2025 Volume: 01 Issue: 05 www.ijrpa.com Review Article

International Journal Research Publication Analysis

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SERUM VITAMIN D AND ASSOCIATED FACTORS AMONG ADULT HIV-POSITIVE PATIENTS IN A TERTIARY HEALTH FACILITY IN SOUTHEAST NIGERIA

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Article Received: 08 August 2025

Article Revised: 28 August 2025

Published on: 18 September 2025

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ABSTRACT

Vitamin D Status and Its Determinants Among HIV-Positive Adults in Nnewi, Nigeria Individuals living with HIV face a heightened risk of opportunistic infections due to compromised immune function. Vitamin D, a key immune modulator, has gained attention for its potential influence on immunity, especially in immunocompromised populations. Deficiency in vitamin D may further impair immune responses in people with HIV. This cross-sectional study assessed vitamin D levels and their associations with sociodemographic, clinical, and lifestyle factors among 56 HIV-positive adults attending the HIV Clinic at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria. Participants' sociodemographic and clinical data were collected alongside serum vitamin D measurements. Statistical analyses included descriptive statistics, chi-square or Fisher's exact tests for categorical variables, independent t-tests for continuous variables, and logistic regression to identify predictors of deficiency. The mean vitamin D concentration in the cohort was 22.88 ± 5.96 ng/ml. Vitamin D deficiency (<20 ng/ml) was present in 26.8% of participants, while 64.3% had insufficient levels (20–29 ng/ml). Significant associations were observed between vitamin D status and both age ($p = 0.02$) and antiretroviral therapy (ART) regimen ($p = 0.002$). Notably, multivariate analysis revealed that individuals on a tenofovir disoproxil fumarate/emtricitabine/dolutegravir regimen had 97% lower odds of vitamin D deficiency compared to those on a tenofovir disoproxil fumarate/lamivudine/dolutegravir regimen. In

conclusion, age and ART regimen significantly influenced vitamin D status among HIV-positive adults in this setting. Routine monitoring of vitamin D may be beneficial in comprehensive HIV care, particularly with consideration of specific ART regimens.

KEYWORDS: Vitamin D deficiency, HIV, Antiretroviral therapy, CD4 count, Viral load, Nigeria.

BACKGROUND

Vitamin D, also known as calciferol, is a fat-soluble vitamin essential for calcium homeostasis and bone health.[1] It can be obtained from limited food sources, fortified products, dietary supplements, and endogenous synthesis triggered by sunlight exposure.[2] Deficient or insufficient vitamin D levels can lead to reduced calcium absorption, which may impact bone metabolism, increase parathyroid hormone (PTH) secretion, and contribute to bone resorption.[3] Globally, vitamin D deficiency is widespread, with women reportedly more affected than men, potentially due to lower dietary intake and differences in sunlight exposure.[4]

The status of vitamin D in the body is primarily assessed via serum 25-hydroxyvitamin D[25(OH)D] concentrations, which reflect both cutaneous synthesis and dietary intake.[5] While the ideal serum levels of 25(OH)D are debated, levels below 20 ng/mL (50 nmol/L) are commonly associated with secondary hyperparathyroidism, osteoporosis, increased fracture risk, and muscle weakness, among other health issues.[6, 7] Moreover, inadequate vitamin D levels have been linked to increased morbidity, with a significant impact on chronic conditions, infections, and mortality rates, especially in immunocompromised populations.[8-10]

Globally, approximately 39 million people live with human immunodeficiency virus (HIV) infection, with an estimated 40.4 million lives lost due to HIV-related complications.[11,12] HIV specifically targets immune cells such as CD4+ T lymphocytes, dendritic cells, monocytes, and macrophages.[13] The early destruction of gut-associated lymphoid tissue (GALT) alters the mucosal barrier, allowing microbial translocation and resulting in chronic immune activation.[14] This ongoing immune response eventually contributes to immune exhaustion and increased susceptibility to opportunistic infections and malignancies, hallmark characteristics of acquired immunodeficiency syndrome (AIDS).[15]

Vitamin D deficiency is prevalent in HIV-infected individuals, with up to 100% of infected individuals presenting suboptimal levels (calcidiol serum <30 ng/mL) and over 30% showing severe deficiency (<20 ng/mL).[16] This deficiency persists even with combination antiretroviral therapy (cART) and has been associated with increased risks of comorbidities, including osteoporosis, cardiovascular disease, type 2 diabetes, and susceptibility to infections such as tuberculosis.[17] These comorbidities may be linked to the immunomodulatory, anti-inflammatory, and antimicrobial functions of vitamin D.[17]

In HIV-positive individuals, disruptions in vitamin D metabolism are observed, leading to increased proinflammatory cytokines, reduced PTH activity, and diminished conversion of calcidiol to its active form (calcitriol), which further impairs immune function.[18] Additionally, specific antiretroviral therapies, including non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs), affect the cytochrome P450 (CYP450) enzyme complex, reducing calcitriol production.[19] Consequently, HIV infection may exacerbate vitamin D deficiency, compounding risks for both skeletal and nonskeletal health outcomes.

MATERIALS AND METHODS

Study Area

This study was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH), a tertiary healthcare facility in Nnewi, Anambra state, southeast Nigeria. Nnewi is a commercial and industrial city and the second most populated city in Anambra state. As a metropolis, Nnewi comprises a single local government area, Nnewi North which consists of four quarters: Otolo, Uruagu, Umudim, and Nnewichi. Geographically, Nnewi falls within the tropical rainforest region of Nigeria. Tmbra state, Nigeria. It has a population of 1,362,000 in 2025 projected from the 2006 census figures based on an annual growth rate of 2.21%.

The research was conducted at the adult HIV clinic of NAUTH Nnewi. This is a specialised clinic that provides comprehensive HIV/AIDS care services for individuals living with HIV in the catchment area of NAUTH. Clinic hours are from 8 am to 4 pm on week days. During this time, a team of experienced healthcare professionals, including doctors, pharmacists and nurses, provide comprehensive care and support to the patients.

Study Design and Population

This study utilized a cross-sectional study design. The study population comprised adult male and female HIV-positive patients receiving care at the NAUTH HIV clinic. The inclusion criteria required that (1) study participants should be between 18 and 65 years old, and (2) on a stable antiretroviral therapy (ART) regimen for at least 6 months before commencement of the study. Patients were excluded if they (1) were pregnant or breastfeeding, (2) had severe comorbidities (such as advanced cancer, end-stage renal disease, or active tuberculosis), which could significantly impact immune function or vitamin D metabolism, (3) were currently taking immunosuppressive medications outside of their HIV treatment regimen and/or (4) were unable to provide informed consent due to cognitive impairment or other reasons.

Sample Size Determination

The sample size for the study (N = 56) was calculated using the Charan and Biswas formula for calculating sample size for cross-sectional studies.[51]

$$\frac{Z_{1-\alpha/2}{}^2 SD^2}{d^2}$$

Where, Z1-α/2 is the standard normal variate at the 95% confidence level = 1.96; SD is the standard deviation of serum vitamin D (ng/ml) = 9.5426; d is the absolute error allowed = 2.5 ng/ml.

Sampling Technique

Consecutive consenting sampling was used to select the study participants. At the start of each clinic day, the first eligible patient was approached and enrolled after obtaining their informed consent. Each subsequent eligible, consenting patient was approached and enrolled in the sequence they arrived, until the sample size was reached.

Data Collection

Participants were provided detailed information about the study to ensure they understood the study purpose and procedure, risk and benefits, the voluntary nature of participation, their freedom to withdraw from the study and the confidentiality of their data. Written informed consent is obtained from all participants. The information provided in study participants was anonymised and protected from unauthorised access, use, disclosure, modification, loss or theft.

The tools for data collection include a proforma was used to collect data on HIV disease stage, antiretroviral therapy (ART) use, CD4 count and HIV viral load from the medical records; a semi-structured questionnaire was used to collect data of sociodemographic information, diet, sun exposure, and medication use; and the PerkinElmer Enzyme Immunoassay Test Kit was used to measure serum vitamin D. Laboratory investigations for CD4 count and viral load NAUTH Nnewi central laboratory using the same standardized machine and technique.

Data Analysis

The study data was coded and analysed using SPSS version 21 (IBM Corp., Armonk, NY, USA). Serum vitamin D level, in ng/mL, are measured as a continuous variable, coded as a categorical variable having three categories, vitamin D sufficiency (≥ 30 ng/mL), vitamin D insufficiency (20-29 ng/ml), or vitamin D deficiency (<20 ng/ml), and further coded as a dichotomous variable (deficient: <20 ng/ml and not deficient: ≥ 30 ng/ml). CD4+ T cell count (cells/mm3) and viral load (copies/ml) were measured as discrete variables. Descriptive data were summarized using mean and standard deviation for continuous variables and frequencies and percentages for categorical variables. The relationships between categorical variables were assessed using chi-square test or Fisher's exact test while independent samples t-test was used for continuous variables. Binary logistic regression analysis was conducted to determine the predictors of vitamin D status. Variables that had a P value of 0.25 or less were included in the regression model. Statistical significance was set at $P < 0.05$.

RESULTS

The mean age of the respondents was 47.23±12.73. The gender distribution was almost equal, 51.8 % males and 48.2 % females. Respondents who had been on ART for 11–20 years had the highest proportion of 37.5%. The distribution of the ART regimen was fairly even with 51.8% being on tenofovir disoproxil fumarate/emtricitabine/dolutegravir (TDF/FTC/DTG) and 48.2% on tenofovir disoproxil fumarate/lamivudine/dolutegravir (TDF/3TC/DTG). Most of the respondents (73.2%) had undetectable viral loads and the mean CD4+ count was 662.5 ± 98.8 cells/mm3 (Table 1). None of the respondents reported having had opportunistic infections or chronic medical conditions.

Table 1: Demographic Characteristics of the Participants.

Variable		Vitamin D < 20 ng/ml	Vitamin D ≥ 20 ng/ml	Total	P-value
Age (years)					0.02a*
	Mean ± SD	40.53±9.66	49.68±12.93	47.23±12.73	
Gender					0.89b
	Male	8 (53.3)	21 (51.2)	29 (51.8)	
	Female	7 (46.7)	20 (48.8)	27 (48.2)	
Time on ART (years)					0.25c
	≤5	5 (33.3)	7 (17.1)	12 (21.4)	
	6 – 10	2 (13.3)	14 (34.1)	16 (28.6)	
	11 – 20	7 (46.7)	14 (34.1)	21 (37.5)	
	>20	1 (6.7)	6 (14.6)	7 (12.5)	
ART Regimen					<0.001b*
	TDF/FTC/DTG	1 (6.7)	28 (68.3)	29 (51.8)	
	TDF/3TC/DTG	14 (93.3)	13 (31.7)	27 (48.2)	
HIV Viral load					0.31c
	Undetectable	13 (86.7)	28 (68.3)	41 (73.2)	
	Detectable	2 (13.3)	13 (31.7)	15 (26.8)	
CD4+ count, cells/mm3					0.61a
	Mean ± SD	651.3 ± 86.8	666.6 ± 103.5	662.5 ± 98.8	
	Minimum, Maximum	500, 810	500, 900	500, 900	
	Median, IQR	620 (600–720)	650 (580–760)	650 (585–730)	

aindependent samples t-test; bPearson chi-square test; cFisher Exact test; TDF, Tenofovir disoproxil fumarate; FTC, Emtricitabine; DTG, Dolutegravir; 3TC, Lamivudine

Table 2 shows the distribution of serum vitamin D level among the respondents. Most of the study participants (64.3%) had vitamin D insufficiency (20 - 29 ng/ml). Deficient vitamin D levels (< 20 ng/ml) were found in 26.8% of the participants while 8.9% had sufficient levels at or above 30 ng/ml. The mean vitamin D level for the study participants was 22.88 ± 5.96 ng/ml.

Table 2: Distribution of Serum Vitamin D Level.

Vitamin D Level	N	%	Mean	SD	Min	Max
Vitamin D Deficiency (<20 ng/ml)	15	26.8	14.67	2.29	10.0	18.0
Vitamin D Insufficiency (20-29 ng/ml)	36	64.3	24.86	2.07	21.0	29.0
Vitamin D Sufficiency (≥30 ng/ml)	5	8.9	33.2	3.35	30.0	38.0
Total	56	100.0	22.88	5.96	10.0	38.0

P <0.001*; N, number of participants; %, percentage; SD, standard deviation; Min, minimum; Max, maximum.

The prevalence of vitamin D status categorised by the gender of the participants is shown in figure 1. The prevalence of deficiency was higher among the HIV-infected male participants (14.3%) than the females (12.5%). In contrast, a higher proportion of HIV-infected females (33.9%) had vitamin D insufficiency than males (30.4%). Furthermore 7.1% of the males had sufficient vitamin D levels compared to 1.8% of the females. The difference in prevalence between males and females was however not statistically significant (P = 0.22).

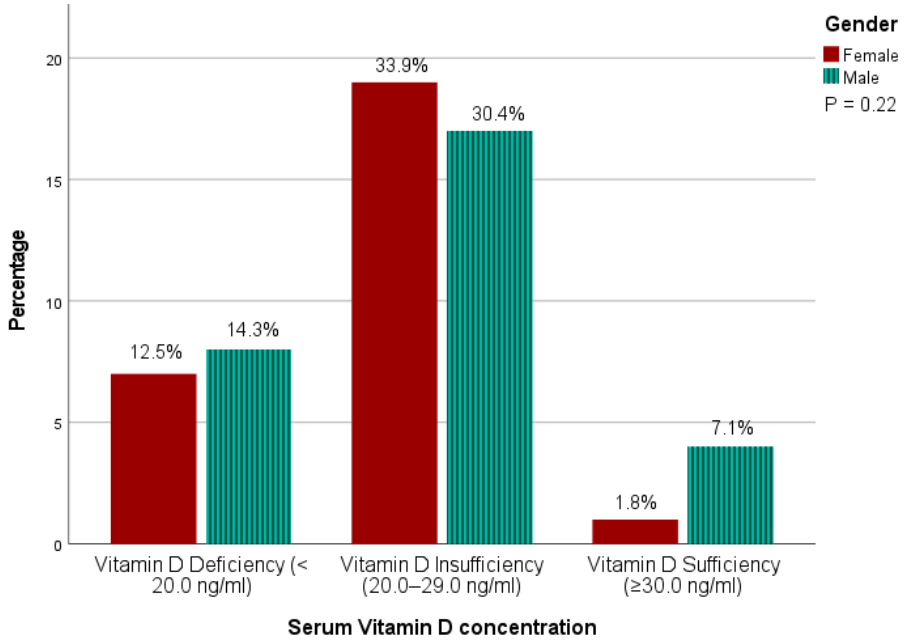

Figure 1. Prevalence of vitamin D deficiency, insufficiency, and sufficiency by gender.

Table 3 presents the results of binary logistic regression model of factors associated with the likelihood of being vitamin D deficient. On univariate analysis, increasing age had lower odds of vitamin D deficiency; for every year decrease in age, the likelihood of being vitamin D deficient increased by 6% (COR = 0.94; 95%CI, 0.88–0.99; P = 0.02). However, following multivariate analysis with other variables held constant, the odds became slightly attenuated and age was no longer a significant predictor of vitamin D status in the study population (AOR = 0.93; 95%CI, 0.84–1.02; P = 0.12). Participants who had spent five years or less on ART had 4-fold increase in the odds of being vitamin D deficient (COR = 4.28; 95%CI, 0.38–47.63; P = 0.23). The odds increased to 7-fold on multivariate analysis (AOR = 7.63; 95%CI, 0.21–276.61; P = 0.27). Time spent on ART was however not a significant predictor of vitamin D status. In contrast, ART regimen was found to be a significant predictor of vitamin D status. Being on TDF/FTC/DTG regimen was associated with 97% lower odds of being vitamin D deficient compared to being on TDF/3TC/DTG (COR = 0.03; 95%CI, 0.01–0.28; P = 0.002). These odds remained constant and statistically significant on multivariate analysis (AOR = 0.03; 95%CI, 0.003 –0.32; P = 0.004)

Table 3: Logistic regression analysis of factors associated with vitamin D status.

Variable		Vitamin D < 20 ng/ml vs. Vitamin D ≥ 20 ng/ml (Ref.)			
		Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age (years)		0.94 (0.88 – 0.99)	0.02	0.93 (0.84 – 1.02)	0.12
Time on ART (years)					
	≤5	4.28 (0.38 – 47.63)	0.23	7.63 (0.21 – 276.61)	0.27
	6 – 10	0.86 (0.07 – 11.36)	0.91	1.92 (0.04 – 83.67)	0.73
	11 – 20	3.00 (0.30 – 30.02)	0.35	11.07 (0.21 – 562.96)	0.23
	>20 (Ref.)	-			
ART Regimen					
	TDF/FTC/DTG	0.03 (0.01 –0.28)	0.002	0.03 (0.003 – 0.32)	0.004
	TDF/3TC/DTG (Ref.)	-			

OR, odds ratio; CI, confidence interval; Ref., reference category

DISCUSSION

The assessment of serum vitamin D levels and associated factors among HIV-positive adults in Nnewi, Nigeria, provides critical insights into the interplay of vitamin D status, antiretroviral therapy (ART), and socio-demographic factors in this population. The study found a high prevalence of vitamin D deficiency (26.8%) and insufficiency (64.3%), with only 8.9% of participants exhibiting sufficient vitamin D levels (≥30 ng/mL). The mean

serum vitamin D concentration was 22.88 ± 5.96 ng/mL, consistent with global reports of suboptimal vitamin D levels among HIV-positive individuals. For instance, a study by Wang *et al.*, (2021) reported that up to 100% of HIV-infected individuals had suboptimal vitamin D levels (<30 ng/mL), with over 30% exhibiting severe deficiency (<20 ng/mL). The high prevalence of vitamin D insufficiency in this study may be attributed to the tropical setting of Nnewi, where, despite ample sunlight, factors such as limited sun exposure, dietary deficiencies, or HIV-related metabolic disruptions could play a role.[52]

Significant associations were observed between vitamin D status and both age and ART regimen. Younger participants were more likely to be vitamin D deficient, with a 6% increased odds of deficiency per year decrease in age on univariate analysis (COR = 0.94; 95% CI, 0.88–0.99; P = 0.02). However, this association was attenuated in multivariate analysis (AOR = 0.93; 95% CI, 0.84–1.02; P = 0.12), suggesting that other factors may confound the relationship. This finding contrasts with some studies, such as a study by Sudfeld *et al.* (2012), which found no significant age-related differences in vitamin D status among HIV-positive individuals, but aligns with others reporting higher deficiency rates in younger populations due to lifestyle factors like reduced sun exposure.[53] The lack of statistical significance in the multivariate model highlights the multifactorial nature of vitamin D status, warranting further investigation into age-related influences in HIV-positive cohorts.

The ART regimen emerged as a significant predictor of vitamin D status. Participants on the tenofovir disoproxil fumarate/emtricitabine/dolutegravir (TDF/FTC/DTG) regimen had a 97% lower odds of vitamin D deficiency compared to those on tenofovir disoproxil fumarate/lamivudine/dolutegravir (TDF/3TC/DTG) (AOR = 0.03; 95% CI, 0.003–0.32; P = 0.004). This finding is notable, as certain ART drugs, particularly non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors, have been shown to interfere with vitamin D metabolism via the cytochrome P450 enzyme complex.[19] While dolutegravir-based regimens are generally associated with fewer metabolic side effects, the difference between TDF/FTC/DTG and TDF/3TC/DTG in this study may be linked to variations in drug interactions or patient-specific factors not fully captured in this analysis. This finding contrasts with a study by[54], which reported no significant association between specific ART regimens and vitamin D levels, but aligns with[33], which suggested that certain ART

combinations may mitigate vitamin D deficiency through less interference with vitamin D metabolism.

Gender did not significantly influence vitamin D status (P = 0.89), despite a slightly higher prevalence of deficiency among males (14.3%) compared to females (12.5%). This finding is in contrast to global trends, where women are often reported to have higher rates of vitamin D deficiency due to lower dietary intake or reduced sun exposure.[55] The lack of a significant gender difference in this study may reflect the relatively balanced gender distribution and similar lifestyle factors among participants in the HIV clinic setting. Similarly, no significant associations were found between vitamin D status and CD4+ count (P = 0.61) or viral load (P = 0.31), despite the mean CD4+ count being relatively high (662.5 ± 98.8 cells/mm³) and 73.2% of participants having undetectable viral loads. This contrasts with studies like[56], which linked vitamin D deficiency to lower CD4+ counts and higher viral loads, suggesting that the stable immune status of participants in this study may have mitigated such associations.

The high prevalence of vitamin D insufficiency and deficiency in this population underscores the need for routine monitoring of vitamin D levels as part of comprehensive HIV care. Vitamin D's immunomodulatory and anti-inflammatory roles are critical for reducing comorbidities such as osteoporosis, cardiovascular disease, and opportunistic infections in HIV-positive individuals[17]. The significant impact of ART regimen on vitamin D status suggests that regimen choice may have broader implications for patient health beyond viral suppression. Clinicians should consider tailoring ART regimens to minimize adverse effects on vitamin D metabolism, particularly in settings with high deficiency prevalence. Moreover, educational interventions to promote sun exposure and dietary sources of vitamin D could address modifiable risk factors in this population.

The study's findings also highlight barriers to optimal vitamin D status, such as potential dietary inadequacies or limited sun exposure. Similar to findings in other low-resource settings[57], socio-economic factors may limit access to vitamin D-rich foods or supplements, exacerbating deficiency. The lack of association between time on ART and vitamin D status (P = 0.27) suggests that duration of treatment may be less critical than the specific regimen used, though the increased odds of deficiency in those on ART for ≤5 years (AOR = 7.63; 95% CI, 0.21–276.61) warrants further exploration in larger studies.

This study has several limitations. The small sample size (n = 56) may have limited the statistical power to detect associations, particularly for variables like CD4+ count and viral load. The cross-sectional design precludes establishing causality between ART regimen and vitamin D status. Future studies should employ larger sample sizes, longitudinal designs, and comprehensive assessments of lifestyle and environmental factors to better elucidate the predictors of vitamin D status in HIV-positive populations.

CONCLUSION

This study revealed a high prevalence of vitamin D deficiency (26.8%) and insufficiency (64.3%) among adult HIV-positive patients in Nnewi, Nigeria, with only 8.9% exhibiting sufficient vitamin D levels. Age and antiretroviral therapy (ART) regimen were significantly associated with vitamin D status, with the tenofovir disoproxil fumarate/emtricitabine/dolutegravir (TDF/FTC/DTG) regimen linked to 97% lower odds of deficiency compared to the tenofovir disoproxil fumarate/lamivudine/dolutegravir (TDF/3TC/DTG) regimen. These findings highlight the critical interplay between ART regimens and vitamin D metabolism in HIV-positive individuals. Routine monitoring of vitamin D levels should be integrated into comprehensive HIV care to address deficiency and mitigate associated health risks, such as osteoporosis and opportunistic infections. Educational programs and clinical interventions should focus on optimizing ART regimens and promoting modifiable factors like dietary intake and sun exposure. It is recommended that healthcare providers play a pivotal role in assessing and managing vitamin D status during routine HIV clinic visits, as many patients may lack awareness of its importance in their overall health. Further research is needed to elucidate the mechanisms by which specific ART regimens influence vitamin D levels and to explore additional factors contributing to deficiency in this population.

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