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Anemia in HIV-Infected Children on ART in Uganda: High Risk Associations with Malaria, Opportunistic Infections, and Poor Adherence

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Purpose: This study aimed to determine the prevalence and associated factors of anemia among HIV-positive children receiving antiretroviral therapy (ART) at two urban clinics in Mbarara City, Southwestern Uganda.

Patients and Methods: We conducted a cross-sectional study among 293 HIV-positive children (<18 years) attending ART clinics at Mbarara Municipal Health Center IV and Holy Innocent Children's Hospital between July and September 2025. A structured questionnaire captured sociodemographic and clinical data. Venous blood were collected for complete blood count (CBC) analysis, and stool samples were examined for intestinal parasites. Data on CD4 count and viral load were abstracted from medical records. Anemia was defined using age-specific WHO criteria. Bivariate and multivariate logistic regression analyses were performed to identify factors associated with anemia, with statistical significance set at $p \leq 0.05$.

Results: The overall prevalence of anemia was 6.14% (95% CI: 3.4% – 8.9%). Normocytic normochromic anemia was the predominant morphological type (72.2%). In the multivariate analysis, a recent episode of malaria (AOR = 9.61; 95% CI: 3.26–28.34), the presence of an opportunistic infection (AOR = 6.78; 95% CI: 1.28–35.83), and poor adherence to ART (AOR = 14.75; 95% CI: 1.31–165.65) were independently and significantly correlated with anaemia.

Conclusion: While the prevalence of anemia in this cohort of HIV-infected children on ART was lower than global averages, it remains a significant clinical concern. The strong associations with malaria, opportunistic infections, and suboptimal ART adherence highlight critical areas for intervention. Targeted strategies, including integrated malaria control, enhanced prevention and management of OIs, and reinforced adherence support, are required to decrease the anaemia burden and optimize health outcomes in this at-risk population.

Keywords: anemia, HIV, children, antiretroviral therapy, ART, Uganda

Introduction

Anemia constitutes a significant global health burden among children living with HIV. Defined according to World Health Organization criteria as hemoglobin concentration below age-specific cut-offs, this condition affected an estimated 1.7 million HIV-positive children worldwide in 2020.¹ The intersection of anemia and HIV is particularly critical in sub-Saharan Africa, the region with the highest burden of pediatric HIV infection. Here, anemia is not merely a common complication but a well-documented, independently associated with faster disease progression and higher mortality.^{2,3}

The etiology of anemia in pediatric HIV is complex and multifactorial. It arises from the interplay of four principal domains: disease-related mechanisms, including advanced immunological stage, high HIV viral load, and concomitant infections like tuberculosis, malaria, and parasitic infestations; treatment-related effects, particularly the use of myelo-suppressive antiretroviral agents such as Zidovudine and the time elapsed since ART initiation; nutritional insufficiencies, most commonly involving iron, vitamin B12, and vitamin A; and socio-demographic determinants, such as low parental education and rural residence, which influence healthcare access and nutritional status.⁴⁻⁶

The widespread availability of antiretroviral therapy (ART) has transformed the hematological profile of children living with HIV. Although ART has markedly decreased the incidence of severe, HIV-associated anemia, a considerable prevalence of mild-to-moderate anemia continues to affect this population despite treatment.⁷ This persistent burden is exemplified by a study in Burkina Faso, which reported anemia prevalence declining from 81.5% prior to ART initiation to 48.3% after one year of therapy, a rate that nevertheless indicates a significant ongoing problem.¹ Contemporary data from Ethiopia further demonstrate considerable heterogeneity in post-ART anemia, with prevalence estimates ranging from 13.3% to 23.8%.^{6,8} This variation highlights the sustained importance of local contextual and co-existing factors in determining hematological outcomes.

Evidence regarding anemia among children with HIV in Uganda remains sparse and highly setting-dependent. Research from the national referral hospital in Kampala reported a prevalence of 50.7% in 2020,⁹ yet findings from this tertiary center are not necessarily applicable to standard care facilities. A significant evidence gap exists for urban primary care clinics in Southwestern Uganda, a region with unique demographic and health profiles. Furthermore, most studies in the area have assessed anemia in the broader child population, not focusing on those receiving antiretroviral therapy (ART).^{10,11}

To address this gap, this study aimed to determine the prevalence and identify the associated factors of anemia among children living with HIV and receiving ART at two urban clinics in Southwestern Uganda. The findings are intended to inform targeted clinical and public health strategies.

Materials and Methods

Study Site

This study was conducted at two ART-providing health facilities in Mbarara City, Southwestern Uganda. The first site, Mbarara Municipal Health Center IV, is a government-aided facility located near the city offices along the main highway. The second site, Holy Innocent Children's Hospital, is a Private Not-for-Profit (PNFP) facility situated approximately 5km from the city center along Isingiro Road. Mbarara City shares borders with Kiruhura, Mbarara, Isingiro, Rwampara, and Sheema districts. Both facilities have comprehensive service departments, including dedicated ART clinics, laboratories, outpatient departments (OPD), and maternity units.

Study Design

We used a cross-sectional study design to determine the prevalence and identify factors associated with anemia in a cohort of pediatric patients attending ART clinics at two health facilities in Mbarara City, Southwestern Uganda: Mbarara Municipal Health Centre IV and Holy Innocent Children's Hospital.

Study Population

The study population comprised all children under 18 years of age receiving ART care at the two health facilities. These facilities were purposively selected as they serve a large, stable cohort of pediatric ART patients in Mbarara City, Southwestern Uganda.

Sample Size Determination

The sample size was calculated using the Kish-Leslie (1965) single population proportion formula to estimate the prevalence of anemia among HIV children on ART.

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where:

n = study sample size

Z= Standard normal deviate corresponding to 95% confidence interval (= 1.96)

P = Prevalence of anemia (22.3% prevalence of anemia, based on a Ethiopian study.¹²

d = margin of error on P (set at 5%)

Calculation:

$$n = \frac{1.96 \times 1.96 \times 0.22.3(1 - 0.777)}{0.05 \times 0.05}$$

n = 266

To compensate for potential participant attrition, the sample size was increased by 10%, yielding a final sample size of 293 study participants.

Sampling Procedure

A systematic sampling technique was employed to ensure each patient had an equal probability of selection. From a total target population of 316 individuals, a calculated sample size of 293 participants was drawn. Proportional allocation was used to distribute the sample across the two study sites based on their respective patient populations. The proportions were calculated as 78/316 (0.2468) for Holy Innocent Children's Hospital and 238/316 (0.7531) for Mbarara Municipal Health Centre IV. Consequently, the final sample consisted of 72 participants (0.2468×293) from the former and 221 participants (0.7531×293) from the latter.

Inclusion Criteria

Children and adolescents (<18 years) with a confirmed HIV diagnosis who were active clients of the ART clinics at either study site were included.

Exclusion Criteria

Participants were excluded if they had received a blood transfusion or iron supplementation therapy within the preceding three months.

Data Collection Procedure

A structured questionnaire was administered to collect socio-demographic data (age, sex, residence, educational level, marital status) and information on factors associated with anemia. These factors included a history of malaria in the preceding month, a family history of anemia, the presence of opportunistic infections (eg, tuberculosis), unsuppressed viral load (VL > 1000 copies/mL), low CD4 count (<200 cells/ μ L), and symptoms suggestive of parasitic infection. All completed questionnaires were checked for completeness before data entry.

Laboratory Methods

For hematological analysis, 4 mL of venous blood was collected from each participant into an EDTA tube. Hemoglobin concentration was measured using an automated hematology analyzer (SYSMEX XN-550). Anemia was defined according to the World Health Organization hemoglobin cutoffs, with thresholds of <11.0 g/dL for children aged 1–5 years, <11.5 g/dL for those aged 5–11 years, and <13.0 g/dL for individuals aged 12–18 years.¹³ For participants identified as anemic, both thick and thin blood films were prepared on microscope slides for morphological classification of anemia and to investigate potential causes, such as malaria. Additionally, a fresh stool sample was collected from each participant for macroscopic and microscopic examination to identify ova, cysts, or trophozoites of intestinal parasites. Data on most recent CD4+ T-lymphocyte counts and viral load results were abstracted from the participants' existing medical records.

Quality Control and Assurance

All laboratory procedures, including complete blood count (CBC) analysis and Giemsa staining for thick and thin blood film examination, followed validated Standard Operating Procedures (SOPs). The SYSMEX XN-550 hematology analyzer was calibrated using manufacturer-supplied calibrators. Furthermore, low, normal, and high internal quality control (IQC) samples were run daily to verify the accuracy and reproducibility of results. For Giemsa staining, known malaria positive and negative reference blood smears were processed concurrently with study samples to validate staining performance. The facility's participation in External Quality Assessment (EQA) schemes was reviewed, and its performance records were found to be satisfactory.

Data Analysis

Following data collection, information was cleaned, coded, and securely archived in both hard and soft copy formats. Data entry were performed using Microsoft Excel and subsequently exported to STATA version 16 for statistical analysis. The prevalence of anemia was calculated as a percentage with a corresponding 95% confidence interval (CI). Categorical variables were compared using the Chi-square (χ^2) test, with statistical significance defined as a p-value < 0.05. To identify factors associated with anemia, logistic regression analysis was employed. All variables demonstrating an association at $p < 0.1$ in bivariate analyses were entered into a multivariable logistic regression model. The strength and significance of associations were expressed as adjusted odds ratios (aOR) with 95% CIs and p-values. Results are presented in summary tables within the manuscript.

Ethical Considerations

The study was conducted in accordance with the principles of the declaration of Helsinki. Ethical approval for this study was granted by the Faculty of Medicine Research Committee (Reference NO: MUST/MLS/25-03M) at Mbarara University of Science and Technology. Administrative clearance was also obtained from the management of Mbarara Municipal Health Center IV and Holy Innocent Children's Hospital prior to data collection. Informed consent and assent were both sought from parents/guardians and consent forms translated into the local language were used. To ensure full comprehension, the consent process was supplemented with a detailed verbal explanation in the participant's preferred language. Participation was entirely voluntary, and participants were assured that withdrawal from the study would not compromise their current or future access to healthcare services. All collected data were anonymized and stored securely, with access limited to the principal investigators to ensure participant confidentiality.

Results

A total of 293 participants were enrolled in the study. The median age was 11 years (IQR: 8–15). As presented in [Table 1](#), the majority were female (54.3%), aged 12–18 years (48.4%), resided in urban settings (68.9%), had attained a primary-level education, and lived in households with four or more members (59.0%).

Prevalence of Anemia Among HIV Positive Children

The overall prevalence of anemia among HIV positive children was (6.14%; 95% CI 3.4% - 8.9%) as shown in [Figure 1](#).

Morphological Types of Anemia Among HIV Positive Children

Normocytic normochromic anemia was the predominant morphological type identified among anemic children, accounting for 13 (72.2%) of cases, followed by microcytic hypochromic anemia 4 (22.2%) and microcytic normochromic anemia 1 (5.6%), as illustrated in [Figure 2](#).

Factors Associated with Anemia Among HIV Positive Children

In the bivariate logistic regression analysis, a recent episode of malaria (AOR = 8.35; 95% CI: 2.41–28.98) and the presence of an opportunistic infection (AOR = 9.02; 95% CI: 1.18–68.82) were significantly associated with anemia among children with HIV, as detailed in [Table 2](#).

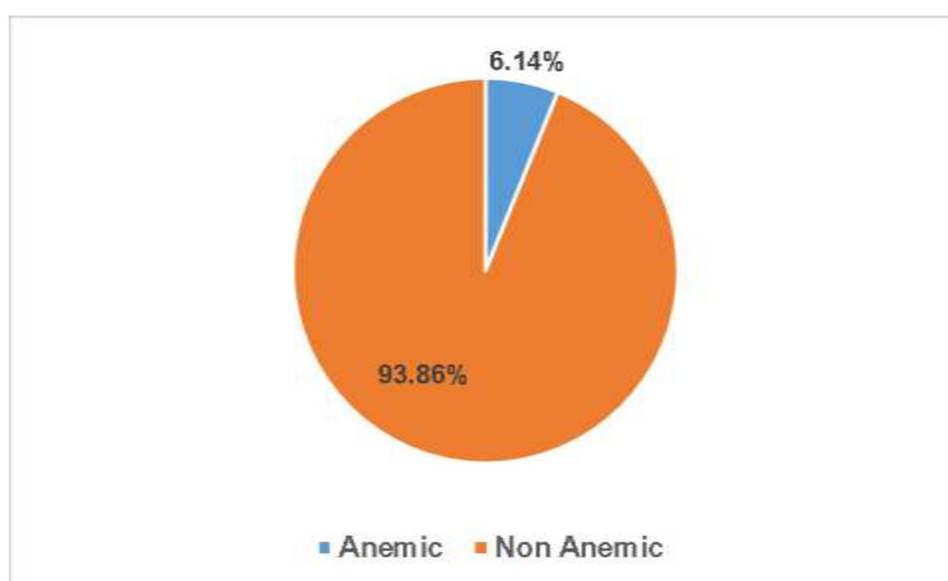
Table 1 Demographic Characteristics of Study Participants (N = 293)

Variable	Category	Frequency (n)	Percentage (%)
Gender	Female	159	54.3
	Male	134	45.7
Age (years)	1–5	33	11.3
	6–11	118	40.3
	12–18	142	48.4
Residence	Rural	91	31.1
	Urban	202	68.9
Education level	No formal education	32	10.9
	Primary	176	60.1
	Secondary	85	29.0
Family size	1–3	120	41.0
	≥4	173	59.0
Occupation (Guardian)	Unemployed	85	29.0
	Casual laborer	82	28.0
	Salaried job	45	15.4
	Self employed	81	27.6

All factors associated with anemia at the bivariate level ($p < 0.05$) and those with p -values ≤ 0.1 were included in a multivariate logistic regression model. As shown in Table 3, recent malaria infection (AOR = 9.61; 95% CI: 3.26–28.34, $P < 0.001$), opportunistic infection (AOR = 6.78; 95% CI: 1.28–35.82, $P = 0.024$), and poor ART adherence (AOR = 14.75; 95% CI: 1.31–165.65, $P = 0.029$) remained independently and significantly associated with anemia.

Discussion

The overall prevalence of anemia among children living with HIV in this study was 6.14%. This finding is consistent with rates reported in similar settings in Ethiopia, such as 6.87% and 7%.^{14,15} This is considerably lower than the rates reported in most previous studies conducted in similar settings in Uganda and across sub-Saharan Africa. A similar study in Mulago national referral hospital and Kayunga regional referral in Uganda reported a higher prevalence of 50.7% and 57.3%^{9,16} respectively. This could be attributed to success of ART regimens, improved nutritional support, and older age profile of the cohort.

**Figure 1** Prevalence of anemia among HIV positive children.

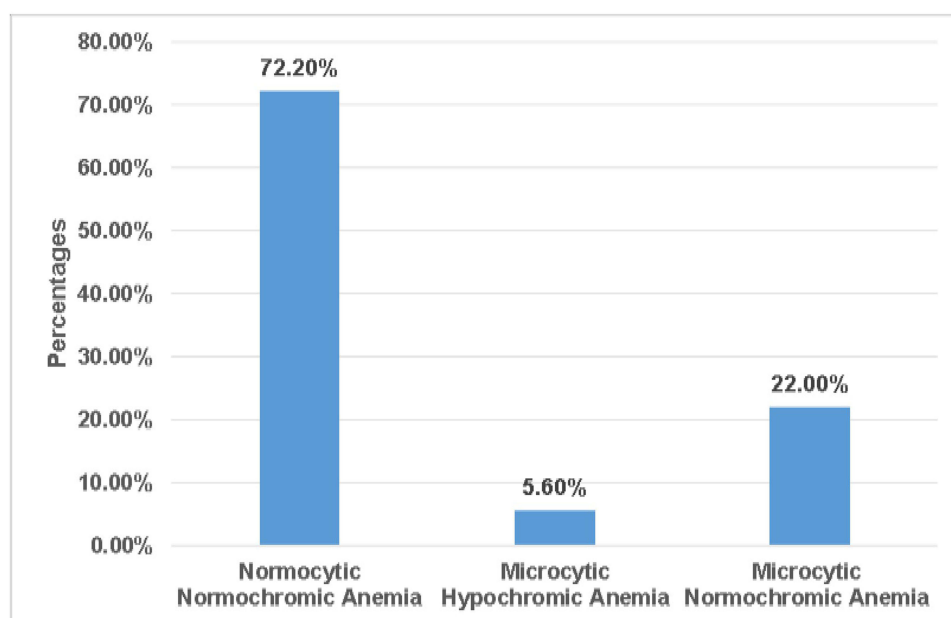


Figure 2 Morphological types of anemia among HIV positive children.

The wide variation in prevalence across studies, from our finding of 6.14% to rates exceeding 50% in some Nigerian pediatric HIV patients¹⁷ and in south east Asia cohorts,¹⁸ underscores the significant role of local context, including differences in ART regimens, nutritional status, co-infection rates (eg, malaria, helminths), and socioeconomic factors.

The most prevalent morphological type of anemia in this study was normocytic normochromic anemia, accounting for 72.2% of all cases. This finding aligns with several other studies conducted among HIV-positive populations in Ethiopia^{4,8,15} and strongly suggests that anemia of chronic disease (ACD), also known as anemia of inflammation, is the primary underlying mechanism. Even with effective ART, people living with HIV experience residual chronic immune activation and inflammation, which are now recognized as key drivers of hematological complications like anemia.^{19,20} This inflammatory state, characterized by elevated cytokines such as IL-6, leads to the sustained overproduction of the key iron regulator, hepcidin.^{21,22} Hepcidin binds to the iron transporter ferroportin, thereby sequestering iron within storage cells and inducing a state of functional iron deficiency, in which iron stores are present but inaccessible for erythropoiesis.²³ In our cohort, this mechanism is particularly relevant given that 72.2% of anemic children presented with normocytic anemia, the classic morphological presentation of hepcidin-mediated iron sequestration.

Concurrently, inflammatory cytokines directly suppress erythroid progenitor cells in the bone marrow and can blunt the erythropoietin response, further impairing erythropoiesis.²⁴ The net result of these processes is the impaired production of new red blood cells, manifesting as the normocytic, normochromic anemia observed in our cohort. This pathophysiology is consistent with our clinical finding that opportunistic infection, a potent inflammatory trigger, was a key predictor of anemia.

The predominance of normocytic anemia, as opposed to microcytic hypochromic anemia (which is characteristic of pure iron deficiency), indicates that the problem is not a lack of iron in the body per se, but rather a functional iron deficiency caused by the body's inflammatory response to the chronic HIV infection, even in the presence of antiretroviral therapy.²⁵ This aligns with our finding that opportunistic infections, a potent trigger of inflammation, were a significant predictor of anemia.

Recent episode of malaria infection was found significant with 9-fold risk of anemia among HIV positive children. This current finding is reinforced by studies carried out in Uganda⁹ and Ethiopia^{8,26} which also found a similar finding. Malaria caused by *Plasmodium* species results in the destruction of both infected and uninfected red blood cells, which are then cleared by the spleen. In addition, the sequestration of infected red blood cells within the microvasculature and the disruption of normal immune responses further contribute to the development of anemia.²⁷

Table 2 Bivariate Analysis of Factors Associated with Anemia Among HIV Positive Children

Variable		COR	95% CI	P value
Gender	Female	Ref		
	Male	1.96	0.58–6.69	0.281
Age (years)	2–5	Ref		
	6–11	2.88	0.25–33.78	0.399
	12–18	1.30	0.10–17.40	0.841
Education level	No formal education	Ref		
	Primary	0.18	0.02–1.48	0.112
	Secondary	0.43	0.05–3.82	0.449
Family size	1–3	Ref		
	≥4	2.81	0.71–11.20	0.143
Occupation (Parent)	Casual laborer	Ref		
	Unemployed	0.72	0.17–3.06	0.656
	Salaried job	0.54	0.08–3.54	0.521
	Self employed	0.55	0.11–2.82	0.471
Residence	Rural	Ref		
	Urban	0.52	0.15–1.76	0.292
Recent malaria episode	No	Ref		
	Yes	8.35	2.41–28.98	0.001*
Intestinal worms	No	Ref		
	Yes	0.59	0.03–11.76	0.727
Other opportunistic infections	No	Ref		
	Yes	9.02	1.18–68.82	0.034*
ART adherence percentage	≥95%	Ref		
	80–94%	12.85	0.84–196.77	0.067
Cotrimoxazole prophylaxis	No	Ref		
	Yes	2.28	0.28–18.33	0.437
ART regimen	ABC/3TC/DTG	Ref		
	TLD	0.27	0.02–3.71	0.329
	TDF/3TC/DTG	0.28	0.01–5.66	0.404
Viral load	Not detectable	Ref		
	Detectable	2.41	0.69–8.44	0.170

Note: *Significant.**Abbreviations:** COR, Crude odds ratio; CI, Confidence interval.**Table 3** Multivariate Analysis of Factors Associated with Anemia Among HIV Positive Children

Variable		Adjusted Odds Ratio	95% CI	P value
Education level	No formal education	Ref		
	Primary	0.34	0.09–1.25	0.105
	Secondary	0.32	0.07–1.48	0.143
Recent malaria episode	No	Ref		
	Yes	9.61	3.26–28.34	<0.001*
Other opportunistic infections	No	Ref		
	Yes	6.78	1.28–35.82	0.024*
ART adherence percentage	≥95%	Ref		
	80–94%	14.75	1.31–165.65	0.029*

Note: *significant.**Abbreviation:** CI, Confidence Interval.

Opportunistic infection was also found associated with anemia among HIV positive children with a 6-fold increase among the children with opportunistic infection. This finding is supported studies from Ethiopia.^{6,26,28} A potential explanation is that opportunistic infections in children can suppress appetite, resulting in malnutrition, which in turn is a major predictor of anaemia²⁹ and Some opportunistic infections are capable of widespread infiltration of the bone marrow, directly affecting hematopoietic progenitor cells.³⁰

HIV-positive children with poor ART adherence had a higher likelihood of being anaemic than their counterparts with good adherence. This finding is supported by a study from Ethiopia where also poor adherence to ART was found significant with high odds of anemia among HIV positive children.²⁶ This could be attributed to the fact that children with poor ART adherence are more prone to developing drug resistance, opportunistic infections (OIs), rapid disease progression, elevated viral load replication, and suppression of the immune system which in turn lead to anemia as reported in various studies.³¹ Caregivers most frequently attributed missed doses to forgetfulness, loss of control over the medication, travel, caregiver changes, falling asleep before the scheduled time.³² It is noteworthy that the confidence intervals for the Adjusted Odds Ratios, particularly for ART adherence, are very wide, reflecting the limited number of anemia cases available for this analysis.

The significance of these findings is twofold, with key implications for clinical application and subsequent research. Firstly, the predominance of normocytic anemia suggests that routine iron supplementation may not be the most appropriate first-line intervention for anemic children living with HIV. Rather than presumptive iron therapy, clinical efforts should prioritize the prompt identification and treatment of underlying inflammatory triggers, such as opportunistic infections and malaria. Secondly, the strong association observed between poor ART adherence and anemia underscores the critical need for interventions designed to support consistent adherence. Such efforts should specifically address the barriers commonly reported by caregivers, including forgetfulness and challenges in accessing medication. Thirdly, malaria prevention strategies particularly the use of insecticide-treated nets and, where appropriate, prophylactic antimalarial should be prioritized within this population. Looking ahead, future studies should utilize longitudinal designs to determine causality and to assess whether therapeutic strategies targeting inflammation (as opposed to iron deficiency) are effective in resolving anemia in this clinical context.

Limitations of the Study

Several methodological limitations should be considered when interpreting these findings. First, the cross-sectional design precludes the drawing of causal inferences, restricting our analysis to the identification of associations rather than definitive relationships. Secondly, the single-center setting As a result, the applicability of our findings to broader populations may be constrained with differing demographic profiles, ART regimens, or co-infection burdens. Third, the modest number of anemia cases [18/293], evidenced by wide confidence intervals surrounding certain adjusted odds ratios (particularly for ART adherence), necessitates cautious interpretation of these effect estimates. Fourth, the assessment of ART adherence relied on caregiver report, a method inherently susceptible to social desirability and recall bias, which may have resulted in overestimation of true adherence levels. Finally, the absence of laboratory biomarkers such as serum ferritin, C-reactive protein, or hepcidin precluded direct confirmation of the proposed pathophysiological mechanism (anemia of chronic disease). Notwithstanding these constraints, the consistency of our findings with established biological pathways and prior research lends credence to the conclusions drawn. The integration of these markers in future research or clinical practice is recommended.

Conclusion

Although this study found a lower prevalence of anemia among HIV-positive children compared to other reports, the condition still represents a public health issue in this setting and was linked to a recent episode of malaria, the presence of opportunistic infections, and poor adherence to ART. Nevertheless, the cross-sectional design prevents any causal conclusions, while the single-center nature, small number of anemic cases, use of caregiver-reported adherence, and absence of laboratory biomarkers (such as ferritin and CRP) restrict the generalizability and precision of the findings. Given these limitations, interventions aimed at preventing anemia are still recommended, along with future prospective, multicenter studies that include objective adherence measures and biomarker confirmation to enhance health outcomes in

this group. Clinical management should focus on proven strategies for malaria prevention, timely diagnosis and treatment of opportunistic infections, and strong adherence support systems, rather than routine iron supplementation. Additional research should involve longitudinal studies to establish causality and interventional trials to assess therapies that target inflammation.

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Disclosure

The authors report no conflicts of interest in this work.

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