SEX VARIATIONS IN CD4 COUNT, SERUM MICROELEMENTS, AND ANTHROPOMETRIC INDICATORS AMONG HUMAN IMMUNODEFICIENCY VIRUS SUBJECTS ON ANTIRETROVIRAL THERAPY IN NIGERIA

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ABTRACT

Background: Human Immunodeficiency Virus infection is frequently accompanied by disruptions in micronutrient balance and progressive immune compromise, which may present differently between males and females. However, evidence describing these differences remains limited, particularly in sub-Saharan Africa. Objective: The present study examined sex-associated differences in CD4+ lymphocyte levels, circulating concentrations of zinc, copper, and selenium, and selected anthropometric measures among adults living with HIV in South Eastern Nigeria. Methods: A cross-sectional analysis was conducted among 110 adults aged 21–70 years, comprising individuals receiving antiretroviral therapy, newly diagnosed HIV-positive individuals not yet on treatment, and HIV-seronegative controls. Serum trace elements were quantified using atomic absorption spectrophotometry, while CD4+ T-cell enumeration was performed via flow cytometry. Body weight, height, body mass index, and blood pressure were measured following standard clinical protocols. Data analysis was carried out using IBM SPSS version 27, with statistical significance defined as p < 0.05. Results: Among participants receiving antiretroviral therapy, females demonstrated higher serum selenium concentrations than males. In the control group, zinc and copper levels differed significantly between sexes. Untreated HIV-positive individuals exhibited marked sex-related differences in body weight and body mass index. Correlation analysis further revealed significant associations between sex, CD4+ T-cell counts, and serum zinc concentrations. Conclusion: These findings indicate that biological sex contributes to variability in immune response, micronutrient distribution, and nutritional status in HIV infection. Incorporating sex-responsive monitoring and nutritional support into HIV care programs may enhance clinical management and therapeutic outcomes.

Keywords: Antiretroviral treatment exposure, Human Immunodeficiency Virus disease status, CD4 lymphocyte count, microelement profile, Biological sex differences.

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1. INTRODUCTION
1.1 BACKGROUND OF THE STUDY

Human Immunodeficiency virus (HIV) has been reported to be a global pandemic due to its devastating effects on human beings in the society and the associated stigma it carries on the victims. It is therefore a global-public health issues [1]. It has affected a lot of people all over the world [1]. Acquired immunodeficiency syndrome (AIDS), being the disease caused by the vector, HIV has been witnessed in so many countries of the world and is known to affect all categories of populations in the society. Human immunodeficiency virus has continued to be a major challenge of the world due to its devastating effects on the population of the world [1]. The infection is chronic and continues to progress with time. So many lives had been destroyed by HIV pandemic in so many countries of the world [2]. Hence, the development of so many therapies including antiretroviral therapy which has remained effective for HIV management and care [2].

A lot of antiretroviral drugs have been found to be effective in the treatment and management of HIV and AIDS infection and therefore has reduced the impact of HIV and AIDS on the population globally [2-4]. Some of these drugs are given in single or combination form. Since the introduction of antiretroviral drugs in HIV care and management system, there had been declining incidence of opportunistic infections and increase in longevity in HIV patients [3, 4]. The immune status of HIV patients has been reported to have improved with the introduction of many types of antiretroviral therapies globally. There has been many emerging trends in the diagnosis, treatment, prevention, care and management of people living with HIV and AIDS [5]. One of the major success recorded in the fight against the Human Immunodeficiency virus (HIV) pandemic is the invention of sophisticated equipment [5], development of accurate diagnostic methods, and introduction of effective antiretroviral therapy which had been reported to be very effective in the treatment, management and care of people living with HIV and AIDS. More new drugs are continued to be discovered recently and more impressive advances made in the areas of diagnosis and research. An improved evaluation may be extended in future for the care and management of HIV and AIDS [5].

Homeostatic control of divalent cations' levels and actions appear to change as HIV-1 infection advances in stages [6, 7] and as changes occur between HIV-1 and the victim [4, 6]. In people living with HIV-1, nutrient supplementation with divalent cations, enzymes in successive stages of HIV infections require the same elements [6, 7]. In HIV-infected organisms, a subsequent coronavirus infection encounters with altered electrolyte balance of the body including relative starvation of divalent cations induced **HIV**-1 integrase (IN) has been validated as an attractive target for the treatment of **HIV** and AIDS [6]. The hallmarks of HIV-1 infection, which advances to AIDS, are chronic immunological activation and reasonable decline of CD4+ T cell count. As a result, low basal immune activation and high anti-HIV-1 molecule production appear to be major HIV-1 resistance mechanisms, reducing target cell vulnerability while reducing viral infectivity. A study results support the hypothesis that several selective strand transfer inhibitors preferentially chelate one cation in solution and that of the metal complexes [8]. The metabolic and immunologic changes associated with the chronic-disease state in HIV-infected patients, not on treatment, predispose them to having suboptimal BMIs [9]. However, there is a significant increase in BMI following initiation of treatment [10]. This implies that the association between the CD4 cell count and hypertension could be different across the various BMI categories [11]. This creates an imperative for further exploration of this relationship as it may represent an avenue for intervention in HIV/AIDS patients to reduce adverse outcomes to both male and female [8].

There have been several reports on the treatment, management and care of HIV and AIDS patients. There has also been several research on the involment of nutrient supplimentation in HIV care system but information is still scanty on the sex variation on CD4+ count, BMI, trace elements, blood pressure and anthropometric indices for HIV/ AIDS patients attending NAUTH, Nnewi Clinic. This study therefore investigated sex variations in CD4+ count, serum microelements, and anthropometric indicators among human immunodeficiency virus-positive subjects on or not on antiretroviral therapy at NAUTH, Nnewi Anambra State, Nigeria.

2. MATERIAL AND METHODS
2.1 METERIALS USED

Materials used include; HIV test kits, Atomic absorption spectrophotometer (AAS), EDTA specimen bottles, plain specimen bottles, Rohren tubes, 5 ml syringes, table top centrifuge machine, deep freezer (-40 ^{O}C), PartecCyflow cytometer (PartecCyflow, Germany) and sphygmomonameter.

2.2. METHODS
2.2.1 STUDY POPULATION

This research was from about 99,296 people living with HIV in Anambra state[3] which involved 30 healthy subjects (males and females) and 30 patients who were diagnosed as primary HIV patients. The mean age was 48.60 ±9.52 years for 50 HIV-infected subjects on antiretroviral therapy (ART) treatment, while 30 were yet to receive ART (just diagnosed of HIV).30 apparently healthy as controls. Total study research subject was 110.

2.2.2 STUDY PROCEDURES AND VARIABLES

A face-to-face oral interview and a physical examination were done for all participants who consented to take part in the study. A structured questionnaire was used for data collection on participants' demographic and clinical characteristics. Data on

HIV infection status and duration, previous and most recent CD4 counts less than 6 months, were collected from the patients and complemented by recorded parameters from their medical records. The physical examination consisted of measuring patient blood pressures, weight and height.

2.2.3 BLOOD COLLECTION
Blood specimens were taken from apparently healthy subjects and people living with HIV and AIDS. Blood (10 ml) was collected from each person, 5ml in EDTA and 5 ml in plain specimen bottles. The samples were labeled appropriately with codes. The blood samples in plain container were centrifuged at 3500 g for 10 min at room temperature to obtain serum. The serum was stored at -40 ^{O}C for micronutrient assay.

2.2.4 SCREENING FOR HUMAN IMMUNODEFICIENCY VIRUS (HIV)
Subjects for control group were screened for HIV-infection using serial algorithm procedure as previously described [12]. This procedure is the WHO standard for HIV testing. It refers to a situation where if the first tested sample indicates non-reactive result, the result is recorded as negative; but if the first tested kit shows reactive, it will be tested with a second test kit, and if the second tested kit shows reactive too, the sample is reported as positive. However, when there is inconsistent result (first test kit reactive, and second test kit non-reactive), a third test kit was used as a tie-breaker to give the final result-retest[8] Unigold and Stat-pak HIV ½ test kits were used for the first, second and tie-breaker respectively. Procedure was carried out according to the manufacturer's description. Classification of HIV stages was done using CD4 counts according to WHO [12].

2.2.5 T-CELLS (CD4^{+} T-CELL) ASSAY
Clusters of differenciation (CD4+ T-cells) count were analyzed using Partexcyflow cytometer by PartecCyflow, Germany according to the method of [6]. After booting the machine, 20 ml of CD4+ T-cell count, PEmAb was added to a Rohren tube followed by 20 l of well-mixed EDTA blood sample. Both were mixed and incubated in the dark at room temperature for 15 min. This was followed by the addition of 800 l of the CD4+ T-cell count buffer. The mixtures were mixed and absorbance taken on the cyflow by plugging the sample tube to the sample port of the cyflow.

2.2.6 MEASUREMENT OF BLOOD PRESSURE
This was done according to the method of [13] using sphygmomanometer

PRINCIPLES
Blood pressure measurement is a fundamental clinical procedure used to assess cardiovascular health. It involves determining the force exerted by circulating blood on the walls of blood vessels, primarily arteries. Accurate measurement is crucial for diagnosing hypertension and monitoring treatment efficacy, it comprises two phases Systolic Pressure: The pressure during heart contraction, when blood is ejected into the arteries and diastolic pressure, the pressure during relaxation, when the heart is filled with blood, the lower value, indicating the pressure in the arteries when the heart is at rest between beats.

An example of normal measure value for a resting healthy adult human is 120 mmHg for systolic and 80 mmHg for diastolic (120/80mmHg). Difference between the systolic and diastolic pressures is referred to as pulse pressure.

2.2.7 DETERMINATION OF SERUM, SELENIUM, ZINC AND COPPER LEVELS IN HUMAN IMMUNODEFICIENCY VIRUS-POSITVE SUBJECTS ON OR NOT ON ANTIRETROVIRAL THERAPY
The above mentioned trace elements were analyzed using the method described by [14].

PRINCIPLE
Atomic Absorption Spectrophotometer's (AAS) principle is based on the sample being aspirated into the flame and atomized when the flame atomic absorption spectrophotometer (FAAS) light beam is directed through the flame into the monochromator and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample procedure

PROCEDURE
The dried samples to be constituted were first digested by heating 2 g of both samples in a furnace for 2 hours at 550 ^{O}C. This was followed by diluting the samples with 20 ml of H_2SO_4 and filtered with filter paper (Whatman 110mm). The digested samples were fed into an air-acetylene flame and the metal's concentration was read from the equipment.

3. RESULTS
Tables 1 - 3 show the results of gender comparison of serum levels of zinc, copper, selenium, weight, height, body mass index, CD4+ count and blood pressure (systolic and diastolic blood pressures) of male and female HIV+ on ART and that not on ART, HIV -ve, and control subjects attending NAUTH, Nnewi, Anambra State, Nigeria. Selenium was significantly higher in female than male HIV positive subjects on ART at p< 0.009. There was no significant variations in values in other parameters. In **table 2**, Zn and Cu were significant at 0.000 and 0.025 in the HIV negative-control

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subjects. Weight (p=0.032) and BMI (p=0.024) were significantly higher in males than females for HIV +ve subjects not on ART **(Table 1)**. Diastolic blood pressure was higher significantly in males than females for HIV +ve subjects not on ART. Analysis done showed that sex had significant variation in serum zinc levels and CD4+ count at $p < 0.05$ **(Table 3)**.

Table 1: COMPARISON ON PARAMETERS STUDIED BETWEEN GENDERS IN HUMAN IMMUNODEFICIENT VIRUS POSITIVE SUBJECTS ON ANTIRETROVIRAL THERAPY

Parameters	Males n=10	Females n=40	t-values	p-values
Zn(ppm)	10.52 ±5.20	11.81 ±6.88	0.410	0.522
Cu(ppm)	24.09 ±4.17	22.71 ±6.90	4.145	0.437
Se(ppm)	6.99 ±2.85	10.70 ±6.26	6.933	0.009
Age(years)	43.70 ±15.84	49.83 ±6.05	20.036	0.260
Wt(kg)	67.70 ±16.15	73.85 ±14.04	0.975	0.288
Ht(m^2)	1.62 ±0.05	1.62 ±0.06	1.527	0.878
BMI(kg/m^2)	25.56 ±6.42	28.26 ±5.51	0.539	0.148
CD4+ Count	541.30 ±14 57	618.30 ±28.26	2.772	0.240
S Bp(Hmmg)	118.00 ±29.36	124.00 ±16.65	1.735	0.547
D Bp(Hmmg)	85.10 ±10.01	81.53 ±13.05	0.813	0.357
HIV +ve Subjects not on ART	Males n=8	Females n=22	t-values	p-values
Zn(ppm)	6.85 ±1.00	2.18±1,38	0.000	0.310
Cu(ppm)	11.20 ±2.15	10.00 ±3.43	1.327	0.215
Se(ppm)	4.15 ±2.00	5.38 ±1.45	0.312	0.334
Age(years)	46.47 ±6.14	48.14 ±5.40	0.000	0.441
Wt(kg)	38.50 ±6.29	32.43 ±3.12	20.00	0.032
Ht(m^2)	81.50 ±10.16	83.82 ±5.50	48.06	0.535
BMI(kg/m^2)	23.60 ±1.12	21.86 ±2.41	5.043	0.024
CD4+ Count	140.52 ±14.62	138.50 ±22.65	2.680	0.778
S Bp(Hmmg)	155.33 ±5.16	147.14 ±8.25	5.295	0.614
D Bp(Hmmg)	147.14 ±8.25	102 ±4.69	0.049	0.913

BMI= body mass index, SBp= systolic blood pressure DBp= diastolic blood pressure

Ht= Height, Wt= Weight,

Table 2: COMPARISON ON PARAMETERS STUDIED BETWEEN GENDERS IN HIV -VE SUBJECTS (CONTROL)

Parameters	Males n=8	Females n=22	t-values	p-values
Zn(ppm)	12.70 ±0.00	6.85 ±1,38	0.000	0.000
Cu(ppm)	24.40 ±3.85	19.99 ±5.63	2.397	0.025
Se(ppm)	9.15 ±4.00	9.88 ±4.45	0.539	0.676
Age (years)	46.50 ±12.29	49.73 ±5.63	40.00	0.494
Wt(kg)	81.50 ±10.16	83.82 ±5.50	48.06	0.535
Ht(m^2)	1.55 ±0.11	1.57 ±0.04	59.60	0.575
BMI(kg/m^2)	33.50 ±0.53	34.00 ±2.81	18.758	0.434
CD4- Count	1097.00 ±18.04	1266.10 ±17.37	8.243	0.000
SBp(Hmmg)	155.33±5.16	147.14±8.25	5.298	0.004
DBp(Hmmg)	147.14±8.25	102.86±4.69	0.049	0.913

BMI= body mass index, SBp= systolic blood pressure DBp= diastolic blood pressure

Ht= Height, Wt= Weight

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Table 3: GENDER COMPARISON OF CD4-T CELL, SERUM SE, ZN CU AND SOME ANTHROPOMETRIC PARAMETERS

Parameters	Pearson r	p-value	P< 0.05
Sex v CD4	0.457	0.011	P< 0.05
Zn v sex	-0.913	0.000	P< 0.05

4. DISCUSSION

The present investigation provides an in-depth evaluation of sex-related changes in immunological indices, serum trace element concentrations, and anthropometric parameters among adults living with Human Immunodeficiency Virus (HIV) in southeastern Nigeria. By examining individuals receiving antiretroviral therapy (ART), untreated HIV-positive subjects, and HIV-seronegative controls, this study offers a comprehensive framework for understanding how biological sex interacts with immune recovery, micronutrient homeostasis, and nutritional status within the context of HIV infection. The findings demonstrate that sex constitutes a relevant biological variable influencing CD4+ T-cell dynamics, trace element distribution, and body composition, thereby reinforcing the need for sex-responsive approaches in HIV care and management system.

One of the key findings of this study is the significantly higher serum selenium concentration observed in female HIV-positive subjects receiving antiretroviral therapy compared with their male counterparts **[Table 1]**. Selenium is an important micronutrient with established antioxidant, anti-inflammatory, and immunomodulatory functions, particularly through its incorporation into selenoproteins such as glutathione peroxidase, which protects immune cells from oxidative damage [4, 15]. Human Immunodeficiency virus infection is characterized by persistent immune activation and oxidative stress, both of which can increase selenium depletion if compensatory mechanisms are not adequate.

The elevated selenium levels among females on ART may reflect sex-dependent differences in micronutrient metabolism, hormonal regulation, dietary intake, or adherence to treatment and nutritional recommendations. Estrogen has been reported to influence antioxidant enzyme expression and trace element bioavailability, potentially enhancing selenium retention in females during chronic disease states. Furthermore, ART itself may improve gastrointestinal absorption and reduce inflammatory micronutrient losses, thereby allowing sex-specific metabolic differences to become more pronounced during treatment. This assertion is collaborated by the findings of [6, 7].

Previous studies have demonstrated that selenium deficiency is associated with faster disease progression, reduced CD4+ T-cell counts, and increased mortality among HIV-infected individuals [6, 16]. The present findings suggest that female patients on ART may experience a more favorable selenium profile, which could contribute to improved immune resilience. This observation aligns with earlier reports indicating that micronutrient repletion during ART is not uniform across sexes and may depend on underlying physiological differences [4, 7]. The absence of a similar sex difference among untreated HIV-positive subjects further underscores the role of ART in modifying micronutrient dynamics rather than sex alone.

Significant sex-based variations in serum zinc and copper concentrations were observed among HIV-negative control subjects, with males exhibiting higher levels of both micronutrients **(Table 2)**. Zinc and copper are essential divalent cations involved in numerous biological processes, including enzyme activity, immune cell signaling, and antioxidant defense [7]. Their homeostasis is tightly controlled, and even subtle imbalances can influence immune competence [7, 11].

The observed differences among HIV-seronegative individuals likely reflect inherent biological and lifestyle-related variations between sexes rather than disease-related effects. Factors such as dietary patterns, occupational exposure, muscle mass, hormonal status, and metabolic rate may contribute to differential trace element distribution. Testosterone has been implicated in modulating zinc metabolism, particularly in relation to muscle mass and protein synthesis, which may partly explain higher zinc levels among males. Copper metabolism, on the other hand, is influenced by ceruloplasmin synthesis, which may vary with sex hormones and inflammatory status [9, 15].

These findings are consistent with reports by [7, 11], who highlighted sex-dependent differences in divalent cation regulation even in non-diseased populations. Importantly, the presence of these differences in HIV-negative controls emphasizes that sex-specific micronutrient variation exists independently of HIV infection and may interact with disease processes once infection occurs. This baseline variability should therefore be considered when interpreting micronutrient deficiencies or excesses in HIV-infected populations.

Among HIV-positive individuals who had not commenced antiretroviral therapy **(Table 2}**, significant differences in body weight and body mass index (BMI) were observed between males and females, with males exhibiting higher values. Anthropometric decline is a well-recognized consequence of untreated HIV infection and is driven by a combination of reduced nutrient intake, malabsorption, chronic inflammation, and increased resting energy expenditure [4, 9].

The lower body weight and BMI observed among female untreated subjects may indicate greater vulnerability to HIV-

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associated wasting or differing sociocultural and economic factors that influence nutritional access. In many low-resource settings, women may experience food insecurity, caregiving burdens, and delayed health-seeking behavior, all of which can exacerbate nutritional depletion during chronic illness. Biologically, differences in fat distribution and muscle mass between sexes may also influence how weight loss manifests during disease progression.

These findings were in agreement with previous studies carried out in Nigeria and other sub-Saharan African countries, which reported more noticeable anthropometric compromise among female HIV-infected individuals before ART initiation [4, 12]. The initiation of ART has been shown to reverse or stabilize weight loss and improve BMI through viral suppression and reduction of systemic inflammation [10]. The observed sex differences in untreated subjects therefore highlight the importance of early diagnosis and timely treatment initiation, particularly among female patients who may be at higher risk of nutritional deterioration.

The correlation analysis (**Table 3**) revealed a significant association between sex and CD4+ T-cell counts, indicating that immune recovery or maintenance may vary between males and females. This agrees with the similar report of [9]. Cluster of differentiation (CD4+) T-cells count serves as the first target of HIV infection and are at the centre of the immune surveillance and coordination. Their depletion is a hallmark of disease progression while their restoration is a key indicator of ART efficacy [6, 11].

Sex-related differences in CD4+ T-cell dynamics have been reported in both HIV-infected and uninfected populations. Females generally exhibit higher baseline CD4+ T-cell counts and stronger immune responses, which may be attributed to genetic factors that is linked to the X chromosome and the immunostimulatory effects of estrogen. However, heightened immune activation in females may also predispose them to increased inflammation during chronic infection [9].

The positive association between sex and CD4+ T-cell counts observed in this study suggests that biological sex influences immune competence in HIV-infected individuals, potentially affecting disease trajectory and treatment response. These findings support earlier reports by [6, 16] who emphasized on the functions of micronutrients and immune control in shaping CD4+ T-cell recovery during ART. Understanding these sex-based immunological differences is essential for optimizing treatment plans and evaluating procedures.

A strong negative association was observed between sex and serum zinc levels, indicating that zinc status differs markedly between males and females in the study population. Zinc plays a critical role in T-cell maturation, cytokine production, and antiviral immunity, and its deficiency has been linked to impaired immune responses and increased susceptibility to opportunistic infections [7, 16].

Sex-dependent differences in zinc metabolism may arise from variations in dietary intake, absorption efficiency, hormonal regulation, and lifestyle behaviors such as alcohol consumption and smoking. Zinc deficiency has been shown to impair chemotaxis and phagocytic function of immune cells, thereby compromising host defense mechanisms [18]. In the context of HIV infection, zinc imbalance may exacerbate immune dysfunction and delay immune recovery even in the presence of ART.

The observed association reinforces the importance of monitoring zinc status in HIV-infected individuals and suggests that sex-specific thresholds or supplementation strategies may be necessary. Previous studies have demonstrated that zinc supplementation can enhance immunological response in HIV-infected patients, particularly when deficiencies are identified early [6, 16]. However, excessive zinc intake may interfere with copper absorption, highlighting the need for balanced and individualized nutritional interventions.

Although blood pressure differences were not consistently significant across all groups, the interaction between BMI, CD4+ T-cell counts, and blood pressure remains clinically relevant. HIV infection and ART exposure have been associated with metabolic alterations, including hypertension and cardiovascular risk, particularly as patient longevity increases [5, 12].

BMI has been identified as a potential confounder in the relationship between immune status and blood pressure, as higher BMI may mask or modify the association between CD4+ T-cell counts and hypertension [17]. The present findings suggest that sex-specific differences in body composition may influence cardiovascular risk profiles among HIV-infected individuals. This underscores the importance of integrating anthropometric assessment into routine HIV care, alongside immunological and biochemical monitoring.

The collective findings of this study highlight the limitations of a uniform approach to HIV management and emphasize the importance of incorporating sex as a biological variable in clinical decision-making. Differences in micronutrient status, immune response, and nutritional indicators suggest that male and female patients may benefit from tailored monitoring and intervention strategies.

From a public health perspective, integrating sex-responsive nutritional screening, micronutrient assessment, and immune monitoring into HIV programs could improve treatment outcomes and reduce long-term complications. Such an approach aligns with global recommendations advocating for personalized and patient-centered HIV care [18]. In resource-limited settings, targeted interventions may also enhance cost-effectiveness by prioritizing high-risk groups for nutritional

and immunological support.

5. CONCLUSIONS

So far, Sex-related differences were shown to exert a measurable impact on immune competence, trace element levels, and body composition among adults living with Human Immunodeficiency Virus, independent of exposure to antiretroviral treatment. Distinct patterns observed in CD4^{+} lymphocyte levels, circulating concentrations of zinc, copper, and selenium, as well as variations in body mass index and body weight, indicate that biological sex contributes to heterogeneity in both nutritional and immunological responses during HIV infection. Elevated selenium concentrations among female participants receiving antiretroviral therapy may reflect sex-specific metabolic or therapeutic interactions, whereas the divergence in zinc and copper levels among HIV-seronegative individuals suggests intrinsic differences in micronutrient regulation. In individuals who had not commenced therapy, disparities in body mass index and weight point to differential susceptibility to nutritional decline as the disease progresses. Moreover, the significant associations identified between sexes, CD4^{+} cell counts, and serum zinc levels reinforce the role of combined physiological and lifestyle-related factors in shaping immune recovery. Taken together, these findings highlight the limitations of uniform treatment approaches and support the integration of sex-conscious nutritional evaluation and immunological monitoring into HIV care frameworks, which may contribute to improved therapeutic response and sustained clinical outcomes.

STUDY LIMITATIONS AND FUTURE DIRECTIONS

While this study provides valuable insights, certain limitations should be acknowledged. The cross-sectional design precludes causal inference, and the sample size, particularly within subgroup analyses, may limit generalizability. Dietary intake, supplementation history, and hormonal status were not assessed, which could further elucidate the mechanisms underlying observed sex differences.

Future studies employing longitudinal designs, larger cohorts, and comprehensive nutritional assessments are warranted to explain temporal relationships and causal pathways. Investigating gene–nutrient and hormone–immune interactions may also enhance understanding of sex-specific responses

AUTHORS' CONTRIBUTIONS:

IP Ezeugwunne: Conceptualisation, Methodology, PN Marv-Ifeobi: Software, PN Marv-Ifeobi: Investigation, CC Dike: Resources, PN Marv-Ifeobi: Writing-original draft, IP Ezeugwunne and CC Dike: Supervision, together with Ede Nnamdi Ede, writing-review and editing, PN Marv-Ifeobi: Visualisation and project administration, IP Ezeugwunne: Formal analysis, Chinonye Goodness Ezeugwunne Stubbs payment for publication.

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