Tracking biomarkers of iron dysregulation across HIV treatment spectrum: Insights from erythroferrone, hepcidin and hephaestin levels in Western Kenya

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ABSTRACT

Anaemia is a frequent comorbidity in HIV infection, driven in part by disruptions in iron regulation. While the role of hepcidin is well documented, the contributions of erythroferrone and hephaestin remain underexplored, especially in African cohorts. This study investigated the differential expression of these biomarkers across defined sub-categories of antiretroviral therapy (ART) exposure and adherence in Western Kenya with an aim to elucidate their contributions to iron deficiency anaemia (IDA) in HIV-infected individuals. 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) study groups. Serum levels of erythroferrone, hepcidin, and hephaestin were quantified using ELISA. Iron status and haemoglobin were assessed using standard hematologic and biochemical methods. Logistic regression models were used to evaluate associations between biomarker levels, ART status, and IDA risk. ART non-adherence and ART-naivety were associated with significantly higher prevalence of IDA (65.4% and 50.0%, respectively) compared to ART adherence (17.6%, $P = 0.009$). Erythroferrone levels were significantly suppressed in ART-naive and non-adherent individuals (median: 21.7 and 31.2 ng/mL, respectively) compared to adherent and healthy controls (38.1 and 50.2 ng/mL, $P < 0.0001$). Elevated hepcidin levels were observed in ART-naive and non-adherent participants (113.0 and 84.1 ng/mL, respectively), aligning with functional iron deficiency. Hephaestin levels were markedly reduced in untreated and non-adherent groups, implicating impaired iron absorption. Binary logistic regression confirmed ART non-adherence (AOR = 9.97, 95% CI: 2.66–37.41), low erythroferrone (AOR = 0.094, 95% CI: 0.01–0.72), elevated hepcidin (AOR = 3.36, 95% CI: 1.36–8.25), and reduced hephaestin (AOR = 1.137 per µg/L decrease, 95% CI: 1.07–1.20) as independent predictors of IDA. ART status exerts a profound influence on iron homeostasis in PLWHIV through modulation of key regulatory proteins. Suppression of erythroferrone, elevation of hepcidin, and depletion of hephaestin underlie a triad of dysregulation that drives iron-restricted erythropoiesis. These findings call for integrative diagnostic frameworks that include iron biomarkers beyond ferritin, and underscore the urgent need to address ART non-adherence as a modifiable determinant of haematologic health in HIV.

Keywords: Anaemia, ART Adherence, Erythroferrone, HIV, Hepcidin, Hephaestin, Iron Deficiency, Inflammation, Western Kenya

1. INTRODUCTION

Among people living with HIV (PLWHIV), anaemia represents a common and debilitating complication, affecting nearly 70 % of the 1.4 million Kenyans living with the virus (Abonyo *et al*., 2020; CDC, 2019). Its occurrence varies with disease progression and antiretroviral therapy (ART) status, contributing significantly to morbidity, decreased quality of life, and increased mortality (Aemro *et al*., 2022; Sah *et al*., 2020). While there are many causes of HIV-related anaemia including nutritional deficiencies, bone marrow suppression, opportunistic infections, effects of ART like zidovudine, coinfections and comorbidities, evidence increasingly implicates dysregulation of iron homeostasis as a central mechanism often manifesting as functional iron deficiency anaemia or iron-restricted erythropoiesis (Abioye *et al*., 2020; Obeagu *et al*., 2024). Iron homeostasis in humans is tightly regulated at both systemic and cellular levels to ensure sufficient iron availability for essential processes such as erythropoiesis, (Ru *et al*., 2024). Normally, this regulation is orchestrated by a coordinated system of hormones, transporters and storage proteins including ferroportin, ferritin, transferrin, hepcidin, erythroferrone, and hephaestin, preventing iron deficiencies and toxicity from overload (Camaschella *et al*., 2020a).

Beyond the well-established actors like ferroportin, ferritin and transferrin, hepcidin, hephaestin and erythroferrone remain poorly characterized in the context of HIV, yet vital and may provide diagnostic and therapeutic utility. Hepcidin, regarded as the master regulator of iron homeostasis, is an active 25-amino acid peptide hormone produced by the liver. Normally, it controls plasma iron by regulating intestinal absorption and storage in specific body tissues like macrophages (Kesharwani *et al*., 2025). On the other hand, erythroferrone, the hormone secreted by erythroid precursors in the bone marrow in response to erythropoietin stimulation, suppresses hepcidin during increased erythropoiesis, thereby permitting more iron to be absorbed and released from stores to meet the high demand for red blood cell production (Chambers *et al*., 2025). In this axis, enterocytes secret hephaestin, a multicopper ferroxidase that converts ferrous iron into its ferric form that readily binds transferrin to enable export to the bone marrow for effective erythropoiesis (Amadei *et al*., 2025). Together, these proteins work as a functional triad integrating signals for red blood cell production, iron availability, and absorption efficiency.

Evidence suggest that HIV-related inflammation disrupts iron regulatory protein balance by altering key regulatory pathways. For example, HIV- induced chronic immune activation leads to elevated interleukin-6, which upregulates hepcidin. Elevated hepcidin traps iron within macrophages and enterocytes, resulting in functional iron deficiency despite normal or elevated ferritin levels (Kamvuma *et al*., 2024). This mechanism limits iron availability for erythropoiesis. Simultaneously, inflammatory cytokines suppress erythropoietin, an erythroblast-derived hormone that physiologically suppresses hepcidin during increased erythropoietic demand (Rohr *et al*., 2023). Low erythropoietin in HIV implies a blunted erythropoietic response with possibly reduced erythroferrone expression, contributing to persistent iron deficiency anaemia. Even so, erythroferrone remains understudied, particularly in this cohort. Another important but minimally analyzed regulator is hephaestin whose dysregulation may lead to poor iron loading onto transferrin predisposing HIV patients to IDA. Experimental models, including the *sla* mouse, have shown that hephaestin deficiency results in intestinal iron malabsorption and microcytic anaemia (Chen *et al*., 2003). While studies on inflammatory bowel disease have demonstrated inflammation-driven downregulation of hephaestin (Doguer *et al*., 2018). In addition, the role of antiretroviral therapy (ART) in modulating iron regulation is well recognized but incompletely understood. ART has been shown to reduce systemic inflammation, normalize hepcidin levels, and partially restore iron mobilization (Chambers *et al*., 2025; Shen *et al*., 2013). However, anaemia persists in a significant proportion of treated patients, suggesting that some dysregulatory mechanisms remain active. With non-adherence to ART remaining a major challenge in sub-Saharan Africa, treatment interruption may perpetuate chronic immune activation, further aggravating iron dysregulation and anaemia (Mwangi *et al.*, 2021). Given the high burden of anaemia among PLWH and its association with poor treatment outcomes, there is a pressing need to explore how ART status influences iron regulation. While hepcidin has been well characterized, the interplay of erythroferrone and hephaestin in HIV-associated anaemia remains poorly understood, especially in African populations. Moreover, the relationship between these biomarkers and the risk of iron deficiency anaemia (IDA) across different ART adherence statuses has not been clearly established. This study tracked biomarkers of iron dysregulation across the HIV treatment spectrum, specifically assessing erythroferrone, hepcidin, and hephaestin levels across ART-naive, ART non-adherent, and ART-adherent individuals in Western Kenya.

1.1 Statement of the Problem

Persistent anaemia in HIV is associated with reduced quality of life, faster disease progression, and elevated mortality. In sub-Saharan Africa, where ART non-adherence and delayed initiation are common, anaemia persists despite widespread ART availability. Conventional diagnostic frameworks relying solely on haemoglobin and ferritin are insufficient to delineate the complex etiologies of HIV-related anaemia, particularly functional iron deficiency driven by chronic inflammation. Although hepcidin has emerged as a key regulator in this process, the roles of erythroferrone and hephaestin remain poorly characterized, especially in Kenyan population. Moreover, the influence of ART exposure and adherence on these biomarkers is largely unstudied. This limits clinicians' ability to differentiate between absolute and functional iron deficiency, hindering targeted interventions. Understanding how ART modulates these iron regulatory pathways is essential to advancing precision diagnostics and effective management of anaemia in HIV.

1.2 Research Objectives

i. To quantify serum levels of erythroferrone, hepcidin, and hephaestin in HIV-infected adults at Busia County Referral Hospital, Western Kenya
ii. To determine the association between ART status, iron regulatory biomarkers, and the risk of iron deficiency anaemia in HIV-infected adults at Busia County Referral Hospital, Western Kenya

II. LITERATURE REVIEW

2.1 Iron Deficiency Anaemia in HIV infection

Anaemia is a common and multifactorial complication among people living with HIV (PLWHIV), particularly in sub-Saharan Africa, where nutritional deficiencies and suboptimal ART adherence are prevalent. Emerging evidence implicates dysregulation of iron homeostasis, rather than inadequate intake, as a central driver of HIV-associated iron deficiency anaemia (IDA), marked by restricted iron availability for erythropoiesis despite preserved or elevated iron stores (Masini *et al.*, 2022; Mwangi *et al*., 2021)

2.2 Regulation of Iron Homeostasis

At the core of systemic iron regulation are three proteins: erythroferrone, hepcidin, and hephaestin. Each of these has a distinct role in balancing iron mobilization, absorption, and transport. Hepcidin, a hepatic hormone upregulated by inflammation and iron overload, degrades ferroportin, thereby inhibiting iron efflux from enterocytes and macrophages. Erythroferrone, secreted by erythroblasts in response to erythropoietin, counter-regulates hepcidin during heightened erythropoietic demand, enhancing iron availability. Hephaestin, a ferroxidase expressed in enterocytes, oxidizes Fe^{2+} to Fe^{3+} for transferrin loading and systemic transport (Chambers *et al*., 2025; Coffey & Ganz, 2018; Sangkhae *et al.*, 2021).

2.3 Effects of HIV on Iron Homeostasis

HIV infection alters iron homeostasis through chronic inflammation (Akase *et al.*, 2019). Elevated IL-6 induces hepcidin, restricting iron absorption and trapping iron in macrophages. Inflammation also suppresses erythropoietin and erythroferrone, removing a key brake on hepcidin, further limiting iron availability (Domingos *et al*., 2020). Though this pathway is known, erythroferrone's role in HIV-associated anaemia remains poorly studied, especially in African contexts. Hephaestin, critical for intestinal iron export, is also understudied in HIV. Its downregulation in inflammatory models suggests similar suppression in untreated HIV. While ART reduces inflammation and partially restores iron regulation, anaemia often persists, particularly in non-adherent individuals. Most existing studies rely on ferritin or transferrin, which are unreliable in inflammatory states. This study assessed erythroferrone, hepcidin, and hephaestin across ART adherence strata in Western Kenya, linking them to iron deficiency anaemia. The findings aim to clarify how ART and immune status influence iron availability and inform better diagnostic and treatment strategies.

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 as per the MMAS-8 adherence score (De las Cuevas & Penate, 2015) and Healthy Control, n = 51. Healthy controls were the HIV antibody seronegative with the rapid immunochromatographic test, Determine^{TM} (Abbot Laboratories, Tokyo, Japan). Participants who had been on ART for 6–12 months at the time of enrolment were included to ensure adequate exposure for measurable effects on iron metabolism, while still representing the early treatment phase when metabolic alterations are most evident as per Abioye *et al*., (2023). 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 haematologic disorders such as sickle cell disease, thalassemia, or leukemia. Comorbidities were ascertained through a combination of medical record review and participant self-report during enrolment interviews.

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. For the healthy control group (HC), 112 HIV-negative individuals were initially recruited. After exclusions due to withdrawal of consent (n = 29), borderline anaemia (n = 25), HBV positivity (n = 5), and sample loss during transport (n = 2), the final HC sample size was 51. 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 (NIH, 2024) 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 Blood Sample Collection

Approximately 10 ml venous blood was collected from each participant by certified experienced phlebotomist as per the (WHO, 2010) guidelines and aliquoted in 5 ml EDTA vacutainers for determination of haemoglobin concentration, viral load (VL) and CD4+ T cells count and 5 ml serum separator tubes (SST) for evaluation of erythroferrone, hepcidin and hephaestin levels. All samples were labelled with unique participant codes to prevent misidentification. Haemoglobin concentrations in fresh EDTA samples were determined immediately. Enumeration of CD4+ T cells and VL determination were done within 6 hours post phlebotomy. Clotted samples in SST were centrifuged at 1500 revolution per minute for 10 minutes and sera were stored at -20^0C awaiting analyses (Valo *et al*., 2022).

3.5 Determination of markers of Iron Deficiency Anaemia

Haemoglobin concentrations were measured using Coulter ACT 5diff analyzer (Beckman Coulter, France), following stringent quality control procedures involving normal, low and high-concentration blood controls to ensure accurate and reliable results. 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). Serum ferritin levels (µg/L) were measured using Beckman Coulter AU 5800 (Brea, California, USA) by turbidimetry as previously described by (Omuse *et al*., 2020a). Iron deficiency was defined as ferritin < 70 µg/L in line with previous studies in inflammatory states (Masini *et al*., 2022; Omuse *et al.*, 2022; WHO, 2024). Anaemic subjects with serum ferritin < 70 µg/L were considered to have iron deficiency anaemia (Omuse *et al*., 2022).

3.6 Determination of Iron Regulatory Proteins

Serum erythroferrone, hepcidin, and hephaestin levels were determined by sandwich ELISA using commercially available kits according to the manufacturers' instructions. Erythroferrone was measured using the Human Protein *fam132b* (erythroferrone) ELISA Kit (Cusabio Biotech Co., Ltd.), hepcidin was quantified using the Hepcidin-25 (bioactive) HS ELISA Kit (DRG International, Inc.), and hephaestin was assessed using the Human *heph* (hephaestin) ELISA Kit (ELK Biotechnology).

3.7 Statistical Analysis

Statistical analyses were conducted in GraphPad Prism version 9 (GraphPad Software, San Diego, California USA) (Motulsky, 2019). Categorical variables (gender, iron and anaemia categories) were analyzed by Chi square test while Continuous variables (age, ferritin, haemoglobin concentration, hepcidin, erythroferrone and hephaestin) were compared across study groups using Kruskal-Wallis test followed by Dunn's *post-hoc* correction for multiple comparisons due to data non-normality. Binary logistic regression was performed to assess the association between iron deficiency anaemia, ART status and iron regulatory proteins. For regression analyses, group exclusions were applied according to the comparison of interest: in models comparing adherent with non-adherent participants, healthy controls and ART -naive individuals were excluded, while in models comparing adherent with ART-naive participants, healthy controls and non-adherent individuals were excluded. Although co-infections were excluded at recruitment, the models were not adjusted for other potential confounders such as nutritional status or subclinical inflammation. All tests were two-tailed and *P* values < 0.05 were considered statistically significant.

IV. FINDINGS & DISCUSSION

4.1 Demographic Characteristics and Markers of Iron Deficiency Anaemia of the Study Participants

The demographic characteristics and markers of iron deficiency anaemia of the study subjects are summarized in Table 1. The current study enrolled 163 participants comprising of 112 PLWHIV sub-categorized by ART exposure and adherence into ART non-adherent (NA, n = 42), ART- naive (n = 23), ART- adherent (A, n = 47), and healthy controls (HC, n = 51). The median age was comparable across the study groups, ranging from 36.5 years in the ART non- adherent group to 38.0 years in the ART- naive group (P = 0.838). Gender distribution showed a male preponderance in the non-adherent group (59.5 %) compared to 39.1% in the ART-naive, 31.9% in the ART-adherent, and 47.1% in the healthy controls, with a trend toward statistical significance (P = 0.065). Serum ferritin levels were significantly lower among non-adherent participants (median: 57.9 µg/L; IQR: 37.5) compared to the ART-adherent group (median: 114.9 µg/L; IQR: 78.9; $P < 0.0001$) and healthy controls (median: 211.8 µg/L; IQR: 121.4; $P < 0.0001$). Similarly, ART-naive individuals had markedly reduced ferritin levels (median: 53.5 µg/L; IQR: 52.4) compared to both the Adherents and healthy control groups ($P < 0.0001$). Ferritin concentrations in the Adherent group were also significantly lower than in the healthy control group (P = 0.0005). Overall, iron deficiency iron deficiency, defined by low serum ferritin levels, was present in 31.3% of the study population. The burden of iron deficiency was highest in the non-adherent (64.3%), followed by the naive groups (56.5%) compared to the Adherent category (23.4%) ($P <$ 0.0001). Haemoglobin concentrations were significantly lower among HIV-infected individuals, particularly in the ART-naive (12.0 g/dL) and non-adherent (12.1 g/dL) groups, compared to healthy controls (13.9 g/dL; $P < 0.0001$). The median haemoglobin level in the ART-naive group was also significantly lower than in the ART-adherent group (12.9 g/dL; $P < 0.05$). Additionally, the ART-adherent group had significantly lower haemoglobin levels than healthy controls ($P < 0.05$). Anaemia prevalence was highest in the non-adherent group (61.9%), followed by the naive (52.2%) and adherent (36.2%) groups. Iron deficiency anaemia (IDA) was almost exclusively concentrated in the HIV-infected groups, with the highest burden among non-adherents at 65.4%, followed by ART-naive at 50.0% and ART-adherent participants at 17.6% (P = 0.009). Overall, these findings demonstrate a clear gradient of iron metabolism disruption and anaemia severity, most pronounced among ART non-adherent and ART-naive individuals, while ART adherence confers a partial protective effect.

Table 1

Demographic Characteristics and Markers of Iron Deficiency Anaemia of the Study Subjects

Characteristics	HC, n = 51	A, 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)	0.065
Male	24 (47.1)	15 (31.9)	9 (39.1)	25 (59.5)	
Ferritin, µg/L	211.8 (121.4)	114.9 (78.9)^{b}	53.5 (52.4)^{c}	57.9 (37.5)^{c}	**<0.0001**
Iron deficiency, n (%)	0 (0.0)	11 (23.4)	13 (56.5)	27 (64.3)	**<0.0001**
Haemoglobin, g/dL	13.9 (2.0)	12.9 (2.0)^{a}	12.0 (2.1)^{a,c}	12.1 (1.0)^{c}	**<0.0001**
Anemic, n (%)	0 (0.0)	17 (36.2)	12 (52.2)	26 (61.9)	**<0.0001**
IDA, n (%)	-	3 (17.6)	6 (50.0)	17 (65.4)	**0.009**

Values are presented as median (interquartile range) for continuous variables and n (%) for categorical variables. HC = healthy controls; A = ART-adherent HIV-infected participants; Naive = ART-unexperienced HIV-infected participants; NA = ART non-adherent HIV-infected participants. *P*-values were derived from the Kruskal–Wallis test for continuous variables and Chi-square test for categorical variables. Ferritin; $^{b}P < 0.001$ vs. HC, $^{c}P < 0.0001$ vs. A and HC; Haemoglobins, $^{a}P < 0.05$ vs. A and HC, $^{c}P < 0.0001$ vs. HC. Iron deficiency was defined as ferritin <30 µg/L. Anaemia was defined as haemoglobin <13 g/dL in males and <12 g/dL in females. Iron deficiency anaemia was diagnosed when both criteria for iron deficiency and anaemia were met.

4.2 The Levels of Iron Regulatory Proteins across Study Groups

The levels of iron regulatory proteins across and between study groups are summarized and presented in Table 2 and Figure 1. The results of this study reveal marked alterations in the iron regulatory pathways, showing a strong association with ART status. Significantly lower erythroferrone levels were observed in the NA group (median: 31.2 ng/mL; IQR: 13.8) compared to the HC group (median: 50.2 ng/mL; IQR: 48.3; $P < 0.0001$). Similarly, ART-naive subjects exhibited reduced erythroferrone concentrations (median: 21.7 ng/mL; IQR: 23.9) compared to both the A group (median: 38.1 ng/mL; IQR: 42.1; P = 0.0246) and HC group ($P < 0.0001$). Conversely, elevated hepcidin levels were noted in the NA group (median: 84.1 ng/mL; IQR: 46.4) relative to the A (median: 41.7 ng/mL; IQR: 21.1; $P <$

0.0001) and HC (median: 30.5 ng/mL; IQR: 18.8; $P < 0.0001$) groups. ART-naïve participants also demonstrated significantly higher hepcidin levels compared to both A and HC groups. Additionally, the A group exhibited elevated hepcidin concentrations compared to the HC group ($P = 0.0262$). Regarding hephaestin, the NA group showed significantly lower levels (median: 14.9 µg/L; IQR: 14.1) compared to the A (median: 25.1 µg/L; IQR: 10.7; $P = 0.0001$) and HC (median: 35.1 µg/L; IQR: 14.5; $P < 0.0001$) groups. Similarly, hephaestin levels were lower in the ART-naïve group (median: 20.4 µg/L; IQR: 10.3) relative to both the A ($P = 0.0349$) and HC ($P < 0.0001$) groups. The A group also had significantly reduced hephaestin levels compared to the HC group ($P = 0.0001$).

Table 2

The Iron Regulatory Proteins of the Study Participants

Characteristics	**HC, n=51**	**A, n=47**	**Naive, n=23**	**NA, n=42**	***P*-value**
Erythroferrone, ng/mL	50.2 (48.3)	38.1 (42.1)	21.7 (23.9)	31.2 (13.8)	**<0.0001**
Hepcidin, ng/mL	30.5 (18.8)	41.7 (21.1)	113.0 (54.8)	84.1 (46.4)	**<0.0001**
Hephaestin, µg/L	35.1 (14.5)	25.1 (10.7)	20.4 (10.3)	14.9 (14.1)	**<0.0001**

Data are presented as median and IQR for erythroferrone, hepcidin and hephaestin. 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 Kruskal-Wallis test followed by Dunn's *post hoc* test for multiple comparison. Significant *P*-values are in bold. *P-Value* <0.05 was considered statistically significant.

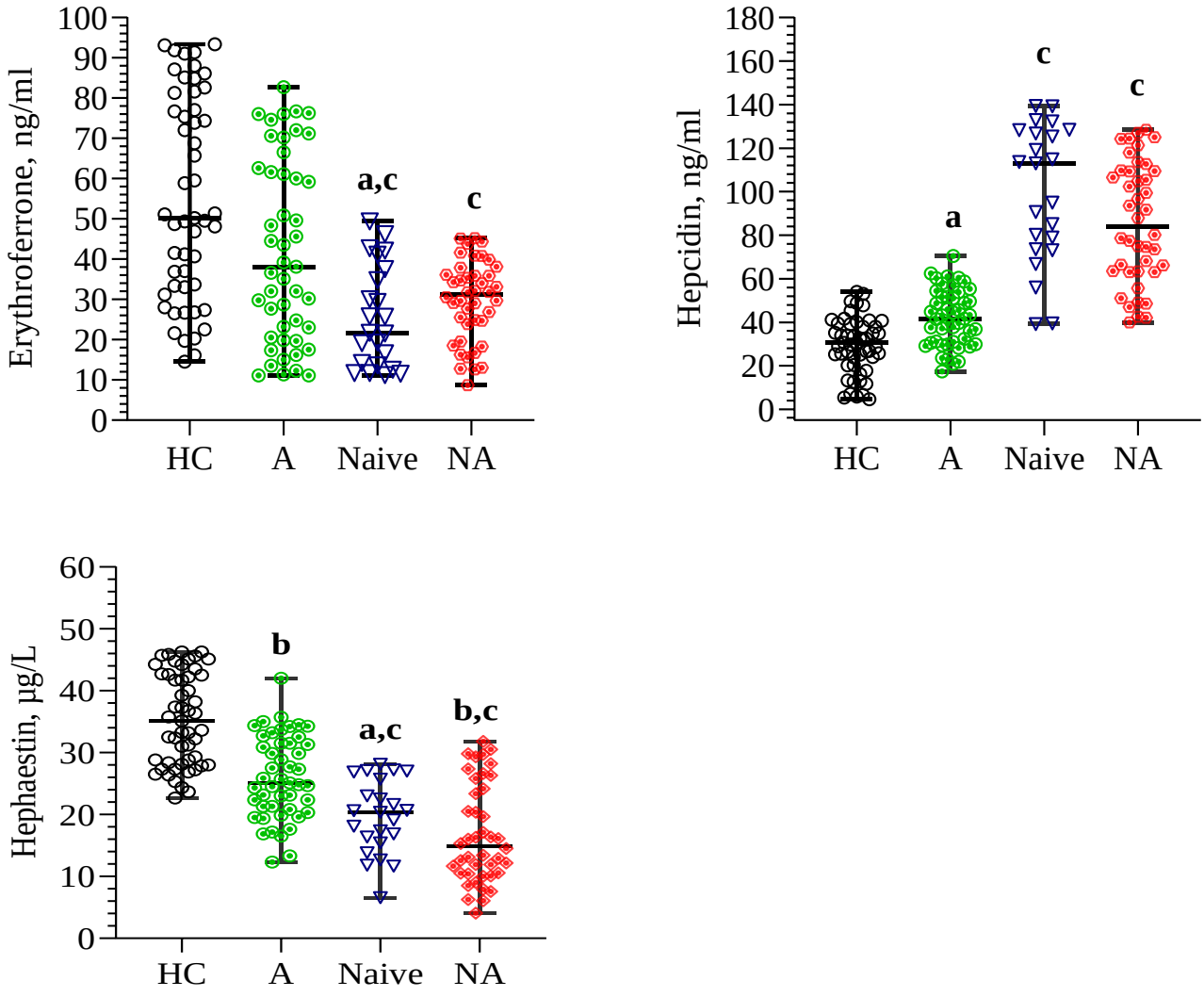

Figure 1

Pairwise Comparisons of Iron Regulatory Proteins between the Study Groups

Data are presented as scatter dot plots. Line through the scatter dot plots represents the median, and the lower and upper error bars indicate 10th and 90th percentiles respectively. Scatter dots beyond the error bars represent outliers. NA, PLWHIV ART non-adherent; Naïve, 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. Erythroferrone; $^{a}P < 0.05$ vs. A, $^{c}P < 0.0001$ vs. HC; Hepcidin; $^{a}P < 0.05$ vs. HC, $^{c}P < 0.0001$ vs. A and HC; Hephaestin, $^{a}P < 0.05$ vs. A, $^{b}P < 0.001$ vs. A and $^{c}P < 0.0001$ vs. HC. *P-value* <0.05 was considered statistically significant. Significant *P*-values are in bold.

4.3 Association of iron deficiency anaemia with ART status, and iron regulatory proteins in HIV infection

The association of iron deficiency anaemia with ART status, and iron regulatory proteins are summarized in Table 3. ART status demonstrated a strong relationship with IDA development in HIV disease. The ART non-adherent individuals were almost 10 times more likely to develop IDA compared to ART-adherent participants (AOR = 9.97; 95% CI: 2.66–37.41; $P = 0.001$). Likewise, the ART-naive participants had a fivefold increased risk of developing IDA compared to their ART-adherent counterparts (AOR = 5.18; 95% CI: 1.16–23.08; $P = 0.031$). On the other hand, higher erythroferrone levels were protective against IDA. Participants with erythroferrone levels above 50 ng/mL had over 90% reduced risk of IDA compared to those with levels ≤50 ng/mL (AOR = 0.094; 95% CI: 0.01–0.72; $P = 0.022$).

Conversely, elevated hepcidin levels significantly increased the likelihood of IDA. Participants with hepcidin concentrations >50 ng/mL were more than three times as likely to develop IDA compared to those with lower levels (AOR = 3.36; 95% CI: 1.36–8.25; $P = 0.008$). Lower hephaestin levels were positively associated with the risk of IDA. For every 1 µg/L decrease below 35 µg/L, there was a 13.7% increased likelihood of IDA (AOR = 1.137; 95% CI: 1.073–1.204; $P < 0.0001$).

Table 3

Association of Iron Deficiency Anaemia with ART Status, and Iron Regulatory Proteins in HIV Infection

Variable	B (SE)	Adjusted Odds Ratios (95% CI)	*P*-value
ART Status Adherent (Ref.) ART NA	 2.300 (0.674)	 1 9.973 (2.659 – 37.405)	 **0.001**
ART Status Adherent (Ref.) ART Naive	 1.644 (0.763)	 1 5.176 (1.161 – 23.076)	 **0.031**
Erythroferrone, ng/mL ≤50 (Reference) >50	 - 2.368 (1.037)	 1 0.094 (0.012 – 0.715)	 **0.022**
Hepcidin, ng/mL ≤50 (Reference) >50	 1.210 (0.459)	 1 3.355 (1.364 – 8.252)	 **0.008**
Hephaestin, µg/L >35 (Reference) ≤ 35	 0.129 (0.029)	 1 1.137 (1.073 – 1.204)	 **< 0.0001**

B = regression coefficient; SE = standard error; Adjusted Odds Ratio (AOR) obtained from multivariate logistic regression; CI = confidence interval; Ref. = reference category. ART Non-adherent = individuals on ART but not adherent; ART Naïve = ART-unexperienced; ART Adherent = reference group. AORs reflect the likelihood of developing iron deficiency anaemia compared to the reference categories after adjusting for all variables in the model.

4.5 Discussion

This study provides a comprehensive analysis of iron regulatory protein dynamics, specifically erythroferrone, hepcidin, and hephaestin, in relation to ART adherence among people living with HIV (PLWHIV) in Western Kenya. The findings reveal distinct biomarker patterns across ART-adherent, non-adherent, and ART-nave individuals, highlighting significant disruptions in iron metabolism that correspond closely with ART status. These disruptions appear to drive the burden of iron deficiency anaemia (IDA),

Anaemia prevalence was highest among ART non-adherent individuals at 61.9 %, followed by ART-naive at 52.2 % and ART-adherent participants at 36.2%. This distribution is consistent with prior studies in similar populations, including those by Aemro *et al*., (2022) and Sah *et al*., (2020), which emphasized that both delayed ART initiation and poor adherence are strong predictors of anaemia. The markedly lower ferritin levels in ART-naive and non-adherent individuals further support the presence of absolute or functional iron deficiency, a pattern that has also been documented by Abioye *et al*., (2020) and Obeagu *et al*., (2024). In contrast, ART-adherent individuals had higher ferritin

and haemoglobin levels, although still below those of healthy controls, suggesting that ART exerts a partial corrective effect on haematologic parameters but does not fully normalize iron metabolism.

A key finding in this study was the suppression of erythroferrone among ART- naive and non-adherent participants. Erythroferrone is a bone marrow-derived hormone that plays a central role in iron mobilization by downregulating hepcidin during active erythropoiesis. Participants with erythroferrone levels above 50 ng/mL had over 90% reduced odds of IDA compared to those with lower levels, indicating a potent protective effect. This supports previous observations by Babar & Saboor, (2024) and (Ru *et al*., 2024), who demonstrated that erythroferrone is an essential intermediary between erythropoietin signalling and iron release from storage. The suppressed erythroferrone levels observed in ART-naive and non-adherent groups may be due to reduced erythropoietin activity, a consequence of chronic HIV-induced inflammation and cytokine interference. These findings underscore the relevance of erythroferrone as both a diagnostic and therapeutic target in HIV-related anaemia.

Concomitantly, this study observed significant elevations in hepcidin among the ART-naive and non-adherent groups. Hepcidin acts as the master regulator of systemic iron homeostasis by inhibiting iron export via ferroportin degradation. Elevated hepcidin in these groups likely reflects persistent immune activation and interleukin-6 upregulation which are hallmarks of untreated or poorly treated HIV. Logistic regression analysis confirmed that hepcidin levels above 50 ng/mL were independently associated with more than a threefold increased likelihood of IDA. These findings align with those from Camaschella *et al*., (2020b) and Kesharwani *et al.*, (2025), which showed that elevated hepcidin under inflammatory conditions leads to iron sequestration within macrophages and enterocytes, thereby limiting its bioavailability for erythropoiesis. Even among ART-adherent participants, hepcidin levels remained higher than in healthy controls, suggesting that ART, although effective in reducing systemic inflammation, may not fully normalize hepcidin regulation. This residual elevation could explain the persistent anaemia observed in a significant proportion of treated individuals.

Hephaestin, a ferroxidase involved in converting ferrous to ferric iron to facilitate transferrin loading in enterocytes, was also significantly suppressed in ART-naive and non-adherent individuals. Participants with lower hephaestin levels were found to have a 13.7% increased likelihood of IDA for every unit decrease below the 35 µg/L reference point. While this protein has not been extensively studied in human HIV cohorts, animal models and inflammatory bowel disease studies have documented its downregulation in chronic inflammatory states. The data presented here suggest that in the context of HIV, reduced hephaestin may contribute to impaired dietary iron absorption and transport, particularly when ART is absent or inconsistently used. These findings expand upon the work of Chen *et al*., (2003) and Doguer *et al*., (2018), and position hephaestin as a novel but crucial player in the pathophysiology of HIV-related anaemia.

Together, these findings highlight the complex interplay between erythropoietic drive, systemic inflammation, and iron transport mechanisms. The biomarker patterns identified in this study illustrate a coordinated disruption of iron metabolism in ART-naive and non-adherent individuals, suppressed erythroferrone impairs hepcidin regulation, elevated hepcidin promotes iron sequestration, and reduced hephaestin limits intestinal iron export. ART adherence appears to mitigate this disruption, though not completely, as evidenced by residual abnormalities in iron biomarkers among adherent individuals. This suggests that while ART is critical for haematologic recovery, adjunctive strategies may be needed to fully restore iron homeostasis.

Clinically, these findings suggest a potential role for expanded iron biomarker panels in the diagnosis and management of anaemia among PLWHIV. The combined assessment of erythroferrone, hepcidin, and hephaestin provides a more nuanced understanding of the underlying causes of anaemia than conventional markers like ferritin or transferrin alone. Furthermore, monitoring these biomarkers could inform individualized treatment strategies, including the careful use of iron supplements, erythropoiesis-stimulating agents, or anti-inflammatory therapies.

Despite its strengths, including the robust biomarker analysis and strict sub-categorization by ART status, this study has some limitations. The cross-sectional design limits causal inference, and the lack of inflammatory cytokine data (e.g., IL-6, TNF-α, CRP) prevents direct correlation between immune activation and biomarker levels. Dietary iron intake, gastrointestinal health, and other comorbidities that might influence iron metabolism were not assessed. Future longitudinal studies are needed to determine the temporal relationship between ART initiation, immune recovery, and normalization of iron regulatory pathways. Additional research is also warranted to validate the utility of erythroferrone and hephaestin as clinical biomarkers or therapeutic targets in HIV-associated anaemia.

V. CONCLUSION & RECOMMENDATION

5.1 Conclusion

Poor adherence and naivety to ART are strongly associated with a higher risk of iron deficiency anaemia in HIV-infected individuals, mediated through suppression of erythroferrone, elevation of hepcidin, and reduction of hephaestin. ART adherence ameliorates these disruptions but fails to achieve complete normalization.

5.2 Recommendation

Incorporation of iron regulatory biomarkers, erythroferrone, hepcidin, and hephaestin, into routine clinical evaluation of anaemia in HIV is warranted to improve diagnostic precision, with sustained ART adherence remaining essential for restoring iron homeostasis and achieving durable haematologic recovery.

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