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Research Article

Evaluation of Calcium Levels Among HIV Patients Attending RSUTH: ART- Naïve Versus ART Experienced

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Abstract

Calcium is an essential mineral involved in bone metabolism, neuromuscular function, and intracellular signaling. HIV infection and antiretroviral therapy (ART) have been associated with disturbances in mineral metabolism and reduced bone health. This study estimated serum calcium levels in ART naïve and ART treated HIV subjects attending the Rivers State University Teaching Hospital, Port Harcourt, Nigeria, and examined effects of sex, ART duration, regimen and lifestyle factors A cross-sectional study design was employed, involving 180 participants (120 HIV-positive and 60 HIV-negative as controls) within the age range of 18-60 years. Serum calcium was determined by the O-cresolphthalein complexone colorimetric method. Group comparison using one –way ANOVA with Tukey's post hoc test (Graph pad prism version 9.0.0, 121). Results with values of $P< 0.05$ was considered significant. Results showed a decline in mean serum calcium levels across the three groups (control, ART-naïve and ART treated). Control males had higher calcium than Control females (9.69 ±0.44 mg/dl: 9.22 ±0.39 mg/dl; $p = 0.0045$). ART-naïve patients had reduced calcium (males: 8.47 ± 0.55; females: 8.23 ± 0.43 mg/dl), ART users exhibited the lowest levels (males: 6.34 ±0.73; females: 6.55 ±0.67 mg/dl; $p<0.0001$ across groups), Longer ART duration correlated with progressively lower calcium (approximately 7.16 mg/dl at 3 months to 5.55 mg/dl beyond 6 months; $p < 0.05$). ART regimen did not affect males but in female's calcium was higher in subjects on TDF+3TC+DTG versus TDF+3TC+EFV (7.05 ± 0.64 mg/dl vs 6.13 ± 0.49 mg/dl; $p = 0.0126$). Lifestyle factors showed no significant association. HIV infection and ART especially on longer duration are associated with significant reductions in serum calcium. Routine monitoring and appropriate nutritional supplementation, therapeutic interventions or regimen choice are recommended to mitigate potential bone-related complications.

Keywords

Calcium, Calcium Metabolism, Bone, HIV, ART, Duration of Therapy

1. Introduction

Calcium is critical in human physiology. It serves structural roles in bone and teeth, acting in intramuscular transmission, muscle contraction, blood coagulation and intracellular signaling for hormone secretion, enzyme activity and cell survival [1, 2]. HIV infection and antiretroviral therapy (ART) have been implicated with disturbances in calcium homeostasis and reduced bone health [3]. Serum calcium is controlled by three factors; parathyroid hormone (PTH), Vitamin D and calcitonin including impacts from renal axis and dietary factors [4, 5]. These disruptions can perturb ionized calcium and lead to some complications in the body metabolism [6].

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Human immunodeficiency virus (HIV) infection remains a major global health challenge, with a global burden in the sub -Saharan African with Nigeria reporting an estimated 1.9 million of people living with HIV [7, 8]. The introduction of antiretroviral therapy (ART) has improved survival rate however with chronic metabolic complications leading to recorded morbidity [7]. Among some of the complications is the effect of persistent immune activation, nutritional deficiencies, weight loss, viral loads on bone cells and ART -related toxicity [3, 9- 10]. Certain antiretroviral agents in the likes of Tenofovir Disproxil Fumarate (TDF) are implicated in proximal renal tubular dysfunction with phosphate wasting and impaired vitamin D metabolism and mechanisms that affects calcium balance and bone mineralization [11, 12]. However, newer formulations like Tenofovir Alafenamide (TAF) and some integrase strand transfer inhibitors (INSTIs) confers more favourable bone safety profiles [13, 14]. It is of note that the HIV related inflammation promotes osteoclastogenesis via cytokines such as TNF- α and IL-6, accelerating bone resorption independent of ART [15, 16].

The significant burden of HIV/AIDS in Nigeria, coupled with the widespread use of ART, necessitates a comprehensive understanding of the long-term metabolic consequences in people living with HIV. Given the interplay of drug exposure, monitoring serum calcium is critical to identifying and mitigating bone and metabolic complications in people living with human immunodeficiency virus (PLHIV). Calcium and vitamin D supplementation, together with lifestyle measures (weight-bearing exercise, smoking cessation, limiting excessive alcohol), can attenuate ART-associated bone loss and support bone health [17]. Therefore, this study evaluates serum calcium in ART-naïve and ART-treated subjects attending the Rivers State University Teaching Hospital, Port Harcourt aiming to check the effect of sex, ART duration, regimen and life style on serum calcium.

2. Materials and Methods

2.1. Study Area

The study was conducted at the Rivers State University Teaching Hospital (RSUTH) HIV comprehensive care clinic in Port Harcourt, Rivers State, Nigeria. RSUTH is a tertiary referral center that provides HIV diagnosis, antiretroviral therapy (ART) initiation and follow-up, and laboratory monitoring to a large and diverse urban and peri-urban population.

2.2. Study Design

A cross-sectional study was carried out to compare serum calcium levels between ART naïve and ART treated people living with HIV (PLHIV) and HIV-negative controls.

2.3. Sample Size

Sample size estimation was performed using G*Power; the calculated minimum was 66 participants. To improve sub group representation and precision, 180 adults (18–60 years) were recruited: 120 HIV-positive (60 ART-naïve and 60 ART-treated) and 60 HIV-negative controls. Informed consent was obtained orally from all subjects and a structured questionnaire was administered to gather relevant information such as demographic data, duration on ART, ART regimen type, and lifestyle factors (especially physical activity, alcohol intake etc.). Clinical records were used to confirm ART regimen and duration.

2.4. Inclusion and Exclusion Criteria

Subjects included in this study were adults aged 18–60 years who attended the RSUTH HIV clinic and consented to participate. Those subjects confirmed with Laboratory analysis of having HIV, ART-naïve subjects with no prior ART exposure, ART-treated subjects who had been on a stable regimen for ≥3 months while those excluded refused consent to the study, female subjects that are pregnant or lactating, subjects having other known primary disorders of calcium metabolism (e.g., primary hyper/hypo parathyroidism), end-stage renal disease on dialysis; malignancies known to affect calcium, severe liver disease or currently on medications that substantially alter calcium metabolism (e.g., high-dose corticosteroids, bisphosphonates, active vitamin D analogues).

2.5. Ethical Approval

The ethical approval was gotten from the Health Research Ethics Committee of Rivers State University Teaching Hospital with reference no. RSUTH/REC/2025475.

2.6. Specimen Collection and Handling

Venous blood (5 ml) was collected by standard aseptic venipuncture into plain tubes. Samples were allowed to clot and were centrifuged at 3,500 rpm for 5 minutes to obtain serum. Aliquot of serum were transferred into plain cryovials and stored at −20°C until analysis.

2.7. Laboratory Analysis

Total serum calcium was measured using the O-cresolphthalein complexone (O CPC) colourimetric method [18]. The manufacturer's instructions were duly followed,

2.8. Quality Control

Analytical runs included internal quality control samples and calibration standards. Standard laboratory procedures were followed for equipment maintenance, reagent storage and sample handling to minimize pre analytical and analytical

variability.

2.9. Statistical Analysis

Results were presented as mean ±standard deviation (SD). Data were analyzed using GraphPad Prism version 9.0.0 (121). Group comparisons were performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Student's t-test was used for pairwise comparisons where appropriate. Results with p-value < 0.05 was considered statistically significant.

3. Results

3.1. Results of Calcium Levels of Control, ART Naive and ART Treated Subjects with Regards to Age and Sex

Table 1. *Mean ±SD of Calcium Levels of Control Subjects (HIV Negative).*

Subjects (Number)	Ca^{2+} (mg/dl)	Age (years)
Control Male (34)	9.69 ±0.44	29.24 ±4.47
Control Female (26)	9.22 ±0.39	22.31 ±2.93
t-value	3.089	4.844
p-value	0.0045	<0.0001
Remark	S	S

Key: S – Significant (p<0.05)

Table 2. *Mean ±SD of Calcium Levels of Subjects with HIV but ART -Na ïve.*

Subjects (Number)	Ca^{2+} (mg/dl)	Age (years)
HIV Naive Male (22)	8.47 ±0.55	40.18 ±10.65
HIV Naive Female (38)	8.23 ±0.43	31.37 ±5.84
t-value	1.386	2.944
p-value	0.1766	0.0064
Remark	NS	S

Key: S – Significant (p<0.05), NS – Non Significant

Table 3. *Mean ±SD of Calcium Levels of Subjects with HIV on ART.*

Subjects (Number)	Ca^{2+} (mg/dl)	Age (years)
HIV Male (28)	6.34 ±0.73	33.00 ±4.72
HIV Female (32)	6.55 ±0.67	34.62 ±7.73
t-value	0.7614	0.5803
p-value	0.4528	0.5663
Remark	NS	NS

Key: NS – Non Significant

Table 4. *Comparison of Calcium Levels between Control, ART- Naïve and ART- Treated Subjects Based on Sex.*

Subjects	Ca^{2+} (Males)	Ca^{2+} (Females)
Control	9.69 ±0.44^{a} (34)	9.22 ±0.39^{a} (26)
ART - Naive	8.47±0.55^{b} (22)	8.23 ±0.43^{b} (38)
ART- Treated	6.34 ±0.73^{c} (28)	6.55 ±0.15^{c} (32)
F-value	108.8	114.4
p-value	<0.0001	<0.0001
Remark	S	S

Key: Mean values with different superscripts are significantly different (p<0.05) from each other in the same column. S – Significant

The results showing the calcium levels of males and females including their respective age brackets are shown in Tables 1-4. The study population reflected more females than males in the ART naive group (females 38, males 22) and ART treated group (females 32, males 28) whereas the control group had more of male subjects than females (34, 26). The mean age for the males in the control group was 29.24 ±4.47 compared to the Females with 22.31 ±2.93 which was significantly different. For the ART naive, the mean age of the males was 40.18 ±10.65 compared to a significant different age of females with 31.37 ±5.84 whereas the mean age for males in the ART treated group was 33.00 ±4.72 which was not significantly different from the females with 34.62 ±7.73. The results also showed a significant difference in the calcium levels among the three groups with a progressive reduction of calcium levels from control to ART naive to ART treated in both males and females (Table 4).

3.2. Results of Calcium Levels in Subjects Considering Physical Activities and Alcohol Consumption

The data from the Control subjects indicated the males were

involved in one physical activity to another, also consumed alcohol whereas the female did not. The calcium levels of those who consumed alcohol compared to those who did not was not significantly different in males (p- 0.6105). For physical activities, 24 subjects were involved with a calcium level of 9.68 ±0.52 which was not significantly different from those that did not engaged in physical activity (10, 9.69 ±0.42). This is shown in Table 5. Meanwhile, in ART naive subjects, the calcium level of those that consumed alcohol was not significantly different from those that did not as seen in Table 6.

Table 5. *Mean ±SD of Calcium Levels in Control Males Considering Physical Activities and Consumption of Alcohol.*

Physical Activity	Ca^{2+} (mg/dl)	Alcohol Consumption	Ca^{2+} (mg/dl)
Yes (24)	9.68 ±0.52	Yes (14)	9.51 ±0.31
No (10)	9.69 ±0.42	No (20)	9.71 ±0.46
t-value	0.0487	F-value	0.5202
p-value	0.9618	p-value	0.6105
Remark	NS	Remark	NS

Key: NS – Non Significant.

Table 6. *Mean ±SD of Calcium Levels in Na ïve HIV Males Consuming Alcohol.*

Alcohol Consumption	Ca^{2+} (mg/dl)
Yes (10)	8.57 ±0.47
No (12)	8.30 ±0.71
t-value	0.7717
p-value	0.4601
Remark	NS

Key: NS – Non Significant.

3.3. Results of Calcium Levels in ART Exposed Subjects Based on Duration and Regimen Given Considering the Sexes

For both males and females, as the duration of receiving ART increases from 3 months to 6 months and greater than 6 months, so are the level of calcium reducing in the serum (Table 7). Table 8 shows that the male subjects in this study mostly took TDF+3TC+EFV and TDF+3TC+DTG ART regimen and that the calcium levels in these subjects were not significantly different on comparison with p value of 0.3312 whereas in the female subjects, the calcium level were significantly different among subjects who took these three different regimen; TDF+3TC+EFV, TDF+3TC+DTG and AZT+TDF+3TC+ATV/r respectively.

Table 7. *Effect of Duration on Calcium Levels in Subjects with HIV on ART Based on Sex.*

Duration	Ca^{2+} (Males)	Ca^{2+} (Females)
3 months	7.16 ± 0.15^{a} (10)	7.44 ± 0.29^{a} (11)
6 months	6.13 ± 0.48^{b} (12)	6.44 ± 0.36^{b} (15)
> 6 months	5.55 ± 0.07^{c} (6)	5.57 ± 0.06^{c} (6)
F-value	14.14	33.10
p-value	0.005	<0.0001
Remark	S	S

Key: Mean values with different superscripts are significantly different ($p<0.05$) from each other in the same column. S – Significant

Table 8. *Effect of ART Regimen on Calcium Levels in HIV Patients Based on Sex.*

ART Regimen	Ca^{2+} (Males)	Ca^{2+} (Females)
TDF+3TC+EFV	6.70 ±0.56 (11)	6.13 ±0.49[a] (9)
TDF+3TC+DTG	6.17±0.78 (17)	7.05 ±0.64[b] (18)
AZT+TDF+3TC+ATV/r	----------	6.91 ±0.61[c] (5)
F-value	t value -1.044	5.630
p-value	0.3312	0.0126
Remark	NS	S

Key: Mean values with different superscripts are significantly different ($p<0.05$) from each other in the same column. S – Significant, NS- Non Significant.

4. Discussion

Proper calcium balance is crucial for maintaining skeletal integrity and metabolic stability. In individuals living with HIV, these calcium disturbances pose a serious challenge due to the combined effects of the viral infection or / and the antiretroviral therapy (ART). In this study, more females were seen in the test group compared to the control group in the age range of 30-35 in consistent with UNAIDS report [7] of a sexually active and productive age.

The study revealed a stepwise decline in mean serum calcium levels: from the HIV-negative control to ART-naive subjects and further to those HIV Subjects on ART as shown in Tables 1-4. This observed pattern strongly suggests that both the infection state and the treatment with antiretroviral drugs contribute to calcium dysregulation and subsequent calcium reduction in the serum of the study subjects. The lower calcium levels observed in ART naive subjects compared to the control subjects align with the findings that chronic HIV infection disrupts mineral metabolism, provoking systemic inflammation which might lead to reduced bone density and increased fracture risk even before treatment commences [19-21].

A progressive decline in the levels of calcium with increasing ART duration confirms that cumulative drug exposure is a risk factor for mineral depletion and subsequent skeletal compromise [22, 23]. If this persistent reduced calcium is sustained in the blood, it directly correlates with increased risk for osteoporosis, osteopenia, bone fractures and fragility [22]. This has been known to exist among people living with HIV [11].

The finding of this study shows that control males had higher calcium levels than control females which is consistent with the study by Lopes *et al* [24], who suggested that higher calcium levels in males may result from differences in dietary intake, body composition, and hormonal regulation. Conversely, Gonz ález-Molero *et al* [25 reported no significant sex differences in calcium among healthy adults, possibly due to population-specific dietary patterns or environmental factors. Such variation highlights that sex and demographic factors can influence calcium homeostasis even in the absence of HIV infection.

Among HIV-na ïve patients, calcium levels were lower compared to controls. This finding corroborates the findings of Kiran *et al* [26], who reported reduced calcium concentrations in untreated HIV-positive individuals and attributed the reduced levels to systemic inflammation and altered renal handling of electrolytes. Also, Overton *et al*. [17] also documented early calcium depletion in HIV-na ïve patients prior to ART initiation. However, there is a contrast of this findings to the study of Abdollahi *et al*. [27], who reported no significant calcium difference between na ïve patients and controls. The disparities may be due to variations in disease stage, nutritional status, population characteristics, difference in the baseline dietary calcium intake or regional disparities in HIV progression.

There were no significant differences observed in the levels of Calcium in both control male and male Naive subjects in this study when the lifestyle factors (physical activities and alcohol consumption) were considered. This observation is in contrast with the study by Godos *et al* [28] that stated the extent alcohol intake can impair both calcium absorption and reduce bone density. The lack of association between lifestyle factors and calcium levels in these study group may be attributed to habits not severe enough to cause measurable differences or the duration of such activity might not have been a chronic case.

Furthermore, the observed sex-specific difference in ART regimen impart, where females on Dolutegravir-based therapy (DTG) maintained higher calcium levels than those on Efavirenz-based regimens (EFV) is of clinically significance pointing to further investigation into the sex specific differences tied to response to ART therapy because there was no significant difference observed in males on different ART regimen. The studies by Chongyangyuenvong & Kiertiburanakul, [29] and Venter *et al* [30] supports emerging evidence that the drug DTG may have more favourable metabolic profile than EFV. These sex differences might be tied to hormonal or pharmacokinetics of some drugs [31]. Regimen-related discrepancies reported in other studies may result from differences in sample size, adherence, or coexisting nutritional deficiencies [32].

5. Conclusion

The findings show significant hypocalcemia in ART naive and ART exposed subjects, more especially in long term ART duration. Routine monitoring of minerals especially serum calcium is advocated for early and prompt detection of mineral abnormalities to enable targeted interventions for proactive management to preserve bone health, prevent future cases or incidence of fractures and reduce long term skeletal morbidity.

Abbreviations

HIV	Human Immunodeficieny Virus
ART	Antiretroviral Therapy
ANOVA	Analysis of Variance
PTH	Parathyroid Hormone
TDF	Tenofovir Disproxil Fumarate
TAF	Tenofovir Alafenamide
INSTIs	Integrase Strand Transfer Inhibitors
TNF	Tumour Necrosis Factor
IL-6	Interleukin -6
AIDS	Acquired Immunodeficiency Syndrome
PLHIV	People Living with Human Immunodeficieny Virus
RSUTH	Rivers State University Teaching Hospital
O CPC	O cresolphthalein Complexone
Ca	Calcium
3TC	Lamivudine
EFV	Efavirenz
DTG	Dolutegravir
AZT	Zidovudine
ATV/r	Atazanavir/Ritonavir
SD	Standard Deviation
UNAIDS	The Joint United Nations Programme on HIV/AIDS

Author Contributions

Helen Anthony Waribo: Conceptualization, Writing – original draft, Methodology, Writing – review & editing

Adulphus Levi Meniel: Formal Analysis, Data curation, Writing – review & editing

Evidence Ogu-Nweke: Software,Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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