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Prevalence of Anemia and Associated Factors among PHIVs Attendants Antiretroviral Therapy Clinics in Public Health Institutions in Dire Dawa Town, East Ethiopia

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Abstract
Background: Anemia is one of the most commonly observed hematological abnormalities and an independent prognostic marker of Human Immunodeficiency virus (HIV) disease progression in people living with HIV. However, there is limited evidence on the magnitude and its correlates among attendants of Antiretroviral Therapy (ART) in low-income countries including Ethiopia. **Objective**: The aim this study was to determine ***Prevalence of anemia and associated factors among PHIVs attendants Antiretroviral Therapy clinics in public health institutions in Dire Dawa Town, East Ethiopia*** **Methods:** An institution based cross - sectional study design was used from mid January to mid February 2014.The study participants were selected by using simple random sampling technique. A pre- tested structured questionnaire was used to collect data. Both bivariate and multivariable logistic regressions were used to identify associated factors. Hematological and immunological data were collected by using blood samples. Odds ratio along with 95% confidence interval was estimated to identify factors associated with anemia among the study population using a multivariable logistic regression. **Results**: The prevalence of anemia was 41.2%, 95% CI (36.7%, 45.9%). In multivariable logistic regression analysis, being female[AOR=1.95, 95% CI (1.22, 3.11)], use of different types of zidovudine (AZT) based Highly Active Antiretroviral Therapy (HAART) : 1c:AZT+3TC+ neverapine [AOR=2.56 , 95% CI (1.28,5.12)] , and 1d: AZT+3TC + Evaferenz [AOR=2.99 , 95% CI(1.27,7.03)] , overall ART category: zidovudine - based HAART [AOR=2.98, 95% CI (1.27,6.99)] , WHO's HIV clinical stage III [AOR=2.49, 95% CI: (1.24, 5.01)] and stage IV [AOR= 5.92, 95% CI (1.26, 27.8)] , and lower CD4 count [AOR=2.34, 95% CI (1.10,4.98)] were independently associated with anemia .**Conclusion**: Macrocytic anemia was common among patients taking Antiretroviral Therapy. The likelihood of developing anemia increases with disease progression associated decreased immunological state and use of zidovudin-based HAART. Therefore, those factors associated with anemia among PHIVs would be emphatically considered comprehensive care and treatment for PHIVs by including anemia treatment and prevention strategies by police makers in collaboration with others responsible bodies.
Keywords: Anemia, Antiretroviral Therapy, Ethiopia, Dire Dawa , Macrocytosis, Zidovudin.

Introduction

Hematological complications have been documented to be the second most common causes of morbidity and mortality among HIV sero-positive patients [1-3]. Anemia is one of the most commonly observed hematological abnormalities and independent prognostic marker of HIV disease progression in people living with HIV [4, 5].

Available literatures have indicated that there is wide variation in the prevalence of anemia among HIV/AIDS patients across the world including Ethiopia. Accordingly, the level of anemia ranges from 10.1% to 77.4% among patients using Highly Active Antiretroviral Therapy (HAART) [1, 6-15] in low- income countries. Few studies reported that macrocytosis or elevated Mean Cell Volume (MCV) is the common form of anemia which might be attributed to the effect of Zidovudine (AZT) regimen and deficiency of vitamin B12 or folate [16].

Anemia in HIV sero- positive people is also associated with an impaired erythropoiesis resulting from the release of inflammatory cytokines and decreased production of hematopoietic growth factors coupled with malabsorption and impaired recycling of iron , malignant bone marrow infiltration and infection with malaria, helminthiasis, and tuberculosis (TB) with the resultant hemolysis , gender, WHO's HIV staging, low CD4 count , and opportunistic infection [9, 11, 16-19].

In Ethiopia, there is Paucity information on the prevalence and correlates of anemia among HIV patients on ART. Few studies reported inconsistently that there is association between anemia and use of Zidovudin (AZT) based ART among attendants of ART clinics without clear understanding of the typology of anemia and of available limited treatment options or categories to plan for the appropriate treatment category for the improvement of Hgb level among cohort of HIV patients. In some of the cases zidovudine –based ART was

found to be protective against anemia than being a risk factor [9-11, 20]. Therefore, this study was conducted to determine the prevalence of anemia and associated factors among ART users in the study area.

Methods

Study setting and design

An Institutional based cross- sectional study design was conducted in Dire Dawa town from January 10 to February 10, 2014. In Dire Dawa town there are 12 health institutions that give ART services: one public Hospital, three private Hospitals and eight health centers.

Study participants and sampling procedure

The study participants were selected 425 HIV positive patients by using simple random sampling technique. A pre- tested structured questionnaire was used to collect data and at least had one visit in public health institutions in Dire Dawa town during the study period, was included in the study. However, HIV sero-positive pregnant women, who were on treatment for anemia in the last 3 months, patients on anti TB drugs and critically ill HIV positive patients were excluded. The sample size was computed using a single population proportion formula to estimate the prevalence of anemia with the following assumptions: prevalence of anemia among ART attendants to be 70% [9] , two sided alpha to be 1.96 , type I error and margin of error to be to be 5% respectively yielding a sample size of 354 including a 10% for non - response On the other hand , an Open epi software was used to determine the sample size for the difference between two population proportions to identify factors associated with outcome variable of interest with the following assumptions : CD4 cell count was considered as an exposure variable [9] to calculate the sample size with an estimated prevalence among the exposed (CD4 count between 350–500 cells/μL) to be 13% and among unexposed (CD4 count greater than 500 cells/μL) to be 4% , 85% of power, ratio of sample size among an unexposed/exposed: to be 1, risk/ prevalence ratio of 3.3 , risk/ prevalence difference of 9 to detect the odds ratio of 3.6 yielding a sample size of 425 including a 10% for non - response . To increase the power of the study the latter sample size was considered in this study.
Samples were selected from nine public health institutions (one hospital and eight health centers) rendering ART services in the town. The hospital is automatically selected while three health centers were selected at random. The sample size was proportionally allocated to each health facility based on the profile of their ART attendants. Systematic random sampling was used to satisfy sample size allocation for facility.

Measurements

The questionnaire was adapted from Ethiopian Demographic and Health Survey document and other related published literatures. It was initially prepared in English and translated to three local languages, “Afan Oromo, Amharic and Somali”, by fluent professionals of the respective languages by taking into account the major ethnic composition of the town and re-translated back to English to check for consistency. Data were then collected using an interviewer administered well structured and pretested questionnaire. Two diploma nurses who had training in ART and one senior laboratory technologist collected the data while one physician was recruited as a supervisor. Clinical and laboratory information including medical history, type of antiretroviral drugs consumption, and duration of antiretroviral therapy, hematologic (hemoglobin and red blood cell (RBC) morphology) and immunologic (CD4 cell count) parameters were determined for all of the study participants. Weight and height was measured by using measurement scale to assess body mass index (BMI) of the study participants. Some clinical information like the clinical staging of HIV and the regimen category that patient is taking was retrieved from the patients’ record.

Moreover, a volume of 3–5 ml venous blood in EDTA vacutainer tube was collected by data collectors from each participant and was sent to the laboratory. The blood sample was used for complete blood count, CD4 count determination and RBC morphology. Hematological parameters: hemoglobin (Hgb), mean corpuscular volume(MCV), mean cell hemoglobin(MCH), mean cell hemoglobin concentration(MCHC) and red cell distribution width(RDW) were determined using the automated blood analyzer CELL DYNE 1800 (Abott Laboratories Diagnostics Division) and CD4 count was measured using BD FACS count machine.

The standard operating procedures (SOPs) were followed during specimen collection and all other laboratory procedures. All reagents used were checked for their expiry date and prepared according to the manufacturer’s instructions to ensure the quality of laboratory data.

In this study, anemia was understood as hemoglobin concentration (Hgb) less than 13 g/dl for males and less than 12 g/dl for females to indicate whether the patient is anemic or not based on WHO’s definition [21]. Age and sex of respondents , WHO’s clinical staging, Type of HAART used and HAART category , duration of ART use in months , BMI , CD4 cell count , and presence or absence of opportunistic infections were independent variables of the study .

Statistical analysis

Data were first checked for completeness manually and entered onto EpiData version 3.11 for cleaning, and exported to SPSS version 16.0 computer software. Data were analyzed by using SPSS version 16.0 statistical packages and presented by frequencies and percentages for categorical variables and means and standard deviations for numerical variables. Bivariate analysis was conducted primarily to check the variables which had an association with the dependent variable individually. Variables associated with the dependent variables at p-value <0.05 were then entered in to multiple logistic regression for controlling the possible effect of confounders. Adjusted Odds ratio along with 95% CI was estimated to identify factors associated with anemia. The results were presented using tables, figures and narratives.

Ethical clearance

The study was cleared from Haramaya University Institutional Research Ethics Review Committee. Then written consent was secured from health institutions to got permission. Verbal informed consent for participation was obtained from each study participants and the collected data were stored in a file, without the name of study participant and password protection of soft data and use of key and lock for hard copy data was employed to guarantee confidentiality.

Results

Socio - demographic characteristics

A total of 425 ART clients were included in this study making a response rate of 100%. Two hundred seventy five (64.3%) of the participants were females and 177(41.6%) were Amhara by ethnicity. One hundred ninety six (46.1%) of the participants were married and one hundred sixty nine (39.8%) were unemployed by occupation. The mean age of the clients was 34 years with (SD±9) years. Two hundred six (48.9%) of the clients were found in the age range between 31 and 45. Among the respondents, 326 (76.7%) were orthodox Christian by religion followed by Muslims which constituted about 72 (16.9%). Eighty five (20%) of the participants had no formal education while 9 (2.1%) had education above 12 grades (Table 1).

Table 1 : Socio-demographic characteristics of ART followers among public health institutions in DireDawa town, Eastern Ethiopia, during February, 2014 (**n = 425)**

Variables		**Number**	**Percentage** (%)
Age (years)	15-30	168	39.5
	31-45	206	48.5
	46-65	51	12.0
Sex	Male	150	35.3
	Female	275	64.7
Marital status	Single	75	17.6
	Married	196	46.2
	Divorced	39	9.2
	Widowed	57	13.4
	Separated	58	13.6
Religion	Orthodox	326	76.7
	Protestant	25	5.9
	Catholic	2	0.5
	Muslim	72	16.9
Ethnicity	Oromo	103	24.2
	Amhara	177	41.6
	Somali	34	8.0
	Tigray	65	15.4
	Harari	39	9.2
	Gurage	7	1.6
Educational status	No formal E	85	20.0
	Primary	240	56.5
	Secondary	91	21.4
	College	9	2.1
Occupational	Unemployed	169	39.8
	student	16	3.8
	Housewife	56	13.2
	House servant	9	2.1
	Daily laborer	74	17.4
	Merchant	27	6.4
	Government employee	45	10.5
	Private employee	29	6.8

Clinical and immunologic characteristics of ART followers

From a total of 425 study participants, 303(71.3%) and 58(13.6%) were in stage one and two of WHO clinical stage respectively and 194(45.6%) are taking zidovudine containing ART regimen (1c or 1d). Consequently, majority of the respondents had CD4 cell count greater than 500cell/µL 172(40.5%) while only 3(.7%) of the respondents had less than 50 cell/µL (Table 2).

Table 2: Clinical and immunological characteristics of ART followers among public health institutions in DireDawa town, Eastern Ethiopia, during February, 2014 **(n = 425)**

Variables		**Number**	**Percentage** (%)
WHO 's clinical staging	Stage 1	303	71.3
	Stage 2	58	13.6
	Stage 3	44	10.4
	Stage 4	20	4.7
Type of ART	1c	146	34.3
	1d	48	11.3
	1e	169	39.8
	1f	62	14.6
CD4 cell	>500	172	40.4
	350-500	101	23.8
	200-349	105	24.7
	100-199	34	8.0
	50-99	10	2.4
	<50	3	0.7
BMI	<18.49	101	23.8
	18.5-24.99	236	55.5
	>25	88	20.7
ART duration in months	3-20	190	44.7
	20-36	94	22.1
	36-60	71	16.7
	>60	70	16.5
OI	Yes	26	6.1
	No	399	93.9
Regimen	AZT user	194	45.6
	Non AZT user	231	54.4

Prevalence of anemia among users of ART

Participant's Hgb level was used to determine the prevalence of anemia. Out of the total 425 respondents, the overall prevalence of anemia was 175 (41.2%). From the total anaemic individuals; 15(3.5%) and 160(37.6%) had sever and mild to moderate anemia, respectively. The type of anemia was determined using red cell indices values and macrocytosis (MCV>100 fl) was found to be more common, 25.6%. The mean (±SD) hemoglobin level of the study participants was 12.52(±1.93) with slight difference between male and female participants (13.34 (±2.08) among male and 12.08 (±1.68) among females with the overall range of (18.8-3.9). The mean(±SD) MCV was 105.21(±12.2) (Figure1).

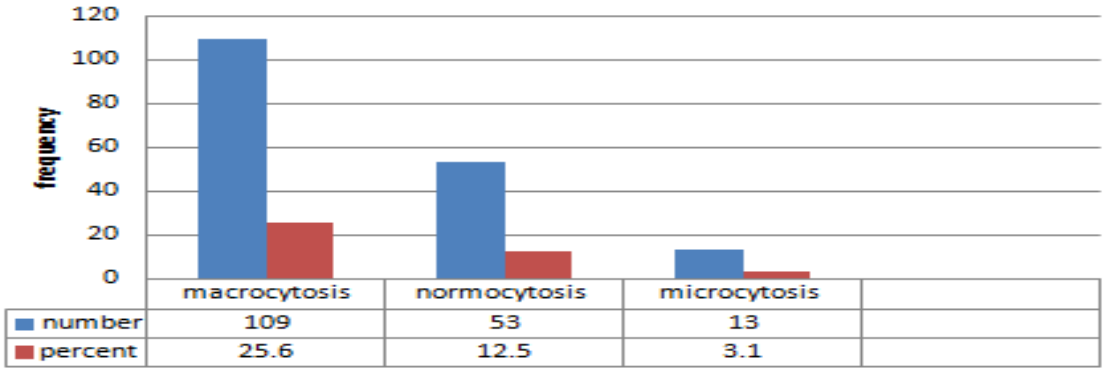

Figure3: Types of anemia among ART followers among public health institutions in Dire Dawa town, Eastern Ethiopia, during February 2014.

Factors Associated with Anemia among ART Clients

In bivariate analyses, age, sex, HAART category and duration of ART use, opportunistic infection, WHO's HIV/AIDS clinical staging, BMI, and CD4 Cell count were associated with anemia at 0.2 confidence level. In multivariable logistic regression analysis, the odds of anemia were nearly twice higher among female participants [AOR=1.95, 95% CI: (1.22, 3.11)] compared with their counterparts, The odds of anaemia were nearly 3 times [AOR=2.99, 95% CI: (1.27, 6.99)] higher among users of zidovudine containing ART (1c = AZT+3TC+neverapine) and id = AZT+3TC +evaferenz [AOR=2.56, 95% CI: (1.27, 6.99)] when compared with stavudine (TDF) based (1e and 1f) ART users , ART category; zidovudine based HAART [AOR=2.98, 95% CI (1.27,6.99)], WHO's HIV clinical staging ; stage III [AOR=2.49, 95% CI: (1.24, 5.01)] and stage IV [AOR= 5.92, 95% CI (1.26, 27.8)] and CD4 cell count of less than 200 [AOR=2.34, 95% CI (1.10,4.98)] were significantly associated with anemia (Table 3).

Table 3: Factors associated with anemia among ART users from public health institution of Dire Dawa town, Eastern Ethiopia, during February, 2014. (**n=425**).

Variables		Anemia		COR (95%CI)	AOR and 95%CI
		Yes	No		
Age (Years)	15-30	73 (17.2%)	95 (22.4%)	1.0	1.0
	31-45	76 (17.9%)	130 (30.6%)	.761(.502, 1.153)	.895(.563, 1.42)
	46-65	26 (6.1%)	25 (5.9%)	1.353(.72,2.536)	1.53(.769,3.04)
Sex	Male	52(12.2%)	98 (23.1%)	1.0	1.0
	Female	123 (28.9%)	152 (35.8%)	1.52 (1.01, 2.30)	1.95(1.22, 3.11)*
Type of ART	1c	73(17.2%)	73 (17.2%)	2.64 (1.38,5.05)	2.56(1.28,5.12)*
	1d	26(6.1%)	22(5.2%)	3.13(1.41,6.94)	2.99(1.27,7.03)*
	1e	59(13.9%)	110(25.9%)	1.42(.74,2.69)	1.11(.55,2.23)
	1f	17(4)	45(10.6%)	1.0	1.0
OI	No	156(36.7%)	243(57.2%)	1	1
	Yes	19(4.5 %)	7(1.6%)	4.23(1.74,10.29)	1.42(.39, 5.11)
WHO's clinical staging	Stage 1	109(25.6%)	194(45.6%)	1.0	1.0
	Stage 2	23(5.4%)	35(8.2%)	1.17 (.66, 2.08)	.93(0.49, 1.76)
	Stage 3	27(6.4%)	17(4%)	2.83(1.48, 5.42)	2.49(1.24, 5.01)*
	Stage 4	16(3.8%)	4 (.9%)	7.12(2.32, 21.83)	5.92(1.26, 27.8)*
ART duration in Months	3-20	75(17.6)	115(27.1)	1	1
	20-36	39(9.2%)	55(12.9%)	1.09(.66,1.80)	1.34(.77,2.46)
	36-60	30(17.1%)	41(16.4%)	1.12 (.65,1.96)	1.08(.57, 2.06)
	>60	31(7.3%)	39(9.2%)	1.22(.70,2.121)	1.62(.83, 3.18)
BMI	<18.49	51(12%)	50(11.8%)	1.88(1.04,3.37)	1.68(.87,3.23)
	18.5-24.99	93(21.9%)	143(33.6%)	1.19 (.72,1.99)	1.10(.62,1.93)
	>25	31(7.3%)	57(13.4%)	1	1
CD4 cell count	<200	27(6.4%)	21(4.9%)	2.18 (1.14,4.18)	2.34(1.10,4.98)*
	200-500	85 (20%)	122 (28.7%)	1.18(.78,1.79)	1.27(.79,2.03)
	>500	63(36%)	107(42.8%)	1	1
ART category	AZT user. Non AZT user.	99(23.3) 76(17.9)	95(22.4) 155(36.5)	2.12(1.43,3.14) 1	2.98(1.27,6.99)* 1

* = p ≤ 0.05 , AOR= Adjusted Odds Ratio , BMI = Body Mass Index , COR = Crude Odds Ratio , OI= Opportunistic Infection , 1c= AZT+3TC+neverapine , 1d = AZT+3TC + evaferenz , 1e = TDF+3TC+evaferenz , 1f= TDF+3TC+neverapine

Discussion

The prevalence of anemia is less than half among the study population in which macrocytic anemia is more common. In multivariable analysis, sex of the participant, use of different types of zidovudine (AZT) containing HAART, ART category, CD4 cell count, and WHO clinical staging remained independent factors associated with of anemia.

In this study prevalence of anemia (41.2%) is less when compared with results of studies from different regions including Ethiopia [6, 8, 9]. This might be attributed to difference in the definition of anemia used; anemia was defined as hemoglobin level less than 12g/dl for both male and female participants in these studies while it was understood as Hgb concentration less than 13 g/dl for males and less than 12 g/dl for females in the present study. However, our finding is almost all similar with other studies conducted previously [1, 7]. This similarity could be due to similarity of the study participants in both cases and of operational definition used to define anemia .

Gender difference in terms of proportion and severity of anemia was observed in this study. Females were found to be more likely anemic and develop severe anemia compared with males study participants. The odds of anemia were nearly twice among female participants compared with their counterparts. The level of mild to moderate anemia (37.6%) in this study was lower when compared with other studies while the observed degree of severity (3.5%) is comparable with some previous studies [6, 9]. This could be due to difference in the definition of anemia in the respective studies.

In this study macrocytic anemia (MCV>100 fl) is more common .The odds having anemia among patients who were taking a zidovudine based - HAART regimen such as AZT, 3TC and neverapine and AZT, 3TC and evaferenz is nearly three times higher compared with patients who were taking stavudine based regimen. Other studies have also indicated that macrocytosis is typically associated with cyanocobalamine or folate deficiency [16] and use of zidovudine (AZT) based HAART than stavudine –based regimen [8, 11, 16, 17, 20, 22, 23]. The commonness of macrocytic anemia among HAART clients might indicate that anemia in ART users is more likely attributed to use of AZT based HAART due to the myelosuppressive effect of zidovudine causing morbidity and increasing treatment cost than anemia in the general population which is often attributed to iron deficiency . Moreover, lack of significant association between body mass index of the study participants and anemia could also suggest that iron deficiency anemia is less likely in this study population.

However, according to reports from different setting of Ethiopia, there are contradicting evidence with this regard that not all AZT combined ART do not increase the risk of anemia . One study indicated that the use of both evaferenz and nevirapine combined AZT can end up in anemia and other complications [20] while another study reported that only evaferenz combined AZT was independently positively associated with greater risk developing anemia than patients taking neverapine combined AZT which has got protective effect [10]. It was also noted that use of HAART for 12 months or more was associated with a protective effect against development of anemia [24] .It seems that the selection of appropriate regimen for the patients is still difficult and requires further attention by initiating large scale study to make more evidence based decision on the selection of conducive treatment approach.

It was found that participants in WHO's stage III and IV of AIDS staging were four times more likely to develop anemia than asymptomatic patients. In agreement with the finding of this study ,in other similar previous studies, it was identified that although anemia occurs at all stages of HIV/AIDS , its prevalence and severity tends to rise with disease progression that is stage III and IV [17, 25, 26]. This could be explained by the fact that viral load is so high during late stage of the disease impaiing the process of erythropoiesis resulting in decline in the level of Hgb during this period of time.

Anemia was also associated with CD4 lymphocyte count < 200 cells/µL compared with a CD4 lymphocyte count > 500 cells/µL which shows that the risk of anemia is higher with more advanced stage of HIV infection. This finding confirmed the findings of prior studies in which low CD4 count has been associated with the occurrence of anemia among users of ART regardless of other factors like WHO's clinical staging [5]. This could also be explained by deterioration in the formation of Hgb due to disrupted erythropoiesis resulting from the release of inflammatory cytokines and decreased production of hematopoietic growth factors coupled with malabsorption and impaired recycling of iron substance secondary to HIV/AIDS.

On this research by considering the main strength of this research lies on its laboratory based study which were generalizable to this populations and its limitations. One is that the study was not able to identify anemia due to deficiency of ferreting and iron binding capacity, micronutrient deficiency like folate, vitamin B12 (cobalamine), and bone marrow depression. The other is that like any measurement Hgb determination might be affected due to error inherent in Hgb determining machine and the measurer although all possible cautions have been taken into account before data collection to overcome such problems.

Conclusions

In this study microcytic anemia is more common than normocytosis and microcytosis indicating the effect of

zidovudine rather than iron deficiency anemia among the study population. Female gender, use of efaferenz and nevarapine combined AZT, late stage of HIV/AIDS, and lower CD4 count were independently impacted the development of anemia.

Health professionals need to routinely investigate and treat hematological abnormalities before and after initiation of ART to improve the quality of life for HIV patients.

Policy designers and planners should also work on the identification of strategies that reduce morbidity and mortality from anemia related to ART use and scale –up research in the area of HAART administration for HIV patients and further causes of anemia among such cohort of population.

Therefore, those factors associated with anemia among PHIVs would be emphatically considered comprehensive care and treatment for PHIVs by including anemia treatment and prevention strategies by police makers in collaboration with others responsible bodies.

It would be better to give great emphasis on plan to reduce and treat microcytic anemia among these population.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DG participated in the design of the study, performed the data collection, performed the statistical analysis and served as the lead author of the manuscript. BS participated in the design of the study and contributed to finalization of the manuscript. GE and DBD participated in the design of the study, statistical analysis and in finalizing the manuscript. All authors have read and approved the final manuscript.

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