Prevalence and Underlying Factors of Anemia Among Adult HIV Patients Undergoing Highly Active Antiretroviral Therapy at Murang'a Level 5 Hospital, Kenya

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Abstract

Background: Anemia is a disorder whereby the body has inadequate healthy erythrocytes. Erythrocytes' function is to carry oxygen throughout the body. Anemia affects over 2billion people worldwide. In Human Immunodeficiency Virus (HIV) infected patients, the highest anemia prevalence in Africa was in Congo at 63%, in Ethiopia, it was as low as 26.2%. In Kenya, among adult pregnant women not initiated on antiretroviral therapy, anemia was at 65.2% while negative control group was 19.6%. In Murang'a County Kenya, there was paucity of data on anemia in HIV patients.

Methods: A cross-sectional study was implemented, 295 study participants were enrolled. Demographic and clinical data were obtained using structured questionnaires. Body mass Index (BMI) calculated. 4ml of blood was collected into an anticoagulant vacutainer for analysis of hematological indices. Baseline CD4, defaulter history, antiviral regimen, prophylaxis and current World Health Organization (WHO) HIV staging were obtained from the Kenya Electronic Medical Record (EMR). Current CD4 value determined using BD FACS PRESTO. Complete blood count (CBC) done using Medonic hemolyzer.

Results: prevalence of anemia was 42.03%. Among n=124 anemic participants mild, moderate and severe anemia was 50.8%, 27.9% and 21.3% respectively. Prevalence related with: year of diagnosis(P-value 0.001) , age(P value 0.012),HIV staging(P-value 0.001), viral load (P value 0.03,BMI(P value=0.002),Defaulter history (P value 0.001)and hematological indices . At CV=0.174: Low HB correlation with HCT was (0.81), MCV (P-value 0.49), MCH (0.40), MCHC (-0.36) and WBC (-0.53), PLT (-0.09) and VL (-0.40). P value=0.008. No linear relation between the CD4 count and HB levels.

Conclusion: There is need for regular monitoring of HB and other related hematological parameters in HIV patients. Collaboration of healthcare workers including hematologists and nutritionists would ensure a more holistic approach in HIV management. Trends like year of diagnosis should be understood and involved in anemia management. Continuous research to determine the complex correlation between anemia and opportunistic infections is necessary

Keywords: Anemia, Immunodeficiency, Hematological parameters, CD4

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1. Introduction

Anemia has been a common complication in chronic illnesses. It is a disease that leads to a poor prognosis in HIV-infected individuals. (Lang et al, 2023).The virus responsible for Acquired Immunodeficiency Syndrome (AIDS) is known as Human Immunodeficiency Virus (HIV). Worldwide, it has consistently had an impact on humanity. (Dziuban et al, 2022). Reduced blood cells (cytopenia) constitute a significant complication in immune-compromised individuals. (Hadgu et al, 2023) Anemia, the most prevalent cytopenia in HIV-positive patients, affects up to 95% of those who have the virus at some point in their lives. (Opie, 2012). Anemia is the most prevalent blood-associated disorder affecting humankind. It is typically expected in chronic illnesses. In chronically ill patients, anemia leads to a poor prognosis and an impaired quality of life (Madu& Ughasoro, 2017)

The determinants of anemia are multi-factorial. HIV has an impact on hematopoietic stem progenitor cells' (HSPCs') ability to endure and proliferate in the bone marrow. (Durandt et al,2019), antiretroviral medications and inflammatory mediators also distress the growth and differentiation of HSPCs. A continuous reduction of HSPCs eventually leads to different types of cytopenia (Durandt et al,2019). Anemia and thrombocytopenia were common cytopenia in HIV patients, with anemia having a prevalence rate of 7.2% to 84% in different study results. (Marchionatti & Parisi, 2021)The multifaceted etiology of anemia and the complex determinants differences in different studies have led to several structures developed to counter its effect. Biomarkers reflecting Inflammation and Nutritional Determinants (BRINDA) project proved that the risks of anemia are diverse and multifarious (Namaste et al,2017)In developed nations, approximately 35% of HIV-infected individuals develop anemia (Berhane et al., 2020).Since developing nations are more likely to suffer from tropical diseases including trichuriasis, TB, hookworm infestation, and malarial parasites, it is imperative to ascertain the prevalence there. Opportunistic infections play a role in HIV/AIDS severity. (Xie et al, 2022)Significant correlations were found between low hematological indices and HIV infection in Kenyan cases of iron deficiency anemia. Compared to the control group, the HIV group frequently experienced low mean corpuscular volume, which increased the risk of anemia.. C-reactive protein (CRP) was elevated in anemic patients which may indicate a significant correlation of anemia to inflammation (Frosch et al,2018). Since some opportunistic infection (OIs) e.g Mycobacterium tuberculosis

are related to inflammation,anemia cannot be addressed without considering OIs.(Xie et al, 2022)

Since no similar research had been done in Murang'a, this was the first of its kind. Murang'a county having a different geographical set-up made the study essential to find out whether the prevalence was consistent with that of the other regions like China 46.6%(Cao & Guiying, 2022), 51.5% in southeast China (Lai et al.,2019), 66.7 % in Nepal(Sah et al.,2020). It was also aimed to confirm or challenge the 26.2% prevalence in Ethiopia (Aynalem et al.,2020) and 56% in Tanzania (Petraro et al., 2016). Ethiopia, Tanzania and Kenya lie in the East Africa region and are separated by a boundary. The discrepancy between the prevalence in Tanzania and Ethiopia prompted this investigation.

Even though HIV was first diagnosed in Kenya in 1984,it remained a threat to the patient's socioeconomic status since the first case. It persists to be a significant cause of high mortality in Kenya. In research to find out the leading cause of mortality in Kisumu County, Kenya, AIDS lead at 23.1% with a mortality case notification rate of 251/100,000 of the population (Waruru et al.,2022).The difference between this study and the others done before is that this study was not only interested in the current prevalence of HIV/AIDS or the extent of Anemia occurrences in HIV/AIDs. The study also established different underlying factors that affect the progression of the disease and recommended different approaches to lower the frequency of the individual progressing to anemia.The results obtained from this study helped draft a conclusion and recommendations that will be shared with the study site and Ministry of Health.Approximately 20% of people worldwide suffer from anemia. (Wouters et al., 2019). Anemia has been allied to depressing the quality of life in ailing individuals suffering from chronic illnesses (Lubega et al, 2022). At Johannesburg, Anemia affected almost half of the adult population suffering Chronic Kidney Disease (CKD); among the Black Africans with CKD, the prevalence was 43.1%, and the black Africans were leading with 46.9%, with the whites having 34.6% while Indians/Asians had 18.2%. Those with mixed races had a prevalence of 45.0% (Nalado et al., 2019)

In adult patients with Tuberculosis infection undergoing treatment, the prevalence of anemia was found to be 61.2%. Patients with anemia and tuberculosis were also found to have reduced Mean corpuscular Hemoglobin (MCH), CD4 count levels, Mean Corpuscular Volume (MCV) and increased viral load copies in those co-infected with HIV. (de Mendonca et al. 2021). The common types of anemia were Iron deficiency anemia, which may result from poor feeding habits in patients with chronic illnesses and chronic inflammation.Anemia of chronic illness (AOC) is a condition caused by prolonged inflammation that results in a cytokines-mediated cascade that renders iron unavailable.(Kim& Cho, 2018). This matches with increased platelet levels observed in tuberculosis and anemia comorbidity (de Mendonca et al., 2021).Anemia is comorbidity related to Human Immunodeficiency Virus (HIV) infection. Most of the studies show that it relates to disease progression and severity. Anemic individuals suffer from fatigue, exhaustion, depression, and weakened mental functions. (Smith, 2010). Nonetheless, there has been a decrease in the infection's ability to proceed into AIDS since the start of highly active antiretroviral therapy (HAART). The rate of patient morbidity and death is influenced by comorbidity and opportunistic infection. Over 1.5 million Kenyans are HIV/AIDs infected, with a new infection of 42000 and 21,000 AIDs related death (UNAIDS, 2019).

Anaemia is a severe worldwide public health issue that impacts people of all ages.(Getu et al,2023) Roughly a one-third of the entire global populace may be impacted by it, and if ignored or misdiagnosed, it can result in multiple organ failure and even death (Chaparro& Suchdev, 2019).Moreover, it can have a detrimental effect on a patient's quality of life in addition to the social and economic development of the country at large.(Brentlinger et al, 2016) Studies shows that global prevalence of anemia was at 32.9 %(Kassebaum et al, 2014). Developments towards mitigating anemia have been slow and rough. From 1990 to 2016 anemia was globally seen to reduce by seven per centum from 40% to 33% (Kassebaum et al, 2014)

Anemia varies in frequency depending on the geographic area. South Asia, the Caribbean, Oceania, and Sub-Saharan Africa. Anemia is a moderate to severe public health concern (Stevens et al,2013).

Among Ethiopian children that were antiretroviral therapy naive, anemia was 42.8% (Geletaw et al, 2017). Anemia across the globe varies greatly and this calls for localization of anemia prevalence rather than use of regional or global statistics to make health related decision (Brittenhamet al, 2023). Under the same anemia definition of HB less than 13.0g/dl in men and less than 12.0g/dl in female population anemia was found to be 54.2% in Myanmar(Minn et al, 2016) while in China it was 27.7% (Li ZhengWei et al, 2017). This was among adult patients living with HIV. Amid the patient being initiated on ART, anemia was found to be 35.04% (95% CI: 30.84% to 39.49%) in Ethiopia,(Aemro et al , 2022). Comparatively those on ART in the same country had anemia of 26.2% (Aynalem et al,2020). This difference in patient under the same geographical region with high similarity of socio-demographic factors signifies the multi factorial role of ART in combating human immune deficiency virus. However, the effect of drugs due to their pharmacokinetics and pharmacodynamics nature cannot be ignored.

Most of the recent research done on the occurrences of anemia in HIV/AID patients are retrogressive meta-analyses based on studies that were done years ago when patients were under ART; with the current improved HAART treatment, there is a need to have more analytical laboratory-based research to examine the current prevalence and underlying causes. In an organized prevalence meta-analysis in China published in January 2022, the pooled magnitude of anemia in PLWHIV was 39.7% for children and 46.6% for adults. (Cao &Guiying, 2022).

By 2020, an estimate of 37.6million people globally were living with HIV and around a 1.5million new infections. (UNAIDS, 2021). Hematological changes are among the

biomarkers that show disease progression and severity (Karn et al, 2022). One of the most specific hematological abnormalities in people with HIV/AIDS has been identified as anemia (Li et al, 2024). It has been associated with poor prognosis and disease severity(Haider, 2019). As documented in different studies, the magnitude of anemia in adult patients undergoing HAART differs significantly. It has a prevalence of 26.2% in Ethiopia (Aynalem et al., 2020), 56% in Tanzania (Petraroet al., 2016),23.8% in Ghana (Obirikoranget al., 2016), 60.61% in Nigeria (Omoregieet al., 2009), 55.8% in Nepal (Martinet al.,2014),55.5% in India (Tiewsohet al., 2019), 41% in Hispanic Puerto Rico (Santiago-Rodriguezet al.,2014), 25.8% in Johannesburg, South Africa(Takuva et al, 2013)

Over 1.5 million Kenyans are HIV/AIDs infected, with a new infection of 42000 and 21,000 AIDs related death (UNAIDs, 2019). On the extent and underlying causes of anemia in HIV patients, there is a lack of data. However, HIV patients were more likely than the negative control group to have anemia in pregnant women. Mean corpuscular volume (MCV) and mean hemoglobin values in the HIV patients were significantly lower. However, serum ferritin and C-reactive protein (CRP) was higher in HIV individuals an evidence of possibilities of inflammation among this group. Iron deficiency anemia was the most common based on the MCV and serum ferritin (Frosch et al, 2018)

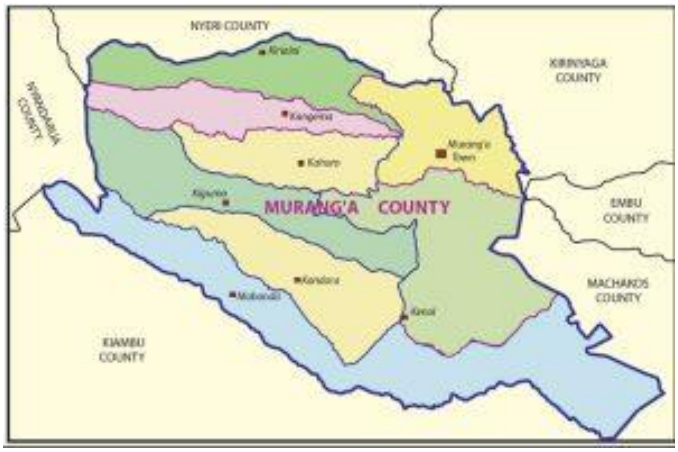
Various studies discuss potential risk factors for anemia in people with Human Immunodeficiency Virus. A low Cluster of differentiation 4(CD4) glycoprotein number was a specific biomarker for Anemia in HIV/AIDs infected people in China(Lai, 2019).Several studies indicates that anemia in PLWHIV is more common in those whose CD4 counts is below 200cell/ul(Soubeigaet al, 2022). Low CD4 lymphocytes lead to decrease in hematopoietic productivity and suppression in the bone marrow. According to a retrospective cohort research, a reduced CD4+ white cell count was linked to severe anemia and females who have anemia and a CD4+ T lymphocyte level of below 100cells/ul were more likely to develop anemia(Harding et al,2020).Viral load copies in the peripheral blood were also defined as a determinants associated to anemia. The magnitude of anemia was higher in those patients that had a viral burden more than 1,000 copies per ml of blood. (Huibers et al,2020).The HAART aims at reducing an individual viral load and viral replication. These drugs may therefore indirectly improve the haemoglobin level because they recover the erythropoietic dysfunction that is caused by the high levels of viral load. (Akdag et al, 2019)However, not all HAART regimen will improve the anemia status of the patient. According to a 2013 study by Takuva et al., the prevalence of anemia was 25.8% prior to the initiation of HAART.. However, after introducing antiretroviral therapy in a sample of 322 subjects, the prevalence doubled. The drug of choice was zidovudine(ZDV) which is known to possibly incapacitate erythroid hematopoietic stem progenitor cells (HSPCs). This will directly result to reduced production of erythrocytes. (Marchionnati & Mariana, 2021). Patients treated on ZDV based HAART are 3.34 times having more chances to develop to anemia than those on non-ZDV regimens. (Tamir et al, 2018)

Opportunistic infections such as Mycobacterium tuberculosis, Histoplasmosis and Leishmaniasis are other rissk factors. 86% of those infected with Mycobacterium tuberculosis had anemia (McDermid et al.,2013), In HIV/AIDS patients diagnosed.Penicillium marneffei, an opportunistic fungal infection most common in Asia, anemia prevalence was found to be 86.89% (Qiu et al.,2015). Cryptococcus neorformans, an opportunistic fungal infection common in the tropical region that is responsible for Cryptococcal meningitis, did not influence the prevalence of anemia (Laiet al., 2019).

2. Materials and methods

2.1 Study site

The investigation was done in Murang'a Level 5 Hospital, Murang'a County GPS Location -0.721699, 37.154779. Murang'a County has a population of 942,581(CHS report, 2017). It has an estimated HIV prevalence of 4.2%, with 27,245 people living with HIV. Murang'a Hospital is a referral hospital tier level five, located in Township ward, Kiharu Sub County, Murang'a County. The facility offers screening and laboratory services as well as therapeutic procedures. It has a high population and is one of the country's largest and most equipped hospitals. It has a bed capacity of three hundred and seventeen. It has a male ward, female ward, children ward, new-born unit and an Intensive care unit(ICU) with a thirty-five beds capacity. It serves over one thousand outpatients on average per day. Seven thousand, five hundred and forty-two (7542) people are on HAART, and 6678 had their viral load suppressed by the year 2017(CHS report, 2017) (Figure 1).



2.2 Study design and sample collection

The study employed a cross-sectional, analytical, and observational research design. It was observational since the research subjects were not manipulated. The illness under investigation was anemia, and the study was an analytical laboratory investigation based on exposure to HIV/AIDS. Since it represents an overview of the anemia that exists and the elements associated with anemia in HIV/AIDS patients at

one time in their lives after exposure, the study was cross-sectional.

2.3Inclusion criteria and exclusion inclusion criteria

All adult HIV/AIDs patients, who consented to take part in this study were included. All adult HIV/AIDs patients who did not consent to participate in the study, HIV/AIDs patients below the age of 18years, pregnant women and those who may not be of sound mind were excluded.

2.4 Laboratory procedures and Quality Assurance

2.4.1 Venous blood Collection procedure

Every item needed for the venipuncture was prepared in advance. A tourniquet, a purple-topped EDTA container, a butterfly with a closed system needle, disposable latex gloves, 70% isopropyl alcohol swabs, cotton wool, and bin liners for non-infectious (coded-black), infectious (color-yellow), and highly infectious (red coded) were among the items included.The vacutainer tubes were labeled with unique study based on number describing the study subjects. The personal identity of the subject and unique number was verified before labeling the tube. Latex gloves were used as part of personal protective equipment. Aseptic techniques were used during phlebotomy. Blood drawing procedure was explained to the client and they were reassured about the safety. The upper extremity's basilica and median ante-cubital veins was the preferred site for phlebotomy. Alternative sites included veins on the hand's dorsum and other veins in the forearm. In order to prevent hematoma, a tourniquet was applied to make the veins more apparent; however it was to be withdrawn right away following vein access. The phlebotomy site was examined with the end of the fingertip. This was done to help determine the precise location of the puncture by allowing one to feel the vein. The phlebotomy site was cleaned by using an alcohol swab or cotton wool dipped in isopropyl alcohol to swab the skin in tiny outward circles. After sanitizing the skin, the prepared puncture site was kept free from finger contact to prevent re-contamination. Employing aseptic methods, the vacutainer device's needle (a butterfly) was put into the vein. Making a counter-fraction far away above the vein can often help to stabilize it before a needle is inserted. The apparatus was permitted to draw 4 milliliters of blood into an evacuated tube at each stage. Following the draw, the blood was repeatedly inverted in tubes holding additives. The samples were then transferred for analysis of blood tests from the phlebotomy section to the haematology section.

2.4.2 CD4 cell count procedure

CD4 cell count enumeration was done using BD FACS Presto system for in vitro diagnosis. Its analysis range is 50cells/ul to 4,000cells/ul. Prior to performing any test, the machine was pressed on the quality control (QC) tab to run an internal quality control(IQC) which would determine the validity, precision and quality of the results to be obtained from the tests. The operator identity (ID) was selected and on pressing the accept display the QC would spontaneously run. Once the QC test is complete the results pops up and on the screen. When the QC passes the machine is ready to proceed with testing.

The study specimens in an Ethylene diamine tetraacetic acid (EDTA) anticoagulant tube would then be mixed thoroughly through gradually inverting the tubes before testing. The test sample was then obtained from the specimen using provided pipettes. The pipette was pressed on the upper end to form a drop on the lower tip. The drop was cautiously dispensed into the sample plug point of the catridge until the fill indicator was full. The cartridge top was then securely closed. The timer was then initiated to start the pre-test countdown. Once the count was over the machine was pressed on the "RUN TEST" tab, the sample ID was typed and the "ACCEPT" screen pop up pressed to initiate test. The Operator ID was selected and accepted. The channel protector was removed without interfering with the cartridge. The cap with the channel was then inserted facing upward into the cartridge tray of the counter machine. The sample processing inside the machine would take 4 minutes then the machine would pop a sound three times and results would display on the screen and would be recorded. All used cartridges, pipettes and gloves were discarded appropriately in biohazard bins.

2.4.3 Complete blood count procedure

Before every day test run a 3panel quality control for low, normal and high values would be run. The control materials are stored at 2-8^{o}C refrigerator after every run. The controls would be obtained from the refrigerator, put at room temperature for a few minutes to stabilize, then they are gently tilted up and down three times to have a homogeneity. On the equipment display the quality control(QC) section would be clicked, then select control-L for low, control N-for normal or control-H for high, manually or using a barcode scanner scan the barcode on the vacutainer. The vacutainer stopper is then opened and the specimen placed under the equipment probe. The equipment automatically picks 20ul of the control sample, run and display the result. The process is repeated for the entire panel and results displayed were printed and put into the control box file pending final analysis. In a span of four hours after being collected, blood specimens placed in EDTA vials were examined every day.K3EDTA specimens of blood were used to evaluate the total blood count (TBC) using a MEDONIC analyzer at ML5HL. The instrument counts Leukocyte differential using 3D laser beam dispersion (fluorescence), Erythrocyte and platelets are counted using light impedance, and hemoglobin using cyano-hemoglobin at 540 nm. To examine hematological variables, the analyzer requires about 20 µl of blood. A total of 18 hematological parameters were examined in this study. These measures comprised Hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), hemoglobin (Hgb), and mean cell hemoglobin concentration (MCHC). Red blood cell count (RBC), platelet count (PLT), and its markers were also incorporated. Absolute and differential counts for lymphocytes and granulocytes were engaged in the white blood cell count (WBC) and its subsets. In the hematology section the blood vacutainer would be inverted three times gently to ensure the

sample mix uniformly. The specimen would then be put into a rotator that ensures more homogeneity. The vacutainer is opened and the specimen placed under the probe ensuring that the probe is inside the blood specimen. The probe automatically suck 20ul of the sample and subject it into laser beams, light impedance and red cell lysing to enumerate leukocytes, red blood cells and hemoglobin respectively. The equipment have set profile ranges for male and females that would be selected before performing the test. The unique number of the patient would be entered in the patient name section. In less than 5minutes the equipment would complete the run and display the results on the equipment screen. The display is clicked on the print section and the results are printed. The daily reports would be collected and put into a box file pending final analysis. All used vacutainer bottles with the blood would then be discarded into a red bin for waste treatment by incineration.

2.4.4 Viral load

The viral load specimens were collected in a K3EDTA plasma preparation tube (PPT). The tube contains an inert gel that separate cells from plasma after centrifugal separation. The viral load (VL) specimens collected were kept on a rack for 30minutes before centrifugal separation to prevent hemolysis. After 30 minutes the specimen were put into the centrifuge and the timer set at 10 minutes and 3000 rotation per minutes (RPM). The separated samples would then be bar coded and remote logged. The samples would then be triple packaged. The specimens were transported together with the other viral load samples. Cold chain protocols were highly observed through the use of cold packs and cool boxes. The samples would then be subjected to Nucleic Acid Amplification Test (NAAT), a molecular method that detects small quantities of HIV genetic materials. The results would then be fed into the EMR (Electronic medical record) as numerical values or LDL (Low than detectable levels). A value equal or below 1000copies/ml or LDL is a proof of viral suppression. Through EMR navigation via the clinician mode the VL values were obtained and put into the excel form containing all the other raw data pending final analysis.

2.5 Electronic medical record (EMR) data abstraction

The subjects' information on the Kenya EMR was obtained by logging in into the dashboard. To get into the dashboard, the given username and password was used. The patient search is done using any of the search tab displayed on the dashboard tiles used to redirect the user to different patient services like registration, triage, clinician, drugs, laboratory testing among others. By clicking to the clinician tab on the display the dashboard would redirect to the clinical content like available visit forms, visit summary, completed, patient summary, drug orders and lab order among others. On click of the patient summary file, a form is displayed containing the year of diagnosis, record of viral load tested, the current ART regimen and prophylaxis in use among others. This information was recorded in the raw data excel form. In the home page the Patient tracing tile display would redirect to a summary of patient's medication default history. This history was collated and inscribed into the excel form containing the rest of the raw data.

2.6 Data analysis

The raw data was filled into Statistical Package for Social Science (SPSS) version 26 software for analysis. Frequency distribution of all variables was obtained and illustrated using pie charts, bar graphs and frequency distribution tables. Comparison between qualitative variables was made using the Z score test, T tests and chi-square and a p-value less than 0.05 was considered statistically significant. Linear regression and Analysis of variance (ANOVA) was used to find out correlation between variables.

2.7 Ethical approval

This research proposal was submitted to the University's Ethics and Research Committee (ERC) for approval. MKU-ERC approved the study (Ref no: MKU/ISERC/2457*)*. The university gave an introductory letter which was used to apply for study license.

Authorization to do the research upon ERC approval was sought from National Commission for Science, Technology and Innovation (NACOSTI). The study was approved and licensed (NACOSTI/P/2022/21936).

3. Results

3.1 Socio demographic characteristics of study population

There were 295 study participants, with 82 (27.8%) men and 72.2% women. The male research participants range in age was 18 to 65 years, with 48.2years being the mean age. Conversely, the female study group age range was18 to 67 years, with an age mean of46.5 years. As presented in table 1, there were more female study participants than male. Most participants were within the age bracket of 38-67years while the age bracket between 18-37years had a lower number. However 48-47years age bracket had the highest prevalence of HIV infection at 38.3% whereas 28-37years had the least at 7.8% only.

3.2Prevalence of Anemia and related underlying factors

The "magnitude of Anemia" study looked at the prevalence of anemia in a group of people with HIV who were receiving anti-retroviral medication. The data showed how anemia severity varied according to age, gender, year of diagnosis, and HIV stages as classified by the World Health Organization. The results showed notable differences in the frequency of anemia among different groups, highlighting the intricate interactions between several factors that lead to anemia in individuals living with HIV.

The patients who had a defined low Hb were 124. Hence the prevalence of anemia in adult HIV patients undergoing HAART at Murang'a level 5 hospital was calculated as below;

$$\text{Prevalence} = \frac{(\text{no offemale resondents with } hb \leq 12.0g/dl) + (\text{no of male with } hb \leq 12.8g/dl)}{\text{total number of respondents}} \times 100$$

$$= \frac{124}{295} \times 100$$

$$= 42.03\%$$

The study found that the prevalence was 42.03% and it was represented as figure 1.

Table 1: The study's demographic presentation

Variable	Category	Frequency	percentage(%)
Sex	Male	82	27.8
	Female	213	72.2
Age(Years)	18-27	24	8.14
	28-37	23	7.8
	38-47	83	28.14
	48-57	113	38.3
	58-67	52	17.62
Religion	Christian	287	97.3
	Muslim	8	2.7

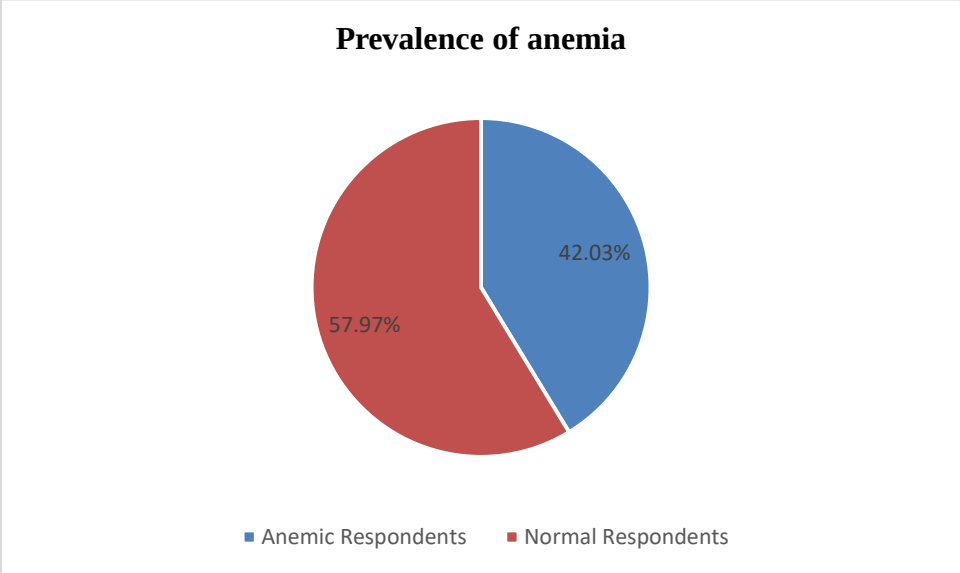


Figure 1: Prevalence of anemia

The prevalence of anemia among HIV-positive patients was examined in the study, and the severity of the condition was divided into three groups: severe, moderate, and mild anemia. The study's findings are shown in table.

Table 2: Prevalence of Anemia among HIV-Positive Patients

		Frequency	Percent
	Severe Anemia	26	8.81%
	Moderate Anemia	34	11.53%
	Mild Anemia	64	21.69%

Anemic male were 26(31.7%) amongst n=82 male, while anemic female were 98(46.0%) among n=213 female as described below.

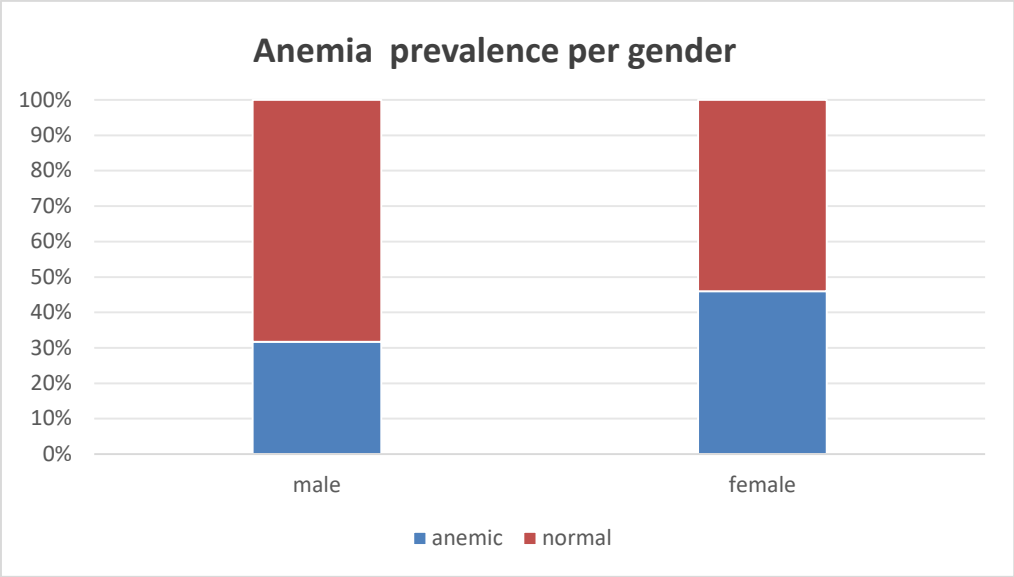


Figure 2: Anemia prevalence per gender

Of the 124 anemic participants in the research, 79.04% were female and 20.96% were male. In gender this distribution indicates a notably higher proportion of females experiencing anemia compared to males. In univariate gender classification the anemic male and female was 31.7% and 46.0% respectively as shown in figure 2 above.

In addition, the levels of mean haemoglobin concentrations were determined in the cohort as shown in figure 3 below. Hb mean concentrations per class for both males and females who were anemic were compared using T-test to test significance and variation. There was no significance difference within the male and female participants (t9=2.26, p=0.41).

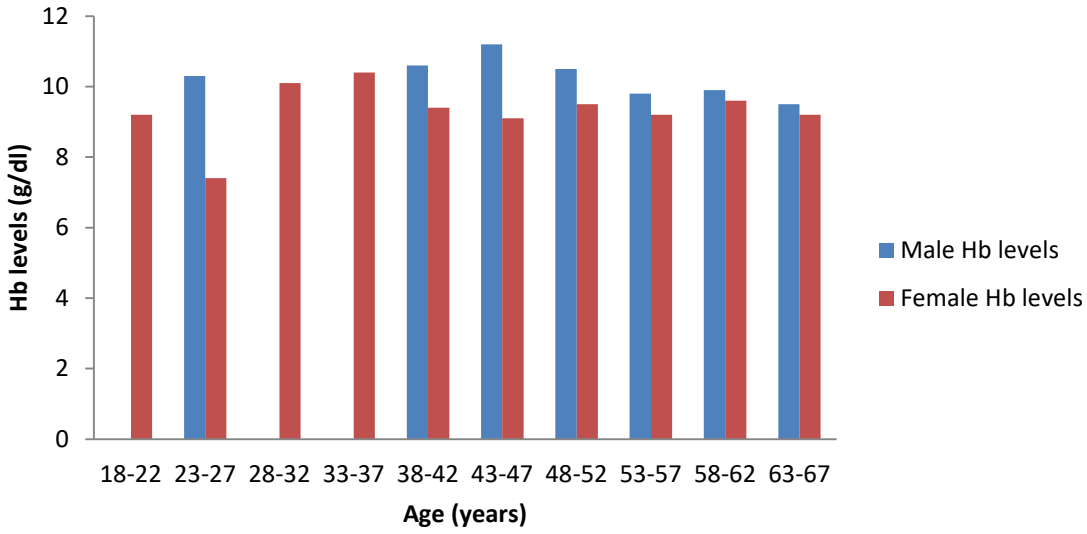


Figure 3: Comparison of HB concentration means against age

The odds of having anemia in regards to different variables were also analyzed as per the table 3 below,

Table 3: Factors associated with Anemia

variable	classification	odd ratio	p value
sex	Male	Ref	
	Female	0.686	0.143
Age	18-27	Ref	
	28-37	1.557	0.061
	38-47	1.07	0.012
	48-57	1.53	0.059
	58-67	1.341	0.041
Duration on HAART	<5years	Ref	
	5-10years	0.37	0.341
	11-15years	1.21	0.253
	above 15years	18.5	0.001
clotrimoxazole	No	Ref	
	Yes	0.014	0.631
TPT	No	Ref	
	Yes	0.08	0.337
HIV STAGE	1	Ref	
	2	0.246	0.576
	3	0.789	0.343
	4	13.421	0.001
Viral load	LDL/<1000 copies	Ref	
	>1000copies	18.98	0.03
BMI	18.5-24.9(Normal)	Ref	
	<18.5(Underweight)	18.66	0.002
	25.0-29.9(Overweight)	0.68	0.476
	>30.0(Obese)	0.27	0.535
HAART REGIMEN	AF2E	Ref	
	AF2B	0.06	0.371
	AF2D	0.25	0.678
	AS5B	8.16	0.001
Defaulter History	<5Months	Ref	
	5-10months	0.217	0.452
	>10Months	18.9	0.001
OIs	No	Ref	
	Yes	0.01	0.891

Key: No-the patient is not in use of the drug, Yes-The patient is in use of the drug, TPT- Mycobacterium tuberculosis preventive therapy, HIV-Human Immunodeficiency Virus, BMI-Body Mass Index, OIs-Opportunistic infections, HAART-Highly Active Antiretroviral Therapy, Ref-Reference variable.AF2B- Tenofovir, Lamivudine and Efavirenz combination, AF2E- Dolutegravir + Lamivudine + Tenofovir, AF2D- Tenofovir, Lamivudin, Atazanavir and Ritonavir combination, AS5B- Abacavir + Lamivudine + Atazanavir + Ritonavir

The HAART regimen categories that participants in this study were taking includes 5 people utilizing AS5B, or 4.0% of the study participants; 4 people in this category, or 3.2% of the participants, had AF2B; and another 4 people in this category, or 3.2% of the participants, had AF2D medicine while the majority of the population(89.6%) were using AF2E. Given the circumstances, the p-value

of having anemia while using AS5B,which is a second line treatment was 0.001(AOR=8.16) as analyzed on table 3.This indicates that the frequency of anemia and this particular HAART category are statistically significantly correlated. This was shown in the figure 4 that follows.

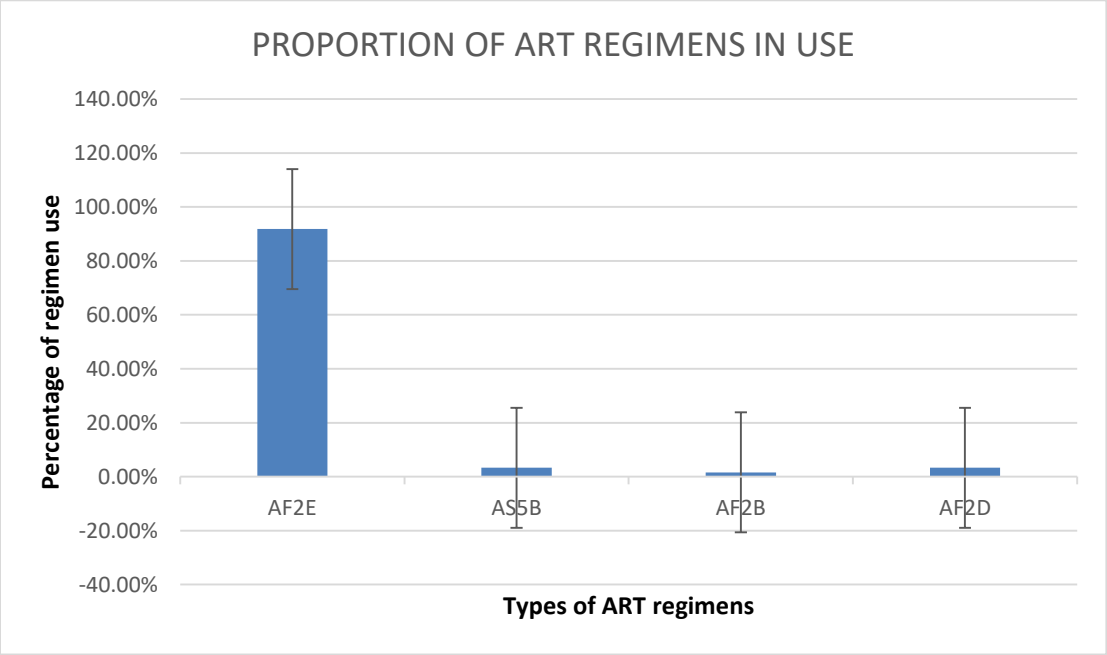


Figure 4: Prevalence of Anemia according to HAART

3.3 Correlation between CD4 cells level and anemia

Both current and baseline CD4 tests were compared. With the term current meaning(at the point of data collection) and baseline(first test after diagnosis).

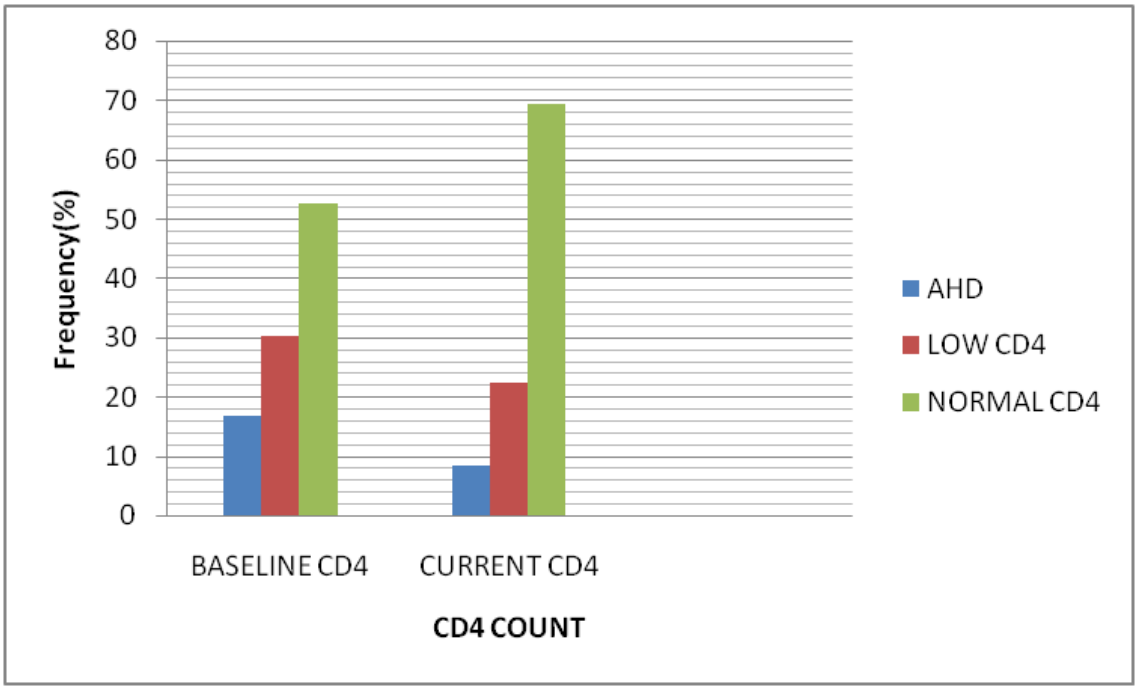


Figure 5: ComparisonCD4 (Current) Count Level and Anemia and CD4 (Baseline) Count Level and Anemia

The two instances showcase variations in the relationship between CD4 counts and anemia prevalence. In one of the instances (CD4 current count), the prevalence of anemia is notably higher among individuals with normal CD4 counts, potentially highlighting the

multi-factorial nature of anemia in HIV patients undergoing HAART. In the another instance (CD4 baseline count), there's a higher prevalence of anemia among those with advanced HIV, possibly reflecting the influence of disease progression on anemia risk. These differences underscore the dynamic interplay between CD4 counts, disease stage, and anemia prevalence, emphasizing the need for tailored interventions. This comparison can be understood by considering the impact of several factors. First, it is likely that the disruption in the production of hemoglobin, which leads to anemia, is a consequence of inflammatory cytokines being released and a decrease in the generation of hematopoietic growth factors. Additionally, the absorption and assimilation of iron in the body may be impaired as a result of HIV/AIDS. Furthermore, the decrease in CD4 cells compromises the immune system of patients, making them more susceptible to opportunistic infections, which in turn can lead to a deficiency in micro nutrients like iron.

The variations in anemia prevalence across CD4 count categories suggest that factors beyond CD4 levels may contribute to anemia risk. These factors could include nutritional status, disease duration, treatment adherence, and overall health status. As such, comprehensive care strategies that consider multiple factors are essential for effective anemia management in HIV patients undergoing HAART. Further analysis of correlation between CD4 and anemia was done to test the significance. With number of anemic (n)=124 (males=26, females=98). The relationship between Low hemoglobin and CD4 counts was analyzed as shown in figure 6 below.

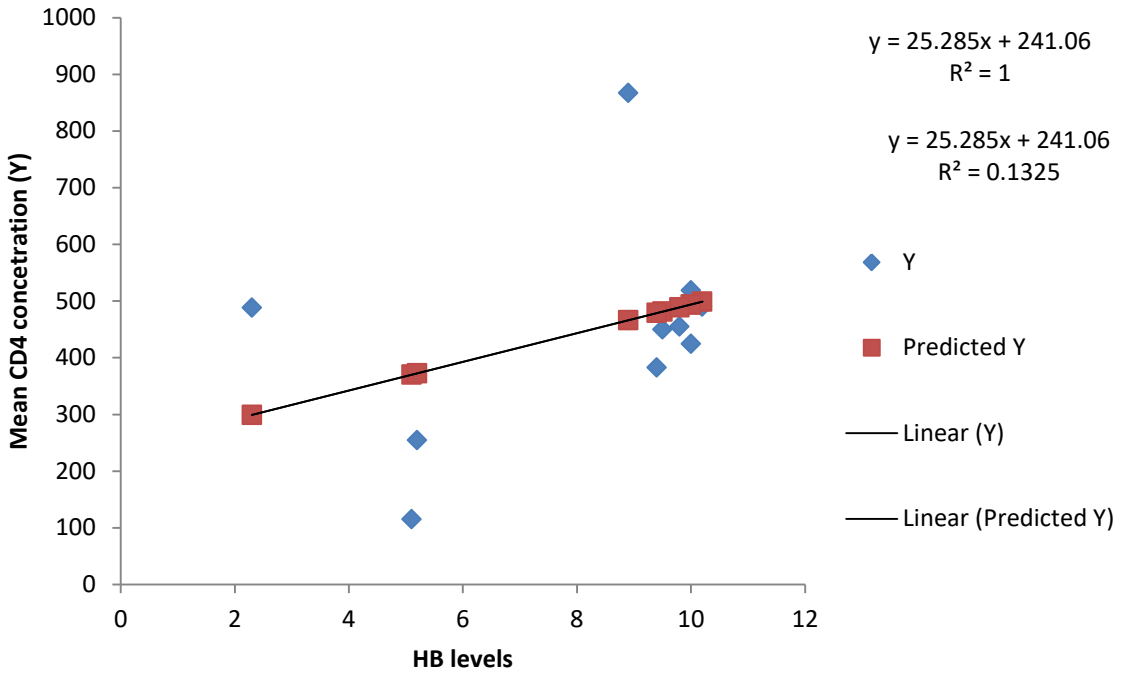


Figure 6: Relationship between CD4 counts and Haemoglobin levels in adult HIV patients undergoing HAART at Murang'a level 5 hospital

Through the use of linear regression analysis, it was established that there was no relationship between Anemia and CD4 mean concentration r=(9)=0.36, F=4.2, P=0.73, CI=95%. More so, sex did not have any influence on the Haemoglobin levels and CD4 counts, p>0.05.

3.4 Correlation between anemia and other hematological indices

In this study, hemoglobin concentration was correlated with other hematological parameters like hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), lymphocyte count (LYMPH), and platelet count (PLT) are collected and analyzed from adult HIV patients receiving HAART. By examining the correlations between anemia and these other indices, researchers aim to understand the broader hematological context of anemia in this population.

Table 4: Frequency Distribution of hematological indices in adult HIV patients undergoing HAART who had anemia

Hematological indices		F	%	P value
HCT	Low HCT	111	91.0%	0.01
	Normal HCT	11	9.0%	
	High HCT	0	0.0%	
MCV	Low MCV	23	18.9%	0.021
	Normal MCV	92	75.4%	
	High MCV	7	5.7%	
MCH	Low MCH	26	21.3%	0.017
	Normal MCH	92	75.4%	
	High MCH	4	3.3%	
MCHC	Low MCHC	15	12.3%	0.036
	Normal MCHC	107	87.7%	
	High MCHC	0	0.0%	
WBC	Low WBC	27	22.1%	0.045
	Normal WBC	87	71.3%	
	High WBC	8	6.6%	
LYMPHOCYTES	Low Lymph	12	9.8%	0.021
	Normal Lymph	84	68.9%	
	High Lymph	26	21.3%	
PLT	Low PLT	15	12.3%	0.011
	Normal PLT	100	82.0%	
	High PLT	7	5.7%	

A scatter plot to determine linear relationship between Haemoglobin levels and other hematological parameters was determined as shown in Figure 6 below. Haemoglobin levels had a linear relationship with HCT, MCV and MCH.

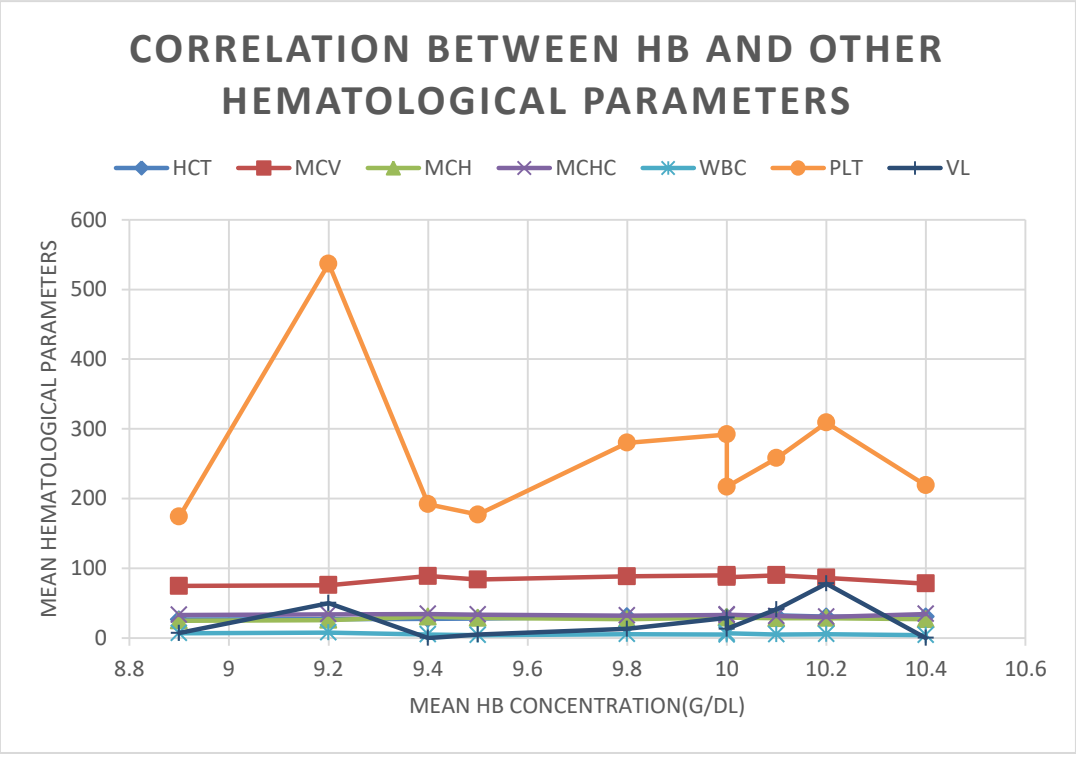


Figure 6: Scatter plot between haemoglobin levels and other hematological parameters.

Table 4below illustrates the link that was found between hemoglobin levels and other hematological markers. Hemoglobin levels showed a positive correlation ($p<0.05$) with HCT, MCV, and MCH.

Table 4: Zero-order correlation co-efficient for all variables in the study

	Hb means	HCT	MCV	MCH	MCHC	WBC	PLT	VL
Hb means	1.00							
HCT	0.81	1.00						
MCV	0.49	0.55	1.00					
MCH	0.40	0.19	0.81	1.00				
MCHC	-0.36	-0.65	-0.40	-0.02	1.00			
WBC	-0.53	-0.51	-0.41	-0.43	0.04	1.00		
PLT	-0.09	-0.06	-0.23	-0.30	-0.11	0.54	1.00	
VL	-0.40	-0.40	-0.50	-0.41	0.24	0.64	0.89	1.00

Note: 1.00 is a perfect correlation. Values within +/- CV (not significant) $p<0.05$ (n=126, Cv=0.174)

4.0 Discussion

Anemia prevalence among persons living with HIV was estimated to be 46.6% (95% CI: 41.9%-51.4%), according to a study by Guiying Cao et al. (2022). These results compared to this study, Guiying study indicates a slightly higher prevalence of anemia among individuals living with HIV. There could be a number of reasons for the variation in prevalence rates, such as variations in the research populations, study sites, study methodology, and study periods.

Table 1 points that the prevalence of anemia among people living with HIV based on their hemoglobin (Hb) levels. Out of a total of 295 patients undergoing evaluation, 8.81% were identified with severe anemia, 11.53% with moderate anemia, and 21.69% with mild anemia. Collectively, the survey found that the anemia overall prevalence in this cohort is substantial, affecting a considerable portion of the HIV-positive patient population. These findings highlight a significant severity of anemia among the HIV-positive patient population under study, with the majority experiencing mild anemia. However, a significant number also suffers severe anemia. In a study conducted by Guiying Cao et al. (2022), the population of adults who have HIV, the combined occurrence rates of anemia severity were as follows: 21.6% (95% confidence interval: 19.9% to 23.3%) for mild anemia, 22.6% (95% confidence interval: 14.8% to 30.4%) for moderate anemia, and 6.2% (95% confidence interval: 4.4% to 8.1%) for severe anemia. Another study by Yinzhong Shen at el. (2013) the occurrence rates of mild, moderate and severe anemia stood at 32.4%, 17.0%, and 2.5%, respectively.

According to a study by Shen et al. (2013), anemia was discovered in 760 (51.5%) of the 1476 male patients and 251 (53.2%) of the 472 female patients. The frequency of anemia did not exhibit a significant difference based on gender (P=0.523) which was insignificant too to our study with a p value of 0.143 which was less than 0.05.

This alignment between the current study and Shen et al.'s findings reinforces the common observation of a higher prevalence of anemia among females within HIV-positive populations undergoing antiretroviral therapy. Such consistencies emphasize the importance of gender-specific considerations in anemia management strategies and the potential need for targeted interventions to address this disparity.

Hemoglobin levels showed notable variations in another study by Saathoff et al. (2011), with lower levels seen in females compared to males and in HIV-positive persons compared to HIV-negative participants.

The study determined the patient's prevalence on anemia according to year diagnosed. By examining the magnitude of anemia in regard to the year the subject was diagnosed or have been o HAART, it will depict the effectiveness of HAART to the patient. Disease progression also changes with time and it is therefore necessary to find out the impact of length after exposure to disease. In this study the its researchers can discern potential trends or changes in anemia's severity over time. The prospect of this study to shed light on the temporal trends of anemia severity and prevalence makes it significant. Those with over 15 years since exposure had a more risk of exposure (AOR=18.5, p value =0.001), compared to those with less than 5 years, used as reference.

The study expounds how the prevalence of anemia was classified based on the patients' ages. According to the breakdown, 10.4% of the participants were between the ages of 18 and 27, 6.5% were between the ages of 28 and 37, 33.9% were between the ages of 38 and 47, 32.3% were between the ages of 48 and 57, and 16.9% were older than 58.

Overall, according to table 2; those aged 28-37 years had more risk of getting anemia (AOR=1.557) than those aged 18-27years (reference). Basically, the age between 28-37 is where majority are at childbearing and physiologically is more predisposed to low hemoglobin. Those aged 48-57(AOR=1.53, p value=0.059) and 58years and above (AOR=1.341, p value 0.041) were also at higher risk compared to reference age (18-27). This distribution sheds light on the variations in anemia prevalence among various age groups. The study by Assefa et al. (2015), which looked into anemia prevalence dependent on patient age, can be used to compare this. In this regard, they discovered in their

research that, at the 6-month mark, older age was a factor that was independently associated with higher chances of having anemia

Moreover, a number of variables were found to be independently linked to a higher risk of anemia at the 12-month mark. Baseline anemia (AOR = 2.01; 95% CI: 1.36-2.97), people in the 25–34 age range (AOR = 5.92; 95% CI: 1.39–25.15), and people in the 45–54 age range (AOR = 4.78; 95% CI: 1.07–21.36) were among these covariates.

Based on the patients' WHO HIV stage, the data set provides an overview of the prevalence of anemia. 55.7% of the individuals were in Stage 1 of HIV, 34.4% were in Stage 2, 8.2% were in Stage 3, and 1.6% were in Stage 4 according to the distribution. This dissection offers valuable insights into the variations in anemia prevalence among various HIV infection stages. According to the study odd ratio in Table 5,Stage 4 are more susceptible of having anemia(AOR=13.421, p value 0.001), stage 3 follows in the category(AOR=0.0789, p value=0.343) , there was a statistical correlation between the prevalence of anemia and HIV Stage 3 and 4, with a p-value of 0.001, which was less than 0.05.Similar findings were found in the study by Taye et al. (2015), which included 123 participants, or 68.3% of the total, who were categorized as HIV/AIDS clinical stage II by the WHO, with clinical stage I being the next most common at 19.4%. Interestingly, anemia was more prevalent in patients in WHO clinical stages II and III, even though there was no evidence that these stages were independent risk factors for anemia (p>0.05).These studies highlight the variation in anemia incidence according to HIV stage and highlight the intricate interactions between treatment plans, patient characteristics, and the course of the illness.

The survey indicated that the prevalence of anemia categorized by viral load levels among the participants. The distribution indicates that among the participants, 79.0% had a viral load of undetectable, (<LDL) 18.6% had a viral load of moderate(<1000copies/ul), and 2.4% had a viral load of high (>1000 copies/ul). According to table 5, those with unsuppressed viral load(>1000 copies/ul) were at more risk of getting anemia(AOR=18.98)Given that the p value obtained was 0.03, which was less than 0.05 at the 95% confidence interval, the link between viral load and anemia prevalence was judged to be statistically significant, as this breakdown illustrates. Similar findings were noted in research by Ageru et al. (2018), where anemia was reported in 85% of patients with an undetectable viral load, 10% with a moderate viral load, and 5% with a high viral load. On the other hand, (O'Mahony et al. 2023) reported contrasting results, with 70% anemia prevalence in patients with a viral load of undetectable, 25% with a viral load of moderate, and 5% with a viral load of high. Study by Thompson et al. (2021) indicated that, the distribution was 75% anemia prevalence in patients with a viral load of undetectable, 20% with a viral load of moderate, and 5% with a viral load of high. These comparisons highlight the intricate relationship between viral load and anemia, suggesting that higher viral loads might be connected with a higher probability of anemia. These results carry significant clinical importance, as they underscore the potential interplay between disease progression, viral load management, and anemia prevalence in HIV-positive patients. Effective management of viral load could be a crucial factor in mitigating anemia's impact on patient outcomes.

The study indicated the BMI among the anemic that the distribution reveals that among the participants, 1.6% had severe wasting, 7.3% had moderate wasting, 54.0% had a normal BMI, 17.5% were overweight, 11.7% were categorized as Obese I, and 7.5% were Obese II. Table 2 discuss further the characterization of risk in various subgroup with those with BMI <18.5 being at more risk (AOR=18.66, p value=0.002). This breakdown highlights the correlation between low BMI (underweight individuals) and anemia prevalence was found to be 0.002 which indicated that there was a statistical relationship between prevalence of anemia and BMI since it was less than 0.05. Similar patterns were seen in a study by Ageru et al. (2018), which found that people with a BMI under 18.5 were 2.96 times more likely to be anemic (AOR = 2.96, 95% CI: -1.37–6.39). Furthermore, compared to those who were already on HAART, those who had not started HAART were 2.23 times more likely to develop anemia (AOR = 2.23, 95% CI: 1.16–4.28). The results are noteworthy because they emphasize how crucial a balanced diet and weight control strategy is for treating anemia in people living with HIV. Maintaining a healthy BMI can be a key component of health interventions aimed at lowering the prevalence of anemia and enhancing patient well-being in general. More so, BMI is a measure that can show disease progression and poor dietary intake or absorption. During advanced stages of a disease, the patient will tend to have poor appetite and sometimes desire to eat is completely lost. Food absorption and assimilation is also affected by the disease state. Therefore, anemia at this stage may be due to poor dietary intake or absorption related malfunctions

Table 3 reveals that the bulk of individuals (87.7%) who have a few cumulative months defaulter history (0 to 5 months) only. Defaulter history in HIV medication is an important information as it points out the level of drugs adherence by the patient. Notably, a large number of the patients adhered to the medication with just a small percentage (5%) having abandoned medication for more than 10months. In table 2, the susceptibility rate test shows that default history of >10months had (AOR=18.9,p value 0.001) The low p-value of 0.001 indicates a statistically significant relationship between defaulter history duration and anemia prevalence, suggesting that the duration of defaulting from treatment may be associated with the likelihood of developing anemia.

A potential nonlinear association between CD4 cells enumeration and anemia prevalence is suggested. The findings highlight the significance of monitoring CD4 counts to predict and manage anemia risk in HIV patients. The data reflects a similar pattern when considering baseline CD4 counts. Notably, variations exist in the relationship between CD4 counts and anemia prevalence, potentially highlighting the multi-factorial causes of anemia in HIV patients. The observed differences suggest that anemia risk is influenced by factors

beyond CD4 levels, such as nutritional status, disease duration, treatment adherence, and overall health. As a result, comprehensive care strategies that encompass various factors are vital for effective anemia management in HIV patients undergoing HAART

In order to investigate the relationship between anemia and other hematological indices in adult HIV patients using HAART, Pearson's chi-square test was employed in the study. Given that the obtained p value of 0.02 was less than 0.05, the study demonstrated a statistically significant link between anemia and the various hematological indices.

Denue et al. (2013) found that in patients who had not started Highly Active Antiretroviral Therapy (HAART), anemia (defined as packed cell volume [PCV] <30%) was observed in 57.5% of cases, leukopenia (defined as WBC <2.5) in 6.1% of cases, and thrombocytopenia (defined as <150 platelets) in 9.6% of cases. These incidence rates were markedly greater than those of their peers who were already on HAART, who had incidence rates of 1.7% for leukopenia, 1.2% for thrombocytopenia, and 24.3% for anemia, respectively.

This suggests that between anemia and a number of hematological indices, such as hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin, there is a statistically significant association in the two studies. These findings underlined the importance of considering these indices in the context of anemia diagnosis and treatment. The strong correlation between anemia and hematological indices highlighted in these studies suggests the need for integrated care strategies. Monitoring and addressing hematological imbalances could potentially contribute to early anemia detection, effective management, and improved quality of life for HIV patients undergoing HAART.

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Authors' contributions

D.K.: conceptualization of the project, data collation, grouping and analysis, formulation of the original draft, evaluation of the manuscript, visualization; S.E.: Visualization, preparation of the original draft, reviewing of the manuscript, supervision, data interpretation; S.K.: conceptualization of the project, methodology, writing the original draft, reviewing of the manuscript, supervision, data interpretation. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that there are no competing interests.

Abbreviations

AIDS-Acquired Immune Deficiency syndrome
CCC-Comprehensive Care Centre
CD4-Cluster of differentiation number 4
HAART- Highly Active Antiretroviral Therapy.
HIV-Human Immunodeficiency Virus

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