PREVALENCE OF ANEMIA AMONG HIV/AIDS PATIENTS WHO ARE TAKING ANTIRETROVIRAL THERAPHY AT WOLKITE HEALTH CENTER GURAGE ZONE,SOUTH WEST, ETHIOPIA

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Prevalence of anaemia among HIV/AIDS patients who are taking antiretroviral therapy at Wolkite health centre Gurage Zone, South West, Ethiopia

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Abbreviations and Acronyms

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Treatment
AZT	Azidothymidine
CD	Cluster of Differentiation
DNA	Deoxy Ribonucleic Acid
EDTA	Ethylene Diamine Tetra acetic Acid
HAART	Highly Active Anti retro viral Therapy
HGB	Haemoglobin
HIV	Human immunodeficiency virus
IDA	Iron Deficiency Anaemia
NO	Nitric Oxide
RBC	Red Blood Cell
WBC	White Blood Cell
WHO	World Health Organization
ZDV	Zidovudine

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ABSTRACT

Background*:* *Anemia is a frequent complication of people living with HIV/AIDS who are taking ART therapy. It is a worldwide public health problem with the global prevalence of about 24.8% affecting both developing and well-developed countries with an impact on health and socio-economic development of nations.*

Objective*: The aim of this study was to determine the prevalence of anaemia among ART users in Wolkite health centre Gurage zone southwest Ethiopia.*

Methods*:* *Institution based cross-sectional study from February to June 2022. Study participants who are visiting Wolkite health centre ART clinic were consecutively included for this study. Data were collected by using structured questionnaires which include socio-demographic and other potential factors. A total of 3mlof blood sample was collected from each study participants for haemoglobin measurement using CELL DYNE 1800 haematology analyser. Quality control samples were tested before running patient samples to check the performance of the instrument. Data were analyzed by using SPSS version 20 and summarized in tables.*

RESULT*:* *A total of 158 patients were enrolled and among them 104 (65.8%) were females and 115 (72.8%) of them were urban residents. The overall prevalence of anemia among study participants was 18(11.4%). Higher proportion of anemia was found in females 11(61.1%) and urban residents 14(77.7%). Moreover, a higher proportion of anemia was observed in patients aged greater than 15 years (96.8% and in a patients who are WHO clinical stage II (p<0.001)*

CONCLUSION *AND RECOMMENDATION:* *The prevalence of anemia in the current study was found to be a mild public health problem.Having advanced WHO clinical stage II, encountering intestinal parasitosis, and using HAART drug regimens were predictors for anemia among participants attending ART in this study. Therefore, it is recommended that preventive strategies like nutritional education, nutritional screening, iron supplementation should be considered. In addition early diagnosis and treatment of anemia, longitudinal studies on foliate, erythropoietin determination are needed to determine the actual etiology of anemia in HIV infected individuals.*

Key words; - Anemia HIV ART users Wolkite

Chapter One; - Introduction

1.1. Background

Anemia is a condition in which hemoglobin concentration and red blood cell (RBC) numbers are lower than normal and insufficient to meet an individual's physiological need (1). Also anemia can be defined as a reduced absolute number of circulating RBCs or a condition in which the number of RBCs (and subsequently their oxygen-carrying capacity) is insufficient to meet physiologic needs. Anemia is most commonly diagnosed by a low HGB concentration or a low hematocrit (in males anemia is diagnosed when HGB is <13 g/dl and HCT is <39% whereas female anemia is when HGB is <12 g/dl and HCT is <36%. Anemia can also be diagnosed using RBC count, mean corpuscular volume, blood reticulocyte count, blood film analysis, or HGB electrophoresis (2, 3).

The human immune deficiency virus (HIV) which is the causative agent of acquired immune deficiency syndrome (AIDS), is retroviruses that possess an enzyme reverse transcriptase that can transcribe single stranded RNA in to double stranded DNA(4). Acquired immune deficiency syndrome (AIDS) is the last stage of HIV infection. It is defined by the occurrence of any of more than 20 opportunistic infection or HIV related cancer. AIDS is a syndrome which is the last stage of HIV infection in which a person immune system is fully compromised leaving the body open to wide range of potentially deadly disease (5,6) .

Anemia is one of the most frequently reported hematological complications in people afflicted with human immunodeficiency virus/acquired deficiency syndrome (HIV/AIDS). Although not well studied, it is estimated that about 60–80% of late-stage HIV patients are anemic. In these patients, 22% of anemia is thought to be caused by HIV treatments including antiretroviral medications Moreover, anemia is considered one of the key independent predictors of HIV disease progression (7,8). The association of HIV/AIDS with anemia has many causes and the major cause of anemia is malnutrition associated with Iron deficiency, foliate deficiency and infections such as malaria and helmenthiasis (9).

In established HIV infection, rate of hemoglobin decrease with falling CD4 count and many studies have found that there is association between anemia during established infection and a faster progression to AIDS and death (10).

The etiology of anemia in HIV infection is multifactorial and from this an obvious cause of anemia in patients with HIV infection is blood loss. Blood loss may be associated with such condition as neoplastic disease (e.g., Kaposi sarcoma in the gastrointestinal tract). Other than blood loss, the pathophysiology of HIV- associated anemia may involve decreased RBC production, increased RBC destruction, and ineffective RBC production (11, 12). Also the use of myelosuppressive medications can be another cause of pathophysiology of HIV-associated anemia(13). If an individual used antiretroviral treatment effectively, there will be an increased level of CD4 cells and hemoglobin concentration, which in turn decrease the occurrence of anemia. Also HIV infected patients on HAART showed lower prevalence of anemia. Azidothymidine (AZT) is a widely used HIV reverse transcriptase inhibitor and one of the first line antiretroviral drugs recommended by WHO for treating HIV infected adults in resource limited countries. Azidothymidine is a well-known cause of drug induced hepatotoxicity. Several studies in developed countries have shown that Azidothymidine alone and Azidothymidine based HAART regimen is associated with significant reduction of HGB level and neutrophil number (15). Zidovudine (ZDV) related anemia usually occurs with three months after initiation of therapy. Risk factors include high Azidothymidine dosage, increased treatment duration, low CD4 count and pre- existing anemia (16).

1.2. Statement of the Problem

Anemia is a frequent complication of People living with HIV/AIDS who are taking ART therapy and associated with an increased risk of disease. It is a worldwide public health problem with the global prevalence of about 24.8%, affecting both developing and developed countries with an impact on health and socio-economic development of nations (17). Hematological complications have been documented to be the second most common causes of morbidity and mortality among HIV sero-positive patients with a significant impact on quality of life and clinical outcomes (18-20).

The most common cause of anemia worldwide is iron deficiency, resulting from prolonged negative iron balance, caused by inadequate dietary iron intake, absorption, and other nutritional deficiencies like folic acid and vitamin B12. Vitamin B12 deficiency may result from malabsorption in the ileum or from gastric pathology caused by an array of infections or other conditions that affect the gastric mucosa in HIV-infected patients; folic acid deficiency is generally caused by either dietary deficiency or jejunal pathology as the disease progress. In addition to the above causes among HIV/AIDS patients, anemia could be associated with a highly active antiretroviral therapy (HAART) like zidovudine based regimen and opportunistic infections (21, 22).

Anemia is a common feature of HIV infection, occurring in about 35% of HIV/AIDS the patients who start antiretroviral treatment (ART) in Europe and North America (23), while a comprehensive figure for the burden of anemia in HIV/AIDS patients in Sub Saharan Africa hasn't been estimated. Their variation in the prevalence of anemia among HIV/AIDS patients ranges from 10.1% to 77.4%, whereas in local areas, particularly in southwest and eastern Ethiopia, its magnitude is 41.2% and 69.6%, respectively (24).

It has been shown that anemia influences the natural history of HIV disease progression and operating as a strong independent predictor of death (25, 26, and 27). Uncorrected anemia results in multisystem disabling symptoms like fatigue, exhaustion, increased risk of HIV dementia, poor quality of life, decreased survival, and possibly even the exacerbation of poverty in countries, like Ethiopia where the prevalence of HIV/AIDS is high.

In addition to variation its variability in magnitude, anemia is influenced by factors which are associated with HIV/AIDS patients on ART, sociodemographic factors, ART regimen, low CD4 count, ZDV use, higher HIV-1RNA levels in plasma have long been recognized as important contributors of anemia in HIV/AIDS patients on ART (28).

1.3 Significance of the study

In Ethiopia, anemia has become a common and serious hematologic complication in both HIV infection and its treatment. This study helps to give updated information on anemia prevalence and its severity which aid to intervene the problem. Furthermore, it will also serves as baseline data for other researchers.

Chapter Two; - literature review

Prevalence of anemia

According to 2006 data from the world health organization, the prevalence of anemia is 24.8% globally. It affects an estimated 2 billion people worldwide. It is the most common hematological abnormality associated with HIV infection although the burden of anemia in HIV/ADS patients is not very well understood, it is estimated that about 60-80% of patients in their late stage of disease are affected worldwide. In these patients, 22% of anemia is thought to be caused by several treatments given to the patients including anti-retroviral medications (29).

A study conducted in 2009 in south India on factors associated with anemia in HIV infected individuals (n=6996) showed that 4431 patients with a CD4+ T cell count values available, and the mean HGB value decline from 12 g/dl at CD4+ T cell count >500 cell/ul to 10.0 g/dl at CD4+ T cell count<100cell/ul (30). The global prevalence of anemia in HIV infected patients with regard to sex; females are disproportionately more affected by the condition as compared to males. In a study conducted in the US, it was reported that women had 71% greater prevalence of anemia than men when anemia is defined as hemoglobin of less than 12 g/dl in women and HGB less than 13 g/dl (17).

A study conducted in South Africa in 2006 showed that the prevalence of anemia among anti-retroviral naïve HIV infected children increased with HIV disease stage from 42% with no evidence of immune suppression to 85% with sever immune suppression (31).

In Ethiopia the prevalence of anemia is not studied extensively; the study was conducted from 2006 to 2013 at the ART clinic of the Arba Minch hospital and health center on total of 411 adult HIV positive patients with complete information on hemoglobin levels and CD4 count were assessed for anemia prevalence and risk factors; anemia was present in 52.3% of the study participants, with 28.1%, 22.9% and 1.3% described as mild, moderate and severe anemia respectively (32). In a cross-sectional study conducted in 2012 at Jimma University specialized hospital showed that from the total 234 HIV positive participants, the overall prevalence of anemia was 54 (23.1%) with 19 (16.2%) of patients on HAART while the remaining 35 (29.9%) where HAART naive. On the other hand from the total anemic individuals 1(1.9%), 14(25.9%), and 39(72.2%) had severe, moderate, and mild anemia respectively (33).

The study conducted in 2013 at Zewditu memorial Hospital in Addis Ababa on 180 pediatric HIV/AIDS patients showed that the prevalence of anemia was higher in females (24.4%) and rural resident (41%); more over anemia showed higher incidence in patients aged 6-11 years (25%). When anemia was characterized by severity, mild, moderate and severe anemia accounts 21(52.5%), 17(42.5%) & 2(5%) respectively. There was a significant increase in severity & prevalence of anemia in those CD4+ T cell count bellow 350 cell/ul (34)

A cross-sectional study conducted in 2013 among 265 HIV infected children attending Gondar university Hospital ART clinic anemia was associated with eating green leafy vegetables being on cotrimoxazole treatment, but there was no significant association with age, sex, residence, and HAART regimen, intestinal parasitic infection, WHO clinical stage and CD4 count percentage (35). Another study conducted in 2014 at Butajira hospital Southern Ethiopia on adult people living with HIV/AIDS receiving ART showed that the effect of malnutrition by itself can decrease CD4+ T cell and contribute to abnormal B-cell response which adversely affect the overall clinical outcomes and exacerbate HIV related immune depression. And also this study found that a significant association between anemia and malnutrition; participant who were anemic were 1.94 times more likely to be malnourished than those with normal HGB level (36).

In cross sectional study that was conducted in 2017 at black lion hospital, among 255 HIV patients on ART, the prevalence of anemia before and after ART initiation was 41.9 and 11.4% respectively. The study shows that there is a significant difference in CD4+ T cell count, RBC count hemoglobin values and RBC indices in HIV patients before and after ART initiation. Among the total number of anemic cases the prevalence of normocytic normochromic anemia before and after ART initiation was 71% and 58.6% respectively. The prevalence of macrocytic normochromic anemia was 4.7% before and 27.6% after the initiation of ART (37). The first study assessing the prevalence of anemia in children living with HIV/AIDS on HAART in the Tigray regional state was 7%, with most patients having mild to moderate anemia (38).

Chapter three; - Objectives

General objective

- To assess the prevalence of anemia among ART users at Wolkite Health Centre in Gurage zone from February 13 to June 26 2022.

Specific Objectives

- To determine the prevalence of anemia among ART users in the study area.
- To determine severity of anemia among ART users in the study area.

CHAPTER four: - Materials and Methods

4.1. Study Area

The study was conducted in Wolkite health center which is located in Wolkite town. The town is found 158 km southwest of Addis Ababa, the capital city of Ethiopia. Wolkite is the capital city of Gurage Zone with an average annual temperature of 18.6 °C and an average rainfall of 1244 mm. According to town municipality, the town has an elevation between 1910 and 1935 meters above sea level (39, 40). The projected Wolkite town total population is 70,796 of whom 53% are males and 47% females, the proportions of the under-five population were 2,169 populations (41). Wolkite health center offers diagnosis and treatment for patients that reside in Wolkite town and nearby neighbor woredas like Kebena, Abeshge and others. This study was conducted from February 13 to June 26 2022.

4.2. Study Design

An institution based cross-sectional study design was used.

4.3. Source Population

All HIV /AIDS patients who are taking ART at Wolkite health center at ART clinic.

4.4. Study Population

All HIV/AIDS patients on ART that visit Wolkite health center during the study period, from February 13 to June 26 2022.

4.5. Inclusion and Exclusion Criteria

4.5.1. Inclusion Criteria

- ✓ Those who volunteered to participate in the study.

4.5.2. Exclusion Criteria

All ART visited HIV/AIDS patients who are:

- ✓ Pregnant women.
- ✓ Those HIV/AIDS patients with recent vitamin supplement.
- ✓ Newborns below the age of six month.

4.6. Sample size Determination

Single population proportion sample size determination formula is used to determine the sample size of the study. The sample size is determined by considering the prevalence of anaemia 16.2% reported from Jimma University specialized hospital (33) and using 95% confidence interval and 5% marginal error.

$$\mathbf{n} = \frac{\left(\boldsymbol{Z}_{\frac{\alpha}{2}}\right)^{2} \boldsymbol{p}(\mathbf{1}-\boldsymbol{P})}{\boldsymbol{d}^{2}}$$

Where:

Z ά/2= 95%confidence interval=1.96

N= Sample Size

D= Margin of sampling error =5%

P= Prevalence rate 16.2%

$$\mathbf{n} = \frac{(\mathbf{1.96})^{2}(\mathbf{0.162})(\mathbf{1}-\mathbf{0.162})}{(\mathbf{0.05})^{2}} = \mathbf{209}$$

n=209

We have corrected it by using the correction formula:-NF=ni/1+ni/N because our population size becomes <10,000 and our population size (N) is 530 ART patients

NF = 209 / 1+209/530 =149.89 approximately 150

5% of non-respondents rate is added= (150)(0.05)=7.5

nf=150+7.5=157.5 approximately 158

Based on the above computation the estimated sample size for this study is 158.

4.7. Sampling Technique

Consecutive sampling was used for the study to include all HIV/AIDS patients who were sent to Wolkite Health Center ART clinic during the study period.

4.8. Study Variables

4.8.1. Dependdent variable

- ✓ **Anemia**

4.8.2. Independant variable

- ✓ Age
- ✓ Sex
- ✓ Drug regimen
- ✓ WHO clinical stage
- ✓ Educational status
- ✓ Presence of intestinal parasitosis.
- ✓ Duration of HAART

4.9. OPERATIONAL DEFINITION

Anemia is defined as pair WHO hemoglobin concentration < 13mg/dl for male and < 12mg/dl for female.,

CD4 is a glycoprotein that found primarily on the surface of helper T cell.

Hemoglobin is a red substance in the blood that carries oxygen and contains iron.

Iron deficiency anemia is type of anemia indicated as prolonged negative iron balances or excess requirement and loss of iron.

To classify severity of anemia we use WHO classification criteria briefly HGB value in g/dl as mild, moderate and severe depending on sex and age.

Study subjects	**Mild**	**Moderate**	**Severe**
Children 6-59 month of age	10-10.9	7-9.9	<7
Child 5-11 years of age	11-11.4	8-10.9	<8

Child 12-14 years of age	11-11.9	8-10.9	<8
Nonpregnant women 15 years and above	11-11.9	8-10.9	<8
Men 15 years and above	11-12.9	8-10.9	<8

4.10. Data Analysis

Data was analyzed by SPSS version 20 software and descriptive statistics were summarized

4.11. Data Collection

4.11.1. Socio-Demographic and other data

During implementation of data collection the study subjects were asked for their willingness in participation of study. Then it was carried out through verbal communication and the soci demographic and clinical data were collected by using structured questionnaires.

4.11.2. Laboratory Data

Specimen collection

A total of 3 ml of blood sample was collected and transferred in to EDTA tube and mixed well to prevent coagulation.

Sample processing

EDTA anti-coagulated whole blood collected from HIV/AIDS patients was run on complete blood cell count analyzer to determine HGB and HCT respectively.

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4.12. Quality Assurance

To ensure the reliability and validity of the study the following activity was applied just before, during and after the actual laboratory test is performed.

Pre-analytical phase

- ✓ Sample was collected properly using standard procedure.
- ✓ Proper reagents and materials were used.
- ✓ Proper anti-coagulant was used.

Analytical phase

- ✓ SOP was followed throughout the study period.
- ✓ Hemoglobin and other parameter were checked using manual technique.

Post - analytical phase

- ✓ All the transactional measures were checked and the result was interpreted
- ✓ The result was accurately recorded, documented and reported
- ✓ Hematological profile test result was attached to each questionnaire

4.13. Ethical Considerations

Ethical clearance and approval was obtained from Wolkite University College of medicine and Health Science, School of Medical Laboratory Science. Verbal, written consent was obtained from each participant and confidentiality of the results was obtained throughout the study.

4.14. Result Dissemination Plan

The result from the study was submitted to the Wolkite University, College of Medicine and Health sciences, School of Medical laboratory science in order to inform the prevalence of anemia among HIV/AIDS patients. Oral presentation of the thesis was made. The work was published in peer reviewed journals.

CHAPTER Five;- RESULT

5.1 Socio-demographic characteristics of the study participants

A total of 158 patients have consented and participated in this study making a response rate of 100%. One hundred four (65.8%) of the participants were females and 115 (72.8%) of them were urban residents. Majority 154(97.5%) of the participants aged greater than 15 years and 75(47.5%) of the participants had attended primary school education. More than half of the participants 126 (79.7%) were married. Regarding their occupation a quarter of the participants 39 (24.6 %) was self-employed respectively.

5.2 Prevalence and severity of anemia

The overall prevalence of anemia among HIV patients on ART was 18(11.4%). Out of anemic patients, 1(5.5%), 14 (77.7%), 3 (16.6%) had mild, moderate, and severe anemia, respectively. As summarized in the table, the magnitude of anemia was higher in females 11(61.1%) and urban residents 14(77.7%). Moreover, a higher proportion of anemia was observed in patients who have aged greater than 15 years (96.8%)

Table1. **Socio-demographic history of respondents.**

Variable	**Frequency**	**Percent**
Age		
1/2-14	4	2.5
>15	154	97.5
Marital status		
Married	126	79.7
Un married	32	20.3
Residance		
Urban	115	72.8
Rural	43	27.2
Educational status		
Illiterate	28	17.7
Primary	75	47.5
Secondary	39	24.5
Tertiary	8	5.1
College/University	8	5.1
Occupation		
Merchant	47	29.7
Daily labor	32	20.3
Government employee	30	19.0
Farmer	15	9.5
Self-employee	34	21.5

Monthly income		
≤1000	13	8.2
1100-3000	81	51.3
3100-5500	46	29.1
>5500	18	11.4
Gender		
Male	54	34.2
Female	104	65.8

Table2. **ART status of respondents.**

Variable	**Frequency**	**Percent**
VIRAL LOAD		
Undetectable	148	93.7
Detectable	10	6
BMI		
<18	40	41.7
>18	92	58.1
WHO CLINICAL STAGE		
I	17	10.8
II	137	86.7
III	4	2.5
HAART REGIMEN		
Yes	158	100
NO	0	0
ART STATUS		
Yes	158	100
No	0	0
Intestinal Parasites		
Not detected	148	93.7
Detected	10	6.4

Table 3**.** Distribution of anaemia by socio demographic characteristics of HIV/AIDS patients who are taking ART at Wolkite Health Center June 2022(n=158)

Variables		**Anemia**				**Total (%)**	
		Yes(n)	**(%)**	**No (n)**	**(%)**		
Age	1/2-14years	0	0	5	3.5	5	3.5
	≥15 years	18	100	135	96.4	153	96.8
Sex	Male	7	38.8	47	33.5	54	34.1
	Female	11	61.1	93	66.4	104	65.8

Residence	Urban	14	77.7	101	72.1	115	72.7
	Rural	4	22.2	39	27.8	43	27.2
Marital status	Married	16	88.8	110	78.5	126	79.7
	Unmarried	2	11.1	30	21.4	32	20.2
Educational status	Illiterate	3	16.6	25	17.8	28	17.7
	Primary	5	27.7	70	50	75	47.4
	Secondary	7	38.8	32	22.8	39	24.6
	Tertiary	2	11.1	6	4.2	8	5.06
	Higher	1	5.5	7	5	8	5.06
Occupational status	Government	2	11.1	28	20	30	18.9
	Daily Laborer	2	11.1	30	21.4	32	20.2
	Merchant	5	27.7	42	30	47	29.7
	Farming	3	16.6	12	8.5	15	9.4
	Self-employee	6	33.3	28	20	34	21.5
	Total	18	100	140	100	158	100

Based on WHO classification Hgb level<7g/dl were considered to be severe anemia,7-10 g/dl were moderate anemia and values of 11g/dl were considered as mild anemia even if it was based on age, sex and status of the patient. According to this study, from the total 18 anemic patients 1(5.5%) of them were categorized under mild anemia, 14 (77.7%) of them were moderate anemic and 3(16.6%) of them were sever anemic respectively.

Table 4.Distribution of anaemia severity by clinical characteristics and ART status of HIV/AIDS patients who are taking ART at Wolkite Health Centre June 2022 (n=158)

Variables		**Anemia severity**							
		Mild	(%)	Moderate	(%)	Severe	(%)	Total	(%)
Age in years	½-14	0		0		0		0	
	≥15	1	5.5	14	77.7	3	16.6	18	100
Sex	Male	1	5.5	6	33.3	0	0	7	38.8

	Female	0		8	44.4	3	16.6	11	61.1
Viral Load	<1000Copies/ml	15	83.3	3	16.6	0		18	100
	>1000Copies/ml	0		0		0		0	
Drug regimen(HAART I)	Yes	1	5.5	14	77.7	3	16.6	18	100
	No	0		0		0		0	
Duration of treatment	<5 year	0		2	11.1	0		2	11.1
	5-10 year	1	5.5	8	44.4	2	11.1	11	61.1
	>10 year	0		4	22.2	1	5.5	5	27.7
WHO clinical stage	I	0		2	11.1	0		2	11.1
	II	1	5.5	11	50	3	16.6	15	83.3
	III	0		1	5.5	0		1	5.5
	IV	0		0		0		0	
BMI(Kg/m^2)	<18.5	0		7	38.8	2	11.1	9	50
	18.5-24.9	1	5.5	5	27.7	1	5.5	7	38.8
	>25	0		2	11.1	0		2	11.1
Intestinal parasite	Detected	0		4	22.2	3	16.6	7	38.8
	Not detected	1	5.5	10	55.5	0		11	61.1

The large proportion of the study participants (77.7%) were in a WHO clinical stage II ($p<0.001$) also 18(100%) of participants uses HAART drug regimen ($p<0.001$) and from this the participants were on the 1st line drug regimen .About 61.1% of the anemic patients were identified with some intestinal parasite. The most commonly reported intestinal parasite was P.falcifarum. . Also 100% of the anemic patients had viral count < 1000 copies/ml ($p<0.164$) respectively.

Table5.Magnitude of anemia among anemic HIV/AIDS patients who are taking ART at Wolkite Health Center from February to June 2022 (n =158)

Variable		Anemia						Chi-Square(X^2)	P-value
		Yes	(%)		(%)	Total	(%)		
Age	½-14 years	0	0	5	3.5	5	3.1	0.219	0.791
	≥15years	18	100	135	96.4	153	96.8		
Sex	Male	7	38.8	47	33.5	54	34.1	0.403	0.002
	Female	11	61.1	93	66.4	104	65.8		
Drug regimen	Yes	18	100	140	100	158	100	0.611	0.000
	No	0		0		0			
Duration of HAART	<5 year	2	11.1	74	52.8	76	48.1	0.924	1.000
	>5 year	16	88.8	66	47.1	82	51.8		
WHO clinical stage	I	1	5.5	32	22.8	33	20.8	0.497	0.001
	II	14	77.7	108	68.3	122	77.2		
	III	3	16.6	0		0			
	IV	0		0		0			
Educational status	Illiterate	3	16.6	25	17.8	28	17.7	0.451	0.775
	Primary	5	27.7	70	50	75	47.4		
	Secondary	7	38.8	32	22.8	39	24.6		
	Tertiary	2	11.1	6	4.2	8	5.06		
	College or University	1	5.5	7	5	8	5.0		
Intestinal Parasite	Detected	7	38.8	3	2.14	10	6.3	0.790	0.000
	Not detected	11	61.1	137	97.8	148	93.6		
Viral Load	<1000Copies/ml	18	100%	134	95%	152	96.2%	0.697	0.164
	>1000Copies/ml	0		6	4.2%	6	3.7%		

CHAPTER SIX;- Discussion

This study aimed to determine the prevalence and severity of anaemia among HIV/AIDS patients who are taking ART. The overall prevalence of anemia obtained in this among study participants was 11.4%; of them 5.5%, 77.7%, 16.6% had mild, moderate, and severe anemia, respectively.

The prevalence of anemia observed in this study among study participants was (11.4%) is very low when compared to a study done in south India (41%) [30], Arba Minch (52.3%) [32], Gondar (70.1%) [35]; but is comparable to a study done in Jimma (23.1%) [33] and Addis Ababa (22.2%)[34]. The possible explanation to this low prevalence might be due to better follow-up, and being urban residents which is based on the data recorded from the ART card number.

The respective 5.5%, 77.7% and 16.6% mild, moderate and severe anemia observed in this study was higher than a study conducted in Arba Minch [32] reported 28.1%, 22.9%, and 1.3% -mild, moderate and severe anemia, respectively. Furthermore 16.6% severe anemia obtained in this study was higher than a study reported from the Zewuditu Memorial Hospital (ZMH) Addis Ababa 5% [34]. This difference might be due to geographic difference as most of them used more than one study area, age difference as some took children's, some took adults but the current study took all age groups. And there is sample size difference as our sample size is smaller than the previous studies which may increase the prevalence.

In this study also we identified that among participants who had low BMI were higher proportion of anemia. This result was in line with the results of the study in the Butjira Hospital showing a significant association between anemia and malnutrition [36].Low BMI is associated with many nutrient deficiencies including iron, folate and B12 that contribute directly to anemia. According to WHO classification stage , the prevalence of anemia in living with HIV/AIDS on HAART in this study was (5.5-77.7%) mild-moderate which is a higher proportion compared to the study done in the Tigray region which is 7% with most of the patient having mild to moderate anemia. This variation could be attributed to the difference in socioeconomic status, geographical factor (Altitude), seasonality, time of the study, or a combination of this factors which are known to affect anemia.

Limitations of this study were; types of anemia, the effects of family history, menstrual disorder, and diet consumption habit was not assessed. In addition the current study did not consider acute and chronic illnesses, hereditary disorders, or other opportunistic infections which may cause anemia.

Chapter SEVEN: Conclusion

The prevalence of anemia in the current study was found to be public health problem. High proportion of anemia was found in female, having advanced WHO clinical stage II, encountering with intestinal parasitosis, and HAART drug regimens users.

Chapter EIGHT: Recommendation

Based on our finding we would like to recommend the following points to Wolkite Health center and other concerned bodies:

- It is recommended that preventive strategies like nutritional education nutritional screening, iron supplementation should be considered targeting females. In addition early diagnosis and treatment of anemia, longitudinal studies on foliate, erythropoietin determination are needed to determine the actual etiology of anemia in HIV infected individuals.

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Annex II- Questionnaire

Code number; _____

WOLKITE UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICAL LABORATORY SCIENCE

Questioner format prepared for the study of prevalence of anemia among HIV/AIDS patients who are on ART at Wolkite Health Centere.

We are fourth year medical laboratory sciences student in Wolkite University Institute of Health. We are undertaking a research on the prevalence of anemia among HIV/AIDS patients who are on ART at Wolkite Health Center, and we want to ask you some questions. Whatever the information you provide will be kept strictly confidential. Are you a volunteer?

Yes ☐ No ☐

Date of interview _____

Questioner

1. **Age in years**_____
2. **Gender** 0. Male 1. Female
3. **Residence** 0. Urban 1. Rural
4. **Marital status** 0. Unmarried 1. Married
5. **Educational status**
   0. Illiterate
   1. Primary (1-8)
   2. Secondary(9-10)
   3. Tertiary (11-12)
   4. College or University
6. **Occupational status**
   0. Merchant
   1. Daily laborer
   2. Farming
   3. Self employee
   4. Government employee
7. **Monthly income in Ethiopian birr** _____
8. **ART (HAART) status** 0. Not on a ART 1. in an ART
9. **HAART duration/month**_____
10. **HAART regimen**_____
11. **WHO clinical stage** 0. I 1. II 2.III 3. IV
12. **BMI (kg/m^2)** _____
13. **Intestinal parasite detected** _____

Complete blood count sheet

Hematological parameters	**Results**
Viral load (copies/mm^3) if any?	
WBC $x10^9$/L	
Total Lymphocyte $x10^9$/L	
Mixed WBC (%)	
Neutrophil (%)	
RBC$X10^{12}$/L	
Hgb (g/dl)	
HCT (%)	
MCV (fl)	
MCH (%)	
MCHC (pg)	
RDW (%)	
PLT $x10^9$/L	
MPV(fl)	

Annex III- Laboratory Procedure

Principle of CELL DYNE 1800

The automated hematology analyzer is CELL DYNE1800 which is a 3-part differential and measures 18 parameters.

The CELLl DYNE 1800 utilizes impedance resistance to measure human blood cell. Counting and volumetric sizing based on the detection and measurement of changes in electrical resistance produced by a particle suspended in conductive liquid as it is drawn through a small aperture. It measures HGB using spectrometric principle .A modified met hemoglobin method is used for the colorimetric determination of HGB. A portion of the lysed diluted sample from the WBC mixing chamber is used for HGB measurement. A low energy light emitting Diode is used as the light source .a filtered photo detector with wave length of 540 nm measures the transmitted light.

A zero or blank reagent is first obtained to provide a reference to which the sample signal is compared. Lyses reagent lyses the diluted RBC and converts the released HGB to a chromogenic. the sample is then transferred to the HGB flow cell where HGB concentration is measured. The sample enters the flow cell from the bottom .The LED shine through the HGB flow and a 540nm narrow bandwidth filter on to a photo detector the HGB concentration is directly proportional to the absorbance of the sample when the HGB measurement is completed, the HGB flow cell is rinsed with detergent. Reference and sample readings are compared to determine the HGB concentration of the sample. The result is expressed in grams of HGB per deciliter of whole blood.

Materials and Reagents

Materials

- Structured questioners
- EDTA anticoagulant tube
- Tourniquet
- Protective glove
- CBC machine

- Syringe

- Microscope
- Microscopic slide
- Gauze and cotton
- Syringe

Reagent

-95% methanol

-wrights stain

Specimen collection

• About 2-3 ml of blood was collected in to an EDTA tube for CBC.

Laboratory Procedure for CBC

1. After specimen is collected, place it in a rack near the CELL-DYN 1800 System
2. After daily startup is completed, go to **MAIN** menu.
3. Select the desired specimen tube from the rack.
4. Confirm specimen ID on the tube
5. From the **MAIN** menu, press **RUN**
6. From the **RUN** menu, press **[SPECIMENTYPE]**, and then press **[PATIENT SPECIMEN]**.
7. Using the PC keyboard, enter specimen ID information and demographics
8. With the cap tightly secured, gently invert the tube 10 to 15 times.
9. Remove the cap from the pre-mixed specimen tube
10. Place tube under sample aspiration probe and raise the tube so that the end of the probe is deeply immersed in the specimen
11. Press touches plate to activate run.
12. After the instrument aspirates the specimen, the probe moves up. There will be an audible beep and the message line displays **REMOVE SPECIMEN**.
13. Remove the specimen and replace the cap.
14. After the cycle is completed, the results will display on the **RUN** screen in terms of histogram and number and will automatically print out the result.
15. Finally record the value of HGB (g/dl)

Annex IV - Laboratory check lists

DATE----------

Identification code----------

Unique ART number----------

1. RBC parameters
    a. HGB value(g/dl)----------
    b. HCT value (%) ----------
    c. MCV----------
    d. MCH----------
    e. MC ----------