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Serum Vitamin D Level Status by Prostate Cancer Grade and Stage Among Native Africans

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Abstract: *Background:* Vitamin D deficiency is widely speculated to be associated with prostate cancer (PCa) incidence, progression, aggressiveness, and metastatic potentials. However, evidence of this is limited among the black population. Hence, this study was spurred by the dearth of data in this regard. *Methods:* This was a prospectively designed/executed case-controlled descriptive study carried out in the University of Port Harcourt Teaching Hospital (UPTH) in the Niger Delta sub-region of Nigeria. Serum Vitamin D level status was determined/compared between the 380 histologically-verified positive PCa cases and the smoking/sex-matched 380 histologically-verified negative controls using descriptive and comparative statistical tools. The relationship/association between PCa grade/stage and Vitamin D level status was ascertained using crude and adjusted regression models. Data were managed and analyzed with the Statistical Package for Social Sciences version 23 and a p-value of <0.05 was deemed statistically significant. *Results:* The histologically-verified positive PCa patients had significantly lower mean Vitamin D level status (PCa patients: 24.55 ± 3.47 vs. controls: 49.73 ± 4.08; $p<0.001$) but higher mean prostate volumes, BMI status, plasma intact PTH levels, and total PSA levels compared to the histologically-verified negative controls. A decreasing trend of serum Vitamin level status was observed with worsening/increasing PCa grade and stage ($p<0.05$) among the biopsy positive PCa cases. An inverse relationship existed between Vitamin D level status and PCa grade/stage among the Vitamin D deficient PCa subgroup ($p<0.05$) but not the sufficient/insufficient PCa subgroups ($p>0.05$). Among the Vitamin D deficient PCa patients, this inverse relationship continued to strengthen with worsening PCa grade/stage. When compared with the PCa patients with the lowest PCa grade (ISUP grade 1) and stage (T1), an increased likelihood of Vitamin D deficiency was significantly associated with worsening PCa grade (ISUP 2 to 5) and stage (T2 to T4) on crude multiple logistic regression model which was subsequently amplified following adjusting for observed confounders. *Conclusion:* The study findings corroborate the epidemiologic evidence of the association of Vitamin D deficiency with PCa grade and stage; factors that define PCa aggressiveness and metastatic potentials. However, more robust studies among populations of the black race are highly recommended to validate conclusions from this current study.

Keywords: Prostate Cancer, Vitamin D, Vitamin D Deficiency, Vitamin D Insufficiency

1. Introduction

Prostate cancer (PCa) is the most diagnosed male cancer and a leading cause of cancer-related morbidity/mortality around the globe [1, 2]. Its incidence continues to take an upward trend as reported from various continents [1-3]. It was a relatively rare cancer in Nigeria decades ago but has risen to the first position of all male cancers, with a prevalence as high as 15.7% reported [4]. The increasing incidence of PCa has been linked to several risk factors besides the well-established risk factors: genetic predisposition, age, African-American ancestry, and family history [5]. Among the risk factors of current interest are the roles played by nutritional factors in PCa biology [5]. A large number of studies have recently provided evidence about the

protective roles exerted by different Vitamin D metabolites against PCa [6].

Vitamin D (calciferol) is photochemically synthesized from its precursor 7-dehydrocholesterol in the skin under the influence of solar ultraviolet B (UVB) radiation in form of Vitamin D3 (cholecalciferol), although the Vitamin can also be obtained through dietary sources in form of Vitamin D2 (ergocalciferol) [7]. Both Vitamin D3 and D2 isoforms are subsequently metabolized to their biologically inactive and storage form 25-hydroxyvitamin D [25(OH)D] in the liver by 25-hydroxylase [7-9]. The 25(OH)D (calcidiol) is bound mostly (85-95%) on Vitamin D-binding protein (VDBP), partly (5-15%) on albumin, while few (<1%) remain free in plasma and reflects both cutaneous synthesis and dietary acquisition of the Vitamin [8, 9]. In the proximal renal tubules, the inactive/storage form is further metabolized to the more physiologically/biologically active 1,25-dihydroxyvitamin D [1,25$(OH)_2$D or calcitriol], an active endocrine hormone, by the 1-alpha-hydroxylase [7-9]. The regulation of the active Vitamin D is under the tight regulatory control of calcium and parathyroid hormone (PTH) [8, 9].

The intra-prostatic cells have also been found to possess the enzymes that locally metabolize 25(OH)D to the biologically active 1,25$(OH)_2$D including Vitamin D receptors (VDR) necessary for the binding/activity of the biologically active Vitamin D [10-13]. The intra-prostatic concentration of the active 1,25(OH)2D is dependent on the circulating inactive 25(OH)D, not under the regulatory influence of calcium and PTH, and exerts potent cellular regulations by enhancing differentiation and apoptosis, while inhibiting proliferation, angiogenesis, invasiveness, and metastasis of normal prostatic and PCa cells/tissues [10-13]. These effects are thought to be mediated through the VDR [12]. Hence, the deficiency of the Vitamin, evidenced by the reduction of its storage form in both serum and intra-prostatic tissues, has been linked with PCa incidence, progression, aggressiveness, and metastatic potentials [10, 13].

The incidence of PCa among men of the Negroid race has been disproportionately high relative to their Caucasian counterparts as consistently reported [10]. This epidemiologic disparity has been linked to the high prevalence of Vitamin D deficiency among the black populations [13]. The high skin melanin pigmentation in populations of the black race tends to diminish the cutaneous UVB-induced vitamin D synthesis [10-13]. This may also partly account for the disparity in PCa morbidity/mortality frequently observed between the Negroid and the Caucasian populations [12, 13].

Most of the studies evaluating the status and influence of Vitamin D on PCa biology have emanated from western populations. Hence, the current study aimed to define the serum Vitamin D level status, assessed using serum 25(OHD), by PCa grade and stage among patients of predominantly of Nigerian background.

2. Materials and Methods

2.1. Study Design and Site

This was a prospectively designed cross-sectional study conducted at the University of Port Harcourt Teaching Hospital (UPTH), Nigeria. UPTH is one of the tertiary public health facilities located in Port Harcourt, Rivers State, within the Southern part of Nigeria, and a major referral center for all the private, primary, and secondary healthcare facilities in the state and other adjoining states in the region. The hospital has various specialist Departments/Units some of which include the Chemical Pathology, Histopathology, and the Urology unit in the Surgical Department where the current study was conducted.

2.2. Ethical Considerations

Study approval was granted by the Institutional Research Ethics Committee before the commencement of the study. All research subjects gave written informed consent to participate in the study before recruitment. The study was conducted with strict adherence to UPTH Research Ethics protocols and within the principles embodied in the Helsinki Declarations of 1964 and as recently revised in 2013.

2.3. Determination of Sample Size

The sample size population was calculated using a sample size formula for evaluating characteristics in a population of greater than 10,000 at 99% confidence interval (z score of 2.58) and 5% margin of error using 15.7% prevalence of PCa documented in a previous population-based study in Nigeria [4, 14]. The calculated minimum sample size was approximately 380 after adding an anticipated 10% attrition. The non-probability convenience sampling method was used to obtain the required number of study populations until the minimum sample size was exceeded.

2.4. Stratifications/Characteristics of Study Population

The study population consisted of two groups of 380 patients with histologically-confirmed positive PCa (Gleason's score ≥6) and 380 histologically-confirmed PCa-free, smoking- and age-matched (matched by ±2 years) control subjects.

2.5. Eligibility Criteria

Patients with the histologically-confirmed positive PCa (group 1) are those incident/newly-diagnosed and treatment-naïve cases who are metabolically stable with non-metastatic PCa, aged ≥50 years and PCa biopsy Gleason's grade score of ≥6 as recommended by the International Society of Urological Pathology (ISUP).

The controls are the histologically-confirmed negative PCa cases (group 2). They included those who had presented for routine screening or visited the hospital due to any prostate-related symptoms/signs and were subjected to prostate biopsy based on suspicious DRE findings, and/or slightly elevated

TPSA, and/or ambiguous trans-rectal ultrasound (TRUS) prostate gland features. Subsequently, these biopsy findings were found negative for PCa.

Their prostate-related symptoms/signs were found related to prostatitis, and following non-hormonal treatment, all the prostate-related clinical features receded including the normalization of their laboratory parameters/imaging studies.

The criteria for exclusion included the followings: non-consenting potential participants, history of previous prostatectomy for benign prostatic hyperplasia or PCa, history of other extra-prostatic benign/malignant tumors, hepatic and chronic renal diseases, endocrine disorders (diabetes, hyperthyroidism, and hyperparathyroidism), and history of inborn error of metabolism involving vitamin D/skin pigmentation (albinos). Other criteria for exclusion included bedridden status, metabolic instability, currently on calcium and /or Vitamin D-containing supplements, on any medications that influence Vitamin D metabolism, malabsorption syndrome, or drugs affecting serum PSA levels.

2.6. Data Collection

The initial clinical and demographic variables of which data was obtained, using a pre-tested patient-administered questionnaire, included: socio-demographic data (depicted in Table 2), clinical data including blood pressure, body mass index (BMI), waist circumference, and clinical examination findings including digital rectal examinations (DRE).

The laboratory variables included serum total 25(OH)D levels (VDBP-bound, albumin-bound, and the free fractions), vitamin D-binding protein (VDBP), intact PTH, TPSA levels, total testosterone (TT), sex hormone-binding globulin (SHBG), plasma creatinine, albumin, calcium, and inorganic phosphate, C-reactive protein (CRP), pro-calcitonin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphate (ALP), gamma glutamyl-transferase (GGT), hemoglobin (Hb) concentration, glycated hemoglobin A1c (HbA1c), and urine albumin creatinine ratio (UACR).

Other variables were prostate volume obtained by calculation with the prolate ellipsoid formula ([height×length×width]×π/6) using prostate dimensions from the TRUS [15], and abdominal USS. For each suspected case following suggestive PCa medical history, significant clinical features, and positive physical examination findings including DRE, the Urologist carried out a trans-perineal prostate biopsy to determine tumor grade via the Gleason's scoring system followed by clinical TNM staging, if PCa is confirmed.

2.7. Specimen Acquisition, Processing, and Laboratory Analysis

The blood specimens were drawn from any accessible peripheral vein irrespective of fasting or dietary restriction. To minimize the influence of UVB radiation/temperature on Vitamin D status, the collecting time was controlled between 8:00-10:00 am during the period of high UV exposure (November-March) to offset the influence of seasonal differences on Vitamin D status.

During the process, ten milliliters (mls) of random venous blood was obtained and aliquoted as follows: 4mls was transferred into lithium heparin tubes, another 3mls transferred into non-anticoagulated plain tubes; 3mls was transferred into ethyl-diamine-tetra-acetic tubes (EDTA) tubes.

The 4mls in plain tubes were allowed to clot for at least 60 minutes undisturbed at room temperature. When fully retracted, it was centrifuged at 2500g for 15 minutes along with the 4mls in lithium heparin tubes and the separated supernatant (plasma/serum) transferred into well-labeled plain (non-anticoagulated) tubes using Pasteur's pipettes. The separated plasma and serum were stored frozen at 4-8°C if the analysis was immediate or at -20°C if the analysis is delayed (but no later than 72 hours). Before analysis, all frozen specimen, including the reagents, were left unassisted to thaw to room temperature.

The lithium heparinized plasma was analyzed for AST, ALT, GGT, ALP, albumin, creatinine, calcium, and inorganic phosphate on an automated chemistry analyzer (BS-800M, Mindray, China). EDTA tube-processed plasma was analyzed for SHBG while the plain tube-processed serum was analyzed for VDBP, intact PTH, TPSA, TT, and CRP, and pro-calcitonin using the enzyme-linked immune-sorbent assay method (with polyclonal antibody reagents sourced from Monobind Inc., California, USA/R&D systems, Minneapolis, USA). Using the EDTA-acquired whole blood, HbA1c was analyzed on an automated HbAIc analyzer (D10, Biorad, UK) while the Hb concentration was determined on an automated hematology analyzer (BC-10, Mindray, Shenzhen, China).

Serum total 25(OH)D (both the D2 & D3 isoforms) was analyzed using the isocratic reverse-phase high-performance liquid chromatography method (Agilent 1100 series HPLC system) with high-grade reagents (Zivak Techn., Turkey).

Three levels of commercial quality materials were used to evaluate the coefficient of variations (CV). The percent intra-CV and inter-CV of serum total 25(OH)D assays were 5.9% and 9.7%, respectively.

Five mls of random urine specimen was also collected into sterile urine containers for immediate determination of UACR using a semi-automated urine chemistry analyzer (Combilyzer-13, Human, Germany).

2.8. Communication of Laboratory Results/Counseling

The study participants were counseled during their subsequent visits about their Vitamin D level status, and options for replenishing deficient stores or to visit any hospital were offered to those with confirmed Vitamin D deficiency status but negative biopsy for PCa. As this was a non-interventional study, the decision to get Vitamin D supplements administered was left to the individual patient's discretion after informing them about the possible benefits and risks. The study participants with both confirmed PCa diagnosis and Vitamin D deficiency were also informed that irrespective of their decision to get Vitamin D supplementation, treatment of the PCa would not be altered

and that there was no conclusive data yet suggestive that administering Vitamin D to Vitamin D deficient PCa patients would improve their outcome.

2.9. Data Definitions/Stratifications

Vitamin D status was defined as deficient (total serum 25(OH)D <30 nmol/L), insufficient (total serum 25(OH)D 20-50 nmol/L), sufficient (total serum 25(OH)D >50 nmol/L) based on the recommendations of the American Institute of Medicine (IOM), now National Academy of Medicine, which has been adopted by the European Food Safety Authority [16]. PCa tissues were graded from 1-5 based on the ISUP recommendations [17]. PCa staging was done clinically (stages 1 to 4) as recommended by the American Joint Committee on Cancer (AJCC) [18].

BMI was categorized as underweight (<18.5), normal weight (18.5-24.9), overweight (25.0-29.9), and obese (≥30) [19]. Normal hepatic and renal status was defined based on evidence of normal laboratory/radiological parameters of hepatic and renal functions/features. Metabolic stability was defined as normal hepatic/renal functions and normal inflammatory markers.

2.10. Data Management/Statistical Analysis

Data were managed using the Statistical Package for Social Sciences (SPSS) software version 25.0 (IBM Co., Armonk, NY, USA). The continuous variables were initially evaluated for conformity to a normal distribution using both visual (histogram/probability plots) and statistical (Kolmogorov-Smirnov/Shapiro-Wilk tests) parameters. The continuous data found not be of normal distribution were logarithmically transformed before analysis and were summarized using means ± standard deviations; the comparison was made with the independent student t-test or analysis of variance (ANOVA), where necessary. The categorical data were summarized using proportions with counts/percentages; a comparison was made with the chi-square test. Linear logistic regression was used to examine the relationship between continuous variables and both crude and adjusted logistic regression models were used to determine the magnitude of association between vitamin D status and the PCa biopsy grade and clinical stage at 95% confidence intervals. An alpha value of <0.05 was chosen as being statistically significant.

3. Results

Table 1 depicts the comparative analysis of basic characteristics of non-categorical data between the biopsy positive PCa patients and biopsy negative controls. From Table 1, the biopsy positive PCa patients had significantly lower mean Vitamin D levels (PCa patients: 24.55 ± 3.47 vs. controls: 49.73 ± 4.08; p<0.001), but higher mean prostate volumes, higher mean BMI status, higher mean intact PTH levels, and higher mean TPSA levels compared to the negative biopsy controls. No significant difference (p>0.05) was observed among the other non-categorical data when compared between the biopsy positive PCa patients and the biopsy negative controls as shown in Table 1.

Table 2 depicts the comparative analysis of the categorical variables between the biopsy positive PCa cases and the biopsy negative controls. The only significant finding observed was the abnormal DRE features that were found to be more pronounced among the biopsy positive PCa patients compared to the biopsy negative controls (Table 2).

Table 3 shows the distribution of serum Vitamin D level status by PCa tumor grade and stage which shows a significant decreasing trend of serum Vitamin level status with worsening and increasing PCa tissue grade and stage (p<0.001) among the biopsy positive PCa cases.

In table 4, the evaluation of the linear relationship between the ISUP PCa grades and stages as continuous variables and that of serum Vitamin D level status showed an inverse relationship among the Vitamin D sufficiency, insufficiency, and deficient subgroups on both the crude and adjusted linear logistic regression analyses. However, a statistically significant threshold (p<0.05) was only observed among those in the Vitamin D deficient subgroup on a crude linear logistic regression model which was subsequently magnified while adjusting for confounders (BMI, intact PTH levels, serum total PSA levels, prostate volume, and abnormal DRE) in the adjusted linear regression model (Table 4).

Among the Vitamin D deficient PCa patients, the inverse relationship between the serum Vitamin D and PCa grade/stage continued to strengthen with increasing/ worsening PCa grade/stage.

Table 5 depicts the association between Vitamin D deficiency and PCa grade and stage. Shown in Table 5, when compared to the PCa patients with the lowest PCa ISUP grade (GS grade 6)/AJCC clinical stage (T1a-c), an increasing likelihood (odds ratio) of Vitamin D deficiency was observed with worsening/increasing PCa ISUP grade and the AJCC PCa clinical stage among the PCa cases on crude multiple logistic regression model which was amplified while adjusting also for confounders (p<0.05) (Table 5).

Table 1. *Comparison of the non-categorical variables among cases/controls.*

	Biopsy +ve PCa n=380	**Biopsy -ve controls n=380**	
Non-categorical Variables	M ± SD	M ± SD	p-value
Age, years	67.64 ± 5.33	66.79 ± 5.12	0.188
BMI, kg/m^2	29.11 ± 4.21	26.67 ± 4.32	0.002*
Waist circumference, cm	100.34 ± 7.76	99.86 ± 6.59	0.060
SBP, mmHg	126.71 ± 8.22	128.08 ± 7.62	0.074
DBP, mmHg	78.56 ± 6.07	79.11 ± 5.68	0.103

	Biopsy +ve PCa n=380	**Biopsy -ve controls n=380**	
Total serum 25(OH)D, nmol/L	24.55 ± 3.47	49.73 ± 4.08	<0.001*
VDBP, µg/mL	370.76 ± 23.4	450.63 ± 25.09	<0.001
Intact PTH, ng/L (10-65) (plasma)	49.45 ± 4.65	27.88 ± 4.33	<0.001*
Serum total PSA, µg/L	64.55 ± 6.13	2.44 ± 0.92	<0.001*
Plasma albumin, g/L	38.49 ± 5.07	37.94 ± 4.78	0.096
Plasma calcium, mmol/L	2.22 ± 0.97	2.31 ± 0.75	0.069
Plasma Inorganic phosphate, mmol/L	1.10 ± 0.23	1.18 ± 0.34	0.057
Serum CRP, nmol/L (2.9-28.3)	11.13 ± 2.44	10.67 ± 2.61	0.144
Serum pro-calcitonin x 10, µg/L (<2.5)	1.7 ± 0.12	1.6 ± 0.11	0.096
Plasma creatinine, µmol/L	85.64 ± 5.06	84.55 ± 4.96	0.183
Serum total testosterone, nmol/L (9-29)	16.62 ± 3.44	15.46 ± 4.01	0.233
SHBG, nmol/L (10-57)	13.34 ± 4.17	12.94 ± 3.56	0.084
ALT activity, U/L (7-56)	10.44 ± 2.97	11.02 ± 2.44	0.110
AST activity, U/L (10-40)	7.66 ± 1.76	7.91 ± 1.54	0.123
GGT activity, U/L (0-30)	8.72 ± 2.33	8.65 ± 2.21	0.076
ALP activity, U/L (44-140)	67.99 ± 7.56	65.13 ± 6.93	0.198
Hb, g/L (138-172)	11.78 ± 2.33	11.94 ± 2.45	0.234
HbA1c, %	4.11 ± 1.34	3.78 ± 1.56	0.093
Urine ACR, mg/g (normal <10)	5.66 ± 1.75	5.47 ± 1.81	0.341
Prostate volume, cm^3 (20-30)	38.94 ± 4.55	26.47 ± 3.67	0.003*
Gleason's score	7.88 ± 2.22	-	NA

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; VDBP: Vitamin D binding protein; PTH: parathyroid hormone; CRP: C-reactive protein; SHBG: sex hormone-binding globulin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; Hb: Hemoglobin; HbA1c: glycated hemoglobin A1c; ACR: albumin-creatinine ration; NA; not applicable.

Table 2. *Comparison of the categorical variables among cases/controls.*

	Biopsy +ve PCa n=380	**Biopsy -ve controls n=380**	
Categorical Variables	n (%)	n (%)	p-value
Age, years			0.206
≤44 (young adults)	5 (1.3)	5 (1.3)	
45-64 (middle-aged)	105 (27.6)	107 (28.2)	
≥65 (elderly)	270 (71.1)	268 (70.5)	
BMI, kg/m^2			0.488
<18.5 (underweight)	5 (1.3)	4 (1.1)	
18.5-24.9 (normal weight)	86 (22.6)	92 (24.2)	
25.0-29.9 (overweight)	147 (38.7)	152 (40.0)	
≥30 (generalized obesity)	142 (37.4)	132 (34.7)	
Waist circumference, cm			0.133
≤102	179 (47.1)	185 (48.7)	
>102 (central obesity)	201 (52.9)	195 (51.3)	
Blood pressure, mmHg			0.506
Normotensive	305 (80.3)	309 (81.3)	
Hypertensive	75 (19.7)	71 (18.7)	
Habitual tobacco consumption			0.644
Never/previous	369 (97.1)	367 (96.6)	
Current	11 (2.9)	13 (3.4)	
Habitual alcohol consumption			0.583
Never/previous	322 (84.7)	319 (83.9)	
Current	58 (15.3)	61 (15.1)	
Marital status			0.603
Married	370 (97.4)	371 (97.6)	
Bereaved	5 (1.3)	4 (1.1)	
Single	5 (1.3)	5 (1.3)	
Educational status			0.479
No formal education	55 (14.5)	54 (14.2)	
Primary	86 (22.6)	88 (23.2)	
Secondary	109 (28.7)	111 (29.2)	
Tertiary	130 (34.2)	127 (33.4)	
PCa history in a 1^{st}-degree relative	139 (36.6)	131 (34.5)	0.147
Abnormal findings on DRE	307 (80.9)	196 (51.6)	<0.001*
Blood draw season (high UVβR)	380 (100)	380 (100)	NA
Skin color (dark)	380 (100)	380 (100)	NA

*Statistically significant; PCa: prostate cancer; UVBR: ultraviolet β radiation; NA: not applicable;
+ve: positive; -ve: negative.

Table 3. *Distribution of vitamin D status by grade and stage among the PCa patients.*

Variables	Biopsy Positive PCa Cases, n (%)	M ± SD
A. PCa ISUP Grades		
1 (GS ≥3+3)	65 (17.1)	42.33 ± 6.78
2 (GS 3+4)	67 (17.6)	37.41 ± 5.67
3 (GS 4+3)	71 (18.9)	23.45 ± 4.79
4 (GS 4+4, 3+5, or 5+3)	75 (19.7)	19.63 ± 3.44
5 (GS 4+5, 5+4, or 5+5)	102 (26.8)	15.84 ± 3.06
p-value for trend	NA	<0.001*
B. PCa AJCC Clinical Stages		
T1a-c	79 (20.8)	39.08 ± 5.73
T2a-c	86 (22.6)	26.34 ± 4.86
T3a-b	98 (25.8)	18.77 ± 3.67
T4 (NxMx)	117 (30.8)	13.96 ± 2.88
p-value for trend	NA	<0.001*

*Statistically significant; PCa: prostate cancer; M±SD: mean ± standard deviation;
ISUP: International Society of Urological Pathology; GS: Gleason's score;
NA: not applicable; AJCC.

Table 4. *Relationship between serum Vitamin D level status and PCa grade/stage among the sufficient, insufficient, and deficient PCa subgroups.*

SERUM VITAMIN D STATUS	Linear Logistic Regression			
	Crude Model		Adjusted Model[a]	
	β coefficient	p-value	β coefficient	p-value
Sufficient Vitamin D Status, n=70				
A. ISUP Grades				
1 (GS 3+3)	-0.111	0.304	-0.094	0.232
2 (GS 3+4)	-0.115	0.234	-0.110	0.124
3 (GS 4+3)	-0.123	0.165	-0.114	0.131
4 (GS 4+4, 3+5, or 5+3)	-0.131	0.130	-0.123	0.133
5 (GS 4+5, 5+4, or 5+5)	-0.143	0.101	-0.129	0.127
B. PCa Stage				
T1a-c	-0.117	0.494	-0.097	0.455
T2a-c	-0.112	0.216	-0.102	0.244
T3a-c	-0.134	0.179	-0.119	0.200
T4 (NxMx)	-0.144	0.127	-0.128	0.166
Insufficient Vitamin D Status, n=118				
A. ISUP Grades				
1 (GS 3+3)	-0.126	0.379	-0.102	0.601
2 (GS 3+4)	-0.134	0.278	-0.117	0.466
3 (GS 4+3)	-0.167	0.165	-0.128	0.347
4 (GS 4+4, 3+5, or 5+3)	-0.174	0.144	-0.143	0.255
5 (GS 4+5, 5+4, or 5+5)	-0.195	0.105	-0.163	0.179
B. PCa Stage				
T1a-c	-0.137	0.244	-0.112	0.309
T2a-c	-0.144	0.191	-0.142	0.233
T3a-c	-0.167	0.145	-0.164	0.159
T4 (NxMx)	-0.188	0.101	-0.181	0.122
Deficient Vitamin D Status, n=192				
A. ISUP Grades				
1 (GS 3+3)	-0.301	0.027*	-0.399	0.003*
2 (GS 3+4)	-0.377	0.019*	-0.418	<0.001*
3 (GS 4+3)	-0.403	<0.001*	-0.435	<0.001*
4 (GS 4+4, 3+5, or 5+3)	-0.457	<0.001*	-0.489	<0.001*
5 (GS 4+5, 5+4, or 5+5)	-0.506	<0.001*	-0.511	<0.001*
B. PCa Stage				
T1a-c	-0.371	0.016*	-0.406	<0.001*
T2a-c	-0.394	0.011*	-0.455	<0.001*
T3a-c	-0.403	<0.001*	-0.517	<0.001*
T4 (NxMx)	-0.412	<0.001*	-0.598	<0.001*

*Standard deviation; ISUP: International Society of Urological Pathology; GS: Gleason's score;
PCa: Prostate cancer; NA: not applicable; [a]adjusted for BMI, intact PTH levels, serum total PSA levels, prostate volume, and abnormal DRE; NxMx: Lymph nodes/Metastasis could not be assessed.

Table 5. *Association between Vitamin D deficiency (serum 25(OH)D <25nmol/L) and PCa grade/stage.*

	Multiple Logistic Regression					
	Crude Model			**Adjusted Model[a]**		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
A. ISUP Grades						
1 (GS 3+3); Reference	1.000	NA	NA	1.000	NA	NA
2 (GS 3+4)	2.304	1.304-4.671	0.013*	2.667	1.342-4.983	<0.001*
3 (GS 4+3)	3.910	1.939-6.763	0.002*	4.556	2.322-7.688	<0.001*
4 (GS 4+4, 3+5, or 5+3)	4.577	2.710-8.084	<0.001*	5.643	3.373-9.100	<0.001*
5 (GS 4+5, 5+4, or 5+5)	6.119	3.674-9.013	<0.001*	7.844	4.571-10.673	<0.001*
B. PCa Stage	OR	95% CI	p-value	OR	95% CI	p-value
T1a-c; Reference	1.000	NA	NA	1.000	NA	NA
T2a-c	1.980	1.106-3.710	<0.001*	2.418	1.344-4.081	<0.001*
T3a-b	2.463	1.471-4.609	<0.001*	3.671	1.711-5.210	<0.001*
T4NxMx	3.401	1.833-6.515	<0.001*	4.061	2.107-8.122	<0.001*

Standard deviation; ISUP: International Society of Urological Pathology; GS: Gleason's score;
PCa: Prostate cancer; OR: odds ratio; CI: confidence interval; NA: not applicable; [a]adjusted for BMI, intact PTH levels, serum total PSA, prostate volume, and abnormal DRE; NxMx: Lymph nodes/Metastasis could not be assessed.

4. Discussion

4.1. Principal Findings

The established role of Vitamin D is on the regulation of bone health in addition to calcium and phosphate metabolism; however, the deficiency and insufficiency of the vitamin have long been associated with metastatic and non-metastatic PCa [20, 21]. The current study evaluated the relationships and associations between Vitamin D level status and PCa grade and stage among Nigerian males with the disease. One of the significant findings in the present study was the marked reduction of mean serum Vitamin D level status among the histologically-verified positive PCa patients relative to the histologically-verified negative controls. In addition, a significantly decreasing trend of serum Vitamin level status was also observed with worsening/increasing PCa grade and stage among the histologically-verified positive PCa cases. Furthermore, a significant inverse relationship existed between Vitamin D level status and PCa grade and stage among the Vitamin D deficient PCa subgroup but not the sufficient/insufficient PCa subgroups. Among the Vitamin D deficient PCa patients, the inverse relationship between the serum Vitamin D and PCa grade/stage continued to strengthen with worsening PCa grade/stage. When compared with the PCa patients with the lowest PCa grade (ISUP grade 1) and stage (T1), increased odd of Vitamin D deficiency was significantly associated with worsening PCa grade (ISUP 2 to 5) and stage (T2 to T4) among the PCa cases on crude multiple logistic regression model which was subsequently amplified following adjusting for confounders.

4.2. Comparison of Current Findings with the Existing Literature

The findings of this current study corroborate a large number of epidemiologic evidence of the role of Vitamin D deficiency in association with PCa biology [22-28]. In a similar local Nigerian study limited by small sample size, Adedapo and colleagues had documented a significant reduction of serum levels of Vitamin D in PCa patients compared to healthy control subjects but no correlation of Vitamin D was made with either the PCa grade or the PCa stage [24].

In a recent cross-sectional study reported from Sub-Sahara Africa among PCa patients of Kenyan origin, the authors had also noted reduced serum Vitamin D level status was associated with PCa Gleason score grade and suggested that Vitamin D be employed as a biomarker of PCa severity, but this very study was also limited by lack of control subjects and no relationship was explored between Vitamin D level status and PCa stage [25]. However, the authors of that Kenyan study had observed that prostate biopsy tissues with Gleason grade scores ≥7 (a cut-off mark used to define PCa severity) were associated with low Vitamin D level status (OR: 2.9; 95%CI 1.5–5.5; p= 0.001).

In a more similar study documented in the United States by Murphy and colleagues, though the authors found no significant difference in serum Vitamin D level status between PCa patients and controls, a significant association was established between low Vitamin D level status and PCa grade and stage among their European-American and African-American study populations [26]. In yet another similar study reported from the United States, low vitamin D status was significantly found to be associated with increased odds of poor Gleason grade scores (OR: 2.20; p=0.04) at the time of radical prostatectomy in men with PCa disease [27].

In a more recent study documented in Turkey by Ozman and colleagues [28], the authors had observed a reduction of serum Vitamin D level status of patients with PCa compared to the non-cancer control group but the difference did not reach a statistically significant threshold (p>0.05). In that Turkish study, the authors had also observed that patients with clinically insignificant PCa (ISUP grade 1) had significantly higher serum Vitamin D level status than other PCa patients with higher ISUP grade and stage. The authors also reported a weak but significant negative correlation between serum Vitamin D level status and the ISUP grades (r = -0.319, p=0.01) [28].

The findings in the current study concur with the reports of

these previous studies [22-28] and corroborate the role played by Vitamin D deficiency in association with worse pathologic features (that is, high grade/stage) of PCa disease [26, 27].

Contrastingly, the recent elaborate MARTINI-Lifestyle Cohort study found no association between serum vitamin D level status and PCa grade/stage. This discordant conclusion may be related to the demographic variations of the study populations in the MARTINI-Lifestyle Cohort study and the present study [29].

4.3. Mechanisms of Vitamin D Deficiency-induced PCa Pathology

The various anti-PCa properties of Vitamin D are orchestrated via the actions of 1,25(OH)D (calcitriol) on VDR within the normal prostate cells and the tumor micro-environment [30-37]. These actions are generally thought to be mediated mainly through Vitamin D-induced cell growth arrest and enhancement of apoptosis but other potential mechanisms also play vital roles [30]. The active Vitamin D also has anti-inflammatory effects, anti-oxidant/DNA damage repair effects, autophagic cell growth promotion, anti-proliferative/pro-differentiating potentials, and anti-angiogenic effects [31-38]. The repression of these vital intra-prostatic functions of Vitamin D, occasioned by its deficiency status, is the cardinal initiating event in PCa adverse pathology (worsening grade/stage) observed in the current study [30-37]. The Vitamin D-induced cell cycle arrest/apoptotic effect is mediated via down-regulation of the anti-apoptotic proteins (Bcl-2, Bcl-XL) and up-regulation of pro-apoptotic proteins (Bax, Bak, Bad) [30-33]. The anti-inflammatory effects are mediated through the inhibitions of prostaglandin synthesis, p38-MAPK signaling, NF-Kb, and modulation of immune-cancer cell interaction [34, 35].

The anti-oxidant/DNA damage repair is initiated by up-regulation of different antioxidant enzymes (SOD1/2, G6PD, NRF2) and DNA damage repair proteins (p53) [36].

Promotion of autophagic cell death is achieved via a switch from cell survival to cell death and the up-regulation of Beclin 1 and the suppression of mTORC1 [34, 35]. The anti-proliferative/pro-differentiating potentials of active Vitamin D are mediated by up-regulating the CDK inhibitors (p21, p27) and the inhibition of Wnt/β-catenin signaling and telomerase activities [32, 37].

Vitamin D-induced anti-angiogenic activity is mediated by the inhibition of vascular endothelial growth factor; a factor known to potentiate metastasis, migration, and invasion of PCa cell lines [30, 37].

4.4. Connotations of Findings to Clinical Practice and Future Research

Observations from this current study support the role of Vitamin D in PCa grade and stage; two factors that define the aggressiveness and metastatic potentials of PCa disease. Since the Vitamin D supplements have a very wide margin of safety and therapeutic index, elaborate clinical trials should be instituted to evaluate their effectiveness in the PCa prevention or its therapeutic potentials in the management of the disease, especially as adjuvant agents.

4.5. Strength and Limitations of the Current Study

The current study was strengthened by its relatively large sample size and the recruitment of only the histologically-verified subjects with and without PCa. However, the study was limited by some factors which are potential areas for further improvement. As in any observation study, its findings do not signify causal inference but merely associations. It is solely a single-institution-based study, so, its conclusions may not represent the larger population within the study area. Due to financial constraints, the serum Vitamin D level status was only determined once after the PCa diagnosis, so we cannot rule out the possibility that the PCa disease status may have affected the Vitamin D level status. We could not also determine the intra-prostatic Vitamin D level status and activity of the VDR, due to limited resources, which may or may not have given a different outcome if assessed.

5. Conclusion

The current study demonstrated a marked reduction of mean serum Vitamin D level status among the histologically-verified positive PCa patients with a decreasing trend of serum Vitamin level status with worsening PCa grade and stage. This finding was also associated with the existence of a significant inverse relationship between Vitamin D level status and PCa grade and stage among the Vitamin D deficient PCa subgroup. Among the Vitamin D deficient PCa patients, the inverse relationship continued to strengthen with worsening PCa grade and stage. Compared with the PCa patients with the more favorable PCa grade and stage, a significantly increased likelihood of Vitamin D deficiency was found in association with worsening PCa grade and stage. Further investigations using more elaborate studies are recommended to evaluate the conclusions from this present study.

Statement of Ethics

The ethical approval of the study was obtained from the Research Ethics Committee of UPTH following the review of the study protocols.

Disclosure Statement

The authors declare that they have no competing interests.

Author Contributions

Both authors were substantially involved in the concept and design of the study, acquisition, analysis and interpretation of the data, drafting the article, revising the article critically for its intellectual content, and in the final approval of the version to be published.

Data Availability

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author (CA) upon reasonable request.

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References

[1] Taitt HE. Global trends and prostate cancer: a review of incidence, detection, and mortality as influenced by race, ethnicity, and geographic location. Am J Men's Health. 2018; 12 (6): 1807-23.

[2] Rawla P. Epidemiology of prostate cancer. World J Oncol. 2019; 10 (2): 63.

[3] Dasgupta P, Baade PD, Aitken JF, Ralph N, Chambers SK, Dunn J. Geographical variations in prostate cancer outcomes: a systematic review of international evidence. Frontiers Oncol. 20198; 9: 238.

[4] Ukoli F, Osime U, Akereyeni F, Okunzuwa O, Kittles R, Adams-Campbell L. Prevalence of elevated serum prostate-specific antigen in rural Nigeria. Int J Urol. 2003; 10: 315–22.

[5] Abib A, Jaffar G, Khalid MS, Hussain Z, Zainab SW, Ashraf Z, Haroon A, Javed R, Khalid B, Habib P. Risk Factors Associated with Prostate Cancer. J Drug Del Ther. 2021; 11 (2): 188-93.

[6] Grant WB. Review of recent advances in understanding the role of vitamin D in reducing cancer risk: breast, colorectal, prostate, and overall cancer. Anticancer Res. 2020; 40 (1): 491-9.

[7] Bikle DD. Vitamin D: Newer concepts of its metabolism and function at the basic and clinical level. J Endocr Soc. 2020; 4 (2): bvz038.

[8] Bikle D, Christakos S. New aspects of vitamin D metabolism and action—addressing the skin as source and target. Nat Rev Endocrinol. 2020; 16 (4): 234-52.

[9] Saponaro F, Saba A, Zucchi R. An update on vitamin D metabolism. Int J Mol Sci. 2020; 21 (18): 6573.

[10] Richards Z, Batai K, Farhat R, Shah E, Makowski A, Gann PH, Kittles R, Nonn L. Prostatic compensation of the vitamin D axis in African American men. JCI Insight. 2017; 2 (2): e91054. doi: 10.1172/jci.insight.91054.

[11] Wagner D, Trudel D, Van der Kwast T, Nonn L, Giangreco AA, Li D, et al. Randomized clinical trial of vitamin D3 doses on prostatic vitamin D metabolite levels and ki67 labeling in prostate cancer patients. J Clin Endocrinol Metab. 2013; 98 (4): 1498-507.

[12] McCray T, Pacheco JV, Loitz CC, Garcia J, Baumann B, Schlicht MJ, Valyi-Nagy K, Abern MR, Nonn L. Vitamin D sufficiency enhances differentiation of patient-derived prostate epithelial organoids. iScience. 2021; 24 (1): 101974.

[13] Ramakrishnan S, Steck SE, Arab L, Zhang H, Bensen JT, Fontham ET, Johnson CS, Mohler JL, Smith GJ, Su LJ, Woloszynska A. Association among plasma 1, 25 (OH) 2D, ratio of 1, 25 (OH) 2D to 25 (OH) D, and prostate cancer aggressiveness. The Prostate. 2019; 79 (10): 1117-24.

[14] Naing L, Winn T, Rusli BN. Practical issues in calculating the sample size for prevalence studies. Arch Orofac Sci. 2006; 1: 9–14.

[15] Jeong CW, Park HK, Hong SK, Byun SS, Lee HJ, Lee SE. Comparison of prostate volume measured by transrectal ultrasonography and MRI with the actual prostate volume measured after radical prostatectomy. Urol Int. 2008; 81: 179–185.

[16] IOM (Institute of Medicine) Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: The National Academies Press; 2011.

[17] Epstein JI, Egevad L, Amin MB, et al. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. Am J Surg Pathol 2016; 40: 244.

[18] Buoyounnouski MK, Choyke PL, Mckennedy JK, Sartor O, Sandler HM, Amin MB, et al. Prostate cancer-Major changes in the American Joint Committee on cancer eight edition cancer staging manual. Ca cancer J Clin. 2017; 67: 243 – 53.

[19] WHO. Physical Status: The Use and Interpretation of Anthropometry: Report of a World Health Organization (WHO) Expert Committee. Geneva, Switzerland: World Health Organization; 1995.

[20] Trump DL, Chadha MK, Sunga AY, Fakih MG, Ashraf U, Silliman CG, Hollis BW, Nesline MK, Tian L, Tan W, Johnson CS. Vitamin D deficiency and insufficiency among patients with prostate cancer. BJU Int. 2009; 104 (7): 909.

[21] Choo CS, Mamedov A, Chung M, Choo R, Kiss A, Danjoux C. Vitamin D insufficiency is common in patients with non-metastatic prostate cancer. Nutri Res. 2011; 31 (1): 21-6.

[22] Kristal AR, Till C, Song X, Tangen CM, Goodman PJ, Neuhauser ML, Schenk JM, et al. Plasma vitamin D and prostate cancer risk: results from the Selenium and Vitamin E Cancer Prevention Trial. Cancer Epidemiol Biomarkers Prev. 2014; 23 (8): 1494-504.

[23] Aytaç Ş, Tuncay T, Musab AK, Ahmet Ü, Çağlar Y, Ayhan V. Is Prostate Cancer Related to Low Vitamin D Level?. Bull Urooncol. 2019; 18: 113-6.

[24] Adedapo KS, Olusanyo TO, Takure AO. Dietary implication of high malondialdehyde, reduced vitamin D and total antioxidant status of prostate cancer subjects in Ibadan. Int J Biomed Res. 2015; 6: 661-68.

[25] Odhiambo AO, Kiarie GW, Ngugi MP, Joshi MD. Serum Vitamin D profile in black African men with prostate cancer at a tertiary referral facility in Sub-Sahara Africa. J Dent Med Sci. 2014; 30: 60-4.

[26] Murphy AB, Nyame Y, Martin IK, Catalona WJ, Hollowel CM, Nadler RB, et al. Vitamin D deficiency predicts prostate biopsy outcome. Clin Cancer Res. 2014; 20: 2289-99.

[27] Bowen DK, Jordan GA, Malers TJ, Kittles RA, Murphy AB. Vitamin D deficiency predicts adverse pathology at time of radical prostatectomy. J Urol. 2015; 193: e641.

[28] Özman O, Türegün FA, Şimşekoğlu MF, Aferin U, Demirdağ Ç. Association Between Prostate Biopsy Results and Serum Vitamin D Levels. Bull Urooncol. 2021; 20 (2): 83-6.

[29] Thederan I, Chandrasekar T, Tennstedt P, Knipper S, Kuehl L, Tilki D, et al. Circulating Vitamin D and Selenium Levels and Outcome in Prostate Cancer Patients: Lessons from the MARTINI-Lifestyle Cohort. Eur Urol Focus. 2021; 7 (5): 973-9.

[30] Fleet JC, DeSmet M, Johnson R, Li Y. Vitamin D and cancer: a review of molecular mechanisms. Biochem J. 2012; 441 (1): 61-76.

[31] Bhoora S, Punchoo R. Policing cancer: Vitamin d arrests the cell cycle. Int Mol Sci. 2020; 21 (23): 9296.

[32] Karlsson S, Cruz MA, Faresjö M, Khamou AP, Larsson D. Inhibition of CYP27B1 and CYP24 Increases the Anti-proliferative Effects of 25-Hydroxyvitamin D3 in LNCaP Cells. Anticancer Res. 202; 41 (10): 4733-40.

[33] Udristioiu A, Farmazon I, Cojocaru M. Preclinical Researches of Vitamin D Role in Preventing Malignant Diseased, a Systematic Review. Int J Surg: Oncology. 2021; 6 (1).

[34] El-Sharkawy A, Malki A. Vitamin D signaling in inflammation and cancer: Molecular mechanisms and therapeutic implications. Molecules. 2020; 25 (14): 3219.

[35] Negri M, Gentile A, de Angelis C, Montò T, Patalano R, Colao A, et al. Vitamin D-induced molecular mechanisms to potentiate cancer therapy and to reverse drug-resistance in cancer cells. Nutrients. 2020; 12 (6): 1798.

[36] Blajszczak CC, Nonn L. Vitamin D regulates prostate cell metabolism via genomic and non-genomic mitochondrial redox-dependent mechanisms. The Journal of steroid biochemistry and molecular biology. 2019; 195: 105484.

[37] McCray T, Pacheco JV, Loitz CC, Garcia J, Baumann B, Schlicht MJ, et al. Vitamin D sufficiency enhances differentiation of patient-derived prostate epithelial organoids. Iscience. 2021; 24 (1): 101974.