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ORIGINAL RESEARCH

Anemia in HIV Patients Attending Highly Active Antiretroviral Therapy Clinic at Hoima Regional Referral Hospital: Prevalence, Morphological Classification, and Associated Factors

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Purpose: To determine the prevalence of anemia, the morphological classification and to assess the factors associated with anemia among HIV patients attending Highly Active Antiretroviral Therapy (HAART) clinic at Hoima Regional Referral Hospital.

Methods: This was a cross-sectional study among 340 participants attending the HAART clinic at Hoima Regional Referral Hospital. Participants were recruited using a simple random sampling technique. A complete blood count (CBC) was performed using the Sysmex XN-550 hematology analyzer. Thick films were made and examined for malaria parasites, while thin films were examined for the morphological classification of anemia. Bivariate and multivariate logistic analyses were conducted using SPSS (version 23).

Results: Out of the 340 study participants, 255 (75%) were females, and the median age was 39 years (range: 6–76 years). The overall prevalence of anemia among the study participants was 16.8% (95% CI 13.1–21.1). Normocytic normochromic anemia was the most prevalent form of anemia (47.4%). The logistic regression at multivariate analysis showed that age groups (18–27 years, p = 0.017; 28–37 years, p = 0.005; and ≥38 years, p = 0.009), divorced marital status (p = 0.024), the presence of chronic disease (p = 0.010), a family history of anemia (p = 0.007), and the presence of malaria in the past one month (p = 0.001), presence of opportunistic infection (OR = 58, p = 0.000), use of antihelminthic drug in the past 3 months (OR = 0.10, p = 0.003) and unsuppressed viral load (OR = 10.74, p = 0.000) had a significant association with anemia.

Conclusion: Anemia is prevalent in HIV/AIDS patients who receive treatment at Hoima Regional Referral Hospital. Age, marital status, the presence of chronic illnesses, a family history of anemia, experiencing malaria in the past 3 months, the presence of opportunistic infections, the use of antihelminthic drugs in the past 3 months, and an unsuppressed viral load were significantly associated with anemia.

Keywords: anemia, prevalence, HIV/AIDS, antiretroviral therapy

Introduction

Anemia is defined by the WHO as a hemoglobin level of less than 13.0 g/dl for male adults and less than 12.0 g/dl for female adults.[1] Anemia still remains one of the major public health burden. In the year 2021, the prevalence of anemia among individuals of all age groups was 24.3%, representing a total of 1.92 billion cases worldwide.[2] An estimated 63–95% of HIV patients get anemic at some point throughout their illness.[3] Anemia affects both developing and developed countries, making it a worldwide public health issue.

Despite a steady but slow decline in the incidence of HIV infection rate over the last two decades, with improved survival due to the expansion of antiretroviral therapies (ART), the majority of people are living with HIV than ever before.[4] However, with increases in life expectancy following the introduction of ART, hematological changes are one of the most common complications among people living with HIV and could impact both the length and quality of their

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lives.[5] As the most frequent hematologic abnormality in people living with HIV, anemia has been identified as a prognostic marker for HIV disease progression and has been linked with lower risk of survival.[6,7]

Anemia among HIV-infected patients has many underlying causes. HIV could directly and indirectly impact the survival and functioning of hematopoietic stem/progenitor cells (HSPCs) that reside in the bone marrow.[8,9] Furthermore, ART medications, inflammatory mediators released by HIV infection, co-infections, or opportunistic infections could all have an impact on how HSPCs proliferate and differentiate during hemopoiesis.[9] Hematologic abnormalities like anemia, thrombocytopenia, and neutropenia may arise from either the progressive depletion of HSPCs or the suppression of their function.[8] High plasma levels of HIV-1 RNA and low CD4 cell counts (<200 cells/l) have both been linked to an increased risk of anemia.[10,11]

The bone marrow, where blood is produced, experiences a combined impact from various factors, including HIV viral infection, AIDS treatment medications, inflammatory agents released during infections, and potential opportunistic pathogens. HIV infection has both direct and indirect effects on hematopoietic progenitor cells, disrupting the equilibrium of the bone marrow and impairing the processes of cell proliferation and differentiation in hematopoiesis. This leads to significant outcomes such as altered cell counts across all blood cell lineages, abnormal changes in erythroid and granulocytic series, megaloblastic irregularities in the erythroid series, and an impediment in reticulum endothelial iron utilization. Additionally, because hematopoietic progenitor cells express CD4 receptors, type 4 C-X-C chemokine receptors, and type 5 chemokine receptors, they are susceptible to HIV infection.[12]

Several studies have reported variations in morphological types of anemia. In a study in Nigeria, Esan et al reported a normochromic normocytic type of anemia as a major type of anemia in HIV patients,[13] and in Kenya, microcytic hypochromic anemia was the commonest type of anemia, followed by normocytic hypochromic anemia,[14] while in another similar study in Kenya, hypochromic and normochromic anemia were the commonest.[15] In Tanzania, Makubi et al reported microcytic anemia as the most predominant morphological type of anemia,[16] while in Uganda,[17] microcytic hypochromic anemia was reported as the commonest type of anemia.

This study therefore determined the prevalence, morphological type anemia and associated factors in HIV patients attending the ART clinic at Hoima Regional Referral Hospital.

Materials and Methods

Study Area

The study was conducted at the ART clinic at Hoima Regional Referral Hospital in Hoima District. It is located in the Bunyoro sub region of western Uganda. The district is located approximately 230 kilometers (140 miles) by road northwest of Kampala, the capital city of Uganda. Its GPS coordinates are: latitude 1.428578, longitude 31.35488. Hoima Regional Referral Hospital has a bed capacity of 280 and mostly serves the districts of Buliisa, Hoima, Kibaale, Kiryandongo, Kagadi, Kakumiro, Kikuube, and Masindi.

Study Design and Period

This was a hospital-based cross-sectional study conducted between the month of March and September 2022.

Sample Size Calculation

The sample size was calculated using the Kish-Leslie formula (1965). This study used the prevalence of anemia (67%) reported at Mbarara Regional Referral Hospital HIV clinic.[18]

Where n is the sample size of our study

Z = is the critical value for a 95% confidence interval which is 1.96

P = is the estimated proportion of HIV patients with anemia in our study population (0.67).

d = is the desired level of precision

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

$$n = \frac{1.96^2 \times 0.67 \times (1 - 0.67)}{0.05^2}$$

$$n = 339.9$$

Sample size = 340

Sampling Procedure

A simple random sampling technique was used to enroll HIV patients attending the ART clinic at Hoima Regional Referral Hospital. A total of 340 study participants were randomly selected and subjected to structured questionnaires to obtain information on socio-demographic characteristics.

Participants were selected through simple randomization method as previously described by Liu et al.[19] In summary, each HIV patient, a parent or legal guardian of those below 18 years were tasked with selecting a number from cards and depositing it into a container during the recruitment process. If a patient, or a parent/guardian chose an even number, they were granted permission to provide consent, and the patient or a child was included in the study. The cards were rearranged each time a selection was made.

Selection Criteria

All HIV positive patients attending the Art Clinic at Hoima Regional Referral Hospital who consented to participate in the study were enrolled in the study, while all HIV patients who were critically ill, those that were anemic and on anemia treatment, and all pregnant women in HIV care were excluded too.

Data Collection

The socio-demographic characteristics (sex, marital status, age, level of education, alcohol intake, employment, level of income, whether they were urban or rural residents) were collected using a structured questionnaire.

Clinical Data

Clinical data, eg, duration on ART, presence of chronic disease, presence of malaria in the last 1 month, family history of anemia, antibiotic use in the last 3 months, defaulted from ART for >3 months, presence of opportunistic infections (Tuberculosis, Cryptococcal meningitis and oral Candidiasis), HIV clinical stage, history of anti-tuberculosis (TB) drug use, history of antiviral drug use, history of antifungal use, history of antihelminthic use, Body Mass Index (BMI), a BMI was defined as <18.5 underweight, 18.5–24.5 normal, 25–29.9 overweight and >30 obese), and viral load performed within the last 6 months were extracted from patient files.

4 mL of venous blood samples were collected into EDTA tubes. A complete blood count (CBC) was done using the Sysmex XNL-550 hematology analyzer (22,848, Bornbarch Norderstedt, Germany). Thin blood films for those with low hemoglobin levels (Hb <12.0g/dl in females and <13.0 g/dl in males) were made and stained using Giemsa stain for the morphological classification of anemia, Thick smears were made, stained using Giemsa stain, and examined for hemoparasites. CD4 counts were run using BD FACSPresto™ (USA).

Quality Control

The reagents were standardized and quality-controlled before use. Hematology and CD4 analyzers were calibrated, and control samples were run to establish the accuracy and reproducibility of the results. The generated data was double-entered into an Excel spreadsheet, double-checked for completeness, cleaned, and then analyzed.

Data Analysis

The data were entered into an Excel spreadsheet and then exported to SPSS statistics software version 23 (IBM Inc., USA) for analysis. Categorical data and continuous variables were presented in the form of percentages and frequencies. Factors associated with anemia were studied using logistic regression. A p-value of ≤0.05 was considered statistically significant.

Ethical Consideration

The approval was obtained from the Faculty of Medicine Research Committee (FRC) of MUST (Approval No. MUST/MLS/30). Permission to conduct the study was also sought from the director of Hoima Regional Referral Hospital.

Written informed consent was obtained from the study participants above 18 years and from parents/guardians of study participants less than 18 years. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (1964). Privacy and confidentiality was followed all through the study process and those with anemia were referred to physicians for management.

Results

Socio-Demographic Characteristics of Study Participants

We recruited 340 study participants into this study. Majority were females (75%), were aged 38 years and above (48.8%), were unemployed (40.9%), had income level less than Ugandan shillings (UGx). 100,000/= (35.6%), had attained secondary education (55%) and were married (79.7%) as shown in (Table 1).

Prevalence of Anemia Among HIV Patients Attending HAART Clinic at Hoima Regional Referral Hospital

The overall prevalence of anemia among the HIV patients attending HAART clinic at Hoima regional referral hospital was 16.8% (57/340) as presented in (Figure 1).

Table 1 Demographic Characteristics of Study Participants

Variable	Category	Frequency (n)	Percentage (%)
Sex	Male	85	25
	Female	255	75
Age (years)	<18	9	2.6
	18–27	38	11.2
	28–37	127	37.4
	≥38	166	48.8
Employment	Unemployed	139	40.9
	Employed	132	38.8
	Business	59	17.4
	Student	10	2.9
Level of income (Ugx.)	<100,000	121	35.6
	100,000–300,000	114	33.5
	300,000–500,000	58	17.1
	>500,000	47	13.8
Education	No formal education	19	5.6
	Primary	104	30.6
	Secondary	187	55.0
	Tertiary	30	8.8
Residence	Rural	99	29.1
	Urban	241	70.9
Marital status	Single	54	15.9
	Married	271	79.7
	Divorced	15	4.4

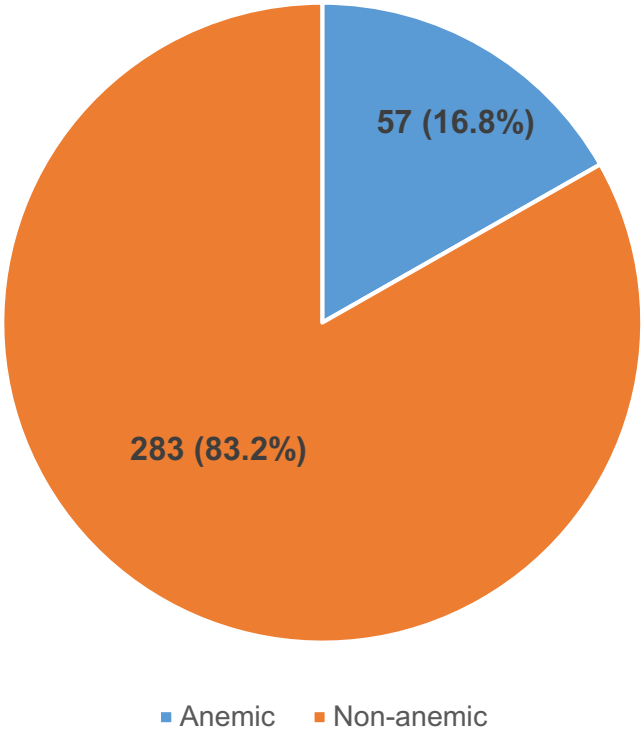

Figure 1 Pie chart showing prevalence of anemia among HIV patients attending HAART clinic at Hoima Regional Referral Hospital.

Morphological Classification of Anemia Among HIV Patients Attending HAART Clinic at Hoima Regional Referral Hospital

The major morphological type of anemia among the HIV patients attending HAART clinic at Hoima regional referral hospital was normocytic normochromic anemia 27 (47.4%) followed by microcytic hypochromic anemia 24 (42.1%), Normocytic hypochromic anemia 4 (7%) and Macrocytic normochromic anemia 2 (3.5%) as shown above (Figure 2).

Factors Associated with Anemia Among Patients Attending ART Clinic at Hoima Regional Referral Hospital

Bivariate Analysis of Factors Associated with Anemia Among Patients Attending ART Clinic at Hoima Regional Referral Hospital

In the bivariate analysis, age (28–37 years, OR = 0.01, p = 0.013; ≥38 years, OR = 0.01, p = 0.008), divorced marital status (OR = 454, p = 0.005), a history of malaria in the past 3 months (OR = 6.44, p = 0.007), a family history of anemia (OR = 14.84, p = 0.007), the presence of opportunistic infections (OR = 65.19, p = 0.000), the use of antihelminthic drugs in the past 3 months (OR = 0.06, p = 0.006), and an unsuppressed viral load (OR = 17.62, p = 0.001) were found to be associated with anemia among HIV patients, as shown in (Table 2).

Multivariate Analysis of Factors Associated with Anemia Among Patients Attending ART Clinic at Hoima Regional Referral Hospital

All the factors that showed an association at the bivariate level were taken for multivariate analysis, as well as other factors with p-values ≤0.1. In the multivariate analysis, age groups (18–27 years, OR = 0.01, p = 0.017; 28–37 years, OR = 0.01, p = 0.005; and ≥38 years, OR = 0.01, p = 0.009), divorced marital status (OR = 98.96, p = 0.024), the presence of chronic disease (OR = 12.10, p = 0.010), a history of malaria in the past 3 months (OR = 7.11, p = 0.001), a family history of anemia (OR = 8.54, p = 0.007), the presence of opportunistic infections (OR = 58, p = 0.000), the use of antihelminthic drugs in the past 3 months (OR = 0.10, p = 0.003), and an unsuppressed viral load (OR = 10.74, p = 0.000) were found to be associated with anemia, as indicated in (Table 3).

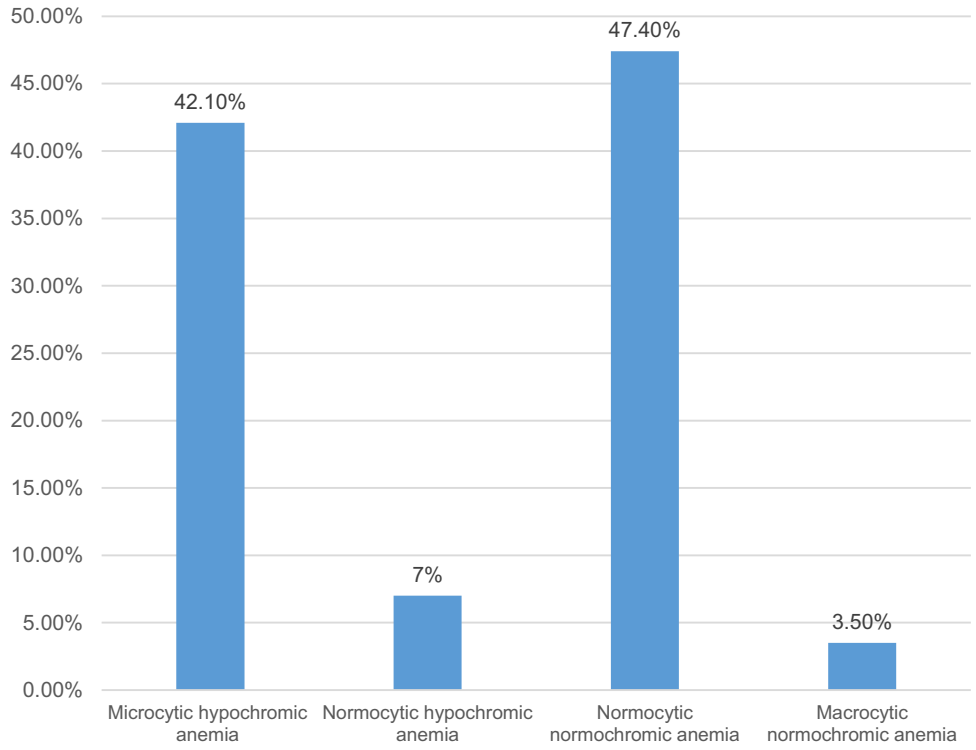

Figure 2 Bar graph showing morphological classification of anemia among HIV patients attending HAART clinic at Hoima Regional Referral Hospital.

Discussion

Anemia is a major concern for individuals suffering from HIV/AIDS, as it is a significant determinant of disease progression. In this study, the overall prevalence of anemia was 16.8%, as defined by the WHO (with a threshold of 12 g/dl for females and 13 g/dl for males). This prevalence is significantly lower than the 47.8% rate of anemia observed among HIV antiretroviral therapy (ART) naive clients attending an immune suppression syndrome clinic at Mbarara Regional Referral Hospital in southwestern Uganda.[20] However, the variations in findings could be explained by the fact that the previous study was conducted on HIV-naive clients, whereas the current study was conducted on HIV patients on

Table 2 Bivariate Analysis of Factors Associated with Anemia Among HIV Patients

Variable		Odds Ratio (OR)	95% Confidence Interval	P value
Sex				
	Male	Ref		
	Female	0.21	0.03–1.31	0.094
Age (years)				
	<18	Ref		
	18–27	0.02	0.00–1.30	0.065
	28–37	0.01	0.00–0.37	0.013*
	≥38	0.01	0.00–0.27	0.008*
Employment				
	Unemployed	Ref		
	Employed	3.18	0.48–21.08	0.230
	Business	5.46	0.42–71.08	0.195
	Student	0.71	0.02–26.64	0.853

(*Continued*)

Table 2 (Continued).

Variable		Odds Ratio (OR)	95% Confidence Interval	P value
Level of income (Ugx.)				
	<100,000	Ref		
	100,000–300,000	0.73	0.11–5.01	0.749
	300,000–500,000	0.11	0.01–2.32	0.155
	>500,000	0.66	0.04–9.80	0.760
Education	None formal	Ref		
	Primary	0.41	0.03–4.94	0.481
	Secondary	0.23	0.02–2.63	0.235
	Tertiary	0.37	0.02–8.72	0.536
Marital status				
	Single	Ref		
	Married	1.49	0.23–9.81	0.677
	Divorced	454.19	6.14–33,586.92	0.005*
Duration on HAART				
	<3 years	Ref		
	3–5 years	0.31	0.02–3.89	0.362
	>5 years	1.61	0.18–14.39	0.671
Presence of chronic disease				
	No	Ref		
	Yes	5.43	0.68–43.61	0.111
History of malaria in the past 3 months				
	No	Ref		
	Yes	6.44	1.68–24.76	0.007*
Family history of anemia				
	No	Ref		
	Yes	14.84	2.13–103.53	0.007*
Presence of opportunistic infection				
	No	Ref		
	Yes	65.19	12.32–344.98	0.000*
Use of antihelminthic drugs in past 3 months				
	No	Ref		
	Yes	0.06	0.01–0.44	0.006*
Viral load				
	Suppressed	Ref		
	Unsuppressed	17.62	3.24–95.71	0.001*

Note: *Significant.

ART. It is unclear how HIV affects the bone marrow microenvironment in vivo, inhibiting hematopoiesis and directly leading to cytopenia.[20]

The prevalence of anemia in this current study was lower than studies in Nigeria at 24.3%,[21] South Africa at 25.8%,[22] Tanzania at 56%,[23] Bayamon, Puerto Rico at 41%,[24] and China at 39.2%.[25] This could be related to geographical differences and food disparities. These disparities in the burden of anemia among HIV-positive patients may be attributable to differences in socio-demographic disparities, study participant characteristics, sample size, and changes in treatment modalities.

In addition, all the study participants were HIV-positive individuals on ART, this may demonstrate the efficacy of HAART in reducing HIV-associated anemia indirectly by lowering the incidence of opportunistic infections and chronic diseases and increasing patients' nutritional status.

Table 3 Multivariate Analysis of Factors Associated with Anemia Among HIV Patients

Variable		Odds Ratio (OR)	95% Confidence Interval	P value
Sex				
	Male	Ref		
	Female	0.27	0.06–1.14	0.074
Age (years)				
	<18	Ref		
	18–27	0.01	0.00–0.45	0.017*
	28–37	0.01	0.00–0.24	0.005*
	≥38	0.01	0.00–0.34	0.009*
Marital status				
	Single	Ref		
	Married	0.79	0.16–3.82	0.767
	Divorced	98.96	1.85–5283.21s	0.024*
Presence of chronic disease				
	No	Ref		
	Yes	12.10	1.82–80.30	0.010*
History of malaria in the past 3 months				
	No	Ref		
	Yes	7.11	2.17–23.30	0.001*
Family history of anemia				
	No	Ref		
	Yes	8.54	1.80–40.51	0.007*
Presence of opportunistic infection				
	No	Ref		
	Yes	58.00	13.64–246.68	0.000*
Use of antihelminthic drugs in past 3 months				
	No	Ref		
	Yes	0.10	0.02–0.46	0.003*
Viral load				
	Suppressed	Ref		
	Unsuppressed	10.74	2.96–39.01	0.000*

Note: *Significant.

The prevalence of anemia among HIV-positive individuals, on the other hand, was marginally equivalent to the finding from the Ethiopian study, with 11.4% prevalence of anemia.[24] Similarly, the findings of this study are comparable to the 16.2% prevalence reported at Jimma University among ART-experienced individuals.[25,26]

According to the current study's findings, normocytic normochromic anemia was the most common type of anemia among HIV patients at HRRH (47.4%), followed by microcytic hypochromic anemia (42.1%) and macrocytic hypochromic anemia being the least common (3.5%). Our finding was comparable with studies in 46.5% in Ethiopia,[26,27] Panwar et al who reported that 46% of the respondents in their study had Normochromic normocytic anemia, followed by 42.85% microcytic hypochromic anemia in New Delhi, India.[28] Our finding defers from two studies, one in Uganda by Nyesigire Ruhinda et al that reported that in HIV-infected children, microcytic-hypochromic anemia (44.9%) was the commonest type of anemia[17] and another in Kenya where microcytic hypochromic anemia was the predominant type of anemia among HIV patients on ART in Kenya.[29]

Factors Associated with Anemia Among Patients Attending ART Clinic at Hoima Regional Referral Hospital

In this study, age groups <18 was found to have increasing odds of developing anemia. This finding is not consistent with studies in China by Shen et al, and Yantao et al, which reported that the prevalence of anemia increases with age (49.6%,

53.5%, and 60.1% among patients aged 18 to 39, 40 to 59, and 60 years, respectively).[30,31] Similar findings were as well reported by Wolde et al, where age, persistent diarrhea, the initial ART treatment, the initial CD4 count, and the initial ALT level all served as independent predictors of incident anemia.[32]

In this study, being divorced was significantly associated with anemia among the study participants, the divorced were 98.96 times likely to develop anemia. This finding is comparable with a study by Guiying et al, in southwestern China that reported that patients who were widowed or divorced had higher odds of having severe anemia than married PLHIV patients.[33] This study is in agreement with a study done in Tanzania that reported that being divorced was associated with anemia in people living with HIV/AIDS.[34] Our results are also comparable to those of a US study that discovered anemia in divorced HIV patients.[35]

In this study, there was an association between chronic infection in HIV and anemia, with a 12.1-fold increase in the odds of anemia among patients with chronic illness. This finding is consistent with a study conducted by Araújo-Pereira et al in 18 outpatient research clinics across 10 low- and middle-income countries, which also observed an association in line with our findings.[36] In a meta-analysis that combined cross-sectional and case-control studies, it was found that the likelihood of a connection between anemia and tuberculosis (TB) increased as the severity of anemia became more pronounced, with a odds ratio of 3.56.[37]

Consistent findings were observed in Ethiopia where the likelihood of developing anemia in patients with a prior history of chronic diarrhea increased significantly compared to patients who did not have chronic diarrhea.[32] In a study conducted across the United States, it was observed that HIV patients co-infected with the hepatitis C virus showed a correlation with the presence of anemia.[38]

Our study observed a significant association between a history of malaria in the past month and anemia in the study participants. This finding is comparable with a study in Tanzania among HIV-positive women on ART that reported current malaria infection and a history of episodes of malaria illness during the index pregnancy, where there was an association between a history of malaria and anemia.[39] The study found higher odds of anemia among patients who reported malaria in the past one month. Malaria parasites tend to destroy red blood cells and therefore deplete the body's iron stores. This could be the reason for the higher odds of anemia in HIV patients who have a history of malaria. These findings are comparable to a study by Menon et al in Uganda who noted that the risk factors for anemia included having malaria parasitemia.[40]

In our present investigation, we found that having a family history of anemia predicts the occurrence of anemia in individuals living with HIV. Interestingly, while most previous research has not specifically emphasized the connection between a family history of anemia and anemia in HIV patients, studies involving different populations have indeed documented associations between a family history of anemia and the development of anemia.[41,42]

In this current study, the presence of opportunistic infections was associated with anemia after initiation of ART; this is consistent with a study in Ethiopia.[43] Opportunistic Infections (OIs) are reported to attack the bone marrow in patients with HIV. These infections may cause marrow changes either directly by the organism itself or indirectly by causing reactive changes.[44] In another study in Ethiopia, women with baseline opportunistic infections and women who were on ART for a long duration were significantly associated with anemia among women living with HIV/AIDS.[45] The above findings are comparable to a study by[46] who reported that factors that often contributed to the risk of developing anemia in HIV infection include the presence of opportunistic infections, sex, and a low CD4+ T-lymphocyte count.[47]

This current study reported unsuppressed viral load and CD4-count less than 200 cells/mm^3 had a significant association with anemia. This finding is consistent with a finding in a study in Tanzania that reported that, the prevalence of anemia increased as the HIV/AIDS advanced from lower to higher WHO clinical stages.[39] This is also consistent with a previous study by Nagawa et al that reported that advanced HIV/AIDS clinical stages (clinical stage III or IV) or CD4 Count of <200 had a strong association with anaemia.[11] The findings are similarly consistent with studies conducted in Kisumu Kenya and Tanzania,[10,48] in Ethiopia,[49] and in Ghana[50] which found that CD4 cell counts of <200 cells per mm^3 and individuals with HIV advanced disease stage III / IV were more likely to develop anemia.

Study Limitations

This study did not collect information on intestinal parasite infections, which are a leading cause of anemia in low- and middle-income nations. Because this was a hospital-based, cross-sectional investigation, the relationship between HIV infection and anemia could not be demonstrated conclusively.

Conclusion

Anemia burden exists among HIV patients at Hoima regional referral hospital, and normochromic normocytic anemia was the commonest type of anemia followed by microcytic anemia among patients. Anemia is prevalent in HIV/AIDS patients who receive treatment at Hoima Regional Referral Hospital. Age, marital status, the presence of chronic illnesses, a family history of anemia, experiencing malaria in the past 3 months, the presence of opportunistic infections, the use of antihelminthic drugs in the past 3 months, and an unsuppressed viral load were significantly associated with anemia. Hemoglobin levels should be monitored routinely, especially among PLWHIV who have one or more of the risk factors for anemia, so that treatment can be initiated if deemed necessary. Further longitudinal studies are needed to determine the association between HIV infection and anemia.

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Disclosure

The authors report no conflicts of interest in this work.

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