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# PREVALENCE OF ANEMIA AND ITS ASSOCIATED FACTORS AT THE TIME OF ART INITIATION AMONG HIV-INFECTED ADULTS AT DEBRE-MARKOS REFERRAL HOSPITAL, NORTHWEST ETHIOPIA: A RETROSPECTIVE CROSS-SECTIONAL STUDY



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# PREVALENCE OF ANEMIA AND ITS ASSOCIATED FACTORS AT THE TIME OF ART INITIATION AMONG HIV-INFECTED ADULTS AT DEBRE-MARKOS REFERRAL HOSPITAL, NORTHWEST ETHIOPIA: A RETROSPECTIVE CROSS-SECTIONAL STUDY

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## ABSTRACT

**Objective**: The aim of this study was to assess the prevalence of anemia and its associated factors at the time of Antiretroviral Therapy (ART) initiation among HIV-infected adults at Debre-Markos Referral Hospital.

**Methods**: an institution-based retrospective cross-sectional study was conducted among 473 patients' charts enrolled from 2014 to 2018 at Debre-Markos Referral Hospital. Patients' chart numbers were selected from the computer using a simple random sampling technique. Data were entered using EPI INFO version 7.2.2.6 and analyzed with Stata 14.0. Anemia prevalence at the time of ART initiation was computed and described using frequency tables. Both bivariate and multivariate logistic regression models were fitted to identify factors of anemia prevalence. Model fitness was checked by using the Hosmer-Lemeshow goodness of fit test**.**

**Results**: from 473 patients' charts, 468 charts were included in the analysis, and a total of 164 anemia cases were recorded. The overall prevalence of anemia among HIV-infected adults at the time of ART initiation was 35.04%. After multivariate analysis, an increased risk of anemia was seen among males ( Adjusted odds ratio (AOR)=2.45; 95% Confidence interval (CI): 1.51-3.98); had no formal education (AOR=2.38; 95% CI: 1.12-5.05); baseline CD4 count ≤ 200 cells/mm$^3$ (AOR=4.67; 95% CI: 2.78-7.85); Body mass index (BMI) <18.5 kg/m$^2$ (AOR=2.43; 95% CI: 1.42-4.16); and ambulatory/bedridden baseline functional status (AOR=2.69; 95% CI: 1.41-5.12).

**Conclusion**: The current study showed that still anemia is a problem among HIV patients at the time of ART initiation. Giving special attention to patients with no formal education and for males is crucial to reduce anemia.


**Keywords**: Anemia; ART; Ethiopia; HIV/AIDS

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study provided baseline information about Anemia status before starting ART.
- To make a representative sample, reviewed charts were selected randomly.
- Since it was based on chart review, this study lacks some variables like, smoking, alcohol consumption, chat chewing, etc.

## INTRODUCTION

Anemia is described as a reduction in the proportion of the red blood cells or the hemoglobin concentration within them. This lower concentration of normal hemoglobin leads to an insufficient oxygen-carrying capacity of red blood cells. It is an important health indicator of both poor nutrition and poor health. Anemia might have a negative effect on the quality of life and also it adversely impacts the social and economic development of a patient with HIV and the country at large [1-6].

Anemia is a serious global public health problem that affects all age groups of the population. It affects up to one-third of the global population and if it is undiagnosed or left untreated for a prolonged period of time, it can lead to multi-organ failure and can even death [6, 7].

Anemia is the most common hematologic abnormality associated with HIV infection, especially among patients with advanced HIV disease [8]. In addition to weakening of the immune system, HIV infection has a negative impact on the hematopoietic system of infected individuals which results in a decreased concentration of hemoglobin in the blood. On the other hand, Anemia contributes to the progression of HIV infection to the Acquired Immunodeficiency Syndrome (AIDS) stage and this, in turn, accelerates progression to mortality [9-13].

Globally in 2019, a total of 1.74 billion anemia cases with an overall prevalence of 22.8% were reported [14]. The burden of anemia is more in developing countries, especially in sub-Saharan Africa, which accounts for more than 89% of the overall Anemia [15, 16]. This anemia prevalence increased more among HIV-infected patients who didn't start ART [8]. In different study settings, the prevalence of anemia at the time of ART initiation among HIV-infected patients was estimated and it was about 55.8%, 51.9%, and 25.8% in Nepal, China, and Johannesburg in South Africa [13, 17, 18], respectively.

Ethiopia is one of the most seriously affected countries by HIV and Anemia is a known predictor of disease progression and death among HIV-infected patients [5, 19]. Studies conducted in Ethiopia showed that the prevalence of anemia at the time of ART initiation among HIV-infected patients was estimated to be between 21.2% and 52.6% [19-25].

Identifying risk factors of anemia prevalence is crucial for developing effective interventions and for monitoring anemia control programs among HIV-infected patients. A cross-sectional survey conducted in China showed that age, low CD4 count, and ethnicity were significant factors of Anemia at the time of ART initiation [13]. Studies conducted in Ethiopia showed that sex, World Health Organization (WHO) clinical stage, TB/HIV co-infection, CD4 cell counts, opportunistic infection (OI), and history of Tuberculosis (TB) were independent predictors of Anemia occurrence at baseline [19-24].

Ethiopia is strongly committed to promote health and wellbeing among HIV-infected patients and the community at large, and anemia control is among its priorities. However, the relative contribution of different risk factors of anemia among HIV-infected patients in Ethiopia still varies from one setting to another. So, knowing of factors at local levels is important to intervene

accordingly. Therefore, this study aimed to assess the prevalence of anemia at the time of ART initiation and its associated factors among HIV-infected patients at Debre-Markos referral hospital.

## METHOD AND MATERIALS

### Study design, setting and period

An institution-based retrospective cross-sectional study was conducted at Debre Markos Referral Hospital from January 1, 2014, to December 31, 2018, among HIV-infected adults. Patients' charts were reviewed to collect the data from randomly selected charts. Debre-Markos Referral Hospital is found at Debre Markos town in East Gojjam Zone of Amhara Nation Regional State (ANRS). It is situated 299 km from Addis Ababa, the capital city of Ethiopia, and 265 km from Bahir Dar, the capital city of ANRS [26]. The study was conducted by reviewing patients' chart from January 1, 2014, to December 31, 2018.

### Source and Study Population

**Source Population:** All adult people living with HIV aged 15 years old and above attending ART Clinic at Debre-Markos Referral Hospital, Northwest Ethiopia.

**Study population:** randomly selected HIV-positive adults age 15 years old and above who were newly enrolled in ART Clinic at Debre-Markos Referral Hospital from January 1, 2014, to December 31, 2018, Northwest Ethiopia.

### Inclusion and exclusion criteria

Adults, age 15 years and above who were newly enrolled in ART Clinic at Debre-Markos Referral Hospital at the time of the study were included in this study. Patients' charts with transferred in record information were excluded because these charts may lack baseline information.

## Sample size and sampling procedure

The Sample size was determined by using a formula to estimate single population proportion with the assumption of 95% level of confidence, 23.4% proportion [22], 4% marginal error, and Power 80%.

$$n_i = \frac{(z\alpha/2)^2.p(1-p)}{w2}$$

Where, $n_i$ = Initial sample size, $\alpha$ = Precision level or level of significance, P = population Proportion of Anemia, w= Marginal error, and $Z_{a/2}$ = the value under the standard normal table. Finally, the sample size was calculated using EPI INFO statistical package version 7.2.2.6 and with consideration of 10% expected incomplete records the final sample size was 473. During the study period, a total of 1264 patients were newly enrolled in Debre-Markos ART Clinic. Of these, 1117 patients' charts fulfill the inclusion criteria. Then by generating a random number on the computer, a total of 473 patients' charts were selected through a simple random sampling technique, and data were collected from them.

## Operational definition

**Anemia** in this study was defined as anemic or non-anemic based on WHO criteria: Hemoglobin concentration < 13 g/dl for males and < 12 g/dl for females [1].

**Functional status** was classified based on WHO criteria as, Working(W) = capable of going out of home and do routine activities including the daily work; Ambulatory (A) = capable of self-care and going to the toilet unsupported; Bed-ridden (B) = can't go even to the toilet unsupported [27].

**Baseline WHO Clinical stages**: taken from chart record at enrolment to ART based on WHO classification criteria, labeled as stage I to IV.

**Body mass index (BMI):** Defined as the weight of the individual in kilograms divided by height in meter square.

## Data collection tools and procedures

First, an extraction tool was developed from HIV/AIDS care monitoring and evaluation tools. Forms used for laboratory request and ART intake were also incorporated into the development of the extraction tool. The tool contains socio-demographic and related baseline clinical variables. Prior to data collection, training was given for three nurses regarding the tool and the way they extract the data from the chart. With the help of medical record number (MRN), patients' charts were selected by computer-generated simple random sampling technique. Patient charts were picked up from the chart room using MRN and then data were extracted from the patient's medical charts by using the tool. Common code was given for each selected chart after data was extracted so that there was no chance of recollection of data from a similar chart. In this way, all selected patients' charts which fulfill the inclusion criteria were reviewed and data extraction was completed.

## Data quality assurance

Pretested data extraction tool was used to maintain data quality. Quality also maintained by extracting data using trained nurses and close monitoring of the procedure by the supervisor. Data clerks were involved in the selection of patients' charts from the computer as well as from the chart room. Before returning of the chart to the shelf, completeness of data extraction tool was checked and a necessary correction was made.

## Data processing and analysis

After the data were extracted from the chart, it was first checked for consistency and completeness. After that it was coded and entered to EPI INFO version 7.2.2.6 and exported to STATA version 14.0 for analysis. A frequency table was used to describe socio-demographic and clinical variables of the study. The prevalence of Anemia at the time of ART initiation was estimated. Model fitness was checked by using the ***Hosmer-Lemeshow goodness of fit test (p-value = 0.6106)*** and the model was fitted well. Bivariate analysis was executed for each variable and those variables with p-value < 0.2 were entered into multivariate binary logistic regression to identify factors associated with anemia prevalence at the time of ART initiation. Odds ratio with 95% CI was computed and variables having p-value < 0.05 in the multivariate logistic regression was considered as statistically independent factors for Anemia at ART initiation.

## Patient and public involvement

Since the study was based on chart review without active involvement of patients, no patient was involved.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical clearance was obtained from the Institutional Review Board (IRB) of the University of Gondar (Ref. No.:-S/N/1600/06/2011). Upon the ethical clearance, a letter of cooperation was obtained from the school of nursing to collect data. Permission was also obtained from Debre Markos Hospital Medical director and ART focal person. Confidentiality was maintained by avoiding the registration of personal identifiers like names to the extraction tool, or it was fully anonymized. Also, no raw data was given for anyone other than the investigator and it was fully anonymized.

# RESULT

## Socio-demographic characteristics of HIV infected adults

Out of all patients enrolled in the ART Clinic from January 1, 2014, to December 31, 2018, a total of 473 charts were selected and reviewed based on the inclusion criteria. From these reviewed charts, 468 charts were included in the analysis and only five charts were excluded due to data incompleteness. Of all patients' charts included in the analysis, 281 (60.04%) were females and about 40.60% of participants were grouped under the category of age 25 to 34 years **(Table 1).**

**Table 1:** Prevalence of Anemia at the time of ART initiation stratified by socio demographic characteristics of HIV infected adults at Debre-Markos Referral Hospital from January 1, 2014 to December 31, 2018 (n = 468)**.**

| Characteristics | Frequency (%) | Anemia status | |
|---|---|---|---|
| | | Anemic (%) | Not Anemic (%) |
| Sex | | | |
| Male | 187 (39.96) | 86 (45.99) | 101 (54.01) |
| Female | 281 (60.04) | 77 (27.40) | 204 (72.60) |
| Age in years | | | |
| 15-24 | 51 (10.90) | 11 (21.57) | 40 (78.43) |
| 25-34 | 190 (40.60) | 65 (34.21) | 125 (65.79) |
| 35-44 | 157 (33.55) | 61 (38.85) | 96 (61.15) |
| ≥45 | 70 (14.96) | 27 (38.57) | 43 (61.43) |
| Religion | | | |
| Orthodox | 447 (95.51) | 158 (35.35) | 289 (64.65) |
| Muslim | 20 (4.27) | 6 (30.00) | 14 (70.00) |
| Protestant | 1 (0.21) | 0 | 1 (100.00) |
| Marital status | | | |
| Single | 56 (11.97) | 18 (32.14) | 38 (67.86) |
| Married | 251 (53.63) | 85 (33.86) | 166 (66.14) |
| Divorced/separated | 125 (26.71) | 47 (37.60) | 78 (62.40) |
| Widowed | 36 (7.69) | 14 (38.89) | 22 (61.11) |
| Educational level | | | |
| No education | 171 (36.54) | 62 (36.26) | 109 (63.74) |
| Primary | 109 (23.29) | 38 (34.86) | 71 (65.14) |
| Secondary | 113 (24.15) | 44 (38.94) | 69 (61.06) |
| College + | 75 (16.03) | 20 (26.67) | 55 (73.33) |
| Residence | | | |
| Rural | 101 (21.58) | 45 (44.54) | 56 (55.45) |
| Urban | 367 (78.42) | 119 (32.43) | 248 (67.57) |

| | | | |
|---|---|---|---|
| Occupation | | | |
| Employed | 153 (32.69) | 50 (32.68) | 103 (67.32) |
| Unemployed | 315 (67.31) | 114 (36.19) | 201 (63.81) |
| Family size | | | |
| < 2 person | 187 (39.96) | 55 (29.41) | 132 (70.59) |
| 3-4 person | 213 (45.51) | 82 (38.50) | 131 (61.50) |
| >4 person | 68 (14.53) | 27 (39.71) | 41 (60.29) |
| HIV disclosure status | | | |
| Disclosed | 426 (91.03) | 149 (34.98) | 277 (65.02) |
| Not disclosed | 42 (8.97) | 15 (35.71) | 27 (64.29) |

## Clinical and Immunological related characteristics of HIV infected adults

Of all study participants included in the analysis, around 29.06% had started ART six months and above after HIV status has confirmed and only 16.67% of study subjects had past OIs. From the group, about 25.85% and 6.20% of patients were grouped under baseline WHO clinical staging of three and four, respectively. One-third of patients included in the analysis had a baseline CD4 count of less than 200 cells/μl and 28.63% of patients were grouped under the category of BMI of less than 18.5kg/m$^2$. Regarding the functional status of HIV-infected patients in this study, around 20.84% had ambulatory or bedridden functional status at enrolment to Highly Active Antiretroviral Therapy (HAART) **(Table 2).**

**Table 2:** Prevalence of Anemia at the time of ART initiation stratified by clinical and immunologic related characteristics of HIV infected adults at Debre-Markos Referral Hospital from January 1, 2014 to December 31, 2018 (n = 468)

| **Characteristics** | **Frequency (%)** | **Anemia status** | |
|---|---|---|---|
| | | **Anemic (%)** | **Not Anemic (%)** |
| Pre-ART duration | | | |
| < 6 month | 332 (70.94) | 122 (36.75) | 210 (63.25) |
| ≥ 6 month | 136 (29.06) | 42 (30.88) | 94 (69.12) |
| Past OI | | | |
| Yes | 78 (16.67) | 34 (43.59) | 44 (56.41) |
| No | 390 (83.33) | 130 (33.33) | 260 (66.67) |
| Past CPT treatment | | | |
| Yes | 93 (19.87) | 32 (34.41) | 61 (65.59) |
| No | 375 (80.13) | 132 (35.02) | 243 (64.80) |
| Past INH prophylaxis | | | |

| | | | |
|---|---|---|---|
| Yes | 15 (3.21) | 3 (20.00) | 12 (80.00) |
| No | 453 (96.79) | 161 (35.54) | 292 (64.46) |
| Past TB treatment history | | | |
| Yes | 14 (2.99) | 8 (57.14) | 6 (42.86) |
| No | 454 (97.01) | 156 (34.36) | 298 (65.64) |
| Baseline clinical staging | | | |
| I | 197 (42.09) | 43 (21.83) | 154 (78.17) |
| II | 121 (25.85) | 35 (28.93) | 86 (71.07) |
| III | 121 (25.85) | 63 (52.07) | 58 (47.93) |
| IV | 29 (6.20) | 23 (79.31) | 6 (20.69) |
| Baseline CD4 count (cells/µl) | | | |
| <100 | 89 (19.02) | 62 (69.66) | 27 (30.34) |
| 100 - 199 | 74 (15.81) | 41 (55.41) | 33 (44.59) |
| 200 - 349 | 124 (26.50) | 34 (27.42) | 90 (72.58) |
| ≥ 350 | 181 (38.68) | 27 (14.92) | 154 (85.08) |
| Baseline BMI | | | |
| < 18.5 | 134 (28.63) | 78 (58.21) | 56 (41.79) |
| 18.5-24.9 | 286 (61.11) | 76 (26.57) | 210 (73.43) |
| >24.9 | 48 (10.26) | 10 (20.83) | 38 (79.17) |
| Baseline functional status | | | |
| Working | 370 (79.06) | 93 (25.14) | 277 (74.86) |
| Ambulatory | 90 (19.23) | 65 (72.22) | 25 (27.78) |
| Bedridden | 8 (1.71) | 6 (75.00) | 2 (25.00) |

**ART**=Antiretroviral Therapy; **OI**=Opportunistic Infection; **CPT**=Cotrimoxazole preventive therapy; **INH**=Isoniazid; **TB**=Tuberculosis; **CD4**=Cluster of Differentiation four; **BMI**=Body Mass Index

## Prevalence of Anemia at the time of ART initiation among HIV infected adults

The overall prevalence of Anemia at the time of ART initiation in this study was 35.04% (95% CI: 30.84 - 39.49). Of this, around 39.63%, 45.12 %, and 15.24% were grouped under the category of mild, moderate, and severe anemia levels, respectively **(Figure 1).**

## Factors that determine Anemia prevalence at the time of ART initiation

After applying bivariate logistic regression, variables with a p-value of 0.2 or less were taken to multivariate logistic regression. Variables included in multivariate analysis were sex, age at enrolment, level of education, residence, family size, pre-ART duration, past OIs, CD4 count at enrollment, Body Mass Index, WHO clinical stage, and functional status at enrollment. After

multivariate logistic regression, five variables including sex, level of education, CD4 count, BMI, and functional status at enrollment were found to be statistically independent predictors of anemia prevalence at the time of ART initiation at a p-value of less than 0.5.

The current study revealed that the odds of being anemic at the time of ART initiation among males was 2.45 times that of females (AOR = 2.45; 95% CI: 1.51 - 3.98). Similarly, the odds of developing anemia among patients with no formal education was 2.38 times that of college and above educational level (AOR = 2.38; 95% CI: 1.12 - 5.05). CD4 count was also another significant factor of anemia at the time of ART initiation. The likely hood of developing anemia at the time of ART initiation among patients with CD4 count less than 200 cells/µl was 4.67 times that of 200 cells/µl or more (AOR = 4.67; 95% CI: 2.78 - 7.85). In the same manner, the odds of being anemic at enrolment to ART was 2.43 times among patients with a BMI of less than 18.5 kg/m$^2$ than patients with normal BMI (AOR = 2.43; 95% CI: 1.42 - 4.16). Lastly, the odds of being anemic among ambulatory and/or bedridden patients was 2.69 times that of working functional status at the time of ART initiation (AOR = 2.69; 95% CI: 1.41 - 5.12) **(Table 3).**

**Table 3**: Bivariate and multivariable logistic regression analysis of factors associated with Anemia at the time of ART initiation among HIV infected adults at Debre-Markos Referral Hospital from January 1, 2014 to December 31, 2018 (n = 468)

| Characteristics | Frequency | | COR (95% CI) | AOR (95% CI ) | P-Value |
|---|---|---|---|---|---|
| | Anemic | Not anemic | | | |
| Sex of the patient | | | | | |
| Male | 86 | 101 | 2.30 (1.56, 3.40) | 2.45 (1.51, 3.98) | **<0.001*** |
| Female | 77 | 204 | 1.00 | 1.00 | |
| Age at enrolment (in year) | | | | | |
| 15-24 | 11 | 40 | 1.00 | 1.00 | |
| 25-34 | 65 | 125 | 1.89 (0.91, 3.93) | 1.96 (0.79, 4.85) | 0.145 |
| 35-44 | 60 | 97 | 2.31 (1.10, 4.85) | 1.65 (0.65, 4.22) | 0.295 |
| 45+ | 27 | 43 | 2.28 (1.003, 5.19) | 1.65 (0.58, 4.67) | 0.346 |
| Level of education | | | | | |
| No education | 62 | 109 | 1.57 (0.88, 2.82) | 2.38 (1.12, 5.05) | **0.024*** |
| Primary | 37 | 72 | 1.32 (0.70, 2.51) | 1.69 (0.76, 3.77) | 0.195 |

| | | | | | |
|---|---|---|---|---|---|
| Secondary | 44 | 69 | 1.87 (1.01, 3.45) | 1.88 (0.87, 4.04) | 0.107 |
| College+ | 20 | 55 | 1.00 | 1.00 | |
| Patient's residence | | | | | |
| Rural | 118 | 249 | 1.67 (1.07, 2.62) | 1.58 (0.91, 2.75) | 0.105 |
| Urban | 45 | 56 | 1.00 | 1.00 | |
| Family size | | | | | |
| ≤ 2 person | 55 | 132 | 1.00 | 1.00 | |
| 3–4 person | 81 | 132 | 1.50 (0.99, 2.28) | 1.30 (0.76, 2.22) | 0.333 |
| ≥ 5 person | 27 | 41 | 1.58 (0.89, 2.82) | 1.41 (0.68, 2.94) | 0.352 |
| Pre-ART duration | | | | | |
| < 6 months | 122 | 210 | 1.00 | 1.00 | |
| ≥ 6 months | 42 | 94 | 0.77 (0.50, 1.17) | 0.93 (0.55, 1.60) | 0.807 |
| Presence of past OI | | | | | |
| Yes | 34 | 44 | 1.55 (0.94, 2.53) | 0.88 (0.46, 1.69) | 0.708 |
| No | 130 | 260 | | | |
| CD4 count at enrolment (cells/µl) | | | | | |
| < 200 | 103 | 60 | 6.86 (4.49, 10.49) | 4.67 (2.78, 7.85) | **<0.001*** |
| ≥ 200 | 61 | 244 | 1.00 | 1.00 | |
| Body Mass Index (kg/m$^2$) | | | | | |
| <18.5 | 78 | 56 | 3.85 (2.49, 5.93) | 2.43 (1.42, 4.16) | **0.001*** |
| 18.5 – 24.9 | 76 | 210 | 1.00 | 1.00 | |
| > 24.9 | 10 | 38 | 0.73 (0.35, 1.53) | 1.26 (0.54, 2.93) | 0.593 |
| WHO Clinical staging | | | | | |
| Stage I | 43 | 158 | 1.00 | 1.00 | |
| Stage II | 35 | 86 | 1.49 (0.89, 2.51) | 0.94 (0.51, 1.73) | 0.850 |
| Stage III/IV | 86 | 60 | 5.27 (3.29, 8.44) | 1.78 (0.96, 3.30) | 0.068 |
| Functional status at enrolment | | | | | |
| Working | 93 | 277 | 1.00 | 1.00 | |
| Ambulatory/bedridden | 71 | 27 | 7.83 (4.74, 12.93) | 2.69 (1.41, 5.12) | **0.003*** |

***significance at a p-value <0.05**

**AOR**=Adjusted Odds Ratio; **ART**=Antiretroviral therapy; **CD4**=Cluster of Differentiation; **COR**=Crude Odds Ratio; **OI**=Opportunistic Infection; **WHO**=World Health Organization

## DISCUSSION

There are varieties of hematologic abnormalities associated with HIV infection, of which anemia remains a public health challenge in HIV-positive patients around the world, particularly in Sub-Saharan African, including Ethiopia. It is a known complication of HIV infection that is associated with an increased risk of morbidity and mortality for patients with HIV.

The overall prevalence of anemia at the time of ART initiation in the current study was 35.04% (95% CI: 30.84 - 39.49). This is higher than studies conducted in Ethiopia, particularly in Hawassa University Referral Hospital, South Ethiopia (23.4%) [22], and University of Gondar Referral Hospital, Northwest Ethiopia (21.2%) [23]. It is also higher than a study done in Johannesburg, South Africa, with a prevalence of 25.8% [18]. However, anemia prevalence in the current study is lower than studies from Addis Ababa, Ethiopia; namely Black Lion Specialized Hospital (41.9%), Zewditu Memorial Hospital (42.9%), Minilik II Hospital (52.6%) [19, 20, 24]. Similarly, the prevalence of anemia at the time of ART initiation is lower than the study conducted at Arba Minch Town, Southern Ethiopia which is 52.3% [21]. Anemia at the time of ART initiation in the current study is also lower than studies conducted from China and Nepal with the prevalence of 51.9% [13] and 55.8% [17], respectively. This lower prevalence of anemia in the current study could be related to differences in socio-demographic characteristics, or it could be due to differences in the time period by which better interventions to reduce anemia were applied recently than in previous times. Additionally, the majority of patients (72.3%) from china had CD4 count less than 200 cells/mm3 [13] compared to the current study, which is about 34.83%. This may be an important biological implication of the current finding in that the lower CD4 count was associated with an increased risk of anemia [11, 28].

In this study, the male sex was found to be an independent predictor of anemia at the time of ART initiation. The odd of being anemic at the time of ART initiation among males is 2.45 times that of females (95% CI: 1.51 - 3.98). This is in line with studies conducted in Zewditu memorial hospital and Arba minchi town, Ethiopia [19, 21]. More alcohol consumption among males than females might contribute to this difference between males and females. As males consume more alcohol, the rate of Vitamin B12 absorption to the circulatory system becomes lower and may

result in pernicious or megaloblastic anemia. In contrast to the current study, the odds of being anemic at the time of ART initiation at Hawassa University referral hospital was higher in females than males [22]. This might be due to the fact that the presence of menstrual blood loss and in the drains on iron stores that occur with pregnancy and delivery might contribute to the development of anemia in women [19]. In addition, in the previous study, more than two-third of the subjects were females which might contribute to the female sex be significant factor.

Similarly, the current study showed that the odds being anemic among patients with no formal education was 2.38 times that of college and above educational status (AOR = 2.38; 95% CI: 1.12 - 5.05). This is supported by a retrospective cross-sectional study from Mizan-Aman General Hospital, Ethiopia [29]. This may be explained by patients with no formal education are less aware of better nutrition and better health care. When HIV infection is observed among those with no formal education, the risk of poor nutrition and occurrence of anemia will be double burdened. Additionally, non-educated patients are not fully aware of anemia symptoms so that they will come to the hospital quite late with high anemic grades.

The current study revealed that the odds of being anemic among patients with CD4 count less than 200 cells/ $mm^3$ was 4.67 times that of CD4 count greater than 200 cells/ $mm^3$ (95% CI: 2.78, 7.85). This is congruent with studies from the University of Gondar referral hospital, Arba minchi town, and Black Lion specialized hospital [21, 23, 24]. It could be due to the fact that bone marrow abnormalities are found at all stages of HIV disease and increases in frequency and severity as the disease advanced more. So, the risk of anemia occurrence increases with progressive immunologic deterioration, and a CD4 cell count of less than 200 cells/mm3 is related to the development of anemia [30].

Body mass index was another variable found to be a significant factor for the occurrence of anemia at the time of ART initiation. The likelihood of developing anemia among patients with a BMI less than 18.5 kg/m$^2$ was 2.43 times that of patients with normal BMI (95% CI: 1.42 - 4.16). This is congruent with a study from Wolaita Sodo University teaching referral hospital, Ethiopia [31]. This might be as a result of deficiencies of micronutrients such as iron, folate, and vitamin B12 in patients with undernutrition. This deficiency of micronutrients directly contributes to the development of anemia among those with a BMI of less than 18.5 kg/m$^2$.

Lastly, the likelihood of developing anemia among HIV patients was statistically associated with baseline functional status. The odds being anemic at the time of ART initiation among HIV patients with Ambulatory and/bedridden functional status were 2.69 times that of patients with working functional status (AOR=2.69; 95% CI: 1.41 - 5.12). Being in ambulatory or bedridden functional status could be an indicator of HIV infection advancement and the occurrence of other opportunistic infections. Patients in ambulatory or bedridden functional status may also be at risk for loss of appetite which may expose them to malnutrition and result in anemia.

## CONCLUSION

The current study showed that still anemia was a problem among HIV patients at the time of ART initiation. Being male sex, having no formal education, CD4 count of less than 200 cells/mm$^3$, undernutrition (BMI < 18.5Kg/m$^2$), and being on ambulatory or bedridden functional status at the time of ART initiation were found to be independent predictors of Anemia. Hence, giving special attention to patients with no formal education and for those males is crucial to reduce anemia. If possible, initiation of ART sooner after status confirmed is crucial especially for those patients who are more at risk of developing low immunity.

## ABBREVIATIONS

AIDS: Acquired Immunodeficiency Syndrome; AOR: Adjusted Odds Ratio; ART: Antiretroviral Therapy; BMI: Body Mass Index; CD4: Cluster of Differentiation Four; CI: Confidence Interval; COR: Crude Odds Ratio; CPT: Cotrimoxazole Prophylactic Therapy; HAART: Highly Active Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; INH: Isoniazid; MRN: Medical Record Number; OI: Opportunistic Infections; TB: Tuberculosis; WHO: World Health Organization.

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## AUTHORS' CONTRIBUTION

All authors made a significant contribution to the work reported. **AA** conceived the idea and design for work, participated in the data collection process, analyze and interpretation of data and also draft the manuscript. **BS, EG, MW** and **BC** approved the designed work with some revisions, participated in data analysis and reviewed the manuscript. All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## COMPETING INTERESTS

The authors have declared that they have no competing interests.

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## AVAILABILITY OF DATA AND MATERIALS

The datasets used during the current study is available from the corresponding author.

## CONSENT FOR PUBLICATION

Not applicable.

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## FIGURE LEGEND:

**Figure 1:** Level of Anemia at the time of ART initiation among HIV infected adults at Debre Markos Referral Hospital.

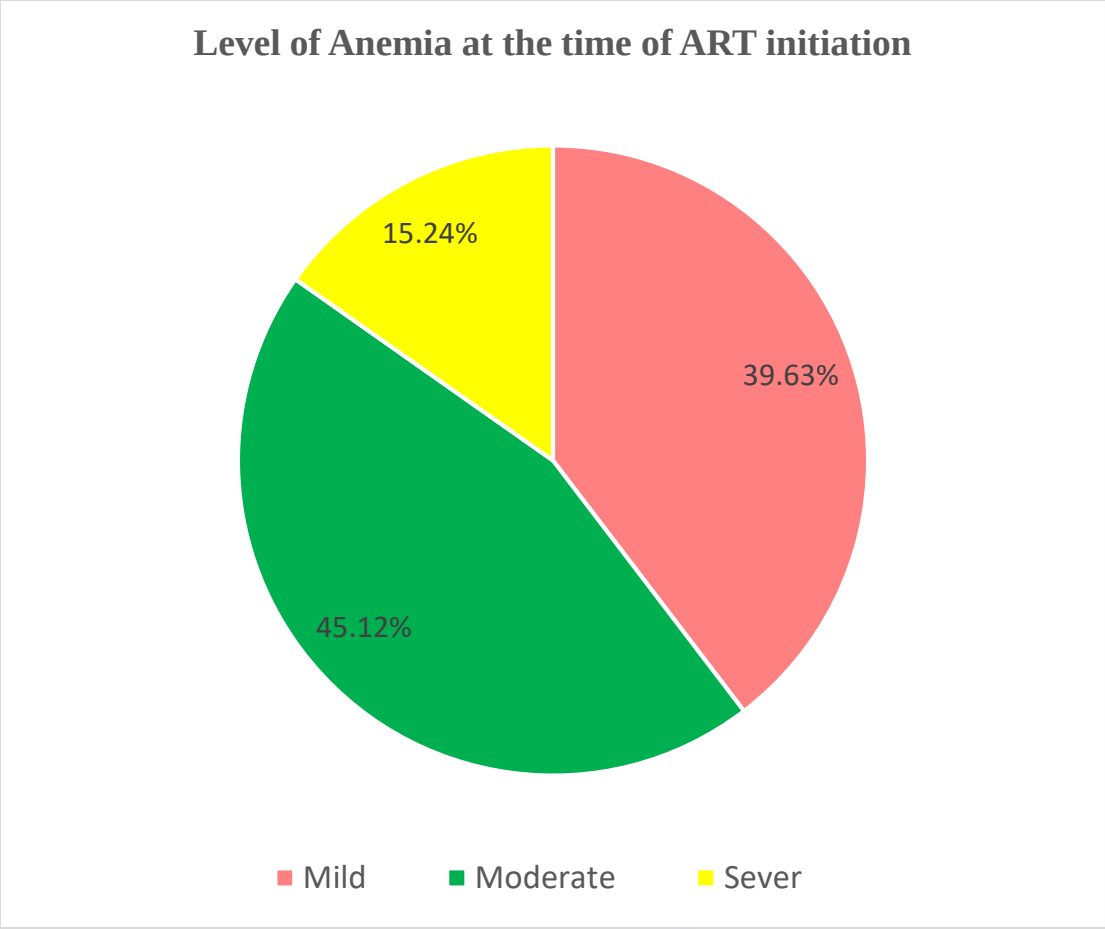


**Figure 1:** Level of Anemia at the time of ART initiation among HIV infected adults at Debre Markos Referral Hospital.

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# Prevalence of anemia and its associated factors among HIV-infected adults at the time of ART initiation at Debre Markos comprehensive specialized hospital, Northwest Ethiopia: a retrospective cross-sectional study

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# Prevalence of anemia and its associated factors among HIV-infected adults at the time of ART initiation at Debre Markos comprehensive specialized hospital, Northwest Ethiopia: a retrospective cross-sectional study

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## Abstract

**Objective**: The aim of this study was to assess the prevalence of anemia and its associated factors at the time of antiretroviral therapy (ART) initiation among HIV-infected adults at Debre-Markos comprehensive specialized hospital.

**Methods**: an institution-based retrospective cross-sectional study was conducted among 473 patients' charts enrolled from 2014 to 2018 at Debre-Markos comprehensive specialized hospital. Patients' chart numbers were selected from the computer using a simple random sampling technique. Data was entered using EPI INFO version 7.2.2.6 and analyzed with Stata 14.0. Anemia prevalence at the time of ART initiation was computed and described using frequency tables. To identify factors for anemia, bivariate and multivariate logistic regression models were fitted. Model fitness was checked using the Hosmer-Lemeshow goodness of fit test**.**

**Results**: From 473 patients' charts, 468 charts were included in the analysis, and a total of 164 anemia cases were recorded. The overall prevalence of anemia among HIV-infected adults at the time of ART initiation was 35.04% (95% Confidence interval (CI): 30.84–39.49). After multivariate analysis, an increased risk of anemia was seen among males ( Adjusted odds ratio (AOR) = 2.45; 95% CI: 1.51–3.98); not attending formal education (AOR = 2.38; 95% CI: 1.12–5.05); baseline CD4+ T-cell count ≤ 200 cells/mm$^3$ (AOR = 4.67; 95% CI: 2.78–7.85); body mass index (BMI) <18.5 kg/m$^2$ (AOR = 2.43; 95% CI: 1.42–4.16); and ambulatory/bedridden baseline functional status (AOR = 2.69; 95% CI: 1.41–5.12).

**Conclusion**: The current study showed that a significant proportion of HIV-infected adults developed anemia at the time of ART initiation. Hence, giving special attention to those who have not attended formal education, males, and late presenters is crucial to reduce the health impact of anemia. The result will provide insight into the development of new anemia preventive strategies.

**Keywords**: Anemia; ART; Associated factors; Ethiopia; HIV/AIDS; Prevalence

## Strengths and limitations of this study

- This study provided baseline information about anemia status before starting ART.
- To make a representative sample, reviewed charts were selected randomly.
- But, due to the retrospective nature of the study, the current study lacks some variables like smoking, alcohol consumption, chat chewing, and type of anemia.
- Additionally, this study was conducted among ART naïve adults, which lacks comparison with anemia after ART initiation.

## Introduction

Anemia is a serious global public health problem that affects all age groups of the population. It affects up to one-third of the global population, and if it is undiagnosed or left untreated for a prolonged period of time, it can lead to multi-organ failure and can even death [1, 2]. It can also have a negative effect on the quality of life and adversely impact the social and economic development of a patient and the country at large [3, 4].

There are varieties of hematologic abnormalities associated with HIV infection, of which anemia remains a public health challenge in HIV-positive patients around the world, particularly in sub-Saharan Africa, including Ethiopia. It is a well-known complication of HIV infection that is linked to an increased risk of morbidity and mortality in HIV patients [5, 6]. Anemia is the most common hematologic abnormality associated with HIV infection, especially among patients with advanced HIV disease [6]. In addition to the weakening of the immune system, HIV infection has a negative impact on the hematopoietic system of infected individuals, which results in a decreased

concentration of hemoglobin in the blood. On the other hand, anemia contributes to the progression of HIV infection to the acquired immunodeficiency syndrome (AIDS) stage and this, in turn, accelerates progression to mortality [5, 7-10].

Globally, in 2019, a total of 1.74 billion anemia cases with an overall prevalence of 22.8% were reported [11]. Anemia is more prevalent in developing countries, particularly sub-Saharan Africa, which accounts for more than 89% of overall anemia [12, 13]. This anemia prevalence increased more among HIV-infected patients who didn't start ART [6]. In different study settings, the prevalence of anemia at the time of ART initiation among HIV-infected patients was estimated to be about 55.8%, 51.9%, and 25.8% in Nepal, China, and Johannesburg, South Africa [10, 14, 15], respectively.

Ethiopia is one of the most seriously affected countries by HIV, and anemia is a known predictor of disease progression and death among HIV-infected patients [3, 16]. Studies conducted in Ethiopia showed that the prevalence of anemia at the time of ART initiation ranged from 21.2% to 52.6% [16-22].

Identifying risk factors for anemia prevalence is crucial for developing effective interventions and monitoring anemia control programs among HIV-infected patients. A cross-sectional survey conducted in China showed that advanced age, low CD4+ T-cell count, and ethnicity were significant factors in increased anemia [10]. Another study conducted in Tanzania revealed that female gender, low body mass index (BMI), lower CD4+ T-cell count, and concurrent tuberculosis treatment were associated with an increased risk of anemia [8]. Studies conducted in Ethiopia reported that female sex, World Health Organization (WHO) clinical stage III/IV, TB/HIV co-infection, Lower CD4+ T-cell counts, presence of opportunistic infection (OI), lower BMI, and

history of tuberculosis (TB) treatment were independent predictors of anemia occurrence at baseline [16-21, 23].

Ethiopia is strongly committed to promote health and wellbeing among HIV-infected patients and the community at large, and anemia control is among its priorities. However, the relative contribution of different risk factors to anemia among HIV-infected patients in Ethiopia still varies from one setting to another. So, knowing the factors at local levels is important to intervene accordingly. Therefore, this study aimed to assess the prevalence of anemia and its associated factors at the time of ART initiation among HIV-infected patients at Debre-Markos comprehensive specialized hospital.

## Method and materials

### Study design, setting and period

An institution-based retrospective cross-sectional study was conducted at Debre Markos comprehensive specialized hospital from January 1, 2014, to December 31, 2018, among HIV-infected adults. Patients' charts were reviewed to collect the data from randomly selected charts. Debre-Markos comprehensive specialized hospital is located in Debre Markos town in the East Gojjam Zone of Amhara Nation Regional State (ANRS). It is located 299 kilometers from Ethiopia's capital, Addis Ababa, and 265 kilometers from Bahir Dar, the capital city of ANRS [24].

### Source and Study Population

**Source Population:** All adult people living with HIV aged 15 years old and above attended the ART Clinic at Debre-Markos comprehensive specialized hospital in Northwest Ethiopia.

**Study population:** Randomly selected HIV-positive adults aged 15 years old and above who were newly enrolled in the ART Clinic at Debre-Markos comprehensive specialized hospital between January 1, 2014, and December 31, 2018, Northwest Ethiopia.

## Inclusion and exclusion criteria

Adults, aged 15 years and above who were newly enrolled in the ART Clinic at Debre-Markos comprehensive specialized hospital at the time of the study were included in this study. Patients' charts with transferred in record information were excluded because these charts may lack baseline information. Additionally, being pregnant at baseline was excluded from the study.

## Sample size and sampling procedure

The sample size was determined by using a formula to estimate single population proportion with the assumption of a 95% level of confidence, 23.4% proportion [19], and a 4% marginal error.

$$n_! = \frac{(z\alpha/2)^2.p(1-p)}{w2}$$

Where, $n_!$ = initial sample size, $\alpha$ = precision level or level of significance, P = population proportion of anemia, w= marginal error, and $Z_{a/2}$ = the value under the standard normal table. Finally, the sample size was calculated using the EPI INFO statistical package version 7.2.2.6 and, with consideration of 10% expected incomplete records, the final sample size was 473. During the study period, a total of 1264 patients were newly enrolled in the Debre-Markos ART Clinic. Of these, 1117 patient charts fulfill the inclusion criteria. Then, by generating a random number on the computer, a total of 473 patients' charts were selected through a simple random sampling technique, and data was collected from them.

## Operational definition of variables

**Anemia** in this study was defined as anemic or non-anemic based on WHO criteria: hemoglobin concentration < 13 g/dl for males and < 12 g/dl for females [25].

**Functional status** was classified based on WHO criteria as: Working (W) = capable of going out of home and doing routine activities, including daily work; Ambulatory (A) = capable of self-care and going to the toilet unsupported; Bed-ridden (B) = can't go even to the toilet unsupported [26].

**Baseline WHO Clinical stages**: taken from the chart record at enrolment to ART based on WHO classification criteria, labeled as stage I to IV.

**Body mass index (BMI):** Defined as the weight of the individual in kilograms divided by their height in meters squared.

## Data collection tools and procedures

First, an extraction tool was developed from HIV/AIDS care monitoring and evaluation tools. Forms used for laboratory requests and ART intake were also incorporated into the development of the extraction tool. The tool contains socio-demographic and related baseline clinical variables. Prior to data collection, training was given to three nurses regarding the tool and the way they extract the data from the chart. With the help of medical record numbers (MRN), patients' charts were selected by a computer-generated simple random sampling technique. Patient charts were picked up from the chart room using MRN and then data was extracted from the patient's medical charts using the tool. A common code was given for each selected chart after the data was extracted, so that there was no chance of recollection of data from a similar chart. In this way, all selected patients' charts that fulfill the inclusion criteria were reviewed and data extraction was completed.

## Data quality assurance

A pretested data extraction tool was used to maintain data quality. Quality is also maintained by extracting data using trained nurses and close monitoring of the procedure by the supervisor. Data clerks were involved in the selection of patients' charts from the computer as well as from the chart room. Before returning the chart to the shelf, the completeness of the data extraction tool was checked and a necessary correction was made.

## Statistical analysis

After the data was extracted from the chart, it was first checked for consistency and completeness. After that, it was coded and entered into EPI INFO version 7.2.2.6 and exported to STATA version 14.0 for analysis. A frequency table was used to describe the socio-demographic and clinical variables of the study. The prevalence of anemia with a 95% confidence interval at the time of ART initiation was estimated. Model fitness was checked by using the ***Hosmer-Lemeshow goodness of fit test (p-value = 0.6106)*** and the model was fitted well. Bivariate analysis was executed for each variable, and those variables with a p-value < 0.2 were entered into multivariate binary logistic regression to identify factors associated with anemia prevalence at the time of ART initiation. An odds ratio with a 95% CI was computed, and variables having a p-value < 0.05 in the multivariate logistic regression were considered as statistically independent factors for anemia at ART initiation.

## Patient and public involvement

Since the study was based on chart review without active involvement of patients, no patients were involved.

## Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Board (IRB) of the University of Gondar (Ref. No. :-S/N/1600/06/2011). Upon the ethical clearance, a letter of cooperation was obtained from the school of nursing to collect data. Permission was also obtained from Debre Markos comprehensive specialized hospital medical director and ART focal person. Confidentiality was maintained by avoiding the registration of personal identifiers like names to the extraction tool. Also, no raw data was given to anyone other than the investigator, and it was fully anonymized.

## Result

### Socio-demographic characteristics of HIV-infected adults

Out of all the patients enrolled in the ART Clinic from January 1, 2014, to December 31, 2018, a total of 473 charts were selected and reviewed based on the inclusion criteria. From these reviewed charts, 468 charts were included in the analysis, and only five charts were excluded due to data incompleteness. Of all the patients' charts included in the analysis, 281 (60.04%) were females, and about 190 (40.60%) of the participants were grouped under the category of age 25 to 34 years **(Table 1).**

**Table 1:** Prevalence of Anemia at the time of ART initiation stratified by socio demographic characteristics of HIV-infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014 to December 31, 2018 (n = 468)**.**

| Characteristics | Frequency (%) | Anemia status | | P-value |
|---|---|---|---|---|
| | | Anemic (%) | Not Anemic (%) | |
| Sex | | | | |
| Male | 187 (39.96) | 86 (45.99) | 101 (54.01) | < 0.001 |
| Female | 281 (60.04) | 77 (27.40) | 204 (72.60) | |
| Age in years | | | | |
| 15-24 | 51 (10.90) | 11 (21.57) | 40 (78.43) | 0.138 |
| 25-34 | 190 (40.60) | 65 (34.21) | 125 (65.79) | |
| 35-44 | 157 (33.55) | 61 (38.85) | 96 (61.15) | |
| ≥45 | 70 (14.96) | 27 (38.57) | 43 (61.43) | |
| Religion | | | | |
| Orthodox | 447 (95.51) | 158 (35.35) | 289 (64.65) | 0.677 |
| Muslim | 20 (4.27) | 6 (30.00) | 14 (70.00) | |
| Protestant | 1 (0.21) | 0 | 1 (100.00) | |
| Marital status | | | | |
| Single | 56 (11.97) | 18 (32.14) | 38 (67.86) | 0.813 |
| Married | 251 (53.63) | 85 (33.86) | 166 (66.14) | |
| Divorced/separated | 125 (26.71) | 47 (37.60) | 78 (62.40) | |
| Widowed | 36 (7.69) | 14 (38.89) | 22 (61.11) | |
| Educational level | | | | |
| No education | 171 (36.54) | 62 (36.26) | 109 (63.74) | 0.365 |
| Primary | 109 (23.29) | 38 (34.86) | 71 (65.14) | |
| Secondary | 113 (24.15) | 44 (38.94) | 69 (61.06) | |
| College + | 75 (16.03) | 20 (26.67) | 55 (73.33) | |
| Residence | | | | |
| Rural | 101 (21.58) | 45 (44.54) | 56 (55.45) | 0.024 |
| Urban | 367 (78.42) | 119 (32.43) | 248 (67.57) | |
| Occupation | | | | |
| Employed | 153 (32.69) | 50 (32.68) | 103 (67.32) | 0.455 |
| Unemployed | 315 (67.31) | 114 (36.19) | 201 (63.81) | |
| Family size | | | | |
| < 2 person | 187 (39.96) | 55 (29.41) | 132 (70.59) | 0.112 |
| 3-4 person | 213 (45.51) | 82 (38.50) | 131 (61.50) | |
| >4 person | 68 (14.53) | 27 (39.71) | 41 (60.29) | |
| HIV disclosure status | | | | |
| Disclosed | 426 (91.03) | 149 (34.98) | 277 (65.02) | 0.924 |
| Not disclosed | 42 (8.97) | 15 (35.71) | 27 (64.29) | |

## Clinical and immunological related characteristics of HIV-infected adults

Of all the study participants included in the analysis, around 136 (29.06%) had started ART six months or above after HIV status had been confirmed, and 78 (16.67%) of the study subjects had past OIs. From the group, about 121 (25.85%) and 29 (6.20%) of patients were grouped under baseline WHO clinical staging of three and four, respectively. One-third of patients included in the analysis had a baseline CD4+ T-cell count of less than 200 cells/μl and 134 (28.63%) of patients were grouped under the category of BMI of less than 18.5kg/m$^2$. Regarding the functional status of HIV-infected patients in this study, around 98 (20.84%) had ambulatory or bedridden functional status at enrolment into highly active antiretroviral therapy (HAART) **(Table 2).**

**Table 2:** Prevalence of anemia at the time of ART initiation stratified by clinical and immunologic related characteristics of HIV-infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014, to December 31, 2018 (n = 468).

| Characteristics | Frequency (%) | Anemia status | | P-value |
|---|---|---|---|---|
| | | Anemic (%) | Not Anemic (%) | |
| Pre-ART duration | | | | |
| < 6 month | 332 (70.94) | 122 (36.75) | 210 (63.25) | 0.227 |
| ≥ 6 month | 136 (29.06) | 42 (30.88) | 94 (69.12) | |
| Past OI | | | | |
| Yes | 78 (16.67) | 34 (43.59) | 44 (56.41) | 0.083 |
| No | 390 (83.33) | 130 (33.33) | 260 (66.67) | |
| Past CPT treatment | | | | |
| Yes | 93 (19.87) | 32 (34.41) | 61 (65.59) | 0.886 |
| No | 375 (80.13) | 132 (35.02) | 243 (64.80) | |
| Past INH prophylaxis | | | | |
| Yes | 15 (3.21) | 3 (20.00) | 12 (80.00) | 0.215 |
| No | 453 (96.79) | 161 (35.54) | 292 (64.46) | |
| Past TB treatment history | | | | |
| Yes | 14 (2.99) | 8 (57.14) | 6 (42.86) | 0.078 |
| No | 454 (97.01) | 156 (34.36) | 298 (65.64) | |
| Baseline WHO clinical staging | | | | |
| I | 197 (42.09) | 43 (21.83) | 154 (78.17) | < 0.001 |
| II | 121 (25.85) | 35 (28.93) | 86 (71.07) | |
| III | 121 (25.85) | 63 (52.07) | 58 (47.93) | |
| IV | 29 (6.20) | 23 (79.31) | 6 (20.69) | |
| Baseline CD4 count (cells/μl) | | | | |
| <100 | 89 (19.02) | 62 (69.66) | 27 (30.34) | < 0.001 |

| | | | | |
|---|---|---|---|---|
| 100 - 199 | 74 (15.81) | 41 (55.41) | 33 (44.59) | |
| 200 - 349 | 124 (26.50) | 34 (27.42) | 90 (72.58) | |
| ≥ 350 | 181 (38.68) | 27 (14.92) | 154 (85.08) | |
| Baseline BMI | | | | |
| < 18.5 | 134 (28.63) | 78 (58.21) | 56 (41.79) | < 0.001 |
| 18.5-24.9 | 286 (61.11) | 76 (26.57) | 210 (73.43) | |
| >24.9 | 48 (10.26) | 10 (20.83) | 38 (79.17) | |
| Baseline functional status | | | | |
| Working | 370 (79.06) | 93 (25.14) | 277 (74.86) | < 0.001 |
| Ambulatory | 90 (19.23) | 65 (72.22) | 25 (27.78) | |
| Bedridden | 8 (1.71) | 6 (75.00) | 2 (25.00) | |

**ART**=Antiretroviral Therapy; **OI**=Opportunistic Infection; **CPT**=Cotrimoxazole preventive therapy; **INH**=Isoniazid; **TB**=Tuberculosis; **CD4**=Cluster of Differentiation four; **BMI**=Body Mass Index

## Anemia prevalence among HIV-infected adults at the time of ART initiation

The overall prevalence of anemia at the time of ART initiation in this study was 35.04% (95% CI: 30.84–39.49). Of these, about 74 (45.12%) were grouped under the category of moderate anemia level **(Figure 1).**

## Factors that determine anemia prevalence at the time of ART initiation

After applying bivariate logistic regression, variables with a p-value of 0.2 or less were taken into multivariate logistic regression. Variables included in multivariate analysis were sex, age at enrolment, level of education, residence, family size, pre-ART duration, past OIs, CD4+ T-cell count at enrollment, Body Mass Index, WHO clinical stage, and functional status at enrollment. After multivariate logistic regression, five variables, including sex, level of education, CD4+ T-cell count, BMI, and functional status at enrollment, were found to be statistically independent predictors of anemia prevalence at the time of ART initiation at a p-value of less than 0.5.

The current study revealed that the odds of being anemic at the time of ART initiation among males was 2.45 times that of females (AOR = 2.45; 95% CI: 1.51–3.98). Similarly, the odds of developing anemia among patients who had not attended formal education was 2.38 times that of

those with a college degree and above educational level (AOR = 2.38; 95% CI: 1.12–5.05). The CD4+ T-cell count was also another significant factor of anemia at the time of ART initiation. The likelihood of developing anemia at the time of ART initiation among patients with a CD4+ T-cell count of less than 200 cells/µl was 4.67 times that of 200 cells/µl or more (AOR = 4.67; 95% CI: 2.78–7.85). In the same manner, the odds of being anemic at enrolment into ART was 2.43 times higher among patients with a BMI of less than 18.5 kg/m$^2$ than among patients with a normal BMI (AOR = 2.43; 95% CI: 1.42–4.16). Lastly, the odds of being anemic among ambulatory and/or bedridden patients was 2.69 times that of working functional status at the time of ART initiation (AOR = 2.69; 95% CI: 1.41–5.12) **(Table 3).**

**Table 3**: Bivariate and multivariable logistic regression analysis of factors associated with anemia at the time of ART initiation among HIV infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014, to December 31, 2018 (n = 468).

| Characteristics | Frequency | | COR (95% CI) | AOR (95% CI ) | P-Value |
|---|---|---|---|---|---|
| | Anemic | Not anemic | | | |
| Sex of the patient | | | | | |
| Male | 86 | 101 | 2.30 (1.56, 3.40) | 2.45 (1.51, 3.98) | **<0.001*** |
| Female | 77 | 204 | 1.00 | 1.00 | |
| Age at enrolment (in year) | | | | | |
| 15-24 | 11 | 40 | 1.00 | 1.00 | |
| 25-34 | 65 | 125 | 1.89 (0.91, 3.93) | 1.96 (0.79, 4.85) | 0.145 |
| 35-44 | 60 | 97 | 2.31 (1.10, 4.85) | 1.65 (0.65, 4.22) | 0.295 |
| 45+ | 27 | 43 | 2.28 (1.003, 5.19) | 1.65 (0.58, 4.67) | 0.346 |
| Level of education | | | | | |
| No education | 62 | 109 | 1.57 (0.88, 2.82) | 2.38 (1.12, 5.05) | **0.024*** |
| Primary | 37 | 72 | 1.32 (0.70, 2.51) | 1.69 (0.76, 3.77) | 0.195 |
| Secondary | 44 | 69 | 1.87 (1.01, 3.45) | 1.88 (0.87, 4.04) | 0.107 |
| College+ | 20 | 55 | 1.00 | 1.00 | |
| Patient's residence | | | | | |
| Rural | 118 | 249 | 1.67 (1.07, 2.62) | 1.58 (0.91, 2.75) | 0.105 |
| Urban | 45 | 56 | 1.00 | 1.00 | |
| Family size | | | | | |
| ≤ 2 person | 55 | 132 | 1.00 | 1.00 | |
| 3–4 person | 81 | 132 | 1.50 (0.99, 2.28) | 1.30 (0.76, 2.22) | 0.333 |
| ≥ 5 person | 27 | 41 | 1.58 (0.89, 2.82) | 1.41 (0.68, 2.94) | 0.352 |
| Pre-ART duration | | | | | |
| < 6 months | 122 | 210 | 1.00 | 1.00 | |

| | | | | | |
|---|---|---|---|---|---|
| ≥ 6 months | 42 | 94 | 0.77 (0.50, 1.17) | 0.93 (0.55, 1.60) | 0.807 |
| Presence of past OI | | | | | |
| Yes | 34 | 44 | 1.55 (0.94, 2.53) | 0.88 (0.46, 1.69) | 0.708 |
| No | 130 | 260 | | | |
| CD4+ T-cell count at enrolment (cells/µl) | | | | | |
| < 200 | 103 | 60 | 6.86 (4.49, 10.49) | 4.67 (2.78, 7.85) | **<0.001*** |
| ≥ 200 | 61 | 244 | 1.00 | 1.00 | |
| Body Mass Index (kg/m$^2$) | | | | | |
| <18.5 | 78 | 56 | 3.85 (2.49, 5.93) | 2.43 (1.42, 4.16) | **0.001*** |
| 18.5 – 24.9 | 76 | 210 | 1.00 | 1.00 | |
| > 24.9 | 10 | 38 | 0.73 (0.35, 1.53) | 1.26 (0.54, 2.93) | 0.593 |
| WHO Clinical staging | | | | | |
| Stage I | 43 | 158 | 1.00 | 1.00 | |
| Stage II | 35 | 86 | 1.49 (0.89, 2.51) | 0.94 (0.51, 1.73) | 0.850 |
| Stage III/IV | 86 | 60 | 5.27 (3.29, 8.44) | 1.78 (0.96, 3.30) | 0.068 |
| Functional status at enrolment | | | | | |
| Working | 93 | 277 | 1.00 | 1.00 | |
| Ambulatory/bedridden | 71 | 27 | 7.83 (4.74, 12.93) | 2.69 (1.41, 5.12) | **0.003*** |

***significance at a p-value <0.05**

**AOR**=Adjusted Odds Ratio; **ART**=Antiretroviral therapy; **CD4+**=Cluster of Differentiation; **COR**=Crude Odds Ratio; **OI**=Opportunistic Infection; **WHO**=World Health Organization

# Discussion

The overall prevalence of anemia at the time of ART initiation in the current study was 35.04% (95% CI: 30.84–39.49), which is higher than studies conducted at the University of Gondar Referral Hospital (21.2%) [20], and Hawassa University Referral Hospital (23.4%) [19]. However, anemia prevalence in the current study is lower than in studies from Addis Ababa [16, 17] and Arba Minch Town [18]. Similarly, anemia prevalence in the current study is lower than in a similar study at Black Lion Specialized Hospital (41.9%) [21]. This could be due to a higher mean of baseline CD4+ T-cell count in the current study (289.5 cells/µl ±167.8) than that of Black Lion Specialized Hospital (162.5 cells/µl ± 108.6). This indicates that the likelihood of anemia will be increased as the HIV infection advances due to immunologic deterioration [27].

Anemia prevalence is also higher than a study done in Johannesburg, South Africa, with a prevalence of 25.8% [15]. The variation may be due to socio-demographic differences. However,

it is also lower than studies conducted in China and Nepal, with a prevalence of 51.9% [10] and 55.8% [14], respectively. This lower prevalence of anemia in the current study could be related to differences in socio-demographic characteristics, or it could be due to differences in the time period in which better interventions to reduce anemia have been applied recently than in previous times. Additionally, the majority of patients (72.3%) from China had a CD4+ T-cell count of less than 200 cells/mm3 [10] compared to the current study, which is about 34.83%. This might have an important biological implication in that the lower CD4+ T-cell count was associated with an increased risk of anemia [5, 28].

In this study, the male sex was found to be an independent predictor of increased anemia at the time of ART initiation. This is in line with studies conducted in Zewditu memorial hospital and Arba Minchi town, Ethiopia [16, 18]. More alcohol consumption among males than females might contribute to this difference between males and females. As males consume more alcohol, the rate of Vitamin B12 absorption into the circulatory system becomes lower and may result in anemia [29]. In contrast to the current study, the odds of being anemic at the time of ART initiation at Hawassa University referral hospital was higher in females than in males [19]. In the previous study, more than two-thirds of the subjects were females, and this might have contributed to increased anemia due to the presence of menstrual blood loss and in the drains on iron stores during pregnancy and delivery [16].

Similarly, the current study showed that the odds of being anemic among patients who had not attended formal education was 2.38 times higher than those with a college degree and above educational status. This is supported by a retrospective cross-sectional study from Mizan-Aman General Hospital, Ethiopia [30]. This may be explained by patients who have not attended formal education are less aware of better nutrition and better health care. When HIV infection is observed

among those who have not attended formal education, the risk of poor nutrition and the occurrence of anemia will be double burdened. Additionally, non-educated patients are not fully aware of anemia symptoms, so they will come to the hospital quite late with high anemic grades [31, 32].

The current study revealed that the odds of being anemic among patients with a CD4+ T-cell count of less than 200 cells/ $mm^3$ was 4.67 times that of a CD4+ T-cell count greater than 200 cells/ $mm^3$. This is congruent with studies from the University of Gondar referral hospital, Arba Minchi town, and Black Lion specialized hospital [18, 20, 21]. It could be due to the fact that bone marrow abnormalities are found at all stages of HIV disease and increase in frequency and severity as the disease advances. So, the risk of anemia occurrence increases with progressive immunologic deterioration and a CD4+ T-cell count of less than 200 cells/mm3 is related to the development of anemia [33].

Anemia was 2.43 times more likely in patients with a lower BMI (18.5 kg/m2) than in those with a normal BMI. This is congruent with a study from Wolaita Sodo University teaching referral hospital, Ethiopia [34]. This might be as a result of deficiencies in micronutrients such as iron, folate, and vitamin B12 in patients with under nutrition. This deficiency of micronutrients directly contributes to the development of anemia among those with a BMI of less than 18.5 $kg/m^2$. Lastly, being in an ambulatory or bedridden functional status increased the likelihood of developing anemia among HIV patients. This might be explained by being in ambulatory or bedridden functional status, which could be an indicator of HIV infection advancement and the occurrence of other opportunistic infections that may also be at risk for loss of appetite, expose them to malnutrition, and result in anemia [35].

In this study, we noted a few limitations. Since the cause of anemia in HIV-infected adults is multi-factorial and the study was based on chart review, we noted that a few variables were missed.

Furthermore, this study did not collect data on dietary habits, substance use status, menstrual habits in females, or the type of anemia. Additionally, this study was conducted among ART-naïve adults, which lacks comparison with anemia after ART initiation.

## Conclusion

The current study showed that a significant proportion of HIV-infected adults developed anemia at the time of ART initiation. Male sex, lack of formal education, a CD4+ T-cell count of less than 200 cells/mm3, under nutrition, and ambulatory or bedridden functional status were discovered to be independent predictors of anemia. Hence, giving special attention to patients not attending formal education, males, and late presenters is crucial to reduce anemia occurrence and its health impact. Finally, the findings of this study will provide baseline information to healthcare providers in order to select ART drugs accordingly, and may provide additional insight into the development of new anemia preventive strategies.

## Abbreviations

AIDS: Acquired Immunodeficiency Syndrome; AOR: Adjusted Odds Ratio; ART: Antiretroviral Therapy; BMI: Body Mass Index; CD4+: Cluster of Differentiation Four; CI: Confidence Interval; COR: Crude Odds Ratio; CPT: Cotrimoxazole Prophylactic Therapy; HAART: Highly Active Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; INH: Isoniazid; MRN: Medical Record Number; OI: Opportunistic Infections; TB: Tuberculosis; WHO: World Health Organization.

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## Competing interests

The authors have declared that they have no competing interests.

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## Availability of data and materials

The dataset used during the current study is available from the corresponding author.

## Consent for publication

Not applicable.

## Authors' contribution

All authors made a significant contribution to the work reported. **AA** conceived the idea and design for the work, participated in the data collection process, analyzed and interpreted the data, and also drafted the manuscript. **BS, EG, MW,** and **BC** approved the designed work with some revisions, participated in data analysis, and reviewed the manuscript. All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Figure legend:

**Figure 1:** Level of anemia at the time of ART initiation among HIV-infected adults at Debre Markos comprehensive specialized hospital.

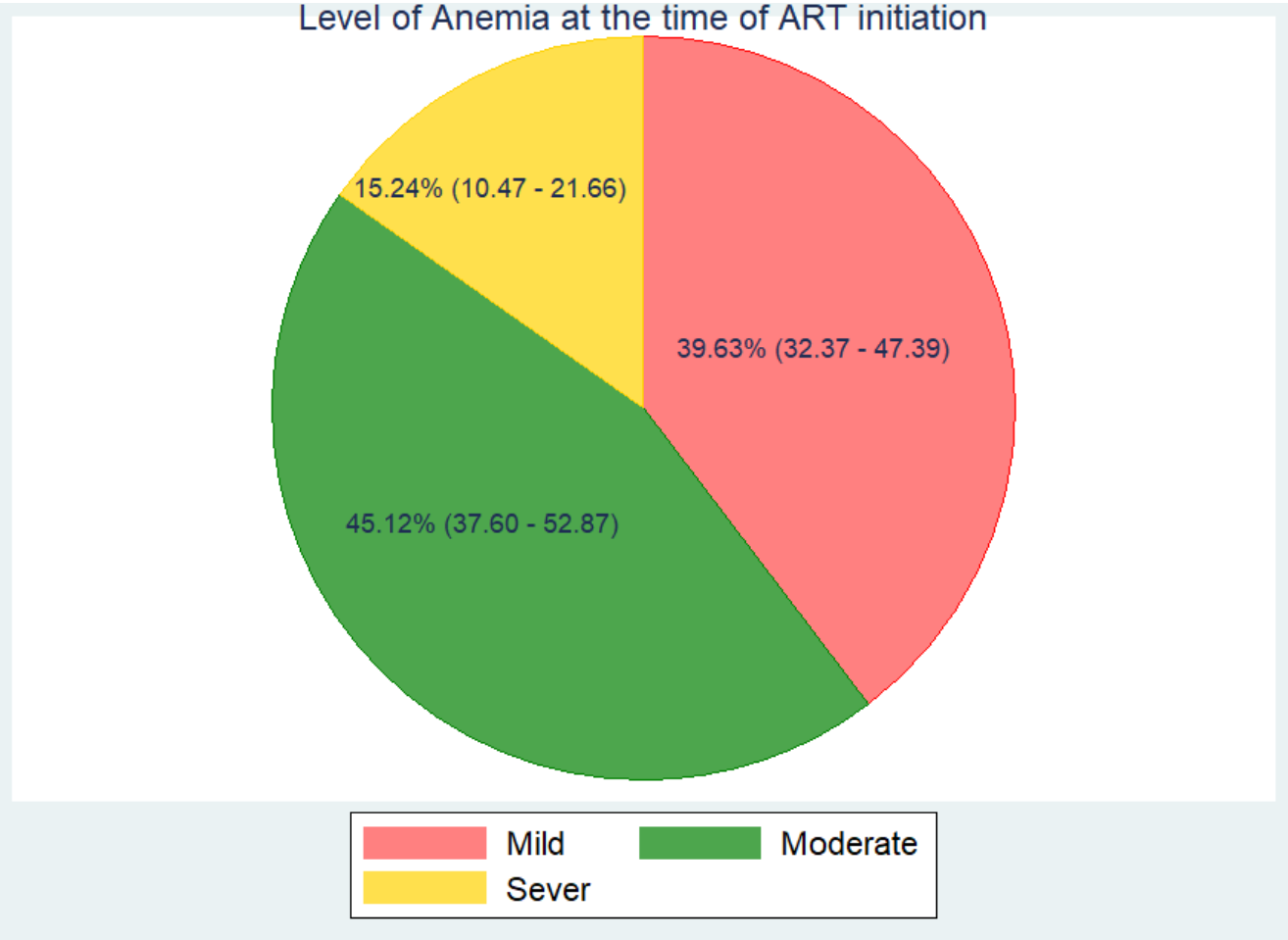


**Figure 1:** Level of anemia at the time of ART initiation among HIV-infected adults at Debre Markos comprehensive specialized hospital.

## STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No. | Recommendation | Page No. | Relevant text from manuscript |
|---|---|---|---|---|
| **Title and abstract** | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract | 2 | Line (27); An institution-based retrospective cross-sectional study was conducted among 472 clients' charts enrolled from 2014 to 2018 at Debre-Markos comprehensive specialized hospital. |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 | Lines (23 – 45); Abstract |
| **Introduction** | | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 3, 4 and 5 | Lines (54 – 94); Introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 | Lines (95 – 97); This study aimed to assess the prevalence of anemia and its associated factors at the time of ART initiation among HIV-infected patients at Debre Markos comprehensive specialized hospital. |
| **Methods** | | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 | Lines (100 – 102); An institution-based retrospective cross-sectional study was conducted at Debre Markos comprehensive specialized hospital from January 1, 2014, to December 31, 2018, among HIV-infected adults. |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 | Lines (199 – 105); Study design, setting and period |

| | | | | |
|---|---|---|---|---|
| Participants | 6 | (a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br><br>Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br><br>Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants | 5<br><br>and<br><br>6 | Lines (106 – 111); Source and study population<br><br>Lines (112 – 116); Inclusion and exclusion criteria (eligibility criteria)<br><br>Lines (126 – 128); By generating a random number on the computer, a total of 473 patient charts were selected through a simple random sampling technique, and data was collected from them. |
| | | (b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed<br><br>Case-control study—For matched studies, give matching criteria and the number of controls per case | N/A | This was a cross-sectional study |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 7 | Lines (131 – 140); Operational definition of variables |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 7 | Lines (141 – 152); Data collection tools and procedures |
| Bias | 9 | Describe any efforts to address potential sources of bias | 8 | Lines (154–159); A pretested data extraction tool was used to maintain data quality. Quality is also maintained by extracting data using trained nurses and close monitoring of the procedure by the supervisor. Data clerks were involved in the selection of patients' charts from the computer as well as from the chart room. Before returning the chart to |

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| | | | | the shelf, the completeness of the data extraction tool was checked and a necessary correction was made. |
| Study size | 10 | Explain how the study size was arrived at | 6 | Lines (117–124); The sample size was determined by using a formula to estimate single population proportion with the assumption of a 95% level of confidence, 25% proportion, and a 4% marginal error. |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8 | Lines (166–171); Bivariate analysis was executed for each variable, and those variables with p-value < 0.2 were entered into multivariate binary logistic regression to identify factors associated with anemia. An odds ratio with a 95% was computed, and variables having a p-value < 0.05 in the multivariate logistic regression were considered as statistically independent factors for anemia at ART initiation. |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding | 8 | Lines (160–171); Statistical Analysis |
| | | (b) Describe any methods used to examine subgroups and interactions | N/A | There were no subgroups. |
| | | (c) Explain how missing data were addressed | N/A | There was no missing data. |
| | | (d) Cohort study—If applicable, explain how loss to follow-up was addressed<br><br>Case-control study—If applicable, explain how matching of cases and controls was addressed<br><br>Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy | N/A | Lines (126–128); By generating a random number on the computer, a total of 473 patients charts were selected through a simple random sampling technique. |
| | | (e) Describe any sensitivity analyses | N/A | |
| **Results** | | | | |

| | | | | |
|---|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | N/A | This was a cross-sectional study with only one stage. |
| | | (b) Give reasons for non-participation at each stage | N/A | |
| | | (c) Consider use of a flow diagram | N/A | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10 and 11 | Line (197); Table 1: Prevalence of Anemia at the time of ART initiation stratified by socio demographic characteristics of HIV-infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014 to December 31, 2018 (n = 468).<br><br>Line (212); Table 2: Prevalence of anemia at the time of ART initiation stratified by clinical and immunologic related characteristics of HIV-infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014 to December 31, 2018 (n = 468). |
| | | (b) Indicate number of participants with missing data for each variable of interest | N/A | There was no missing data |
| | | (c) Cohort study—Summarise follow-up time (eg, average and total amount) | N/A | This was a cross-sectional study |
| Outcome data | 15* | Cohort study—Report numbers of outcome events or summary measures over time | N/A | This was a cross-sectional study |
| | | Case-control study—Report numbers in each exposure category, or summary measures of exposure | N/A | This was a cross-sectional study |
| | | Cross-sectional study—Report numbers of outcome events or summary measures | 12 | Lines (218–219); The overall prevalence of anemia at the time of ART initiation in this study was 35.04% (95% CI: 30.84–39.49). |

| | | | | |
|---|---|---|---|---|
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 12, 13 and 14 | Lines (218–219); The overall prevalence of anemia at the time of ART initiation in this study was 35.04% (95% CI: 30.84–39.49).<br>Lines (241); Table 3: Bivariate and multivariable logistic regression analysis of factors associated with anemia at the time of ART initiation among HIV infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014, to Dec 31, 2018 (n = 468). |
| | | (b) Report category boundaries when continuous variables were categorized | N/A | There was no continuous variable. |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | N/A | There was no estimates of relative risk. |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | N/A | There was no sub group and sensitivity analysis. |
| **Discussion** | | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 14–16 | Lines (247–307); Discussion |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 16 | Lines (303–307); Since the cause of anemia in HIV-infected adults is multi-factorial and the study was based on chart review, we noted that a few variables were missed. Furthermore, this study did not collect data on dietary habits, substance use status, menstrual habits in females, or the type of anemia. Additionally, this study was conducted among ART-naïve adults, which lacks comparison with anemia after ART initiation. |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 14–16 | Lines (247–307); Discussion |
| Generalizability | 21 | Discuss the generalizability (external validity) of the study results | 17 | Lines (308–316); The current study showed that a significant proportion of HIV-infected adults developed anemia at the time of ART |

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|---|---|---|---|---|
| | | | | initiation. Male sex, lack of formal education, a CD4+ T-cell count of less than 200 cells/mm3, under nutrition, and ambulatory or bedridden functional status were discovered to be independent predictors of anemia. Hence, giving special attention to patients not attending formal education, males, and late presenters is crucial to reduce anemia occurrence and its health impact. Finally, the findings of this study will provide baseline information to healthcare providers in order to select ART drugs according to and may provide additional insight into the development of new anemia preventive strategies. |
| **Other information** | | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 18 | This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

BMJ Open

# Prevalence of anemia and its associated factors among HIV-infected adults at the time of ART initiation at Debre-Markos comprehensive specialized hospital, Northwest Ethiopia: a retrospective cross-sectional study

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# Prevalence of anemia and its associated factors among HIV-infected adults at the time of ART initiation at Debre-Markos comprehensive specialized hospital, Northwest Ethiopia: a retrospective cross-sectional study

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**Word count:** 3,715 (Introduction to conclusion; excluding tables and figures).

## Abstract

**Objective**: The aim of this study was to assess the prevalence of anemia and its associated factors at the time of antiretroviral therapy (ART) initiation among HIV-infected adults at Debre-Markos comprehensive specialized hospital.

**Methods**: An institution-based retrospective cross-sectional study was conducted among 473 patients' charts enrolled from 2014 to 2018 at Debre-Markos comprehensive specialized hospital. Patients' chart numbers were selected from the computer using a simple random sampling technique. Data was entered using EPI INFO version 7.2.2.6 and analyzed with Stata 14.0. Anemia prevalence at the time of ART initiation was computed and described using frequency tables. To identify factors for anemia, bivariate and multivariate logistic regression models were fitted. Model fitness was checked using the Hosmer-Lemeshow goodness of fit test**.**

**Results**: From 473 patients' charts, 468 charts were included in the analysis, and a total of 164 anemia cases were recorded. The overall prevalence of anemia among HIV-infected adults at the time of ART initiation was 35.04% (95% Confidence interval (CI): 30.84–39.49). After multivariate analysis, an increased risk of anemia was seen among males ( Adjusted odds ratio (AOR) = 2.45; 95% CI: 1.51–3.98); not attending formal education (AOR = 2.38; 95% CI: 1.12–5.05); baseline CD4+ T-cell count ≤ 200 cells/mm$^3$ (AOR = 4.67; 95% CI: 2.78–7.85); body mass index (BMI) <18.5 kg/m$^2$ (AOR = 2.43; 95% CI: 1.42–4.16); and ambulatory/bedridden baseline functional status (AOR = 2.69; 95% CI: 1.41–5.12).

**Conclusion**: The current study showed that a significant proportion of HIV-infected adults developed anemia at the time of ART initiation. Hence, giving special attention to those who have not attended formal education, males, decreased baseline CD4+ T-cell count, lower BMI, and patients with ambulatory/bedridden baseline functional status is crucial to reduce the health

impact of anemia. The result will provide insight into the development of new anemia preventive strategies.

**Keywords**: Anemia; ART; Associated factors; Ethiopia; HIV/AIDS; Prevalence

## Strengths and limitations of this study

- This study provided baseline information about anemia status before starting ART.
- To make a representative sample, reviewed charts were selected randomly.
- But, due to the retrospective nature of the study, the current study lacks some variables like smoking, alcohol consumption, chat chewing, and type of anemia.
- It is also difficult to determine the temporal link between the outcome and exposure variables.
- Additionally, this study was conducted among ART naïve adults, which lacks comparison with anemia after ART initiation.

## Introduction

Anemia is a serious global public health problem that affects all age groups of the population. It affects up to one-third of the global population, and if it is undiagnosed or left untreated for a prolonged period of time, it can lead to multi-organ failure and can even death [1, 2]. It can also have a negative effect on the quality of life and adversely impact the social and economic development of a patient and the country at large [3, 4].

There are varieties of hematologic abnormalities associated with HIV infection, of which anemia remains a public health challenge in HIV-positive patients around the world, particularly in sub-Saharan Africa, including Ethiopia. Anemia is a well-known complication and the most common

hematologic abnormality associated with HIV infection, especially among patients with advanced HIV disease [5, 6]. In addition to the weakening of the immune system, HIV infection has a negative impact on the hematopoietic system of infected individuals, which results in a decreased concentration of hemoglobin in the blood. On the other hand, anemia contributes to the progression of HIV infection to the acquired immunodeficiency syndrome (AIDS) stage and this in turn accelerates progression to mortality [5, 7-10].

Globally, in 2019, a total of 1.74 billion anemia cases with an overall prevalence of 22.8% were reported [11]. Anemia is more prevalent in developing countries, particularly sub-Saharan Africa, which accounts for more than 89% of overall anemia [12, 13]. This anemia prevalence increased more among HIV-infected patients who didn't start ART [6]. In different study settings, the prevalence of anemia at the time of ART initiation among HIV-infected patients was estimated to be 55.8%, 51.9%, and 25.8% in Nepal, China, and Johannesburg, South Africa [10, 14, 15], respectively.

Ethiopia is one of the most seriously affected countries by HIV, and anemia is a known predictor of disease progression and death among HIV-infected patients [3, 16]. Studies conducted in Ethiopia showed that the prevalence of anemia at the time of ART initiation ranged from 21.2% to 52.6% [16-22].

Identifying risk factors for anemia prevalence is crucial for developing effective interventions and monitoring anemia control programs among HIV-infected patients. A cross-sectional survey conducted in China showed that advanced age, low CD4+ T-cell count, and ethnicity were significant factors in increased anemia [10]. Another study conducted in Tanzania revealed that female gender, low body mass index (BMI), lower CD4+ T-cell count, and concurrent tuberculosis treatment were associated with an increased risk of anemia [8]. Studies conducted in

Ethiopia reported that female sex, World Health Organization (WHO) clinical stage III/IV, TB/HIV co-infection, Lower CD4+ T-cell counts, presence of opportunistic infection (OI), lower BMI, and history of tuberculosis (TB) treatment were independent predictors of anemia occurrence at baseline [16-21, 23].

Ethiopia is strongly committed to promote health and wellbeing among HIV-infected patients and the community at large, and anemia control is among its priorities. However, the relative contribution of different risk factors to anemia among HIV-infected patients in Ethiopia still varies from one setting to another. So, knowing the factors at local levels is important to intervene accordingly. Therefore, this study aimed to assess the prevalence of anemia and its associated factors at the time of ART initiation among HIV-infected patients at Debre-Markos comprehensive specialized hospital.

## Method and materials

### Study design, setting and period

An institution-based retrospective cross-sectional study was conducted at Debre Markos comprehensive specialized hospital from January 1, 2014, to December 31, 2018, among HIV-infected adults. Patients' charts were reviewed to collect the data from randomly selected charts. Debre-Markos comprehensive specialized hospital is located in Debre Markos town in the East Gojjam Zone of Amhara Nation Regional State (ANRS). It is located 299 kilometers (km) from Ethiopia's capital, Addis Ababa, and 265 km from Bahir Dar, the capital city of ANRS [24].

### Source and Study Population

**Source Population:** All adult people living with HIV aged 15 years old and above attended the ART Clinic at Debre-Markos comprehensive specialized hospital in Northwest Ethiopia.

**Study population:** Randomly selected HIV-positive adults aged 15 years old and above who were newly enrolled in the ART Clinic at Debre-Markos comprehensive specialized hospital between January 1, 2014, and December 31, 2018, Northwest Ethiopia.

## Inclusion and exclusion criteria

Adults aged 15 years and above who were newly enrolled in the ART Clinic at Debre-Markos comprehensive specialized hospital at the time of the study were included in this study. Patients' charts with transferred in record information were excluded because these charts may lack baseline information. Additionally, being pregnant at baseline was excluded from the study.

## Sample size and sampling procedure

The sample size was determined by using a formula to estimate single population proportion with the assumption of a 95% level of confidence, 23.4% proportion [19], and a 4% marginal error.

$$n_! = \frac{(za/2)^2.p(1-p)}{w2}$$

Where, $n_!$ = initial sample size, $a$ = precision level or level of significance, P = population proportion of anemia, w= marginal error, and $Z_{a/2}$ = the value under the standard normal table. Finally, the sample size was calculated using the EPI INFO statistical package version 7.2.2.6 and, with consideration of 10% expected incomplete records, the final sample size was 473. During the study period, a total of 1264 patients were newly enrolled in the Debre-Markos ART Clinic. Of these, 1117 patient charts fulfill the inclusion criteria. Then, by generating a random number on the computer, a total of 473 patients' charts were selected through a simple random sampling technique, and data was collected from them.

## Operational definition of variables

**Anemia** in this study was defined as anemic or non-anemic based on WHO criteria: hemoglobin concentration < 13 g/dl for males and < 12 g/dl for females [25].

**Functional status** was classified based on WHO criteria as: Working (W) = capable of going out of home and doing routine activities, including daily work; Ambulatory (A) = capable of self-care and going to the toilet unsupported; Bed-ridden (B) = can't go even to the toilet unsupported [26].

**Baseline WHO Clinical stages**: taken from the chart record at enrolment to ART based on WHO classification criteria, labeled as stage I to IV.

**Body mass index (BMI):** Defined as the weight of the individual in kilograms divided by their height in meters squared.

## Data collection tools and procedures

First, an extraction tool was developed from HIV/AIDS care monitoring and evaluation tools. Forms used for laboratory requests and ART intake were also incorporated into the development of the extraction tool. The tool contains socio-demographic and related baseline clinical variables. Prior to data collection, training was given to three nurses regarding the tool and the way they extract the data from the chart. With the help of medical record numbers (MRN), patients' charts were selected by a computer-generated simple random sampling technique. Patient charts were picked up from the chart room using MRN and then data was extracted from the patient's medical charts using the tool. A common code was given for each selected chart after the data was extracted, so that there was no chance of recollection of data from a similar chart. In this way, all selected patients' charts that fulfill the inclusion criteria were reviewed and data extraction was completed.

## Data quality assurance

A pretested data extraction tool was used to maintain data quality. Quality is also maintained by extracting data using trained nurses and close monitoring of the procedure by the supervisor. Data clerks were involved in the selection of patients' charts from the computer as well as from the chart room. Before returning the chart to the shelf, the completeness of the data extraction tool was checked and a necessary correction was made.

## Statistical analysis

After the data was extracted from the chart, it was first checked for consistency and completeness. After that, it was coded and entered into EPI INFO version 7.2.2.6 and exported to STATA version 14.0 for analysis. A frequency table was used to describe the socio-demographic and clinical variables of the study. The prevalence of anemia with a 95% confidence interval at the time of ART initiation was estimated. Model fitness was checked by using the ***Hosmer-Lemeshow goodness of fit test (p-value = 0.6106)*** and the model was fitted well. Bivariate analysis was executed for each variable, and those variables with a p-value < 0.2 were entered into multivariate binary logistic regression to identify factors associated with anemia prevalence at the time of ART initiation. An odds ratio with a 95% CI was computed, and variables having a p-value < 0.05 in the multivariate logistic regression were considered as statistically independent factors for anemia at ART initiation.

## Patient and public involvement

Since the study was based on chart review without active involvement of patients, no patients were involved.

## Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Board (IRB) of the University of Gondar (Ref. No. :-S/N/1600/06/2011). Upon the ethical clearance, a letter of cooperation was obtained from the school of nursing to collect data. Permission was also obtained from Debre Markos comprehensive specialized hospital medical director and ART focal person. Confidentiality was maintained by avoiding the registration of personal identifiers like names to the extraction tool. Also, no raw data was given to anyone other than the investigator, and it was fully anonymized.

## Result

### Socio-demographic characteristics of HIV-infected adults

Out of all the patients enrolled in the ART Clinic from January 1, 2014, to December 31, 2018, a total of 473 charts were selected and reviewed based on the inclusion criteria. From these reviewed charts, 468 charts were included in the analysis, and only five charts were excluded due to data incompleteness. Of all the patients' charts included in the analysis, 281 (60.04%) were females, and about 190 (40.60%) of the participants were grouped under the category of age 25 to 34 years **(Table 1).**

**Table 1:** Prevalence of Anemia at the time of ART initiation stratified by socio demographic characteristics of HIV-infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014 to December 31, 2018 (n = 468)**.**

| Characteristics | Frequency (%) | Anemia status | | P-value |
|---|---|---|---|---|
| | | Anemic (%) | Not Anemic (%) | |
| Sex | | | | |
| Male | 187 (39.96) | 86 (45.99) | 101 (54.01) | < 0.001 |
| Female | 281 (60.04) | 77 (27.40) | 204 (72.60) | |
| Age in years | | | | |
| 15-24 | 51 (10.90) | 11 (21.57) | 40 (78.43) | 0.138 |
| 25-34 | 190 (40.60) | 65 (34.21) | 125 (65.79) | |
| 35-44 | 157 (33.55) | 61 (38.85) | 96 (61.15) | |
| ≥45 | 70 (14.96) | 27 (38.57) | 43 (61.43) | |
| Religion | | | | |
| Orthodox | 447 (95.51) | 158 (35.35) | 289 (64.65) | 0.677 |
| Muslim | 20 (4.27) | 6 (30.00) | 14 (70.00) | |
| Protestant | 1 (0.21) | 0 | 1 (100.00) | |
| Marital status | | | | |
| Single | 56 (11.97) | 18 (32.14) | 38 (67.86) | 0.813 |
| Married | 251 (53.63) | 85 (33.86) | 166 (66.14) | |
| Divorced/separated | 125 (26.71) | 47 (37.60) | 78 (62.40) | |
| Widowed | 36 (7.69) | 14 (38.89) | 22 (61.11) | |
| Educational level | | | | |
| No education | 171 (36.54) | 62 (36.26) | 109 (63.74) | 0.365 |
| Primary | 109 (23.29) | 38 (34.86) | 71 (65.14) | |
| Secondary | 113 (24.15) | 44 (38.94) | 69 (61.06) | |
| College + | 75 (16.03) | 20 (26.67) | 55 (73.33) | |
| Residence | | | | |
| Rural | 101 (21.58) | 45 (44.54) | 56 (55.45) | 0.024 |
| Urban | 367 (78.42) | 119 (32.43) | 248 (67.57) | |
| Occupation | | | | |
| Employed | 153 (32.69) | 50 (32.68) | 103 (67.32) | 0.455 |
| Unemployed | 315 (67.31) | 114 (36.19) | 201 (63.81) | |
| Family size | | | | |
| < 2 person | 187 (39.96) | 55 (29.41) | 132 (70.59) | 0.112 |
| 3-4 person | 213 (45.51) | 82 (38.50) | 131 (61.50) | |
| >4 person | 68 (14.53) | 27 (39.71) | 41 (60.29) | |
| HIV disclosure status | | | | |
| Disclosed | 426 (91.03) | 149 (34.98) | 277 (65.02) | 0.924 |
| Not disclosed | 42 (8.97) | 15 (35.71) | 27 (64.29) | |

## Clinical and immunological related characteristics of HIV-infected adults

Of all the study participants included in the analysis, around 136 (29.06%) had started ART six months or above after HIV status had been confirmed, and 78 (16.67%) of the study subjects had past OIs. From the group, about 121 (25.85%) and 29 (6.20%) of patients were grouped under baseline WHO clinical staging of three and four, respectively. One-third of patients included in the analysis had a baseline CD4+ T-cell count of less than 200 cells/µl and 134 (28.63%) of patients were grouped under the category of BMI of less than 18.5kg/m$^2$. Regarding the functional status of HIV-infected patients in this study, around 98 (20.84%) had ambulatory or bedridden functional status at enrolment into highly active antiretroviral therapy (HAART) **(Table 2).**

**Table 2:** Prevalence of anemia at the time of ART initiation stratified by clinical and immunologic related characteristics of HIV-infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014, to December 31, 2018 (n = 468).

| Characteristics | Frequency (%) | Anemia status | | P-value |
|---|---|---|---|---|
| | | Anemic (%) | Not Anemic (%) | |
| Pre-ART duration | | | | |
| < 6 month | 332 (70.94) | 122 (36.75) | 210 (63.25) | 0.227 |
| ≥ 6 month | 136 (29.06) | 42 (30.88) | 94 (69.12) | |
| Past OI | | | | |
| Yes | 78 (16.67) | 34 (43.59) | 44 (56.41) | 0.083 |
| No | 390 (83.33) | 130 (33.33) | 260 (66.67) | |
| Past CPT treatment | | | | |
| Yes | 93 (19.87) | 32 (34.41) | 61 (65.59) | 0.886 |
| No | 375 (80.13) | 132 (35.02) | 243 (64.80) | |
| Past INH prophylaxis | | | | |
| Yes | 15 (3.21) | 3 (20.00) | 12 (80.00) | 0.215 |
| No | 453 (96.79) | 161 (35.54) | 292 (64.46) | |
| Past TB treatment history | | | | |
| Yes | 14 (2.99) | 8 (57.14) | 6 (42.86) | 0.078 |
| No | 454 (97.01) | 156 (34.36) | 298 (65.64) | |
| Baseline WHO clinical staging | | | | |
| I | 197 (42.09) | 43 (21.83) | 154 (78.17) | < 0.001 |
| II | 121 (25.85) | 35 (28.93) | 86 (71.07) | |
| III | 121 (25.85) | 63 (52.07) | 58 (47.93) | |

| | | | | |
|---|---|---|---|---|
| IV | 29 (6.20) | 23 (79.31) | 6 (20.69) | |
| Baseline CD4 count (cells/µl) | | | | |
| <100 | 89 (19.02) | 62 (69.66) | 27 (30.34) | < 0.001 |
| 100 - 199 | 74 (15.81) | 41 (55.41) | 33 (44.59) | |
| 200 - 349 | 124 (26.50) | 34 (27.42) | 90 (72.58) | |
| ≥ 350 | 181 (38.68) | 27 (14.92) | 154 (85.08) | |
| Baseline BMI | | | | |
| < 18.5 | 134 (28.63) | 78 (58.21) | 56 (41.79) | < 0.001 |
| 18.5-24.9 | 286 (61.11) | 76 (26.57) | 210 (73.43) | |
| >24.9 | 48 (10.26) | 10 (20.83) | 38 (79.17) | |
| Baseline functional status | | | | |
| Working | 370 (79.06) | 93 (25.14) | 277 (74.86) | < 0.001 |
| Ambulatory | 90 (19.23) | 65 (72.22) | 25 (27.78) | |
| Bedridden | 8 (1.71) | 6 (75.00) | 2 (25.00) | |

**ART**=Antiretroviral Therapy; **OI**=Opportunistic Infection; **CPT**=Cotrimoxazole preventive therapy; **INH**=Isoniazid; **TB**=Tuberculosis; **CD4**=Cluster of Differentiation four; **BMI**=Body Mass Index

## Anemia prevalence among HIV-infected adults at the time of ART initiation

The overall prevalence of anemia at the time of ART initiation in this study was 35.04% (95% CI: 30.84–39.49). Of these, about 74 (45.12%) were grouped under the category of moderate anemia level **(Figure 1).**

## Factors that determine anemia prevalence at the time of ART initiation

After applying bivariate logistic regression, variables with a p-value of 0.2 or less were taken into multivariate logistic regression. Variables included in multivariate analysis were sex, age at enrolment, level of education, residence, family size, pre-ART duration, past OIs, CD4+ T-cell count at enrollment, Body Mass Index, WHO clinical stage, and functional status at enrollment. After multivariate logistic regression, five variables, including sex, level of education, CD4+ T-cell count, BMI, and functional status at enrollment, were found to be statistically independent predictors of anemia prevalence at the time of ART initiation at a p-value of less than 0.05.

The current study revealed that the odd of being anemic at the time of ART initiation among males was 2.45 times that of females (AOR = 2.45; 95% CI: 1.51–3.98). Similarly, the odd of developing anemia among patients who had not attended formal education was 2.38 times that of those with a college degree and above educational level (AOR = 2.38; 95% CI: 1.12–5.05). The CD4+ T-cell count was also another significant factor of anemia at the time of ART initiation. The likelihood of developing anemia at the time of ART initiation among patients with a CD4+ T-cell count of less than 200 cells/µl was 4.67 times that of 200 cells/µl or more (AOR = 4.67; 95% CI: 2.78–7.85). In the same manner, the odds of being anemic at enrolment into ART was 2.43 times higher among patients with a BMI of less than 18.5 kg/m$^2$ than among patients with a normal BMI (AOR = 2.43; 95% CI: 1.42–4.16). Lastly, the odds of being anemic among ambulatory and/or bedridden patients was 2.69 times that of working functional status at the time of ART initiation (AOR = 2.69; 95% CI: 1.41–5.12) **(Table 3).**

**Table 3**: Bivariate and multivariable logistic regression analysis of factors associated with anemia at the time of ART initiation among HIV infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014, to December 31, 2018 (n = 468).

| Characteristics | Frequency | | COR (95% CI) | AOR (95% CI ) | P-Value |
|---|---|---|---|---|---|
| | Anemic | Not anemic | | | |
| Sex of the patient | | | | | |
| Male | 86 | 101 | 2.30 (1.56, 3.40) | 2.45 (1.51, 3.98) | **<0.001*** |
| Female | 77 | 204 | 1.00 | 1.00 | |
| Age at enrolment (in year) | | | | | |
| 15-24 | 11 | 40 | 1.00 | 1.00 | |
| 25-34 | 65 | 125 | 1.89 (0.91, 3.93) | 1.96 (0.79, 4.85) | 0.145 |
| 35-44 | 60 | 97 | 2.31 (1.10, 4.85) | 1.65 (0.65, 4.22) | 0.295 |
| 45+ | 27 | 43 | 2.28 (1.003, 5.19) | 1.65 (0.58, 4.67) | 0.346 |
| Level of education | | | | | |
| No education | 62 | 109 | 1.57 (0.88, 2.82) | 2.38 (1.12, 5.05) | **0.024*** |
| Primary | 37 | 72 | 1.32 (0.70, 2.51) | 1.69 (0.76, 3.77) | 0.195 |
| Secondary | 44 | 69 | 1.87 (1.01, 3.45) | 1.88 (0.87, 4.04) | 0.107 |
| College+ | 20 | 55 | 1.00 | 1.00 | |
| Patient's residence | | | | | |
| Rural | 118 | 249 | 1.67 (1.07, 2.62) | 1.58 (0.91, 2.75) | 0.105 |
| Urban | 45 | 56 | 1.00 | 1.00 | |

| | | | | | |
|---|---|---|---|---|---|
| Family size | | | | | |
| ≤ 2 person | 55 | 132 | 1.00 | 1.00 | |
| 3–4 person | 81 | 132 | 1.50 (0.99, 2.28) | 1.30 (0.76, 2.22) | 0.333 |
| ≥ 5 person | 27 | 41 | 1.58 (0.89, 2.82) | 1.41 (0.68, 2.94) | 0.352 |
| Pre-ART duration | | | | | |
| < 6 months | 122 | 210 | 1.00 | 1.00 | |
| ≥ 6 months | 42 | 94 | 0.77 (0.50, 1.17) | 0.93 (0.55, 1.60) | 0.807 |
| Presence of past OI | | | | | |
| Yes | 34 | 44 | 1.55 (0.94, 2.53) | 0.88 (0.46, 1.69) | 0.708 |
| No | 130 | 260 | | | |
| CD4+ T-cell count at enrolment (cells/µl) | | | | | |
| < 200 | 103 | 60 | 6.86 (4.49, 10.49) | 4.67 (2.78, 7.85) | **<0.001*** |
| ≥ 200 | 61 | 244 | 1.00 | 1.00 | |
| Body Mass Index (kg/m$^2$) | | | | | |
| <18.5 | 78 | 56 | 3.85 (2.49, 5.93) | 2.43 (1.42, 4.16) | **0.001*** |
| 18.5 – 24.9 | 76 | 210 | 1.00 | 1.00 | |
| > 24.9 | 10 | 38 | 0.73 (0.35, 1.53) | 1.26 (0.54, 2.93) | 0.593 |
| WHO Clinical staging | | | | | |
| Stage I | 43 | 158 | 1.00 | 1.00 | |
| Stage II | 35 | 86 | 1.49 (0.89, 2.51) | 0.94 (0.51, 1.73) | 0.850 |
| Stage III/IV | 86 | 60 | 5.27 (3.29, 8.44) | 1.78 (0.96, 3.30) | 0.068 |
| Functional status at enrolment | | | | | |
| Working | 93 | 277 | 1.00 | 1.00 | |
| Ambulatory/bedridden | 71 | 27 | 7.83 (4.74, 12.93) | 2.69 (1.41, 5.12) | **0.003*** |

***significance at a p-value <0.05**

**AOR**=Adjusted Odds Ratio; **ART**=Antiretroviral therapy; **CD4+**=Cluster of Differentiation; **COR**=Crude Odds Ratio; **OI**=Opportunistic Infection; **WHO**=World Health Organization

# Discussion

The overall prevalence of anemia at the time of ART initiation in the current study was 35.04% (95% CI: 30.84–39.49), which is higher than studies conducted at the University of Gondar Referral Hospital (21.2%) [20], and Hawassa University Referral Hospital (23.4%) [19]. However, anemia prevalence in the current study is lower than in studies from Addis Ababa [16, 17] and Arba Minch Town [18]. Similarly, anemia prevalence in the current study is lower than in a similar study at Black Lion Specialized Hospital (41.9%) [21]. This could be due to a higher mean of baseline CD4+ T-cell count in the current study (289.5 cells/µl ±167.8) than that of

Black Lion Specialized Hospital (162.5 cells/μl ± 108.6). This indicates that the likelihood of anemia will be increased as the HIV infection advances due to immunologic deterioration [27].

Anemia prevalence is also higher than a study done in Johannesburg, South Africa, with a prevalence of 25.8% [15]. The variation may be due to socio-demographic differences. However, it is also lower than studies conducted in China and Nepal, with a prevalence of 51.9% [10] and 55.8% [14], respectively. This lower prevalence of anemia in the current study could be related to differences in socio-demographic characteristics, or it could be due to differences in the time period in which better interventions to reduce anemia have been applied recently than in previous times. Additionally, the majority of patients (72.3%) from China had a CD4+ T-cell count of less than 200 cells/mm3 [10] compared to the current study, which is about 34.83%. This might have an important biological implication in that the lower CD4+ T-cell count was associated with an increased risk of anemia [5, 28].

In this study, the male sex was found to be an independent predictor of increased anemia at the time of ART initiation. This is in line with studies conducted in Zewditu memorial hospital and Arba Minchi town, Ethiopia [16, 18]. More alcohol consumption among males than females might contribute to this difference between males and females. As males consume more alcohol, the rate of Vitamin B12 absorption into the circulatory system becomes lower and may result in anemia [29]. In contrast to the current study, the odds of being anemic at the time of ART initiation at Hawassa University referral hospital was higher in females than in males [19]. In the previous study, more than two-thirds of the subjects were females, and this might have contributed to increased anemia due to the presence of menstrual blood loss and in the drains on iron stores during pregnancy and delivery [16].

Similarly, the current study showed that the odds of being anemic among patients who had not attended formal education was 2.38 times higher than those with a college degree and above educational status. This is supported by a retrospective cross-sectional study from Mizan-Aman General Hospital, Ethiopia [30]. This may be explained by patients who have not attended formal education are less aware of better nutrition and better health care. When HIV infection is observed among those who have not attended formal education, the risk of poor nutrition and the occurrence of anemia will be double burdened. Additionally, non-educated patients are not fully aware of anemia symptoms, so they will come to the hospital quite late with high anemic grades [31, 32].

The current study revealed that the odds of being anemic among patients with a CD4+ T-cell count of less than 200 cells/ $mm^3$ was 4.67 times that of a CD4+ T-cell count greater than 200 cells/ $mm^3$. This is congruent with studies from the University of Gondar referral hospital, Arba Minchi town, and Black Lion specialized hospital [18, 20, 21]. It could be due to the fact that bone marrow abnormalities are found at all stages of HIV disease and increase in frequency and severity as the disease advances. So, the risk of anemia occurrence increases with progressive immunologic deterioration and a CD4+ T-cell count of less than 200 cells/mm3 is related to the development of anemia [33].

Anemia was 2.43 times more likely in patients with a lower BMI (18.5 kg/m2) than in those with a normal BMI. This is congruent with a study from Wolaita Sodo University teaching referral hospital, Ethiopia [34]. This might be as a result of deficiencies in micronutrients such as iron, folate, and vitamin B12 in patients with under nutrition. This deficiency of micronutrients directly contributes to the development of anemia among those with a BMI of less than 18.5 $kg/m^2$. Lastly, being in an ambulatory or bedridden functional status increased the likelihood of

developing anemia among HIV patients. This might be explained by being in ambulatory or bedridden functional status, which could be an indicator of HIV infection advancement and the occurrence of other opportunistic infections that may also be at risk for loss of appetite, expose them to malnutrition, and result in anemia [35].

In this study, we noted a few limitations. Since the cause of anemia in HIV-infected adults is multi-factorial and the study was based on chart review, we noted that a few variables were missed. Furthermore, this study did not collect data on dietary habits, substance use status, menstrual habits in females, or the type of anemia. Additionally, this study was conducted among ART-naïve adults, which lacks comparison with anemia after ART initiation.

## Conclusion

The current study showed that a significant proportion of HIV-infected adults developed anemia at the time of ART initiation. Male sex, lack of formal education, a CD4+ T-cell count < 200 cells/mm3, decreased BMI, and ambulatory or bedridden functional status were discovered to be independent predictors of anemia. Hence, giving special attention to patients not attending formal education, males, and late presenters is crucial to reduce anemia occurrence and its health impact. Finally, the findings of this study will provide baseline information to healthcare providers in order to select ART drugs accordingly, and may provide additional insight into the development of new anemia preventive strategies.

## Abbreviations

AIDS: Acquired Immunodeficiency Syndrome; AOR: Adjusted Odds Ratio; ART: Antiretroviral Therapy; BMI: Body Mass Index; CD4+: Cluster of Differentiation Four; CI: Confidence Interval; COR: Crude Odds Ratio; CPT: Cotrimoxazole Prophylactic Therapy; HAART: Highly

Active Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; INH: Isoniazid; MRN: Medical Record Number; OI: Opportunistic Infections; TB: Tuberculosis; WHO: World Health Organization.

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## Competing interests

The authors have declared that they have no competing interests.

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## Availability of data and materials

The dataset used during the current study is available from the corresponding author.

## Consent for publication

Not applicable.

## Authors' contribution

All authors made a significant contribution to the work reported. **AA** conceived the idea and design for the work, participated in the data collection process, analyzed and interpreted the data, and also drafted the manuscript. **BS, EG, MW,** and **BC** approved the designed work with some revisions, participated in data analysis, and reviewed the manuscript. All authors gave final

approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Figure legend:

**Figure 1:** Level of anemia at the time of ART initiation among HIV-infected adults at Debre Markos comprehensive specialized hospital.

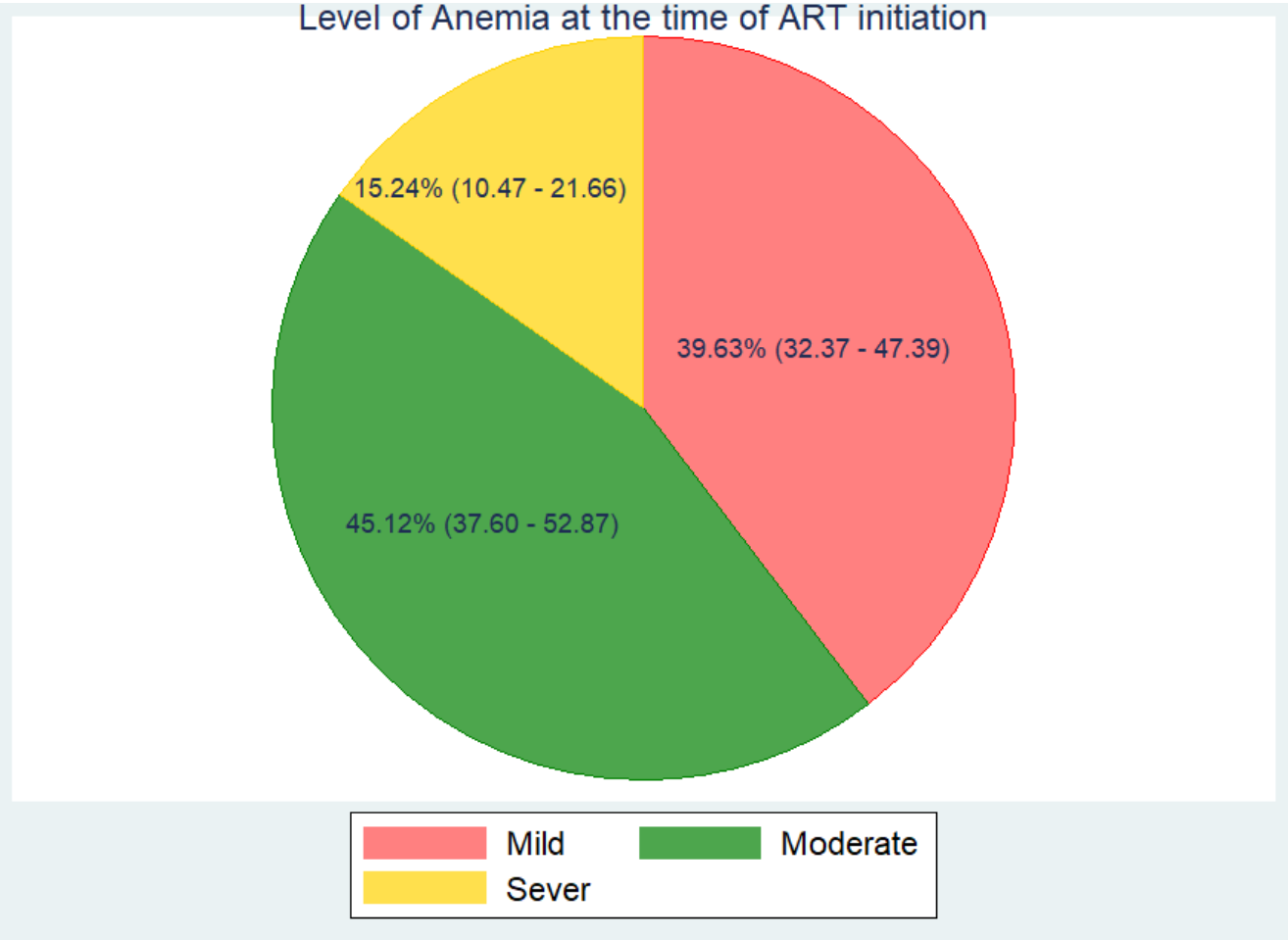


**Figure 1:** Level of anemia at the time of ART initiation among HIV-infected adults at Debre Markos comprehensive specialized hospital.

## STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No. | Recommendation | Page No. | Relevant text from manuscript |
|---|---|---|---|---|
| **Title and abstract** | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract | 2 | Line (27); An institution-based retrospective cross-sectional study was conducted among 472 clients' charts enrolled from 2014 to 2018 at Debre-Markos comprehensive specialized hospital. |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 | Lines (23 – 45); Abstract |
| **Introduction** | | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 3, 4 and 5 | Lines (54 – 94); Introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 | Lines (95 – 97); This study aimed to assess the prevalence of anemia and its associated factors at the time of ART initiation among HIV-infected patients at Debre Markos comprehensive specialized hospital. |
| **Methods** | | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 | Lines (100 – 102); An institution-based retrospective cross-sectional study was conducted at Debre Markos comprehensive specialized hospital from January 1, 2014, to December 31, 2018, among HIV-infected adults. |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 | Lines (199 – 105); Study design, setting and period |

| | | | | |
|---|---|---|---|---|
| Participants | 6 | (a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br><br>Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br><br>Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants | 5<br><br>and<br><br>6 | Lines (106 – 111); Source and study population<br><br>Lines (112 – 116); Inclusion and exclusion criteria (eligibility criteria)<br><br>Lines (126 – 128); By generating a random number on the computer, a total of 473 patient charts were selected through a simple random sampling technique, and data was collected from them. |
| | | (b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed<br><br>Case-control study—For matched studies, give matching criteria and the number of controls per case | N/A | This was a cross-sectional study |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 7 | Lines (131 – 140); Operational definition of variables |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 7 | Lines (141 – 152); Data collection tools and procedures |
| Bias | 9 | Describe any efforts to address potential sources of bias | 8 | Lines (154–159); A pretested data extraction tool was used to maintain data quality. Quality is also maintained by extracting data using trained nurses and close monitoring of the procedure by the supervisor. Data clerks were involved in the selection of patients' charts from the computer as well as from the chart room. Before returning the chart to |

| | | | | |
|---|---|---|---|---|
| | | | | the shelf, the completeness of the data extraction tool was checked and a necessary correction was made. |
| Study size | 10 | Explain how the study size was arrived at | 6 | Lines (117–124); The sample size was determined by using a formula to estimate single population proportion with the assumption of a 95% level of confidence, 25% proportion, and a 4% marginal error. |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8 | Lines (166–171); Bivariate analysis was executed for each variable, and those variables with p-value < 0.2 were entered into multivariate binary logistic regression to identify factors associated with anemia. An odds ratio with a 95% was computed, and variables having a p-value < 0.05 in the multivariate logistic regression were considered as statistically independent factors for anemia at ART initiation. |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding | 8 | Lines (160–171); Statistical Analysis |
| | | (b) Describe any methods used to examine subgroups and interactions | N/A | There were no subgroups. |
| | | (c) Explain how missing data were addressed | N/A | There was no missing data. |
| | | (d) Cohort study—If applicable, explain how loss to follow-up was addressed<br><br>Case-control study—If applicable, explain how matching of cases and controls was addressed<br><br>Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy | N/A | Lines (126–128); By generating a random number on the computer, a total of 473 patients charts were selected through a simple random sampling technique. |
| | | (e) Describe any sensitivity analyses | N/A | |
| **Results** | | | | |

| | | | | |
|---|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | N/A | This was a cross-sectional study with only one stage. |
| | | (b) Give reasons for non-participation at each stage | N/A | |
| | | (c) Consider use of a flow diagram | N/A | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10 and 11 | Line (197); Table 1: Prevalence of Anemia at the time of ART initiation stratified by socio demographic characteristics of HIV-infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014 to December 31, 2018 (n = 468).<br><br>Line (212); Table 2: Prevalence of anemia at the time of ART initiation stratified by clinical and immunologic related characteristics of HIV-infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014 to December 31, 2018 (n = 468). |
| | | (b) Indicate number of participants with missing data for each variable of interest | N/A | There was no missing data |
| | | (c) Cohort study—Summarise follow-up time (eg, average and total amount) | N/A | This was a cross-sectional study |
| Outcome data | 15* | Cohort study—Report numbers of outcome events or summary measures over time | N/A | This was a cross-sectional study |
| | | Case-control study—Report numbers in each exposure category, or summary measures of exposure | N/A | This was a cross-sectional study |
| | | Cross-sectional study—Report numbers of outcome events or summary measures | 12 | Lines (218–219); The overall prevalence of anemia at the time of ART initiation in this study was 35.04% (95% CI: 30.84–39.49). |

| | | | | |
|---|---|---|---|---|
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 12, 13 and 14 | Lines (218–219); The overall prevalence of anemia at the time of ART initiation in this study was 35.04% (95% CI: 30.84–39.49).<br>Lines (241); Table 3: Bivariate and multivariable logistic regression analysis of factors associated with anemia at the time of ART initiation among HIV infected adults at Debre-Markos comprehensive specialized hospital from January 1, 2014, to Dec 31, 2018 (n = 468). |
| | | (b) Report category boundaries when continuous variables were categorized | N/A | There was no continuous variable. |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | N/A | There was no estimates of relative risk. |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | N/A | There was no sub group and sensitivity analysis. |
| **Discussion** | | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 14–16 | Lines (247–307); Discussion |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 16 | Lines (303–307); Since the cause of anemia in HIV-infected adults is multi-factorial and the study was based on chart review, we noted that a few variables were missed. Furthermore, this study did not collect data on dietary habits, substance use status, menstrual habits in females, or the type of anemia. Additionally, this study was conducted among ART-naïve adults, which lacks comparison with anemia after ART initiation. |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 14–16 | Lines (247–307); Discussion |
| Generalizability | 21 | Discuss the generalizability (external validity) of the study results | 17 | Lines (308–316); The current study showed that a significant proportion of HIV-infected adults developed anemia at the time of ART |

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| | | | | initiation. Male sex, lack of formal education, a CD4+ T-cell count of less than 200 cells/mm3, under nutrition, and ambulatory or bedridden functional status were discovered to be independent predictors of anemia. Hence, giving special attention to patients not attending formal education, males, and late presenters is crucial to reduce anemia occurrence and its health impact. Finally, the findings of this study will provide baseline information to healthcare providers in order to select ART drugs according to and may provide additional insight into the development of new anemia preventive strategies. |
| **Other information** | | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 18 | This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.