



**Prevalence and Associated Factors of Anemia in Human
Immunodeficiency Virus Infected Patients on Anti-retroviral
Treatment at St. Paul's Hospital Millennium Medical College**

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**Prevalence and Associated factors of Anemia in Human
immunodeficiency virus infected patients on ART at St.
Paul's Hospital in adult ART follow up clinic**

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Table of Contents

Acknowledgement	I
List of figures and tables	iv
List of figures	iv
List of tables	iv
Abbreviations	vi
Abstract	viii
1. Introduction	1
1.1 Background	1
1.2 Statement of the Problem	2
1.3 Significance of the study	3
2. Literature review	4
2.1. Anaemia	4
2.2. HIV/AIDS	4
2.3. Prevalence of anaemia in HIV patients	5
2.4. Associated factors of anaemia in HIV patients	6
2.5 Conceptual framework	7
3. Objective	8
3.1 General objective	8
3.2. Specific objectives	8
4. Methods	9
4.1 Description of study setting and study period	9
4.2 Study design	9
4.3 Source population	9
4.4 Study population	9
4.5 Inclusion and Exclusion criteria	9
4.5.1 Inclusion criteria	9
4.5.2 Exclusion criteria	10
4.6 Sampling	10
4.6.1 Sample size determination	10
4.6.2 Sampling technique	10
4.8 Study Variables	11
4.8.1 Dependent variables	11

4.8.2 Independent Variables	11
4.9 Operational Definition	12
4.10 Data management and analysis	12
4.11 Data quality control	12
4.12 Ethical considerations	13
4.13 Dissemination of results.....	13
5. Result.....	14
5.1 Socio-demographic characteristics	14
5.2 Clinical characteristics of patients.....	15
5.3. Prevalence of anaemia	18
5.4 Factors associated with anaemia among HIV positive patients on ART.....	19
5.4.1 Bivariable analysis	19
5.4.2 Multivariable logistic regression analysis	22
6. Discussion.....	23
7. Limitations of the study.....	24
8. Conclusion	25
9. Recommendations.....	26
References	27
Annex: check List	31

List of figures and tables

List of figures

Figure 1 Relationships between associated risk factors and anemia	7
Figure 2 schematic representation of data collection procedure	14
Figure 3 BMI (in Kg/m ²) of study subjects of HIV patients on ART in SPHMMC, Addis Ababa, Ethiopia, 2019.....	17
Figure 4 Type of ART regimens in HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019.....	18

List of tables

Table 1 Socio-demographic characteristics of HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019.....	15
Table 2 Clinical characteristics of HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019.....	16
Table 3 RBC parameters of HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019	17
Table 4 Anemia status of HIV patients on ART at SPHMMC, Addis Ababa, Ethiopia, 2019.....	19
Table 5 Bivariable analyses of factors associated with anemia by socio-demographic variables in HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019	20
Table 6 Bivariable analyses of factors associated with anemia by clinical characteristics in HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019	21
Table 7 Multivariable analysis of factors associated with anemia in HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019	22

Abbreviations

AOR-adjusted odds ratio

ART-Antiretroviral therapy

BMI-body mass index

CBC-complete blood count

CI-confidence interval

CM-centimeter

E.C-Ethiopian calendar

Fl-femtoliter

GC-Gregorian calendar

HAART-highly active antiretroviral therapy

Hgb-hemoglobin

HIV-human immunodeficiency virus

ICU-intensive care unit

KG-kilogram

MCV-mean corpuscular volume

MCHC-mean corpuscular hemoglobin concentration

MM-millimeter

PLWHA-people living with HIV AIDS

RBC-red blood cell count

RDW-red cell distribution width

RVI-retroviral infection

SPHMMC-saint Paul's hospital millennium medical college

WHO-world health organization

ZDV -zidovudine

TB-tuberculosis

TDF-tenofovir disoproxil fumarate

Abstract

Background: Anemia is a common complication of infection with the human immunodeficiency virus (HIV) and may have various causes. It is the most common hematologic abnormality among HIV-infected individuals; this includes those who are taking antiretroviral treatment (ART) and those who are ART-naïve.

Objective: the objective of this study was to assess the prevalence and associated factors of anemia in HIV infected patients who are on ART and have follow-up at SPHMMC ART clinic.

Methods: A hospital based cross-sectional analytic study was conducted in 150 patients who were seen from January-June, 2019 G.C. Data on demographic characteristics, related factors of anemia, the latest complete blood count profile, CD4 and ART regimen was collected using a checklist. All HIV patients who fulfilled the inclusion criteria were randomly selected. Data was collected by internal medicine residents and interns. Data was first checked manually for completeness and then was cleaned and analyzed using SPSS version 25. Results were reported by tables, graphs and charts. Level of significance ($p < 0.05$) and CI (95%) was reported.

Results: Among the 150 HIV positive patients on ART, the prevalence of anemia was 14 %. Among the patients with anemia, majority (57.1%) had mild anemia and more than half (57.2%) of patients with anemia had macrocytic type. Sex, BMI, CD4 count and ART regimens were significantly associated with anemia.

Conclusion and recommendation: Anemia is still a health problem in patients who are taking ART with proper follow-up. Therefore, HIV positive patients on ART in the follow-up clinic should have routine screening by CBC for anemia and those taking AZT based regimen needs particular attention.

Key words: HIV, Anemia, ART, SPHMMC, Ethiopia

1.Introduction

1.1 Background

Complications of human immunodeficiency virus (HIV) infection include hematological abnormalities manifested by pancytopenia, anemia being the leading abnormality [1-3]. However, the prevalence of anemia in HIV patients varies considerably, ranging from 1.3% to 95% [4]. Several factors including stage of HIV, age and sex are said to account for the variations in the prevalence of anemia in HIV patients [4]. The causes of anemia have been reported to be multifactorial. Direct effects of HIV and its viral proteins as well as immune dysregulations during HIV infection were found to be responsible for bone marrow suppression [5, 6]

Normocytic normochromic is the most prevalent morphological type of anemia in HIV, followed by microcytic hypochromic and macrocytic in variable percentages. [7,8] Anemia of chronic disease is known to be the most common etiology of normocytic normochromic and microcytic hypochromic anemia in HIV while iron deficiency is known to be a relatively infrequent cause of microcytic hypochromic anemia. [7] Macrocytic anemia is reported to have a low prevalence in HIV. [9]

Anemia has been reported to influence the natural history of HIV disease by increasing the rate of disease progression and mortality in both developed and developing countries [10]. Mocroft et al. have demonstrated that severe anemia is linked with faster rate of HIV disease progression. They have also shown that anemia is a strong predictor of death [11]. The consequences of untreated anemia may lead to multisystem disabling symptoms and fatigue, exhaustion, increased risk of HIV dementia, poor quality of life and possibly even exacerbates poverty in communities with a high HIV prevalence [12]. On the other hand, survival time in HIV-infected persons may be enhanced after recovery from anemia [13].

Anemia is a major health problem globally, which can cause a vicious impediment on quality of life, mortality, morbidity, and socioeconomic progress of a country. It affects more the developing than the developed countries. [14]

Anemia is the most common hematologic abnormality among HIV-infected individuals; this includes those who are taking antiretroviral treatment (ART) and those who are ART-naïve. [15]

1.2 Statement of the Problem

Anemia is a major public health problem in HIV patients around the world. The prevalence of anemia in HIV patients is significantly high. A study conducted in the very early years of HIV discovery had shown the overall prevalence of anemia to be about 28% among people with HIV infection in the pre-AIDS stage of the disease, whereas it could reach as high as 71% in the advanced or AIDS stage of the HIV disease [29]. A study done in Tanzania has shown the prevalence of moderate to severe anemia was 40.46% in adult HIV positive patients receiving first line HAART. [24] A study done in the southwest part of Ethiopia has shown the prevalence of anemia to be 16.2% among ART-experienced HIV patients. [30]

Anemia has a negative effect on the quality of life of HIV patients and progression of the HIV disease [16]. It can lead to impaired physical functioning, psychological distress, poor quality of life, accelerated disease progression, and shorter life expectancy. [31]

Various studies have identified different factors to have association with anemia among adult HIV patients including gender, residence, marital status, educational status, income, duration of ART, type of ART regimen, history of anti-tuberculosis (TB) drug treatment, presence of opportunistic infections (OIs), advanced stage of the HIV disease, CD4+ T lymphocyte count <200 cells/ μ l. [23,28,30] However, the reported magnitudes and associated factors are different from one geographic area to another.

Although anemia can occur at any stage of HIV infection, its severity is correlated with progression of the HIV disease stage. Drugs used to treat HIV infection and its complications are also known to cause bone marrow suppression [17, 10]. It is widely known that AZT alone and AZT based HAART regimen is associated with significant reduction of hemoglobin (Hgb) level [17, 19]. In the sub-Saharan African setting, including Ethiopia both HIV infection and under-nutrition are expected to be high. [16]

Different studies were conducted to assess the prevalence of anemia in HIV infected individuals [20], but there are only few published reports in Ethiopia on the assessment of anemia among HIV positive patients, particularly on those who are on HAART. In our setup the prevalence and related factors of anemia is not studied in patients on HAART having regular follow up at ART clinic.

1.3 Significance of the study

Despite the fact that anemia is a major cause of morbidity and mortality in patients with RVI , data on anemia and associated risk factors among RVI patients are generally insufficient from low-resource countries such as Africa, including Ethiopia. Even with HAART which is initiated as a standard of care of patients with HIV intended to reverse both the immunological and non-immunological complications of HIV, moderate to severe anemia is still a big problem in ART experienced patients especially in resource limited setting like ours.

Anemia is still a common problem among patients taking ART; so that identification and timely interventions to correct anemia may lead to improved health and survival potential of HIV-infected person. Identification and correction of associated factors will help prevent the development of anemia; which in turn will improve quality of life and survival of the patients.

This study will determine the magnitude of anemia in HIV patients on ART and also identifies factors associated with development of anemia and suggests early preventive or correction measures, create awareness to health professionals and policy makers. It will help health professionals working at ART clinic pay attention to identification and proper workup of anemia. It also helps as a stepping stone for future interventional studies.

2.Literature review

2.1. Anaemia

Anemia is a condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs. Specific physiologic needs vary with a person's age, gender, residential elevation above sea level (altitude), smoking, Behavior and different stages of pregnancy. [33]

According to WHO, hemoglobin level is used to diagnose anemia at sea level. The hemoglobin cut-offs (in g/dl) to diagnose anemia in men (age 15 and above), pregnant women and non-pregnant women (age 15 and above) is <13, <11 and <12, respectively. The severity of anemia classified based on hemoglobin level as: mild (men 11-12.9, pregnant women 10-10.9, non-pregnant women 11-11.9), moderate (men 8-10.9, pregnant women 7-9.9, non-pregnant women 8-10.9), severe <8 for both men and non-pregnant women, <7 for pregnant women. [33]

Kassebaum et al estimate that the global anemia prevalence in 2010 was 32.9%, resulting in 68.4 million years lived with disability (YLD). The results emphasize the important contribution made by anemia to the overall global burden of disease. South Asia accounted for 37.5% of the global anemia burden, whereas sub-Saharan Africa contributed 23.9%. [34] Data on the prevalence of anemia in Ethiopia in the general population is lacking.

2.2.HIV/AIDS

HIV is a virus that targets and alters the immune system, increasing the risk and impact of other infections and diseases. There are two types of HIV: HIV-1 and HIV-2. HIV-1 is responsible for the vast majority of HIV infections globally. Without treatment, the infection might progress to an advanced disease stage called AIDS. [32]

About 37 million people currently living with HIV globally. According to the 2017 new spectrum estimate, 722,248 Ethiopians are currently living with human immunodeficiency virus (HIV) and all of them require ART. However, only 420,000 individuals are currently taking antiretroviral (ARV) drugs. [35]

2.3. Prevalence of anaemia in HIV patients

Anemia is the most common hematologic abnormality among HIV-infected individuals; this includes those who are taking antiretroviral treatment (ART) and those who are ART-naïve. [15]

According to reports, the prevalence of anemia among adult HIV patients taking ART ranges between 23% and 50% globally and 24% and 58% in Africa. [1, 27, 28] Generally, the prevalence of anemia tends to be higher in ART-naïve patients compared to ART users.

One retrospective observational cohort study that was done in China included 3452 HIV-infected patients attending Beijing Ditan Hospital. The result of the study showed overall prevalence of anemia was 9.8% (7.6% mild, 1.9% moderate, and 0.2% severe anemia). [21]

A cross-sectional study done in India enrolled 103 consecutive HIV patients attending medical emergency, outpatient department, wards, and ART center showed 86.4% (89/103) patients were found to be anemic. There was no significant difference in prevalence of anemia in ART-naïve patients from those who were on ART. Normocytic- normochromic was the most common type of anemia in males (46%) as well as females (42%). [22]

Mohsen Meidani, Farshid Rezaei, Mohammad Reza Maracy, conducted a study on anemia prevalence and risk factors of 212 HIV positive patients were assessed, at the behavioral disease consulting center in Isfahan, Iran. In this study, the overall prevalence of anemia was 71%, with the majority of patients having mild to moderate anemia. Mild to moderate anemia and severe anemia occurred in 67% and 4% of patients, respectively. Of the 212 HIV positive patients enrolled, 17 (8%) had a positive history of tuberculosis. [23]

In a cross sectional clinic based study conducted at Bugando Medical Centre Care and Treatment Center, in northwestern Tanzania, a total of 346 adult HIV patients on first line Highly Active Antiretroviral Therapy were involved. The patients' data were analyzed using STATA version 11 to determine the prevalence of moderate to severe anemia and risk factors that could predict occurrence of anemia. In this study 346 patients on HAART were enrolled, of whom 100 (40.46%) had moderate to severe anemia. [24]

In a study carried out in Benin City, Nigeria, a total 457 HIV patients attending HIV clinics were recruited for this study. The patients consisted of 217 (86 males and 131 females) HIV patients on HAART for 3 – 6 months and 240 (106 males and 134 females) HAART naïve HIV patients. An overall prevalence of 60.61% (277 out of 457 patients) of anemia was observed in this study. HIV patients on HAART had a significantly lower prevalence of anemia compared with

their HAART naïve counterparts (51.15% vs.69.17%). Generally, there was no significant difference in the prevalence of anemia between both genders (male = 61.98%; female = 59.62%). [25]

Owiredu WK, Quaye L, Amidu N, Addai-MensahO conducted a cross-sectional study at the antiretroviral (ART) clinic in Ghana, a total of 442 PLWHA consisting of 166 patients on HAART (28 males and 138 females) and 276 HAART naïve patients (76 males and 200 females) were recruited. The incidence of anemia (Hbg less or equal to 10.5 (63%) and PCV < 30% (37.6%)) in HAART-naïve patients was significantly higher compared to their counterparts on HAART (46%, 15.2%) respectively. [1]

In a hospital-based cross-sectional study done among adult HIV patients in Debre- Tabor Hospital, Ethiopia, a total of 377 patients' charts were reviewed. Most of the participants (n=237, 62.9%) were taking antiretroviral treatment (ART). The overall prevalence of anemia was 23%. [16]

In a Cross sectional study conducted at ART clinic of Black Lion Specialized Hospital, Addis Ababa, Ethiopia, a total of 255 HIV positive patients were involved in this study. About 95 (37.3%) participants were under WHO clinical stage III and the most widely used ART regimen (62.7%) in this study was 1e (TDF- 3TC-EFV). The prevalence of anemia in HIV patients was 41.9 % (107/255) before ART initiation and 11.4% (29/255) after ART initiation. From anemic patients after ART initiation, about 22(75.9%) had mild anemia and 7 (24.1%) had moderate anemia. Severe anemia was not found in this study. [20]

A retrospective cohort study conducted in Zewditu Memorial Hospital, Addis Ababa, Ethiopia included records of 1061 patients on HAART were selected using simple random sampling technique. The prevalence of anemia at baseline was 42.9%. However, the prevalence significantly decreased to 20.9% at 6 months and to 14.3% at 12 months after HAART initiation. [26]

2.4. Associated factors of anaemia in HIV patients

In a retrospective observational cohort study that was done in China which included 3452 HIV-infected patients attending Beijing Ditan Hospital, the multivariate logistic regression model indicated that female sex, age 40-59 years, age > 60 years, and BMI < 18.5 kg/m², compared with normal BMI and overweight), were associated with increased odds of anemia. Laboratory tests indicated that baseline HIV viral load > 10⁵copies/ ml, baseline CD4 count ≤ 50 × 10⁹/L,

and CD4 count $51-199 \times 10^9/L$ were risk factors for anemia. Anemia was not associated with route of HIV transmission, WHO clinical stage, baseline HIV viral load, SMZ-TMP co-administration, or baseline HBV/HCV co-infection status [21].

In a cross sectional clinic based study conducted at Bugando Medical Centre Care and Treatment Center, in northwestern Tanzania, a total of 346 adult HIV patients on first line HAART were included. The odds of being anemic were strongly predicted by Zidovudine (ZDV) based regimen, low baseline CD4 count (< 200 cells/ μl) and HIV stage 3&4 at enrollment. Most of the anemic patients had macrocytic anemia. [24]

In a hospital-based cross-sectional study done among adult HIV patients in Debre- Tabor Hospital, Ethiopia, a total of 377 patients' charts were reviewed. Being ART-naïve, having treatment history with anti-tuberculosis (TB) drug, taking Zidovudine (ZDV)-containing ART regimen and having recent CD4+ T-lymphocytes count of <200 cells/ μl were associated with occurrence of anemia among adult HIV patients. [16]

A retrospective cohort study conducted in Zewditu Memorial Hospital, Addis Ababa, Ethiopia included records of 1061 patients on HAART were selected. At baseline, male sex, clinical stage III/IV and TB co-infection were independently associated with the odds of being anemic. After 6 months of HAART, male sex, baseline anemia and TDF-based HAART were independently associated with the odds of being anemic [26].

2.5 Conceptual framework

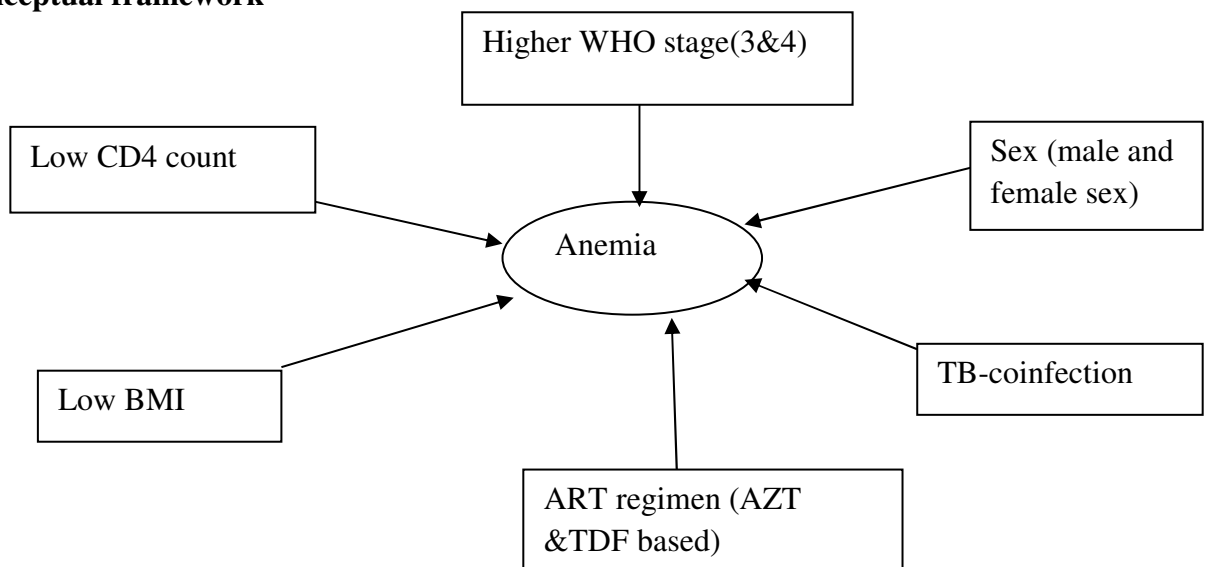


Figure 1 Relationships between associated risk factors and anemia

Source: From different literatures reviewed in this study(16, 21, 24, 26).

3. Objective

3.1 General objective

- To assess the prevalence and related factors of anemia in HIV patients on ART on follow up at ART clinic in SPHMMC from January-June 2019 G.C.

3.2. Specific objectives

- To determine the prevalence of anemia in HIV patients
- To identify factors related to anemia in HIV patients

4. Methods

4.1 Description of study setting and study period

This study was conducted in Saint Paul hospital millennium medical college (SPHMMC), ART clinic. SPHMMC is one of the two biggest specialized referral hospitals in Addis Ababa and in the country. It was established in 1968. It is a rapidly expanding hospital especially after it started working as a medical college since 2000 E.C/2007G.C. It currently runs residency and subspecialty programs in many fields. It is a center of excellence in medical services for renal center providing a dialysis and also the first hospital in the country with renal transplantation center. It also opens a new burn, emergency and trauma center. It has also outreach services to fulfill the community demand in health. The hospital has over 45 beds in medical ward and 6 beds in ICU, with over 1200 admissions per year. It has adult and pediatric ART follow up clinic separately.

The study was conducted by reviewing patients' charts that were seen in ART clinic from January –June, 2019. The data was collected and analyzed from July –August, 2019.

4.2 Study design

Hospital based cross-sectional analytic study was done in ART follow-up clinic on patients who visited the clinic from January-June, 2019

4.3 Source population

All HIV positive patients on ART in St Paul hospital millennium medical college in 2019.

4.4 Study population

All HIV positive patients on ART in St Paul hospital millennium medical college in 2019 that satisfy the study inclusion criteria.

4.5 Inclusion and Exclusion criteria

4.5.1 Inclusion criteria

- ✓ HIV positive patients age 18 years or above on ART for at least 06 months
- ✓ Those who have regular follow up
- ✓ Patients with latest laboratory investigation (CBC within 3-6 months and CD4 count within 6-12 months).

4.5.2 Exclusion criteria

- ✓ Patients transferred from other setup to SPHMMC ART clinic on their first visit
- ✓ pregnant women
- ✓ women in the post-partum period
- ✓ Those adult patients with incomplete information for hemoglobin (Hgb) status on the chart during follow-up
- ✓ Patients with malignancy (both hematologic and solid tumors)

4.6 Sampling

4.6.1 Sample size determination

From study done on prevalence of anemia before and after initiation of ART among HIV infected patients at Black Lion Specialized Hospital (prevalence after initiation of ART was 11.4%), with margin of error 5% and 95% confidence interval, [20] the sample size required for this study is 155 patients.

$$n = Z^2 p (1-p) / w^2$$

$$n = 1.96^2 (0.114(0.886)) / 0.05^2 = 155$$

Where n = number of sample size

$Z_{\alpha/2}$ = Z score at 95% confidence interval $Z = 1.96$

P = anticipated population proportion

W = margin of error = 0.05

Because the source population is <10,000, there is a need to use population correction formula which is $n = n_0 / (1 + n_0 / N)$, where n_0 = initial sample size (155)

N = source population (5000)

This gives a sample of 150; by adding 10% nonresponse rate, the final sample size is 165.

4.6.2 Sampling technique

The chart No of HIV positive patients on ART during the study period were taken from ART clinic; and among those charts, the study subjects were selected randomly by simple random sampling using a computer.

4.7 Data collection method and tools

The data was collected using a document review by interns and internal medicine residents. The collected information includes socio-demographics, clinical characteristics, and immune hematological profile of patients. Data on socio-demographic and clinical characteristics of the study participants was collected by review of medical records. Weight and height measurements were taken from patients' charts and BMI was calculated manually by dividing weight in Kg for height in m². The BMI cut-off value according to WHO classification (in Kg/m²) will be <18.5 (underweight), 18.5-24.99 (normal), overweight (25-29.99) and obese (≥ 30). [36] The principal investigator had a supervisory role. Document review was done by data collectors and principal investigator.

4.8 Study Variables

4.8.1 Dependent variables

- Anemia status

4.8.2 Independent Variables

- Socio-demographic factors-age, sex, level of education, place of residence, occupation, marital status
- WHO stage of HIV
- CD4 count
- Nutritional status (BMI)
- ART regimen
- ART duration
- Duration of HIV since diagnosis
- Cotrimoxazole prophylaxis
- Tuberculosis
- Chronic diarrhea

4.9 Operational Definition

- ✓ Anemia- defined as Hgb concentration less than 13 g/dl for adult males and less than 12 g/dl for adult females. [33]
- ✓ Mild anemia --defined as (Hbg 11–11.9 g/dl for women and 11–12.9 g/dl for men).[33]
- ✓ Moderate (Hbg 8–10.9 g/dl) and severe (Hgb< 8 g/dl) for both sexes.[33]
- ✓ Microcytosis- -defined as MCV < 80 fL, macrocytosis as MCV > 100 FL
- ✓ Hypochromic is defined as MCHC value < 31 g/dl.
- ✓ Regular follow up -defined as when the patient visits ART clinic according to appointment given by health professional (physician/nurse) working at ART clinic.
- ✓ chronic diarrhea—passage of loose stools for more than 01 month

4.10 Data management and analysis

Data was first checked manually for completeness and then entered into a computer and analyzed using SPSS version 25. Bivariable analyses between dependent and independent variables were performed using binary logistic regression. Variables with P-value 0.2 and below were taken into multivariate analysis. Multivariable analysis was done to control (adjust) for possible confounding variable. During the analyses P-value < 0.05 with 95% confidence interval (CI) for OR (odds ratio) was used in judging the significance of the associations. Results are presented in text, tables, charts and graphs.

4.11 Data quality control

To ensure the quality of data, preceding data collection, training of the data collectors was carried out for one day by the principal investigator on the objective, relevance of the study and confidentiality of information.

The checklist was prepared in English. About 5% of the data were verified by the principal investigator during the initial stage of data collection and appropriate instruction was given to the data collectors. The collected data were reviewed and checked for completeness and consistency by principal investigator on daily basis at the spot during the data collection time.

4.12 Ethical considerations

Ethical clearance and permission letter was obtained from the Institutional Review Board (IRB) of Saint Paul's Millennium Medical Collage. A formal letter was written by the Medical Service vice Provost Office of the Hospital and Department of Internal medicine to the concerned units. Permission was asked from the responsible body of the units. Information gained from the charts was also used only for research purpose.

4.13 Dissemination of results.

The results of the study will be submitted to the department of internal medicine and it will be presented as one of the partial fulfillments of residency program in internal medicine department. It will be presented to the college community using presentation. It will be also presented on the annual research conference of the college. All efforts will be made to publish the results to the local and international journals and to present it in national scientific conferences.

5. Result

5.1 Socio-demographic characteristics

There were 5000 HIV Positive patients on ART and among those, the charts of 165 patients were selected by simple random sampling. Among the charts revised, 15 patients were excluded because they didn't satisfy the inclusion criteria and finally 150 patients were included in the study. Eighty nine (59.3%) and 61(40.7%) of participants were female and male respectively. The mean age of the participants was 42.7 years with (SD±10.69), 125(83.3%) of participants were urban dwellers. Eighty (53.3%) and 30(20%) of participants were married and divorced, respectively. Sixty one (40.7%) of participants completed secondary school (Figure 2, Table 1).

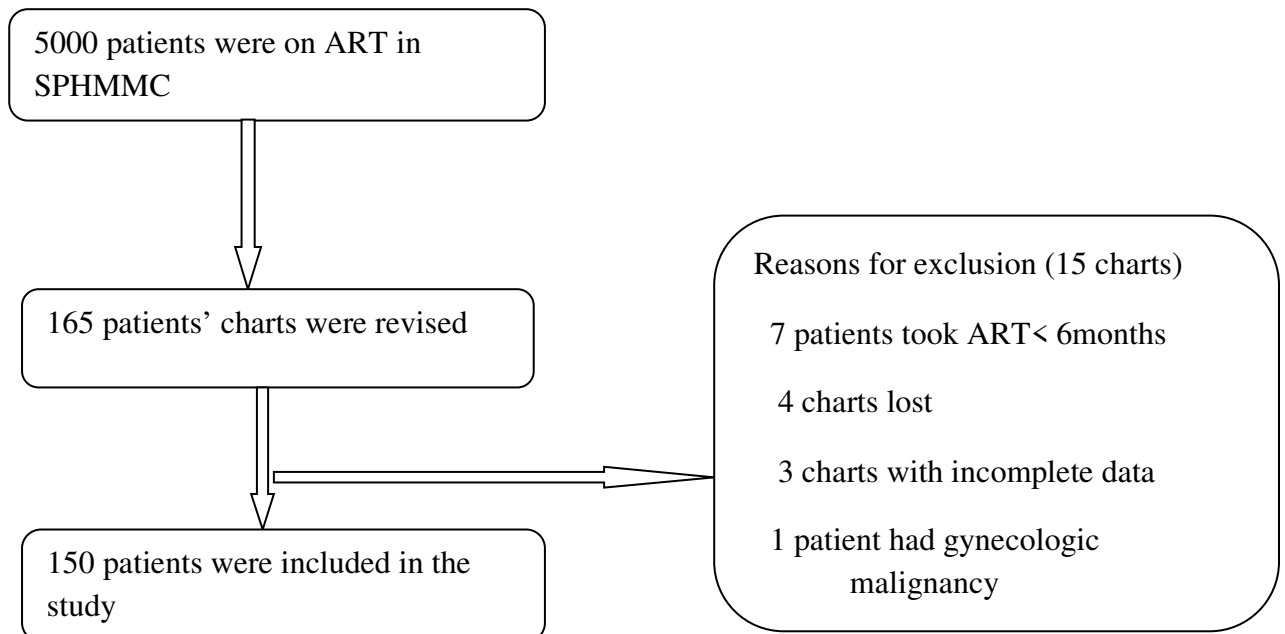


Figure 2 schematic representation of data collection procedure

Table 1 Socio-demographic characteristics of HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019(n =150)

Variables		Frequency	Percent (%)
Age(in years)	18-29	17	11.3
	30-39	41	27.3
	40-49	52	34.7
	50-59	28	18.7
	≥ 60	12	8.0
Sex	Male	61	40.7
	Female	89	59.3
Marital status	Single	17	11.3
	Married	80	53.3
	Divorced	30	20.0
	Widowed	23	15.3
Place of Residence	Urban	125	83.3
	Rural	25	16.7
Educational level	Illiterate	8	5.3
	Can read and write	1	0.7
	Primary school	39	26.0
	Secondary school	61	40.7
	Higher level education	41	27.3
Religion	Protestant	25	16.7
	Orthodox	105	70.0
	Muslim	19	12.7
	Wakefeta	1	0.7

5.2 Clinical characteristics of patients

Eighty (53.3%) of participants were having WHO stage T1 disease. One hundred two (68%) of participants were taking Tenofovirbased regimen (Figure 2). The mean duration of HIV since diagnosis is 9.47 years with (SD± 4.6) and 62 (41.3%) of respondents had HIV for more than 10 years. Seventy (46.7%) of participants took ART for more than 10 years. Eighty nine (59.3%) of participants had normal weight (Figure 1). The CD4 count of 77(51.3%) of participants were more than 500 cells/mm³. There were 2(1.3%) patients who were taking anti- tuberculosis medications. None of the participants had chronic diarrhea (Table 2).

Table 2 Clinical characteristics of HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019(n = 150)

Variables		Frequency	Percent (%)
WHO Stage	Stage T1	80	53.3
	Stage T2	33	22
	Stage T3	30	20
	Stage T4	7	4.7
HIV duration since diagnosis	< 1 Year	8	5.3
	1-5 Year	18	12
	>5-10 Year	38	25.3
	> 10 Year	62	41.3
	Un-known	24	16
Type of ART	AZT + 3TC + NVP	19	12.7
	AZT + 3TC + EFV	20	13.3
	TDF + 3TC + EFV	63	42
	TDF + 3TC + NVP	31	20
	ABC + 3TC + EFV	3	2
	ABC + 3TC + NVP	1	0.7
	AZT + 3TC + ATV/r	2	1.3
	TDF + 3TC + LPV/r	3	2.0
	TDF + 3TC + ATV/r	5	3.3
	ABC + 3TC + ATV/r	3	2
Type of ART regimen	TDF based	102	68
	AZT based	42	28
	ABC based	6	4
Duration of ART	<1 Year	9	6
	1-5 Year	23	15.3
	>5-10 Year	48	32
	>10 Year	70	46.7
Cotrimoxazole	Yes	43	28.7
	No	107	71.3
Tuberculosis	Yes	2	1.3
	No	148	98.7
CD4 count	<200	22	14.7%
	200-349	24	16%
	350-500	27	18%
	>500	77	51.3%

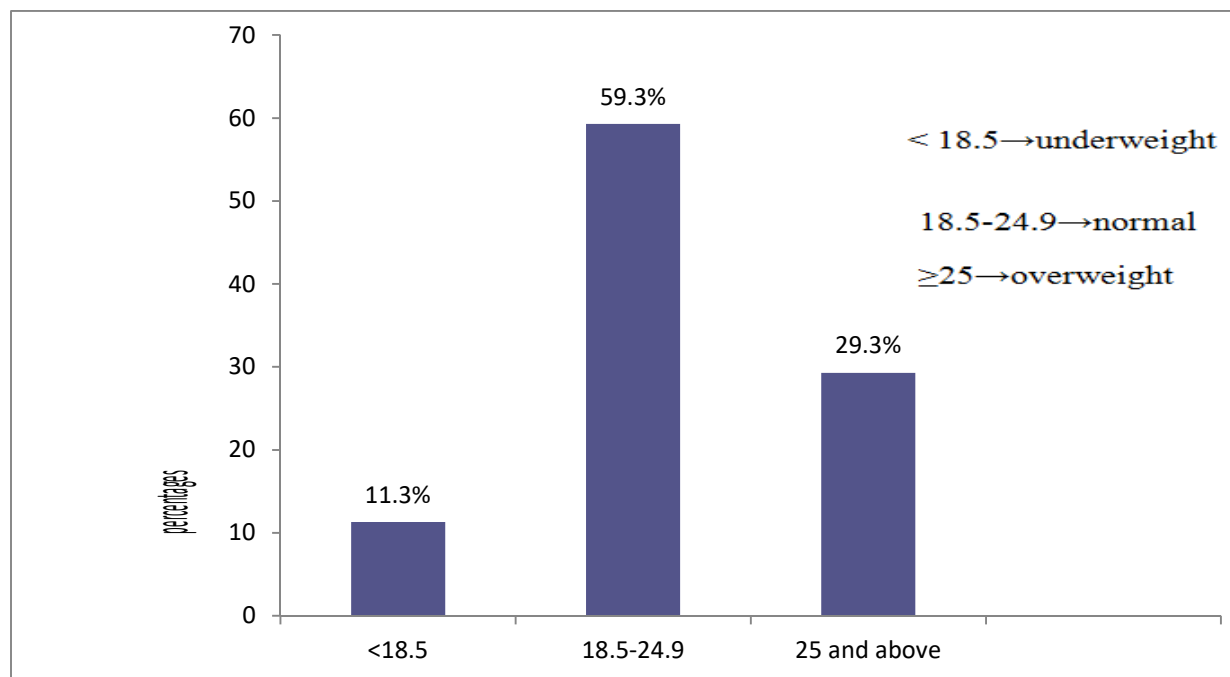


Figure 3 BMI (in Kg/m²) of study subjects of HIV patients on ART in SPHMMC, Addis Ababa, Ethiopia, 2019

Table 3 RBC parameters of HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019(n = 150)

Parameters	RBC($\times 10^6/\mu\text{l}$)	Hgb(g/dl)	MCV (fl)	MCHC (g/dl)	MCH (pg.)	RDW (%)
Mean	4.4	14.3	97.1	33.8	33.02	13.78
Std. Deviation	.68	2.12	10.05	1.87	3.83	1.45
Minimum	2	5.5	77.1	28.6	24.50	11.2
Maximum	6	18.9	126	41.9	45.30	21.2

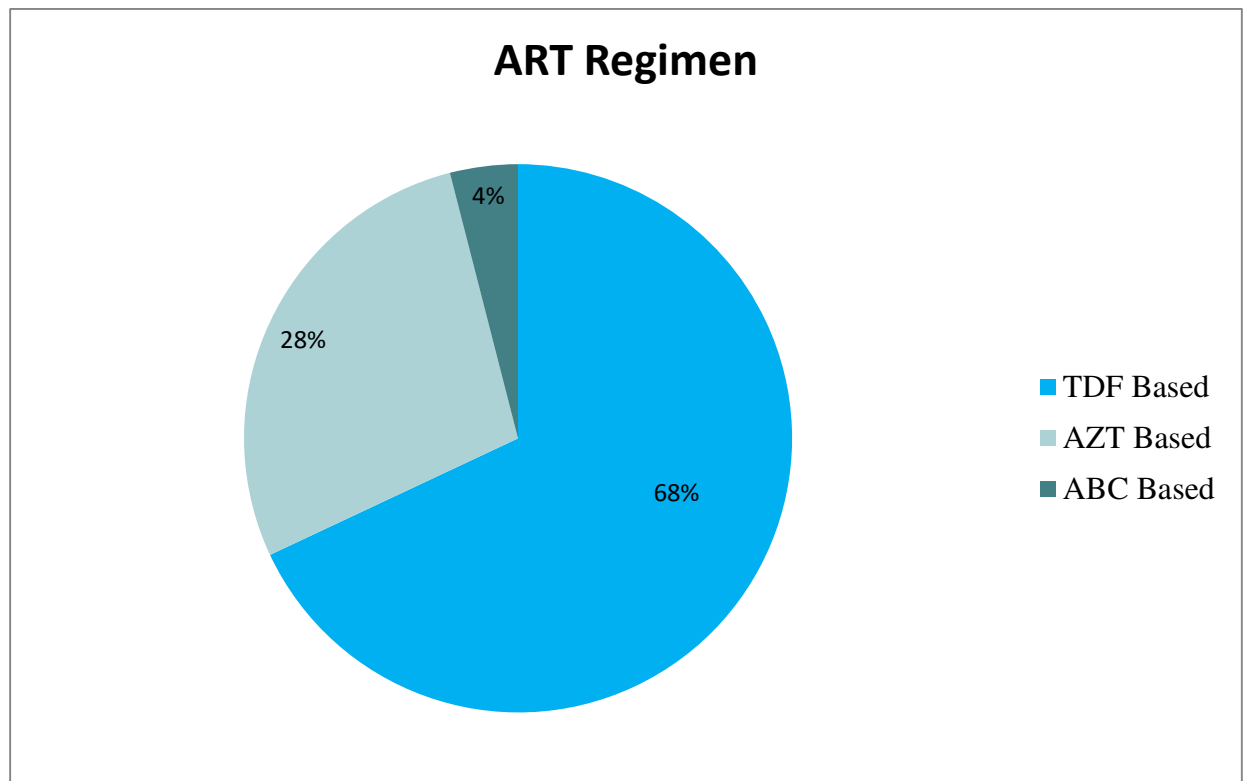


Figure 4 Type of ART regimens in HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019

5.3. Prevalence of anaemia

The prevalence of anemia in HIV patients on ART was 14% (21). About 12 (57.2%) had mild anemia and 7(33.3%) had moderate anemia .Severe anemia was found in 2(9.5%) of patients. Among the patients with anemia, more than half (57.2%) had macrocytic anemia, 38% had normocytic anemia and 4.8% had microcytic anemia. Morethan two third (76.2%) of the patients had normochromic anemia and 23.8% had hypochromic anemia. The mean values for RBC count,Hg,MCV,MCH,MCHC,and RDW were $4.4 \pm 0.68 \times 10^6/\mu\text{l}$, $14.3 \pm 2.12 \text{ g/dl}$, $97.1 \pm 10.05 \text{ fl}$, $33.02 \pm 3.83 \text{ pg}$, $33.8 \pm 1.87 \text{ g/dl}$, and $13.78 \pm 1.45\%$, respectively. (Table 3)

Table 4 Anemia status of HIV patients on ART at SPHMMC, Addis Ababa, Ethiopia, 2019(n= 150)

		Frequency	Percent (%)
Anemia	No	129	86
	Yes	21	14
Anemia Grading	Mild	12	57.2
	Moderate	7	33.3
	Severe	2	9.5
MCV	Microcytic	1	4.8
	Normocytic	8	38
	Macrocytic	12	57.2
MCHC	Hypochromic	5	23.8
	Normochromic	16	76.2

5.4 Factors associated with anaemia among HIV positive patients on ART

5.4.1 Bivariable analysis

In a bivariable analysis of this study, the factors associated with anaemia are BMI, ART regimen and CD4 count. Anaemia was not associated with other clinical factors. The odds of having anaemia in patients who were taking Zidovudine based regimen was 6 times (COR 6.1, 95% CI: 2.2-16.7, p-value <0.001) as compared to those who were taking TDF-based regimen. The odds of having anaemia in patients with BMI < 18.5 kg/m² was 8.9 times (COR 8.9; 95% CI: 2.2-36, p-value 0.002) higher compared with those with BMI ≥ 25 kg/m². The odds of being anaemic in patients with CD4 count < 200/μl, was 8.2 times (COR 8.2, 95% CI: 2.5-26.9, p-value 0.001) as compared with those with CD4 count of > 500 /μl. None of the socio-demographic factors were associated with anaemia. (Table 5 and 6)

Table 5 Bivariable analyses of socio-demographic variables that can be associated with anemia in HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019 (n = 150)

Variables		Anemia				COR (95%CI)	P-Value
		No		Yes			
		N	%	N	%		
Age group	18-29	14	9.3	3	2	1	
	30-39	34	22.7	7	4.7	1.0(0.2-4.2)	0.96
	40-49	43	28.7	9	6	1.0(0.2-4.1)	0.97
	50-59	27	18	1	0.7	0.2(0.02-1.8)	0.14
	≥60	11	7.3	1	0.7	0.4(0.04-4.7)	0.48
Sex	Male	55	36.7	6	4	1	
	Female	74	49.3	15	10	1.8(0.7-5.1)	0.2
Marital Status	Single	14	9.3	3	2	1	
	Married	72	48	8	5.3	0.5(0.1-2.2)	0.37
	Divorced	25	16.7	5	3.3	0.9(0.2-4.5)	0.93
	Widowed	18	12	5	3.3	1.3(0.3-6.4)	.75
Place of Residence	Urban	109	72.7	16	10.7	1	
	Rural	20	13.3	5	3.3	1.7(0.6-5.2)	0.4
Educational Level	Illiterate	7	4.7	1	0.7	1.0(0.1-10)	0.98
	Read and write	1	0.7	0	0	0	0.99
	Primary school	30	20	9	6	2.1(0.7-7.1)	0.2
	Secondary school	55	36.7	6	4	0.8(0.2-2.7)	0.7
	Higher level education	36	24	5	3.3	1	

Table 6 Bivariable analyses of factors associated with anemia by clinical characteristics in HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019(n = 150)

Variables		Anemia					
		No		Yes		COR(95%CI)	P-value
		N	%	N	%		
WHO Stage of Patients	Stage T1	69	46	11	7.3	1	
	Stage T2	28	18.7	5	3.3	1.1(0.4-3.5)	0.8
	Stage T3	25	16.7	5	3.3	1.3(0.4-4.0)	0.7
	Stage T4	7	4.7	0	0	0	0.99
HIV duration since diagnosis	< 1 Year	8	5.3	0	0	1	0.99
	1-5 Year	16	10.7	2	1.3	1.0	0.99
	>5-10 Year	34	22.7	4	2.7	1.0	0.99
	> 10 Year	51	34	11	7.3	1.0	0.99
	Un-known	20	13.3	4	2.7	1.0	0.99
Duration of ART	<1 Year	9	6	0	0	1	
	1-5 Year	19	12.7	4	2.7	1.0	0.99
	>5-10 Year	42	28	6	4	1.0	0.99
	>10 Year	59	39.3	11	7.3	1.0	0.99
ART regimen	TDF based	95	63.3	7	4.7	1	
	ABC based	5	3.3	1	0.7	2.7(0.3-26.5)	0.4
	AZT based	29	19.3	13	8.7	6.1(2.2-16.7)	<0.001
Cotrimoxazole	Yes	34	22.7	9	6	2.1(0.8-5.4)	0.1
	No	95	63.3	12	8	1	
Tuberculosis	Yes	1	0.7	1	0.7	6.4(0.4-106.5)	0.25
	No	128	85.3	20	13.3	1	
CD4 count	<200	13	8.7	9	6	8.2(2.5-26.9)	0.001
	200-349	22	14.7	2	1.3	1.1(0.2-5.7)	0.9
	350-500	23	15.3%	4	2.7%	2.1(0.5-7.9)	0.3
	>500	71	47.3%	6	4.0%	1	
BMI	< 18.5	9	6%	8	5.3%	8.9(2.2-36)	0.002
	18.5-24.9	80	53.3%	9	6%	1.1(0.3-3.9)	0.8
	≥ 25	40	26.7%	4	2.7%	1	

5.4.2 Multivariable logistic regression analysis

In a multivariable analysis of this study, factors associated with anemia in HIV patients were sex of patient, CD4 count, BMI and ART regimen at a p-value of <0.05. The odds of having anemia in females was 6 times (AOR 6; 95% CI:1.4-25.6) higher when compared with male sex. The odds of having anemia among respondents with CD4 count <200 have 17 times(AOR: 17; 95% CI: 3.1-91) higher when compared with patients who have CD4 count greater than 500. Likewise, the odds of anemia in patients whose BMI < 18.5 was 10 times (AOR: 10; 95% CI: 1.6-62.4) higher when compared with patients whose BMI was ≥ 25 . The odds of having anemia were 12.7 times (AOR: 12.7; 95% CI: 3.3-48.4) higher in patients who took Zidovudine based regimen as compared to Tenofovir based regimen. (Table 7)

Table 7 Multivariable analysis of factors associated with anemia in HIV infected patients on HAART at SPHMMC, Addis Ababa, Ethiopia, 2019(n = 150)

Variables		Anemia status					
		No	Yes	COR(95%CI)	P-value	AOR(95%CI)	P-value
		N (%)	N (%)				
Sex	Male	55(36.7)	6(4)	1		1	
	Female	74(49.3)	15(10)	1.8(0.7-5.1)	0.2	6.0(1.4-25.6)	0.02*
Cotrimoxazole	Yes	34(22.7)	9(6)	2.1(0.8-5.4)	0.1	0.7(0.2-2.6)	0.6
	No	95(63.3)	12(8)	1		1	
CD4 count	<200	13(8.7)	9(6)	8.2(2.5-26.9)	0.001	17(3.1-91)	0.001*
	200-349	22(4.7)	2(1.3)	1.1(0.2-5.7)		1.0(0.14-8.1)	0.95
	350-500	23(15.3)	4(2.7)	2.1(0.5-7.9)		2.2(0.4-12.0)	0.4
	>500	71(47.3)	6(4)	1		1	
BMI	<18.5	9(6)	8(5.3)	8.9(2.2-36)	0.002	10.0(1.6-62.4)	0.01*
	18.5-24.9	80(53.3)	9(6)	1.1(0.3-3.9)		1.9(0.4-9.1)	0.4
	≥ 25	40(26.7)	4(2.7)	1		1	
ART regimen	TDF based regimen	95(63.3)	7(4.7)	1		1	
	ABC based regimen	5(3.3)	1(0.7)	2.7(0.3-26.5)		1.4(0.1-20.2)	0.8
	AZT based regimen	29(19.3)	13(8.7)	6.1(2.2-16.7)	<0.001	12.7(3.3-48.4)	<0.001*

* -- p-value < 0.05

6. Discussion

In this hospital based cross sectional study, prevalence and factors associated with anemia among HIV positive patients on ART that have follow-up at ART clinic in SPHMMC have been studied. Complications of human immunodeficiency virus (HIV) infection include hematological abnormalities manifested by pancytopenia, anemia being the leading abnormality [1-3]. It is the most common hematologic abnormality among HIV-infected individuals; this includes those who are taking antiretroviral treatment (ART) and those who are ART-naïve. [15]

The prevalence of anemia among HIV positive patients, as addressed in several studies, differs in different countries worldwide. This study revealed that the prevalence of anemia was 14%. This finding is in agreement with the study done in Black lion specialized hospital in Addis Ababa, Ethiopia, which noted the prevalence of 11.4% after ART initiation. [20] It is also in agreement with another study done in one of public hospital in Ethiopia, which was 20.9% and 14.3% at 6 month and 1 year, respectively. [26]

The prevalence found in this study is lower than that was showed in a study done in Debre Tabor, Ethiopia, which was 34%. [37] It is also lower as compared to finding in a study done in Tanzania in which 40.46% had moderate to severe anemia. [24] The variation in the burden of anemia between the two study settings could be related to differences in WHO stage, duration of ART and immunity. In the study of Tanzania, more than 50% of study participants were in WHO clinical stage 3&4 with a median time on ART of about 4 years and a mean baseline CD4 163.8 cells/ μ l; while in this study more than half (53.3%) participants were under WHO stage 1, 46.7% took ART for >10 years and more than half (51.3%) had CD4 count >500 cells/ μ l.

In this study, females were more likely to be anemic than men. This finding is consistent with the study done in Debre-tabor, Ethiopia [37] and china [21], in both studies female sex was independently associated with anemia. A study done in Iran also showed similar finding. [23] In the contrary, the study done in one of public hospital in Addis Ababa showed that male sex was more likely associated with anemia [26]. In the study that was done in Nigeria, there was no significant difference in both genders. [25] The proposed reason was the presence of menstrual blood loss and to the drains on iron stores that occur with pregnancy and delivery in women.

Patients with low BMI ($<18.5\text{kg/m}^2$) in this study were more likely to have anemia as compared to normal weight and overweight. This is supported with the studies done in china and Debre tabor, Ethiopia.[21, 37] This might be due to chronic inflammatory state resulting in release of inflammatory cytokines that might affect absorption and recycling of micronutrients .This may lead to malnutrition.

In this study patients with low CD4 count ($< 200\text{cells/mm}^3$) were more likely to have anemia compared to patients with CD4 count $> 500\text{cells/mm}^3$.This finding is in line with studies done in Black Lion specialized hospital and Debre tabor which showed low CD4 count is strongly associated with anemia. [20,16] Another study that was done in Debre tabor identified similar finding.[37] This is also supported by studies from India and Tanzania .[22,24] This might be due to patients with low CD4 cells has decreased immunity and expose them to opportunistic infections that leads to deficiency of micronutrients and may affect bone marrow.

In this study, patients who took AZT containing ART regimens were more likely to be anemic than those who took TDF containing regimens. This finding is consistent with findings of studies that were done in Debre tabor, Ethiopia (two studies) and Tanzania.[16, 24, 37] This may be explained by myelosuppressiveeffect of AZT. However,this finding is in contrast to finding of study done in Zewditu hospital which showed that patients who took TDF based regimen were more likely to be anemic.[26]

7. Limitations of the study

This study couldn't asses the nutritional diversity of the patients .It also couldn't asses the serum level of micronutrients (iron and vitamin B12) because these are not routinely available and not routinely done in our setup.The anemia classification was based on RBC parameters, not supported by peripheral morphology.

8. Conclusion

Anemia is still a health problem in patients who are stable and taking ART with proper follow-up. This might have an impact on quality of life of these patients. This study showed overall prevalence of anemia is 14%. Sex, BMI, CD4 count and ART regimens were significantly associated with anemia.

9. Recommendations.

1. Patients taking AZT based regimen needs particular attention.
2. Physicians and nurses working in ART clinic should be aware and advice patients on nutritional aspect to improve their BMI.
- 3.The department of internal medicine should plan further study to asses types of anemia by incorporating peripheral morphology and serum level of micronutrients(iron studies, vitamin B12 and folate),which might have treatment implications.

References

1. Owiredun WK, Quaye L, Amidu N, Addai-Mensah O. Prevalence of anemia and immunological markers among Ghanaian HAART-naïve HIV-patients and those on HAART. *Afr Health Sci.* 2011; 11(1):2–15.
2. Dikshit B, Wanchu A, Sachdeva RK, Sharma A, Das R. Profile of hematological abnormalities of Indian HIV infected individuals. *BMC Blood Disord.* 2009; 9:5.
3. Dhurve SA, Dhurve AS. Bone marrow abnormalities in HIV disease. *Mediterr J Hematol. Infect Dis.* 2013; 5(1): e2013033
4. Belperio PS, Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature *Am J Med.* 2004; 116 (Suppl 7A):27S–43
5. Re MC, Zauli G, Furlini G, Giovannini M, La Placa M. HIV-1 infection and hematologic picture. *Microbiologic.* 1991; 14 (2):165–76.
6. Bain BJ. Pathogenesis and pathophysiology of anemia in HIV infection. *Curr Opin Hematol.* 1999; 6(2):89–93.
7. Kasthuri A, Sanjeevan S, Kar P. A study of hematological manifestations of HIV infection. *Indian J Sex Transm Dis* 2006; 27:9-16
8. Tripathi AK, Misra R, Kalra P, Gupta N, Ahmad R. Bone marrow abnormalities in HIV disease. *J Assoc Physicians India* 2005; 53:705-10.
9. Mata-Marín JA, Gaytán-Martínez JE, Martínez-Martínez RE, Arroyo-Anduiza CI, Fuentes-Allen JL, Casarrubias-Ramirez M. Risk factors and correlates for anemia in HIV treatment-naïve infected patients: A cross-sectional analytical study. *BMC Res Notes* 2010; 3:230
10. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ward JW. Epidemiology of anemia in human immunodeficiency virus (HIV)-infected persons: results from the multistate adult and adolescent spectrum of HIV disease surveillance project. *Blood.* 1998; 91(1):301–8.
11. Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, Colebunders R, Pradier C, Darminio Monforte A, Ledergerber B, Lundgren JD: Anemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. *AIDS* 1999, 13:943–950.

12. Semba RD, Martin BK, Kempen JH, Thorne JE, Wu AW: The impact of anemia on energy and physical functioning in individuals with AIDS. *Arch Intern Med* 2005, 165:2229–2236
13. Semba RD: Iron-deficiency anemia and the cycle of poverty among human immunodeficiency virus-infected women in the inner city. *Clin Infect Dis* 2003, 37:105-111.
14. McLean E, Cogswell M, Egli I, Wojdyla D, De Benoist B. Worldwide prevalence of anemia, WHO vitamin and mineral nutrition information system, 1993–2005. *Public Health Nutrition*. 2009; 12(4):444–454
15. Pande A, Bhattacharyya M, Pain S, et al. Anemia in antiretroviral naïve HIV/AIDS patients: a study from Eastern India. *Online J Health All Sci*. 2011; 10(4):1–5.
16. Hermela Melese, Molla Mesele Wassie, Haile Woldie, Abilo Tadesse, Nebiyu Mesfin. Anemia among adult HIV patients in Ethiopia: HIV/AIDS - Research and Palliative Care 2017; 9: 25–30.
17. Moyle G. Anemia in persons with HIV infection: prognostic marker and contributor to morbidity. *AIDS Rev*. 2002; 4(1):13–20.
18. Moh R, Danel C, Sorho S, Sauvageot D, Anzian A, Minga A, et al. Hematological changes in adults receiving a Zidovudine-containing HAART regimen in combination with Cotrimoxazole in Cote d'Ivoire. *Antivir Ther*. 2005; 10(5):615–24.
19. Fornai F, Moore D, Mermin J, Brooks JT, Were W, Buchacz K, et al. Hematologic changes associated with Zidovudine following single-drug substitution from stavudine in a home-based AIDS care program in rural Uganda. *J Int Assoc Physicians AIDS Care*. 2009; 8(2):128–38
20. Gashaw Garedew Woldeamanuel and Diresibachew Haile Wondimu; Prevalence of anemia before and after initiation of antiretroviral therapy among HIV infected patients in Ethiopia; *BMC Hematology* (2018) 18:7
21. Guorui Dai, Jiang Xiao, Guiju Gao, Xuejing Chong, Fang Wang, Hongyuan Lian, Liang Ni, Di Yan, Fengting Yu, Ling Xu, Di Wang, Junyan Han, Hui Zeng, Hongxin Zhao; Anemia in combined antiretroviral treatment-naïve HIV-infected patients in China: *Bioscience Trends*. 2016; 10(6):445-453.

22. Panwar A, Sharma SC, Kumar S, Sharma A. A study of anemia in human immunodeficiency virus patients: Estimating the prevalence, analyzing the causative effect of nutritional deficiencies, and correlating the degree of severity with CD4 cell counts. *Med J DY Patil Univ* 2016; 9:312-8.
23. Meidani M, Rezaei F, Maracy MR, Avijgan M, Tayeri K. Prevalence, severity, and related factors of anemia in HIV/AIDS patients. *J Res Med Sci* 2012; 17(2): 138-42.
24. Daniel Wilfred Gunda, & Semvua Bukheti Kilonzo, Bonaventura Cornel Mpondo; Magnitude and correlates of moderate to severe anemia among adult HIV patients receiving first line HAART in Northwestern Tanzania: *Pan African Medical Journal*. 2016; 23:26.
25. R. OMOREGIE, E.U. OMOKARO, O. PALMER, H.O. OGEFERE, A. EGBEOBAUWAYE, J.E. ADEGHE, S.I. OSAKUE and V. IHEMEJE; Prevalence of anemia among HIV-infected patients in Benin City, Nigeria: *Tanzania Journal of Health Research, Vol. 11, No. 1, January 2009*.
26. Muluken Assefa, Woldaregay Erku Abegaz, Aster Shewamare, Girmay Medhin and Mulugeta Belay; Prevalence and correlates of anemia among HIV infected patients on highly active anti-retroviral therapy at Zewditu Memorial Hospital, Ethiopia: *BMC Hematology* (2015) 15:6.
27. Bhattad D, Kulkarni V, Bhawe A, Balasubramanian M, Upase DP, Khude S. Refractory anemia in an immunocompromised patient— What is it? *J Assoc Physicians India*. 2013; 61(9):673–675.
28. Wisaksana R, Sumantri R, Indrati AR et al. Anemia and iron homeostasis in a cohort of HIV-infected patients in Indonesia. *BMC Infect Dis*. 2011; 11:213.
29. Zon LI, Groopman JE. Hematologic manifestations of the human immunodeficiency virus (HIV). *Semin Hematol*. 1988; 25(3):208–218.
30. Gedefaw L, Yemane T, Sahlemariam Z, Yilma D. Anemia and risk factors in HAART naïve and HAART experienced HIV positive persons in south west Ethiopia: a comparative study. *PLoS One*. 2013; 8(8): e72202.
31. Volberding PA, Levine AM, Dietrich D, Mildvan D, Mitsuyasu R, Saag M. Anemia in HIV infection: clinical impact and evidence-based management strategies. *Clin Infect Dis*. 2004; 38(10):1454–1463.

32. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection; WHO, 2nd edition; 2016.
33. WHO. *Hemoglobin concentrations for the diagnosis of anemia and assessment of severity*. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1)
34. Kassebaum NJ, Jasrasaria R, Naghavi M, et al. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. 2014; 123(5):615-624
35. National guidelines for comprehensive HIV prevention, care and treatment, Ethiopia, 2018.
36. Global database on body mass index, World Health Organization, 2006.
37. Kassaw Wubneh Zerihun, Gashaw Andargie Bikis and Esmael Ali Muhammad. Prevalence and associated factors of anemia among adult human immune deficiency virus Positive patients on anti-retroviral therapy at Northwest Ethiopia. *BMC*. 2019; 12:168

Annex: check List

Data Collection Formats

Date_____

Code _____

Part I: Baseline demographic characteristics

S.R	Question	Response category
1	Age(in years)	_____
2	Sex	1. Male 2. Female
3	Place of Residence	1. Urban 2. Rural
4	Educational Level	1. Illiterate 2. Can read/write 3. Primary education(1-8) 4. Secondary education(9-12) 5. Higher level education (College/University)
5	Marital status	1.single 2.married 3.divorced 4.widowed
6	Religion	1.Protestant 2.Orthodox 3.Muslim 4.Others(specify)_____

Part II: Baseline clinical characteristics.

S.R	Question	Response category
1	WHO clinical stage	1.Stage I 2.Stage II 3.Stage III 4.Stage IV
2	Type of ART regimens	_____
3	Duration of ART	_____ (in years)
4	Duration of HIV since diagnosis	_____ (years)
5	Cotrimoxazole prophylaxis	1, Yes 2,No
6	CD4 cell count (Cells/mm3)	_____
7	BMI (Kg/m2)	Height(cm)_____ Weight(kg) _____ BMI_____
8	Red blood cell parameters	1. RBC ($\times 10^6/\mu\text{l}$)_____ 2.Hgb (g/dl) _____ 3.MCV (fl) _____ 4.MCH (pg.)_____ 5.MCHC (g/dl) _____ 6. RDW (%)_____
9	Tuberculosis	1.Yes 2.No
10	Chronic diarrhea	1,yes 2,No