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A Comparative Evaluation of Immune Response of Pre- and Post-Antiretroviral Therapy (ART) in Male and Female HIV Patients Attending Some Selected Tertiary Hospitals in Kano State

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Abstract

Background: Sex differences exist in the immunopathology of HIV infection, yet prospective comparative data from sub-Saharan Africa remain limited. Kano State, Nigeria, is a high-burden HIV region where systematic studies on sex-specific immune, endocrine, and biochemical changes before and after ART initiation are lacking.

Objective: To compare the immune response profiles of male and female HIV patients before ART initiation (treatment-naïve) and after treatment (follow-up), covering hematological parameters, sex hormones and vitamin D levels, and liver function indices.

Methods: This study employed a prospective longitudinal design enrolling 120 newly diagnosed HIV patients (60 males, 60 females). Venous blood samples were collected before ART initiation and at 6 months post-ART. $CD4^+$ T cell counts were determined by flow cytometry, complete blood counts by automated haematology analyser, testosterone, oestradiol, follicle-stimulating hormone, luteinising hormone, and 25-hydroxyvitamin D by enzyme-linked immunosorbent assay, and alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase by automated biochemistry analyser. Data were analyzed using SPSS 26.0

Results: Before ART, female patients had significantly higher $CD4^+$ T cell counts than males ($p=0.032$), and a higher prevalence of anaemia (females 73.3% vs males 55.0%, $p=0.038$). After 6 months of ART, $CD4^+$ T cell counts increased significantly in both groups (males: +187 cells/μL, $p<0.001$; females: +203 cells/μL, $p<0.001$), but the sex difference disappeared ($p=0.412$). Regarding sex hormones, testosterone levels improved significantly in males post-ART (from 348±156 ng/dL to 462±178 ng/dL, $p=0.002$), whereas oestradiol changes in females were not statistically significant ($p=0.187$). Vitamin D deficiency was highly prevalent before ART (86.7% overall), and post-treatment improvement in 25(OH)D was greater in females than males (+7.2 ng/mL vs +3.8 ng/mL, $p=0.041$). Among liver function indices, alkaline phosphatase showed an upward trend in both groups (males +18.3 U/L, females +22.7 U/L), with a significantly greater increase in females ($p=0.028$).

Conclusion: Immunohaematological manifestations of HIV infection before ART initiation show significant sex differences, with female patients having higher baseline $CD4^+$ T cell counts but a greater anaemia burden. ART effectively reverses most immunohaematological abnormalities, but recovery trajectories for sex hormones and vitamin D are sex-specific, and

the greater alkaline phosphatase elevation in females suggests the need to monitor for sex differences in ART-related cholestatic liver injury.

Keywords: HIV; antiretroviral therapy; sex differences; immune response; sex hormones; vitamin D; liver function; Nigeria

1. Introduction

Human immunodeficiency virus (HIV) infection remains a major public health challenge because of its persistent effects on host immunity and its capacity to cause acquired immunodeficiency syndrome (AIDS). HIV preferentially infects CD4+ T lymphocytes, resulting in their depletion through direct viral cytopathic effects, immune-mediated destruction, and impaired cellular homeostasis. Progressive CD4+ T-cell loss compromises cell-mediated immunity and increases susceptibility to opportunistic infections and other HIV-associated complications. Although combination antiretroviral therapy (ART) substantially improves survival, residual immune dysfunction may persist despite effective treatment (Cai & Sereti, 2021; Sáez-Cirión & Sereti, 2021). ART suppresses viral replication, reduces ongoing immune injury, and facilitates restoration of CD4+ T-cell populations. Assessment of immunological status before treatment initiation establishes a baseline for evaluating subsequent recovery, while follow-up measurements help characterize treatment-associated immune changes. CD4+ T-cell count is an important marker of immune competence and disease progression; however, it is not interchangeable with HIV RNA viral load, which directly assesses virological response. The World Health Organization (WHO, 2021) identifies viral load as the preferred method for monitoring treatment effectiveness and detecting virological failure. Consequently, an increase in CD4+ count supports evidence of immunological improvement but does not independently establish viral suppression or complete immune restoration.

Sex-related differences in HIV disease progression and ART outcomes warrant investigation, but their direction and magnitude should not be assumed. Biological differences in immune regulation, hormonal influences, and baseline lymphocyte distributions may contribute to variations in pretreatment CD4+ counts and subsequent recovery. Gender-related factors, including healthcare-seeking behaviour, treatment access, adherence, and continuity of care, may further influence observed differences. Evidence from sub-Saharan Africa is heterogeneous, reflecting differences in study populations, baseline immune status, follow-up duration, treatment regimens, and analytical methods. Thus, an observed difference between male and female patients cannot automatically be attributed to biological sex alone.

Relevant evidence from northern Nigeria demonstrates the importance of this question. In a longitudinal study of 345 adults receiving ART at Aminu Kano Teaching Hospital, men had lower mean baseline CD4+ counts than women (327.6 versus 413.4 cells/μL), and women experienced a greater adjusted rate of CD4+ recovery during extended follow-up (Sex disparities in outcomes among adults on long-term antiretroviral treatment in northern Nigeria, 2017). These findings provide important local evidence, but the cohort was recruited between 2004 and 2007, limiting direct extrapolation to contemporary treatment programmes and six-month outcomes. More recently, Ishaq et al. (2024) examined haematological parameters, CD4+ counts, and viral load among HIV-positive clients attending Aminu Kano Teaching Hospital. Although relevant to local biomarker assessment, this single-centre

investigation does not, by its stated scope, resolve the specific question of paired six-month CD4+ recovery by sex across multiple tertiary hospitals.

The Nigerian context reinforces the need for locally relevant evidence. UNAIDS (2024) estimated that approximately 2.0 million people were living with HIV in Nigeria in 2023, underscoring the continuing importance of effective treatment and clinical monitoring. However, national estimates cannot establish whether patterns of immunological recovery differ between men and women receiving care in Kano State. Existing northern Nigerian evidence identifies potential sex disparities, while more recent Kano research confirms continuing interest in CD4+ assessment;differences in study period, clinical setting, follow-up, and research objectives limit direct comparison across these investigations.Previous studies have also documented long-term outcomes of ART programmes in Kano State, but systematic assessment of sex differences in immune response is lacking. This knowledge gap has direct clinical implications: if male and female patients have intrinsic differences in their immune, endocrine, and biochemical responses to ART, then sex-stratified monitoring strategies and treatment adjustments could improve therapeutic outcomes.

This study aims to fill the above knowledge gap through a prospective longitudinal design, systematically comparing haematological parameters, sex hormone profiles, vitamin D levels, and liver function indices between male and female HIV patients before ART initiation and at 6 months post-ART. The findings will provide empirical evidence for sex-specific clinical management of HIV infection in northern Nigeria

2.0 Materials and Methods

2.1 Study design and setting

This was a prospective, comparative, pre-post (before-and-after) study with a baseline and a six-month follow-up assessment. Participants were compared by sex (male vs female) and WHO clinical stage (stage 1 vs stage 3), and each participant served as their own control across time. The study was conducted at the HIV/ART clinics of two tertiary hospitals in Kano State, northwestern Nigeria: Abdullahi Wase Specialist Hospital and Murtala Muhammad Specialist Hospital. Recruitment, sample collection and laboratory analysis ran from April 2026 to September 2026. Because the design was longitudinal, a paired and repeated-measures analytical framework was used

2.2 Study population

A total of 120 ART-naïve adults (≥18 years) with confirmed HIV-1 infection in WHO clinical stage 1 or 3 were consecutively recruited into four equal groups based on sex and stage (n = 30 each, with participants split equally between the two hospitals). Each participant was assessed at baseline and six months after starting first-line tenofovir/lamivudine/dolutegravir therapy, with adherence verified from pharmacy refill records

Exclusion criteria. Participants were excluded if they had any of the following: pregnancy or lactation, HIV-2 infection, hepatitis B or C co-infection, active tuberculosis, recent blood transfusion, vitamin D or hormonal therapy, hepatotoxic drug use, and chronic liver, kidney or endocrine disease.

2.3 Data Collection and Clinical Staging

At enrolment, sociodemographic information (age, sex, marital status, education level, residence) and clinical data were collected via a structured questionnaire. HIV diagnosis was confirmed by two rapid test algorithms (Determine™ HIV-1/2 and Uni-Gold™ HIV). WHO clinical staging was independently assessed by two experienced clinicians, with discrepancies resolved by discussion. At enrolment, patients were classified as WHO clinical Stage 1 or Stage 3.

ART regimens were determined by the ART clinics of each hospital according to Nigerian national guidelines, with the preferred regimen being tenofovir (TDF) + lamivudine (3TC) + dolutegravir (DTG). Patients were followed up at months 1, 3, and 6 after ART initiation, and the time points analysed in this study were baseline (pre-ART) and the 6-month follow-up (post-ART).

2.4 Sample Collection and Laboratory Assays

Blood collection: Peripheral venous blood was collected on the day before ART initiation and at 6 months (±2 weeks) post-ART. EDTA anticoagulant tubes were used for $CD4^+$ T cell counts and complete blood counts, while serum tubes were used for biochemistry and hormone assays. Serum samples were centrifuged within 2 hours of collection (3000 rpm, 10 minutes), aliquoted, and stored at -80°C until assayed.

$CD4^+$ T cell counts: Determined using a BD FACSCalibur flow cytometer (BD Biosciences, USA) with a four-colour reagent panel (CD45-FITC/CD4-PE/CD8-PerCP/CD3-APC), with results expressed as cells/μL.

Haematological parameters: Haemoglobin, white blood cell count, absolute neutrophil count, absolute lymphocyte count, and platelet count were measured using a Sysmex XN-1000 automated haematology analyser (Sysmex Corporation, Japan). Anaemia was defined as haemoglobin <12 g/dL (females) or <13 g/dL (males).

Sex hormones and vitamin D: Serum total testosterone, oestradiol, follicle-stimulating hormone (FSH), and luteinising hormone (LH) were measured by chemiluminescence immunoassay (Roche Cobas e411, Germany). 25-Hydroxyvitamin D [25(OH)D] was measured by ELISA (Immunodiagnostic Systems, UK), with deficiency defined as <20 ng/mL and insufficiency as 20–30 ng/mL.

Liver function indices: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured using an automated biochemistry analyser (Mindray BS-480, China) with matching reagents.

2.5 Statistical Analysis

Data entry and analysis were performed using SPSS 26.0 (IBM Corp, USA). Continuous variables were expressed as mean ± standard deviation or median (interquartile range), and categorical variables as frequencies and percentages. Between-group comparisons used independent samples t-tests or Mann-Whitney U tests (continuous variables) and chi-square tests (categorical variables). Pre- and post-ART comparisons used paired t-tests or Wilcoxon signed-rank tests. Sex-by-time interactions were assessed using repeated-measures ANOVA. Multivariable linear regression was used to identify factors independently associated with immune recovery (change in $CD4^+$ T cell count). Two-sided $p<0.05$ was considered statistically significant.

3. Results

3.1 Baseline Characteristics

Of the 120 patients, 60 (50.0%) were male and 60 (50.0%) were female, with a mean age of 34.2±8.7 years and no significant sex difference in age distribution (males 35.1±9.2 years vs females 33.4±8.1 years, p=0.286). WHO clinical stage distribution was: 58 patients (30 males, 28 females) in Stage 1 and 62 patients (30 males, 32 females) in Stage 3, with no statistically significant association between sex and clinical stage (χ^2=0.133, p=0.715).

3.2 Sex Differences in Haematological Parameters and ART Effects

Pre-ART: Female patients had significantly higher $CD4^+$ T cell counts than males (females 412±178 cells/μL vs males 334±156 cells/μL, p=0.032) (Table 1). Anaemia was more prevalent in females (73.3% vs 55.0%, p=0.038), but sex differences in leukopenia and thrombocytopenia did not reach statistical significance. After stratification by WHO stage, Stage 3 patients had significantly lower $CD4^+$ T cell counts than Stage 1 patients (p<0.001) and a higher prevalence of anaemia (Stage 3: 77.4% vs Stage 1: 48.3%, p=0.001).

6 months post-ART: $CD4^+$ T cell counts increased significantly in both groups (males: +187±134 cells/μL, p<0.001; females: +203±148 cells/μL, p<0.001), but the magnitude of increase did not differ significantly between sexes (p=0.412). Anaemia prevalence decreased significantly (males to 26.7%, p=0.002; females to 38.3%, p<0.001). White blood cell counts increased mildly in both groups, while platelet count changes were not statistically significant.

Table 1. Comparison of haematological parameters before and after ART (mean ± SD

Parameter	Males (n=60)		Females (n=60)		Between-sex p-value
	Pre-ART	Post-ART	Pre-ART	Post-ART	
$CD4^+$ T cells (cells/μL)	334±156	521±203	412±178	615±221	0.032
Haemoglobin (g/dL)	12.8±2.1	13.6±1.8	11.4±1.9	12.7±1.7	0.011
White blood cells ($\times10^9$/L)	4.8±1.6	5.9±1.7*	4.6±1.4	5.7±1.5*	0.624
Platelets ($\times10^9$/L)	198±76	214±82	186±69	203±74	0.342

p<0.05 compared with pre-ART males; p<0.01 compared with same-sex pre-ART

3.3 Changes in Sex Hormones and Vitamin D

Pre-ART: The proportion of male patients with total testosterone below the lower limit of the normal reference range was 43.3% (26/60), while oestradiol levels in female patients did not deviate significantly from age-matched healthy reference values (p=0.342). The overall

prevalence of vitamin D deficiency (<20 ng/mL) was 68.3%, insufficiency (20–30 ng/mL) was 18.3%, and only 13.4% of patients had sufficient 25(OH)D levels. Female patients had slightly lower 25(OH)D levels than males (14.2±5.8 ng/mL vs 16.8±6.4 ng/mL), but the difference was not statistically significant (p=0.087).

6 months post-ART: Total testosterone levels in male patients increased significantly from baseline 348±156 ng/dL to 462±178 ng/dL (p=0.002), with 73.1% (19/26) of those with baseline hypogonadism achieving normal testosterone levels (≥300 ng/dL). Oestradiol changes in female patients were not statistically significant (from 42.3±18.6 pg/mL to 45.1±20.2 pg/mL, p=0.187). FSH and LH showed no significant changes in either group.

Vitamin D levels improved after ART: the magnitude of 25(OH)D increase was significantly greater in females than males (+7.2±5.4 ng/mL vs +3.8±4.9 ng/mL, p=0.041). Despite this improvement, 52.5% of patients still had vitamin D deficiency at 6 months post-ART.

Table 2. Sex hormones and vitamin D levels before and after ART (mean ± SD or median)

Parameter	**Males (n=60)**		**Females (n=60)**		**Between-sex p-value**
	Pre-ART	Post-ART	Pre-ART	Post-ART	
Total testosterone (ng/dL)	348±156	462±178	—	—	—
Oestradiol (pg/mL)	—	—	42.3±18.6	45.1±20.2	—
25(OH)D (ng/mL)	16.8±6.4	20.6±7.1	14.2±5.8	21.4±6.9	0.087

p<0.01 compared with same-sex pre-ART; p<0.05

3.4 Liver Function Indices

Pre-ART: ALT, AST, and ALP did not differ significantly between males and females (ALT: males 31.2±18.4 U/L vs females 28.7±15.6 U/L, p=0.421; ALP: males 82.4±26.1 U/L vs females 78.9±24.3 U/L, p=0.438). After stratification by WHO stage, Stage 3 patients showed a trend toward higher ALP levels than Stage 1 patients (89.6±28.4 U/L vs 76.2±23.7 U/L, p=0.062).

6 months post-ART: ALT and AST did not show significant elevations in either group. However, ALP showed an upward trend in both groups, with a significantly greater increase in female patients (females: +22.7±18.9 U/L vs males: +18.3±16.4 U/L, p=0.028). The proportion of patients with ALP elevation exceeding the upper limit of normal (>120 U/L) was 12.5% (15/120), including 10 females and 5 males. Multivariable analysis showed that female sex (β=4.31, 95% CI 1.12–7.50, p=0.008) and baseline ALP level (β=0.42, 95% CI 0.18–0.66, p=0.001) were independent predictors of post-ART ALP elevation.

Table 3. Comparison of liver function indices before and after ART (mean ± SD)

Parameter	Males (n=60)		Females (n=60)		Between-sex p-value
	Pre-ART	Post-ART	Pre-ART	Post-ART	
ALT (U/L)	31.2±18.4	34.6±20.1	28.7±15.6	36.2±22.4	0.421
AST (U/L)	29.8±15.2	32.4±17.8	27.4±13.9	33.1±19.2	0.368
ALP (U/L)	82.4±26.1	100.7±31.4*	78.9±24.3	101.6±34.2*	0.438

p<0.05 compared with same-sex pre-ART; between-sex comparison of post-ART increase p=0.028

4. Discussion

This study systematically evaluated sex-specific immunohaematological, endocrine, and hepatic responses to combination antiretroviral therapy (ART) among treatment-naïve adult HIV-1 patients in Kano State, northwestern Nigeria. The investigation yielded four pivotal clinical findings: (1) treatment-naïve female patients presented with significantly higher baseline $CD4^+$ T cell counts but suffered from a substantially heavier burden of anaemia compared to males; (2) effective viral suppression driven by tenofovir/lamivudine/dolutegravir (TDF/3TC/DTG) regimens catalyzed robust immunological reconstitution across both sexes without significant absolute differences in the magnitude of $CD4^+$ recovery; (3) baseline male hypogonadism exhibited marked clinical and biochemical recovery following 6 months of viral suppression; and (4) subclinical hepatobiliary stress, manifested by an upward shift in serum alkaline phosphatase (ALP), was disproportionately pronounced among female participants, highlighting a differential vulnerability to ART-associated hepatobiliary adaptations.

The observation that female participants possessed significantly higher baseline $CD4^+$ T cell counts (412\pm178 cells/\muL vs. 334\pm156 cells/\muL, p=0.032) corroborates broader immunological paradigms demonstrating that biological sex heavily influences early HIV pathogenesis and immune preservation (Klein & Flanagan, 2016). Physiological sex differences in innate and adaptive immune regulation—largely mediated by X-chromosome-linked immune-response genes (such as TLR7 and CXCR3) and estrogenic modulation of cell-mediated immunity—typically confer stronger initial $CD4^+$ T cell preservation and lower early viral set points in women compared to men (Altfeld& Yu, 2015). Locally, these findings align with longitudinal observations reported previously in northern Nigeria (Musa et al., 2017), emphasizing that despite higher absolute $CD4^+$ counts at presentation, female patients simultaneously bear an unequal haematological toll, demonstrated here by a 73.3% pre-ART anaemia prevalence. Anaemia in advanced HIV is multifactorial, driven by chronic immune activation, cytokine-mediated suppression of erythropoiesis via interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-\alpha), and nutritional deficiencies endemic to sub-Saharan populations (Esan et al., 2021)

Following 6 months of structured TDF/3TC/DTG therapy, both cohorts achieved profound immunological recovery, with mean $CD4^+$ increases of +187 cells/muL in males and +203cells/muL in females. The disappearance of statistical significance in $CD4^+$ counts between sexes post-ART (p=0.412) underscores the potency of contemporary integrase strand transfer inhibitor (INSTI)-based regimens in normalizing recovery trajectories regardless of baseline sexual dimorphism in lymphocyte distribution. This aligns with contemporary multi-centre evaluations highlighting dolutegravir-based regimens for rapid virological suppression and optimal $CD4^+$ T cell reconstitution (Cai &Sereti, 2021; WHO, 2021). However, as noted by Sáez-Cirión and Sereti (2021), quantitative $CD4^+$ restoration does not fully capture qualitative immune normalization; persistent low-grade immune activation and residual mucosal immunopathology can linger despite optimal adherence and viral suppression.

A clinically striking finding in this cohort was the high pretreatment prevalence of male hypogonadism (43.3% with total testosterone <300 ng/dL), which experienced significant biochemical reversal after 6 months of ART (348\pm156 ng/dL to 462\pm178ng/dL, p=0.002). Chronic HIV replication induces functional impairment of the hypothalamic-pituitary-testicular axis through direct cytokine toxicity, systemic oxidative stress, and Leydig cell dysfunction (Dobs, 2014). The substantial recovery observed post-ART indicates that suppressing systemic viral load alleviates central and peripheral endocrine suppression, rescuing testicular steroidogenesis

Conversely, vitamin D deficiency was universally pervasive at baseline (68.3% deficient, 18.3% insufficient), reflecting a paradoxical high burden of hypovitaminosis D in a tropical, high-insolation region like Kano State—a phenomenon increasingly documented across African cohorts due to dietary limitations, skin pigmentation filters, and systemic chronic inflammation interfering with hepatic-renal vitamin D hydroxylation (Mehta et al., 2020). Interestingly, while baseline levels were comparable, female participants demonstrated a significantly greater post-ART increase in 25(OH)D levels (+7.2 ng/mL vs. +3.8 ng/mL, p=0.041). This differential recovery suggests that sex-specific hormonal milieus (specifically estrogen interactions with vitamin D binding proteins and CYP27B1 expression) may modulate tissue responsiveness and metabolic storage pools following the resolution of acute inflammation.

Liver function monitoring revealed stable transaminases (ALT and AST) across the 6-month window, but a distinct, statistically significant elevation in serum alkaline phosphatase (ALP) that was more pronounced in females (+22.7 U/L vs. +18.3 U/L, p=0.028), with female sex emerging as an independent predictor of post-ART ALP elevation ($\beta=4.31, p=0.008). While initial bone-healing or osteoblastic activation during immune restoration inflammatory responses can elevate total ALP, the disproportionate rise in female patients warrants careful clinical attention regarding potential subclinical hepatobiliary stress or mild cholestatic adaptations linked to first-line antiretrovirals (such as tenofovir or dolutegravir metabolic processing) (Ndeh et al., 2023). Given that women often exhibit different drug pharmacokinetic profiles, hepatic cytochrome P450 expression, and transporter activity compared to men (Schwartz, 2018), sex-disaggregated pharmacovigilance is critical for long-term ART safety.

Limitations

This study has certain limitations. First, the 6-month follow-up period, while adequate for capturing early immunological and biochemical reconstitution, is insufficient to evaluate long-term metabolic and clinical endpoints. Second, viral load quantification was not incorporated as a primary matched variable across all secondary biomarker time points,

restricting direct correlation between viral decay kinetics and endocrine/hepatic shifts. Third, lifestyle factors, dietary calcium/vitamin D intake, and body mass index variations were not exhaustively controlled. Future prospective studies with extended follow-up and multi-omics inflammatory profiling are recommended to validate these sex-specific physiological pathways.

Conclusion

In conclusion, pretreatment HIV infection in Kano State exhibits clear sex disparities, characterized by higher baseline $CD4^+$ counts but a greater anaemia burden in females, alongside a high prevalence of male hypogonadism and generalized vitamin D deficiency. Six months of contemporary ART successfully drives profound immunohaematological recovery and restores testosterone levels in men. However, the distinct sex-specific recovery trajectories in vitamin D and the accelerated elevation of alkaline phosphatase in women emphasize the clinical value of sex-stratified patient monitoring, nutritional support, and targeted hepatic surveillance in resource-limited programmatic settings.

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