

The current study findings indicate the need to evaluate the clinical implications of a VitD-based regimen to inhibit the contribution of intracrine pathways involving the adrenal-derived hormones within the PCa tissues toward CRPC emergence. Further studies are recommended in this regard.

4.5. Strength and Limitations

The strength of the current study is hinged in the use of a relatively large sample size population but was also limited by certain factors which are areas for improvement in further studies. As with most observational study designs, the observations here do not infer causality but mere association. The study was also a single-center study, so, its findings may not reflect the larger population within the studied region and so, must be interpreted with caution.

5. Conclusion

At the attainment of CRPC status, most of those who developed CRPC had VitD deficiency with associated higher serum levels of TPSA, TT, and adrenal-derived androgens compared to the VitD insufficient and VitD sufficient cohorts. The CRPC cohorts also had higher serum levels of ACTH, TT, TPSA, FT, BT, and adrenal-derived androgens but lower VitD status than the non-CRPC cohorts. An inverse relationship was observed between VitD and all the adrenal-derived androgens among the overall CRPC cohorts which were more amplified among the VitD-deficient CRPC cohorts. Current findings indicate the role of VitD in CRPC through its influence on the adrenal-derived androgens. However, further studies are recommended to verify these findings and their clinical implications.

Abbreviations

5-adiol	5-Androstenediol
ACTH	Adrenocorticotropic Hormone
A-dione	Androstenedione
BT	Bioavailable Testosterone
CRPC	Castration-resistant Prostate Cancer
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone-sulfate
DHT	Dihydrotestosterone
DRE	Digital Rectal Examination
E2	17-β-Estradiol
ELISA	Enzyme-linked Immunosorbent Assay
FSH	Follicle-stimulating Hormone
FT	Free Testosterone
GS	Gleason Score
ISUP	International Society of Urological Pathology
11-KT	11-keto-testosterone
LH	Luteinizing Hormone
PADT	Primary Androgen Deprivation Therapy
PCa	Prostate Cancer
PSA	Prostate-specific Antigen
RSUTH	Rivers State University Teaching Hospital
T	Testosterone
TT	Total Testosterone
TPSA	Total Prostate-specific Antigen
TRUS	Trans-rectal Ultrasound Scan
VitD	Vitamin D

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Data Availability Statement

The data supporting this study's findings are not publicly available due to containing information that could compromise the privacy of research participants, but they are available

from the corresponding author (CA) upon reasonable request.

Conflicts of Interest

The authors have no conflict of interest to declare.

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