Caught between infection and deficiency: The iron anaemia nexus in HIV-infected adults in Western Kenya

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ABSTRACT

Anaemia remains a pervasive complication among people living with HIV (PLWHIV), with multifactorial origins that include poor antiretroviral therapy (ART) adherence, immune dysfunction, and iron dysregulation. This study investigated the interplay between nutritional status, immune markers, and iron biomarkers in contributing to anaemia and iron deficiency anaemia (IDA) in HIV-infected adults in Western Kenya. A cross-sectional study was conducted at Busia County Referral Hospital among 163 adults comprising HIV-infected ART-adherent (n = 47), ART-naive (n = 23), non-adherent (n = 42), and healthy control (n = 51) participants. Demographic, clinical, immunologic, and biochemical data were collected through interviews, physical measurements, and laboratory analyses. Iron indices (ferritin, serum iron, and transferrin), haemoglobin concentration, CD4+ T cell counts, HIV viral load, and body mass index (BMI) were measured using standardized protocols. Group comparisons were performed using Kruskal-Wallis and chi-square tests. Binary logistic regression was used to assess predictors of IDA. The highest prevalence of anaemia (61.9%) and iron deficiency anaemia (65.4%) was observed among non-adherent individuals, followed by ART-naïve (52.2% and 50.0%) and adherent participants (36.2% and 17.6%). Haemoglobin concentrations and iron levels were significantly lower ($P < 0.0001$), while transferrin levels were elevated ($P < 0.0001$) in ART non-adherent and naïve groups compared to controls. CD4+ T cell counts were markedly suppressed, and viral loads elevated in these groups, underscoring immune compromise. Logistic regression identified unsuppressed viral load (AOR = 10.83; $P = 0.023$), CD4+ T cell count <500 cells/μL (AOR = 4.01; $P = 0.010$), and elevated transferrin (AOR = 2.72; $P = 0.047$) as independent predictors of IDA. The findings suggest that poor ART adherence exacerbates inflammation, impairs iron metabolism, and increases anaemia risk. Integrating viral suppression, immune recovery, and iron biomarker monitoring in HIV care may improve early identification and management of IDA. Future studies should explore longitudinal trajectories of iron indices and anaemia in PLWHIV across different ART regimens.

Keywords: Anaemia, Iron Deficiency, ART Adherence, CD4+ T cell Count, HIV, Ferritin, Transferrin, Viral Load, Western Kenya

I. INTRODUCTION

Globally, approximately 39 million people live with Human Immunodeficiency Virus (HIV), with sub-Saharan Africa bearing two-thirds of this burden as per the United Nations Programme on HIV/AIDs (UNAIDS, 2020). In Kenya alone, over 1.4 million people live with HIV, and Busia County stands out with a prevalence of 7.7 % (CDC, 2019). Despite incredible advances in HIV management, anaemia remains a persistent threat among people living with HIV (PLWHIV), with prevalence estimates ranging from 30% to over 70%, depending on disease stage and access to treatment (Cao *et al*., 2022a). It is often overlooked, yet it quietly undermines health, contributing to fatigue, diminished physical performance, impaired cognitive functions, weakened immunity, and worse clinical outcomes even in patients optimally receiving antiretroviral therapy (ART) (Mwakishalua *et al.*, 2024; Shrivastav *et al.*, 2025). Understanding and managing persistent anaemia in this population remains a challenge, even in the context of ART availability (Obeagu & Kanu, 2024). For instance, in clinical practice, anaemia is commonly reduced to a number on a haemoglobin report, but behind that number lies a complex interplay of viral activity, immune function, nutrition, and iron metabolism, each silently influencing the trajectory of patient health (Abonyo *et al*., 2020).

While the etiology of anaemia in HIV is multifactorial, encompassing nutritional deficiencies, bone marrow suppression, chronic inflammation and effects of ART (Obeagu & Obeagu, 2024), its silent partner is often iron dysregulation (Babar & Saboor, 2024). Iron is essential for red blood cell production, immune competence, and cellular

energy (Qin *et al*., 2022). However, in the milieu of PLWHIV, iron metabolism becomes distorted (Obeagu, 2025). HIV-driven upregulation of inflammation, particularly in poor ART adherence, alters how the body stores and utilizes iron (12) ambiguously manifesting as anaemia of chronic disease or functional iron deficiency/ iron restricted erythropoiesis with diagnostic confusion (Babar & Saboor, 2024).

Serum ferritin, the traditional marker of iron storage, may appear deceptively normal or elevated despite a patient being iron deficient (Abioye *et al*., 2020). This is because ferritin also rises in response to inflammation (Liao *et al*., 2025). On the other hand, serum iron and transferrin levels may drop, not from dietary deficiency, but from immune-driven iron sequestration (Obeagu & Obeagu, 2024). These overlapping signals make it difficult to distinguish whether a patient truly lacks iron or is simply unable to utilize iron, which is an important distinction with major treatment implications. Immunological parameters equally add another layer of intricacy. The CD4+ T cell count and viral load are not just markers of HIV progression, they are active players in the inflammatory milieu that disrupts iron handling and erythropoiesis (Garrido-Rodríguez *et al*., 2022). A low CD4+ T cell count or a high viral load often signals immune dysfunction and inflammation, both of which suppress erythrocyte production, activity and stability subsequently worsening anaemia (Mitterstiller *et al*., 2022). Also, malnutrition which is common in resource-limited settings like Busia, is both a cause and consequence of HIV progression (Melku *et al*., 2020; Rezazadeh *et al*., 2023). Body Mass Index (BMI), a simple yet powerful measure of nutritional status reflects this reality (Mohajan & Mohajan, 2023). Low BMI occurs in PLWHIV with highest rates reported among ART naive and non-adherents (Kalinjuma *et al*., 2023), a phenomenon frequently linked to poor dietary intake, micronutrient deficiencies, and impaired haematopoiesis (Fuseini *et al*., 2021; Isabirye *et al*., 2020). Nonetheless BMI is seldom examined in conjunction with additional biochemical and clinical markers like ferritin, iron, transferrin, viral load and CD4 + T cell counts when evaluating anaemia risk in HIV care (Acharya *et al*., 2024).

Despite these intersected factors, clinical approaches to anaemia prediction, classification and management in HIV care remain fragmented. Haemoglobin is often assessed in isolation, while iron indices, immune markers, and nutritional status are evaluated separately only in advanced cases in resource limited settings (Acharya *et al*., 2024). As a result, many patients fall through the diagnostic cracks, and interventions are often non-specific or ineffective (Abioye *et al*., 2020). In Busia County, where the triple burden of HIV, undernutrition and ART non-adherence is particularly pronounced, gaps in understanding carry real-world consequences. Nonetheless, few studies have systematically explored how iron biomarkers (ferritin, iron, and transferrin) interact with immune function (CD4, VL) and nutritional status (BMI) to shape anaemia profiles in this high-risk population.

This study compared haemoglobin levels, alongside serum ferritin, iron, transferrin, CD4+ T cell counts, viral loads, and BMI across HIV-infected adults sub-categorised based on treatment status into ART- naive, adherent and non-adherent and healthy controls at Busia County Referral Hospital. This research offers a comprehensive look at the often-overlooked contributors to anaemia with an aim to move beyond routine metrics to a more integrated, evidence-based framework useful in identification and management of iron deficiency anaemia in HIV care.

1.1 Statement of the Problem

Anaemia among HIV-infected adults continues to be underdiagnosed and inadequately managed in many clinical settings, especially in resource-limited regions. In Busia County, a region characterized by high HIV prevalence, poor ART adherence, and widespread undernutrition, complications of anaemia is intensified. Although haemoglobin measurement is routine in HIV care, it offers a limited picture and fails to uncover underlying contributors. The absence of integrated diagnostic protocols that include patient nutritional status and biomarkers of iron homeostasis to the routine assessment of immune suppression and viral activity leads to suboptimal management of iron deficiency anaemia, which remains a major contributor to poor quality of life and clinical outcomes in PLWHIV.

1.2 Research Objectives

i. To assess the prevalence of anaemia and iron deficiency anaemia among HIV-infected adults at Busia County Referral Hospital, Western Kenya
ii. To evaluate the association between anaemia and biomarkers of iron status (serum ferritin, iron, and transferrin), immune function (CD4+ T cell counts and HIV viral load), and nutritional status among HIV-infected adults at Busia County Referral Hospital, Western Kenya
iii. To identify independent predictors of iron deficiency anaemia in HIV-infected adults at Busia County Referral Hospital, Western Kenya

II. LITERATURE REVIEW

2.1 Anaemia in HIV Infection

Anaemia is one of the earliest and most common hematologic manifestations of HIV infection, occurring across all stages of disease progression (Abonyo *et al*., 2020) . It has been consistently associated with faster disease

progression and increased mortality, independent of CD4+ T cell count and viral load (Obeagu *et al*., 2024). The mechanisms are multifaceted, including direct effects of HIV on bone marrow progenitors, opportunistic infections, nutritional deficiencies, and adverse drug effects. Anaemia may also result from immune activation leading to cytokine-mediated suppression of erythropoiesis (Cao *et al*., 2022b).

2.2 Iron Metabolism and Biomarkers

Babar & Saboor, (2024) highlighted that iron metabolism in individuals living with HIV is disrupted by the persistent inflammatory environment. They further implicated inflammation-mediated hepcidin upregulation secondary to HIV infection to be central to this dysregulation. Elevated hepcidin levels inhibit gut iron absorption and promote its sequestration within macrophages, thereby limiting its bioavailability for erythropoiesis despite sufficient or even elevated iron stores. This phenomenon, termed as functional iron deficiency, complicates clinical interpretation. Liao *et al*., (2025) notes that markers such as serum ferritin may be misleading in HIV due to its acute-phase reactivity, often remaining elevated regardless of true iron status. They further argued that serum iron and transferrin may be more reliable indicators of iron bioavailability and provide clearer insights into iron-restricted erythropoiesis in the context of HIV-related inflammation.

2.3 Nutritional Status, Immune Function and Inflammatory Burden in HIV

Garrido-Rodríguez *et al*., (2022) and Mitterstiller *et al*., (2022) report that unchecked HIV replication is linked to sustained inflammation. Inflammation related pro-inflammatory cytokines such as IL-6 and TNF-α inhibit renal and hepatic erythropoietin synthesis thereby shortening red blood cell lifespan, promoting anaemia. While ART adherence promotes viral suppression and immune recovery, non-adherence or ART-naive status are susceptible to sustained inflammatory effects, heightening the risk of immune-driven anaemia. Mohajan & Mohajan, (2023) linked undernutrition in HIV to deficiencies in iron, folate, and B12, which impair erythropoiesis. BMI, though basic, reflects nutritional status and has been correlated with anaemia severity (Kalinjuma *et al*., 2023). Yet, Acharya *et al*., (2024) observed overreliance on haemoglobin alone in anaemia diagnosis overlooking key drivers like iron status and nutritional imbalances in such an inflammatory setting. In places like Busia, this limited approach leads to misdiagnosis and poor outcomes. Abioye *et al*., (2020) highlight the lack of integrated studies in Western Kenya, where anaemia, HIV, and malnutrition intersect. This study addresses examined how clinical, immune, and nutritional factors jointly influence anaemia.

III. METHODOLOGY

3.1 Study Design, Site and Population

This was a cross-sectional study conducted at Busia County Teaching and Referral Hospital, Western Kenya. Upon obtaining written informed consent, the study enrolled HIV-positive adults sub-categorised based on treatment status as ART-naive, n = 23 as per (NIH, 2020), adherent, n = 47 and non-adherent, n = 42 through a combination of pharmacy refill records and self-reports in accordance with MMAS-8 adherence score (De las Cuevas & Peñate, 2015) and Healthy Control, n = 51. Healthy controls were the HIV antibody seronegative with the rapid immunochromatographic test, Determine^{TM} (Abbot Laboratories, Tokyo, Japan). Exclusions related to anaemia were menstruation, pregnancy, lactation, iron supplements, recent transfusion, acute non-HIV infections, and chronic comorbidities like chronic kidney disease, TB, cancer, or hematologic disorders such as sickle cell disease, thalassemia, or leukemia.

3.2 Sample Size and Sampling Technique

Systematic random sampling design was employed in this study where the first case was selected randomly among the PLWHIV seeking treatment in the hospital. The 11th case after the starting point followed a systematic selection. The 11th interval was calculated by dividing 7.7 % (prevalence of HIV in Busia County) of 16 752 newly infected Kenyans with HIV in 2023 by the sample size (n) of 112 until a sample size of 112 was reached.

The sample size was determined by (Charan & Biswas, 2013)

$n= (Z^2* p * (1−p)) / e2$

$n = ((1.96)^2 * 0.27*(1−0.077)) / (0.05)^2$

Where:

n is the sample size

Z is normal deviation at desired confidence interval (1.96)

p is the proportion of the cases in Busia County (7.7 %),

e^2 is the degree of precision (5%)

= 110

3.3 Ethical Considerations

Ethical approval and permit to conduct the study were obtained from Masinde Muliro University of Science and Technology Scientific and Ethics Review Committee (Ref: MMU/COR: 40312 Vol. 6 (01)) and National Commission for Science, Technology and Innovation (License No: NACOSTI/P/24/34958). Study respondents were thoroughly educated in accord with the international recommended guidelines ((Tsan & Puglisi, 2023)) and written informed consent obtained prior to enrolment into the study. Confidentiality was ensured by using unique participant codes, with data stored on password - protected systems accessible only to the investigators. Study subjects with anaemia, iron deficiency and iron deficiency anaemia were referred for treatment.

3.4 Determination of Age, Sex, Weight, Height and BMI

Age and sex data of study subjects were collected using a questionnaire (Singh *et al*., 2024). Weight was measured in kilograms using electronic weight scale, height in meters using a stadiometer and BMI calculate as weight (Kg) divided by height (m) square by erudite and skillful nurses as per (Casadei & Kiel, 2024; Warrier *et al*., 2024). Study subjects with BMI <18.5 Kg/m^2 were categorized as underweight while those with ≥18.5 to 24.9 Kg/m^2 as normal weight (Weir & Jan, 2024). The rest of the categories; overweight (BMI ≥25.00 < 30.00 kg/m^2) and obese (BMI ≥ 30.00 kg/m^2) were not observed (Weir & Jan, 2024).

3.5 Blood Sample Collection

A closed system of phlebotomy was used to draw blood from the median cubital vein of the antecubital fossa of each participant by certified experienced phlebotomist as per the (WHO, 2010) guidelines. Samples included, 4-5 ml whole blood in 5 ml EDTA vacutainers for determination of haemoglobin concentration, viral load (VL) and CD+ T cell count, 4-5 ml blood in 5 ml serum separator tubes (SST) for evaluation of ferritin, iron and transferrin. All samples were labelled with unique participant identifiers to avoid misidentification. Estimation of haemoglobin concentrations were performed immediately using the fresh EDTA samples. Enumeration of CD+ T cells and VL were done within 6 hours of sample collection. Clotted samples in SST were centrifuged at 1500 revolution per minute for 10 minutes for separation of sera. Sera were stored at ≤ -20^0C awaiting analyses (Valo *et al*., 2022).

3.6 Determination of HIV-1 Viral Load

HIV-1 viral load was evaluated using the automated Abbott m2000 Real Time HIV-1 System according to the manufacturer instructions (Abbott Molecular Inc., Illinois, USA). Selection of the equipment was informed by its previously reported good performance in detection of HIV-1. It is shown to be reliable, minimizes contamination through automation, and has a rapid turnaround time (Scott *et al*., 2009). HIV-1 RNA was extracted from 0.2 mL plasma samples. HIV RNA was further reverse-transcribed into complementary DNA (cDNA). HIV-1 specific internal control primers were used to amplify the cDNA. HIV-1 oligonucleotide probes linked to fluorescent dye were used to detect the HIV-1 cDNA amplicons. The analyzer converted the intensity of fluorescence into VL. Actual HIV-1 RNA copies per millilitre of subject samples recorded and further classified as suppressed (≤ 1000 copies/ml) and unsuppressed (> 1000 copies/ml) according to (WHO, 2023).

3.7 Enumeration of CD4+ T Cells

The CD4+ T cells of the study subjects were measured using the BD FACSCalibur™ flow cytometer (Becton-Dickinson, Franklin Lakes, USA). Concisely, 5µl of EDTA blood samples were placed in tubes. The red blood cells (RBC) were lysed by addition of RBC lysis buffer followed by incubation for a period of 5 minutes. Thereafter, lysed cells were washed off and fluorescent-tagged anti-CD3, anti-CD4 and anti-CD45 monoclonal antibodies added to the remaining formed elements (WBC). The cells were then incubated for 30 minutes, washed and fluorescent labelled CD4+ T cells enumerated using the flow cytometer. Study subjects were categorized based on their CD4+ T cell counts as immune competent (≥ 500 cells/µl) and immune compromised (< 500 cells/µl) according to WHO, (2023).

3.8 Measurement of Haemoglobin Concentration, Anaemia Rates and Levels

Coulter ACT 5diff analyzer (Beckman Coulter, France) was controlled using the normal, low and high blood controls for quality outcomes. Thereafter, subjects' haemoglobin (Hgb) concentrations were determined and assessed for derangements. Male and female study subjects with Hgb <13 g/dL and <12 g/dL, respectively, were considered anemic (WHO, 2019). Anaemic subjects with serum ferritin <70µg/L were considered to have iron deficiency anaemia (Omuse *et al*., 2022a).

3.9 Determination of Serum Ferritin, Iron and Transferrin Levels

Determination of ferritin, iron and transferrin levels were performed on Beckman Coulter AU 5800 (Brea, California, USA). Serum iron levels (µmol/L) were determined using the TPTZ [2, 4, 6-Tri-(2-pyridyl)-5-triazine] as the chromogen while transferrin (mg/dL) and ferritin (µg/L) by turbidimetry as previously described by (Omuse *et al*.,

2020). Iron deficiency was defined as ferritin < 70µg/L in line with previous studies (Masini *et al*., 2022; Omuse *et al*., 2022b; WHO, 2024). Serum iron reference was set at 10.6-32 µmol/L according to Xia *et al*., (2019) while transferrin at 204-360 mg/dl (Ogun & Adeyinka, 2024).

3.10 Statistical Analysis

Statistical analyses were conducted in GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, California USA) (Motulsky, 2019). Chi square test was used to determine the association between categorical variables (sex, BMI, Viral load, CD4+ T Cell counts, ferritin, iron and transferrin categories), and HIV and ART use statuses. Continuous variables (age, weight, height, BMI, VL, CD4+ T cell counts, ferritin, iron, transferrin levels and haemoglobin concentration) were compared across study groups using Kruskal-Wallis test followed by Dunn's *post-hoc* correction for multiple comparisons to control for overall type 1 error. Association of iron deficiency anaemia with nutritional status, immune status and iron biomarkers was determined using binary logistic regression. All tests were two-tailed and *p* values < 0.05 were considered statistically significant.

IV. FINDINGS & DISCUSSION

4.1 Demographic, Anthropometric and Clinical Characteristics of the Study Participants

Demographic, anthropometric and clinical characteristics of the study participants are summarized in Table 1. A total of 163 adults (females, n = 90 and males, n = 73) comprising of PLWHIV (n = 112) sub-categorised as ART non-adherent (NA, n = 42); ART unexperienced (Naive, n = 23) and ART adherent for between 6 and 12 months (A, n = 47), and healthy control (HC, n = 51) were recruited into the study. Analysis of demographic characteristics found similar average ages across groups (36.5–38 years, $P = 0.838$), with marginally more males in the non-adherent group and female predominance in the adherent and naive groups, $P = 0.065$. Anthropometric data showed significantly lower weight and BMI in the non-adherent and naïve groups compared to adherent patients and healthy controls, $P < 0.0001$. Although not statistically significant, $P = 0.099$, a notable number of individuals in the non-adherent and naive groups had BMI values below 18.5 kg/m^2. Examination of anthropometrics showed non-adherent and naive patients to have higher viral loads (log_{10} 4.6 and 4.5, respectively) with low viral suppression rates (7.1% and 17.4 %), while adherent patients had a lower viral load (log_{10} 3.4) and higher suppression (42.6 %). Also, CD4+ T cell counts were lowest in the non-adherent and naive groups, moderately preserved in adherent patients, and highest in healthy controls, $P < 0.0001$. Moreover, immune assessment revealed higher proportion of immunocompromised (CD4 < 500 cells/ µL) individuals in the non-adherent (73.8 %) and naive (73.9 %) groups, compared to the adherent group (38.3 %), $P = 0.001$. Altogether, indicating the importance of ART adherence in achieving viral control and promoting immune recovery in HIV infection.

Table 1

Demographic, Anthropometric and Clinical Characteristics of the Study Participants

Characteristics	HC, n = 51	Adherent, n = 47	Naive, n = 23	NA, n = 42	*P*-value
Age, years	37.0 (6.0)	37.0 (7.0)	38.0 (7.0)	36.5 (9.3)	0.838
Gender, n (%)					
Female	27 (52.9)	32 (68.1)	14 (60.9)	17 (40.5)	
Male	24 (47.1)	15 (31.9)	9 (39.1)	25 (59.5)	0.065
Height, m	1.69 (0.1)	1.68 (0.1)	1.66 (0.1)	1.68 (0.1)	0.465
Weight, kg	67.8 (18.0)	66.6 (14.7)	56.9 (11.0)[b,c]	59.2 (11.8)[a,c]	**<0.0001**
BMI, kg/m^2	24.5 (5.3)	23.5 (5.5)	20.4 (5.1)[a]	21.1 (3.7) [a,c]	**<0.0001**
BMI status, n (%)					
<18.5	-	3 (6.4)	4 (17.4)	9 (21.4)	
≥ 18.5	51 (100.0)	44 (93.6)	19 (82.6)	33 (78.6)	0.099
Log_{10} HIV-1 RNA copies/mL	-	3.4 (1.9)	4.5 (1.6)[a]	4.6 (1.1)[c]	**<0.0001**
Viral suppression status					
< 1000 cps/mL	-	20 (42.6)	4 (17.4)	3 (7.1)	
≥ 1000 cps/mL	-	27 (57.4)	19 (82.6)	39 (92.9)	**<0.0001**
CD4+ T cells/µL	1407 (1303)	576 (374)[c]	396 (349)[a,c]	423 (252)[a,c]	**<0.0001**
Immune status, n (%)					
< 500	-	18 (38.3)	17 (73.9)	31 (73.8)	
≥ 500	51 (100.0)	29 (61.7)	6 (26.1)	11 (26.2)	**0.001**

Data are presented as medians and IQR for age, height, weight, BMI, Log_{10} HIV-1 RNA and CD4+ T cells, and as number and proportion of subjects for gender, BMI status, viral suppression status and immune status. BMI, body mass index; HIV-1, human immunodeficiency virus type 1; RNA, ribonucleic acid; CD4+ T cell, Cluster of

differentiation 4 positive T cell; HC, healthy control; Naive, PLWHIV ART unexperienced; Adherent, PLWHIV ART adherent for between 6 to 12 months; NA, PLWHIV ART non-adherent. Chi square test was used to analyze categorical data while Kruskal-Wallis test was performed on continuous data. Dunn's *post hoc* tests were run for continuous data that were significantly different across study groups. Weight, $^{a}P < 0.05$ vs. A, $^{b}P < 0.001$ vs. A, $^{c}P < 0.0001$ vs. HC; BMI, $^{a}P < 0.05$ vs. A and HC, $^{c}P < 0.001$ vs. HC; Log_{10} HIV-1 RNA, $^{a}P < 0.05$ and $^{c}P < 0.0001$ vs. A; CD4+ T cells, $^{a}P < 0.05$ vs. A and $^{c}P < 0.0001$ vs. HC. The *P* value 0.001 was for the NA, Adherent and Naïve study groups only since the HC group had no CD4+ T cell counts < 500/µL. *P*- value < 0.05 was considered statistically significant. Significant *P - value*s are shown in bold.

4.2 Iron and Anaemia Profiles of the Study Participants

Iron and anaemia profiles of the study participants are summarized in Table 2, with *post hoc* analyses presented in figure 1. Ferritin levels were lowest in the non-adherent (57.9 µg/L) and naive (53.5 µg/L) compared to the adherent (114.9 µg/L) and healthy control groups (211.8 µg/L), $P < 0.0001$. Levels in the adherent group were equally lower relative to healthy controls', $P < 0.001$. Consequently, iron deficiency was most prevalent in the non-adherent (64.3%) and naive (56.5%) groups, followed by the adherent group (23.4%), while none in the healthy control group. Likewise, serum iron levels were lower in the non-adherent (9.9 µmol/L) compared to adherent (13.4 µmol/L) groups $P < 0.05$. Further comparisons revealed levels in non-adherent, naive (10.5 µmol/L) and adherent groups to be significantly lower compared to healthy controls (21.1 µmol/L), $P < 0.0001$. As a result, hypoferremia occurred in over half of the non-adherent and naive participants, nearly a quarter of the adherent group, but absent in healthy controls. Conversely, transferrin levels were highest in the non-adherent (343.2 mg/dL) and naive (345.2 mg/dL) groups, $P < 0.0001$, but comparable between the adherent group (282.9 mg/dL), and healthy controls (272.3 mg/dL), $P > 0.05$. Hypertransferrinaemia was observed in 40.5 % of non-adherent and 26.1 % of naive individuals, but was absent in adherent and healthy control subjects.

Generally, haemoglobin concentrations were significantly lower in HIV-infected individuals, especially in the naive (12.0 g/dL) and non-adherent (12.1 g/dL) groups, compared to the healthy controls (13.9 g/dL; $P < 0.0001$). Also, the median haemoglobin concentration of the naive group was significantly lower compared to the Adherent's (12.9 g/dL), $P< 0.05$. Moreover, haemoglobin concentration in the Adherent subjects was significantly lower than that of the healthy controls, $P < 0.05$. Anaemia was most prevalent in the non-adherent group at 61.9 %, followed by the naive group at 52.2 % and the adherent group at 36.2 %, while no cases were observed among healthy controls. Iron deficiency anaemia, defined by the presence of low haemoglobin levels alongside depleted iron stores, was particularly pronounced among the anemic non-adherent individuals, affecting 65.4 %, followed by the naive group at 50.0 % and only 17.6 % of the adherent group, $P = 0.009$.

Table 2

Iron and Anaemia Profiles of the Study Participants

Characteristics	**HC, n=51**	**A, n=47**	**Naive, n=23**	**NA, n=42**	***P*-value**
Ferritin, µg/L	211.8 (121.4)	114.9 (78.9)	53.5 (52.4)	57.9 (37.5)	**<0.0001**
Iron deficiency, n (%)	0 (0.0)	11 (23.4)	13 (56.5)	27 (64.3)	**<0.0001**
Normoferritinaemia, n (%)	51 (100.0)	36 (76.6)	10 (43.5)	15 (35.7)	
Iron, µmol/L	21.1 (10.8)	13.4 (7.0)	10.5 (9.6)	9.9 (6.2)	**<0.0001**
Hypoferraemia, n (%)	0 (0.0)	11 (23.4)	12 (52.2)	23 (54.8)	**<0.0001**
Normoferraemia, n (%)	51 (100.0)	36 (76.6)	11 (47.8)	19 (45.2)	
Transferrin, mg/dL	272.3 (31.8)	282.9 (51.8)	345.2 (54.8)	343.2 (61.7)	**<0.0001**
Hypertransferrinaemia, n (%)	0 (0.0)	0 (0.0)	6 (26.1)	17 (40.5)	
Normotransferrinaemia, n (%)	51 (100.0)	47 (100.0)	17 (73.9)	25 (59.5)	_
Haemoglobin, g/dL	13.9 (2.0)	12.9 (2.0)	12.0 (2.1)	12.1 (1.0)	**<0.0001**
Anemic, n (%)	0 (0.0)	17 (36.2)	12 (52.2)	26 (61.9)	**<0.0001**
Non anemic, n (%)	51 (100.0)	30 (63.8)	11 (47.8)	16 (38.1)	
Iron deficiency anaemia, n (%)	-	3 (17.6)	6 (50.0)	17 (65.4)	**0.009**

Data are presented as median and IQR for ferritin, iron, transferrin and haemoglobin concentration, and as frequency and proportion for iron deficiency, normoferritinemia, hypoferremia, normoferremia, hypoferremia, normotransferinaemia, hypertransferrinaemia, anaemic, non-anaemic and iron deficiency anaemia. NA, PLWHIV ART non-adherent; Naive, PLWHIV ART unexperienced; A, PLWHIV ART adherent for between 6 to 12 months; HC, healthy control. Data analysis was performed using chi square for categorical data and Kruskal-Wallis test followed by Dunn's *post hoc* test for multiple comparison of numerical data. *P*-value < 0.05 was considered statistically significant. Significant *P*-values are in bold.

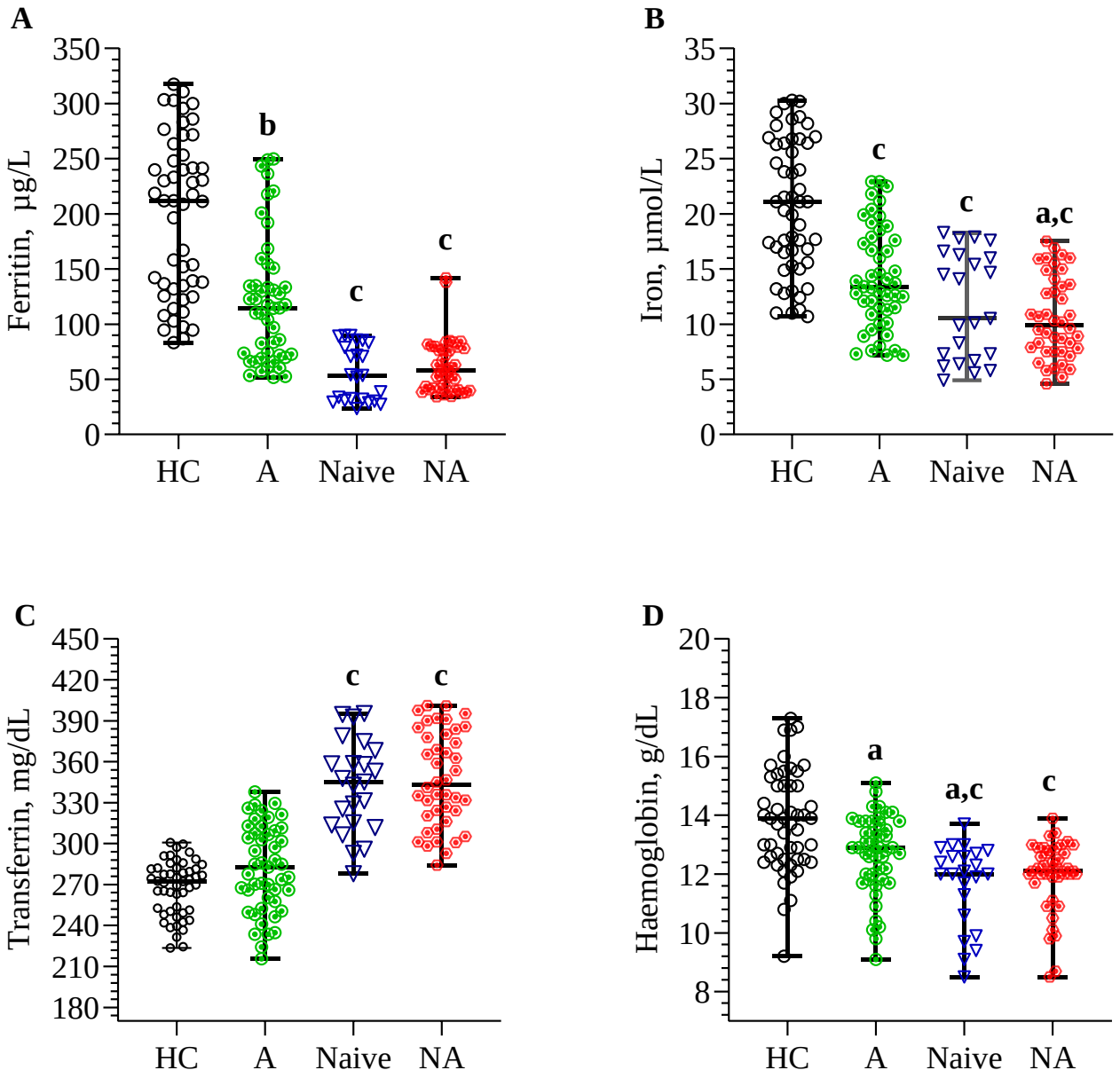

Figure 1
Dunn's Post hoc between Group Comparison of Ferritin, Iron and Transferrin Levels of Study Subjects

Data are presented as scatter dot plots, where the line through the scatter plots represents the median, and the error bars indicate the 10th and 90th percentile. The scatter dots beyond the error bars represent outliers. NA, PLWHIV ART non-adherent; Naive, PLWHIV ART unexperienced; A, PLWHIV ART adherent for between 6 to 12 months; HC, healthy control. Statistical analyses were conducted using Dunn's *post hoc* test. Ferritin; $^{b}P < 0.001$ vs. HC, $^{c}P < 0.0001$ vs. A and HC; Iron, $^{a}P < 0.05$ vs. A, $^{c}P < 0.0001$ vs. HC; Transferrin, $^{c}P < 0.0001$ vs. A and HC; Haemoglobins, $^{a}P < 0.05$ vs. A and HC, $^{c}P < 0.0001$ vs. HC. *P*-value < 0.05 was considered statistically significant. Significant *P*-values are in bold.

4.3 Association of Iron Deficiency Anaemia (IDA) with Nutritional Status, Immune Status and Iron Biomarkers in HIV Infection

Association of iron deficiency anaemia with nutritional status, immune status and iron biomarkers are summarized in Table 3. Nutritional status, as measured by body mass index (BMI), showed that participants with BMI less than 18.5 kg/m² were 1.8 times more likely to have IDA compared to those with normal BMI (≥ 18.5 kg/m²), although association did not reach statistical significance ($P = 0.323$). In contrast, immune status and viral load demonstrated strong relationships with IDA. Individuals with CD4+ T cell counts below 500 cells/µL were four times more likely to have IDA compared to those with counts above 500 (AOR = 4.009; 95% CI: 1.384–11.607; $P = 0.01$). Similarly, participants with a high viral load (> 3.0 $\log_{10}$ HIV-1 RNA copies/mL) had a tenfold increased likelihood of

developing IDA compared to the virally suppressed individuals (AOR = 10.833; 95% CI: 1.393–84.241; *P* = 0.023). Serum iron levels also demonstrated a positive association with IDA, those with low serum iron (<10.6 µmol/L) having 2.4 times greater odds of anaemia with the association approaching statistical significance (*P* = 0.053). Likewise, transferrin levels provided additional insight. Participants with iron deficiency anaemia had significantly higher odds presenting with elevated transferrin levels (>360 mg/dL) compared to those with normal levels (AOR = 2.723; 95% CI: 1.011–7.329; *P* = 0.047), elevation that likely reflects a compensatory response to depleted iron stores.

Table 3

Association of Iron Deficiency Anaemia with Nutritional Status, Immune Status and Iron Biomarkers in HIV Infection

Variable	B (SE)	Adjusted Odds Ratios (95% CI)	*P*-value
BMI, Kg/m^2			
≥18.5 (Reference)		1	
<18.5	0.593 (0.601)	1.810 (0.558 – 5873)	0.323
CD4+ T cell/µL			
≥500 (Reference)		1	
<500	1.389 (0.542)	4.009 (1.384 – 11.607)	**0.010**
Log_{10}HIV-1 RNA copies/mL			
≤3.0 (Reference)		1	
>3.0	2.383 (1.046)	10.833 (0.1.393 – 84.241)	**0.023**
Iron, µmol/L			
≥10.6 (Reference)		1	
<10.6	0.884 (0.456)	2.419 (0.990 – 5.915)	0.053
Transferrin, mg/dL			
≤360 (Reference)		1	
>360	1.002 (0.505)	2.723 (1.011 – 7.329)	**0.047**

Data are presented as regression coefficients (B) with standard errors (SE), adjusted odds ratios (AOR) with 95% confidence intervals (CI), and corresponding *P - value*s. Reference categories for comparison are indicated for each variable. *P*-value < 0.05 was considered statistically significant and is shown in bold. BMI, body mass index; CD4+ T cells, cluster of differentiation 4 positive T lymphocytes; HIV-1, human immunodeficiency virus type 1; RNA, ribonucleic acid.

4.4 Discussion

This study examined the connection between iron deficiency and anaemia in HIV-infected adults in Western Kenya. It focused on how adherence to antiretroviral therapy (ART) affects iron levels, the prevalence of anaemia, and interactions between the immune system and nutrition. The results show that untreated and sub-optimally treated HIV is linked to disrupted iron metabolism, anaemia and a high rate of iron deficiency anaemia (IDA). Contrariwise, ART adherence offers some protection, though does not fully restore normal blood health.

The study population was age matched. Gender distribution showed more HIV-infected males in the ART non-adherent group and more females among adherent and naive individuals. This finding coincides with studies from Malawi and Limpopo County, South Africa which noted that women are more likely to test their HIV status and less likely to stop HIV treatment post initiation relative to men, often linked to poor health seeking behaviours (Nhlongolwane & Shonisani, 2023). This is likely attributed to stigma, job mobility and societal views towards men, particularly those that put emphasis on strength and stoicism as highlighted by Chavalala *et al.*, (2025).

Anthropometric data showed lower body weight and BMI with notably higher rates of underweight in non-adherent (21.4 %) and naive (17.4 %) participants compared to the adherent (6.4 %) and healthy control indicating nutritional and metabolic burden of untreated and suboptimally treated HIV, where progressive immune suppression drives weight loss and muscle wasting. This aligns with findings from Lahai *et al.*, (2022) and Kheswa (2017), who indicated that untreated or poorly managed HIV infection contributes to muscle loss, malnutrition, and weight decline primarily attributed to persistent inflammatory activities, hyper-metabolism, and HIV-related enteropathy that hinder nutrient uptake. Pro-inflammatory cytokines like interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are raised in uninhibited HIV infection, leading to enhanced proteolysis, anorexia and nutrient deficits.

Immunologically, ART-naive subjects appeared severely immunocompromised (73.9 %) with only 17.4 % having viral load below 1000 copies per millilitre of blood, indicating rapid immune deterioration in untreated HIV infection. These findings are consistent with studies by N'Takpe *et al.*, (2020) and the NIH, (2025) which reported that untreated and sub-optimally treated HIV promotes its progression and host immunosuppression. Nonetheless, ART-adherent participants demonstrated markedly improved clinical and immunological profiles. Their immune reconstitution was evident with 61.7 % achieving CD4 + T cell counts ≥ 500 cells/µL with substantial viral suppression

of up to 42.6 % underscoring the beneficial effects of consistent ART as demonstrated in the DART and HPTN 052 trials (Floyd *et al*., 2020). Interestingly, the ART non-adherent subjects closely resembled ART-naive participants. Majority (73.8 %) were immunocompromised with only 7.1% of them virally supressed, confirming viral rebound and immune decline on ART noncompliance illustrating the consequences of treatment interruption as previously reported by Odubela *et al*., (2025).

The current study revealed significant alterations in iron balance and anaemia indicators among HIV-infected individuals, particularly in the ART non-adherent and naive groups. The ART-naive study group demonstrated the most severe disruptions in iron metabolism and hematologic indices compared to the rest of the study groups. Their median ferritin level was the lowest (53.5 µg/L), with equally higher rate of iron deficiency (56.5 %), indicative of severe HIV infection associated with depleted iron stores. Likewise, their serum iron levels were significantly reduced (10.5 µmol/L), with 52.2% cases of hypoferremia, indicating combined absolute and functional iron deficiency. Conversely, serum transferrin levels were strikingly elevated (345.2 mg/dL), with 26.1% subjects in this group presenting with hypertransferrinaemia, suggestive of a compensatory response to low iron stores. Hematologic analysis also found the ART - naive participants with low haemoglobin concentration (12.0 g/dL) and high rate of anaemia (52.2 %), half of whom with iron-deficiency anaemia. These findings mirror previous African studies reporting that untreated HIV infection is associated with inflammation-driven hepcidin dysregulation and nutritional iron depletion (Obeagu *et al*., 2024). Inflammation - induced hyperhepcidinemia promotes internalization and degradation of the key iron exporter ferroportin, leading to iron sequestration in ferritin, decreased intestinal iron absorption, and reduced iron availability for erythropoiesis (Kesharwani *et al*., 2025). The ART-adherent participants showed partial recovery of iron stores and hematological parameters compared to the naive subjects. Ferritin levels were significantly higher (114.9 µg/L), and iron deficiency less common (23.4%) relative to the naïve subjects. Their serum iron concentrations averaged 13.4 µmol/L, with hypoferremia occurring in only 23.4%. In addition, their transferrin levels (282.9 mg/dL) were closer to normal, with no cases of hypotransferrinemia. Equally, haemoglobin concentrations were improved to 12.9 g/dL, and anaemia prevalence reduced to 36.2 %, with only 17.6 % its anemic members having iron deficiency anaemia. Altogether, indicate ART-associated restoration of erythropoiesis enhanced by improved iron absorption and mobilization from stores. These findings support the evidence that optimal use of ART suppresses HIV-induced inflammation, thereby lowering hepcidin levels and enhancing gut iron absorption and mobilization from the stores hence available to erythroid precursors for haemoglobin synthesis (Obeagu *et al.*, 2024). Concomitantly, down-regulation of proinflammatory cytokines enhance erythropoietin production by the kidney and liver while up regulating the expression of erythropoietin receptors on erythroblasts promoting uptake (Peng *et al*., 2020). The ART non-adherent group showed serious iron imbalance, similar to what was observed in ART-naive participants. This emphasizes the harmful effect of uncontrolled HIV replication along with ongoing inflammation. Their ferritin levels were notably low at 57.9 µg/L, with the highest at prevalence of iron deficiency anaemia (64.3 %) observed in this group compared to all other clinical categories, suggesting depleted iron stores plausibly exacerbated by chronic inflammation and poor iron absorption. Serum iron levels were markedly reduced (9.9 µmol/L), with over half of the participants 54.8 % exhibiting hypoferremia, indicating a substantial disruption in iron availability likely driven by pronounced inflammatory state and impaired mobilization from stores. Contrariwise, serum transferrin levels were elevated at 343.2 mg/dL with hypertransferrinemia observed in 40.5% of the participants in this study group. This likely indicates a compensatory response to iron deficiency and an increased iron-binding capacity attributable to depleted stores. Further, this group demonstrated a median haemoglobin concentration only 0.1 g/dL higher than the ART-naive group. It also had the highest anaemia cases at 61.9%, with iron-deficiency anaemia accounting for 65.4% of these cases implying widespread iron-related hematological impairment. These results are consistent with studies by Mulaudzi *et al*., (2020) and Ringshaw *et al*., (2025), which reported similar patterns in South Africans living with HIV, where discontinuation of ART was linked to exacerbated iron depletion and higher anaemia rates.

Iron deficiency anaemia was strongly associated with circulating HIV RNA copies, immune functions and biomarkers of iron homeostasis. Participants with high viral load demonstrated a tenfold increased risk of developing iron deficiency anaemia, highlighting the impact of uncontrolled HIV replication on inflammation-related hepcidin imbalance, blunted renal and hepatic erythropoietin secretion, and downregulation of erythropoietin receptor expression on erythroid precursors resulting in ineffective erythropoiesis as previously reported by Obeagu *et al*., (2024). Obeagu and co-authors implicated high viremia in the hypersecretion of hepatic interleukin-6 that stimulates production of a key regulator of iron homeostasis, hepcidin which, in turn, inhibits intestinal iron absorption while promoting its retention in macrophages ultimately contributing to iron deficiency anaemia. Current research findings also indicated that HIV infected patients with CD4+ T cell counts below 500 cells/ µL had four times higher odds of having iron deficiency anaemia. Our findings coincide with Xie *et al*., (2022) who reported immunocompromised HIV patients within Guangxi, China to have more than twice the risk of developing anaemia. They also matched a multilevel analysis of HIV infected women in 18 counties of Africa by Tilahun *et al*., (2024) who reported iron deficiency, a condition whose dysregulation is closely linked to degree of inflammation (Rosenblum, 2023), to be the most prevalent cause of anaemia in HIV patients. In addition, serum iron levels under 10.6 µmol/L were associated with a 2.4-fold increased

risk of iron deficiency anaemia by borderline statistical significance. Surprisingly, BMI under 18.5 kg/m² was not significantly linked to iron deficiency anaemia. This indicates that immune and inflammatory processes may play a larger role in HIV related iron deficiency anaemia than nutritional status, a trend previously reported among HIV patients from a neighbouring Ugandan cohort (Obeagu & Obeagu, 2024). Nonetheless, iron deficiency anaemia was associated with 2.7 times greater odds of elevated transferrin, suggesting a liver response to depleted iron stores.

Overall, these findings highlight the key role of ART adherence in reducing HIV-related anaemia and iron deficiency. They suggest that effective HIV treatment significantly lowers viral replication, virus-driven immune activation, and inflammation. This, in turn, reduces IL-6 mediated hyperhepcidinemia, which improves iron absorption and red blood cell production. However, low-grade inflammation may continue, along with possible ART-related toxicity to bone marrow. This can occur even with strict adherence to ART, leading to anaemia. The differences in ferritin, iron and transferrin values among groups support the idea of a changing iron-anaemia spectrum in HIV. As the disease advances, malnutrition potentially sets in and true iron deficiency develops, characterized by low ferritin in stores, low iron in circulation and high transferrin as a compensatory response. Even so, ART appears to help patients approach a normal iron state, but it does not completely restore it. Our results align with Muhie and Tegegne, who noted better haemoglobin and iron indicators in patients adhering to ART (Abioye *et al*., 2024; Muhie & Tegegne, 2024; Obeagu *et al*., 2024). The significantly high transferrin levels observed concurred with a cross sectional study by Obirikorang *et al*., (2016), but differed from a research in Rwanda (Masaisa *et al*., 2012) which found lower transferrin levels in women with advanced HIV.

Despite the notable strength of current findings, the cross-sectional design limits causal inference. The absence of inflammatory cytokine data precludes direct assessment of immune activation. Moreover, nutritional status was evaluated using BMI alone, which may not capture subclinical deficiencies. Dietary iron intake and gastrointestinal health were also not assessed. Although major co-infections were excluded, residual confounding from subclinical inflammation cannot be ruled out. Future longitudinal studies are necessary to track changes in iron parameters and anaemia status over time in relation to ART adherence, which would provide stronger evidence on temporal patterns and causal relationships than is possible in a cross - sectional design.

V. CONCLUSION & RECOMMENDATIONS

5.1Conclusion

HIV infection affects the balance of iron and haemoglobin, especially in people who are not on antiretroviral therapy (ART) and those who do not follow their treatment. These individuals show clear signs of iron-deficiency anaemia, such as low ferritin and serum iron levels, along with high transferrin levels. On the other hand, individuals who stick to their ART see improvements in their iron levels and a decrease in anaemia cases. This emphasizes how ongoing ART can help reduce blood-related issues linked to HIV.

5.2 Recommendation

Clinical practice should incorporate routine monitoring of iron status and haemoglobin levels in HIV care to enable early detection and timely management of iron deficiency anaemia.

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