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Anaemia Phenotypes in Highly Active Antiretroviral Treatment Defaulting Adults at the Comprehensive Care Clinic at the Siaya County Teaching and Referral Hospital

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Abstract

Background: Anaemia is a major cause of morbidity and mortality among people living with human immunodeficiency virus (HIV) infection. While adherence to antiretroviral treatment (ART) reduces the incidence of anaemia, non-adherence (defaulting) to ART is associated with a number of haematologic derangements including anaemia. However, the mechanisms and predictors of anaemia have not been examined among ART defaulters in western Kenya. This study, therefore, aimed at evaluating the anaemia prevalence and classification of the anaemia based on haemoglobin (Hb) concentrations and erythrocyte morphology as well as suppression of erythropoiesis in ART-defaulting adults at a comprehensive care clinic in western Kenya.

Methods: This cross-sectional study was conducted among HIV-infected adults (≥18 years) at Siaya County Teaching and Referral Hospital Comprehensive Care Clinic from January to July, 2018. All blood samples were collected between 8.00-10.00 hours and automated haematology analyzer used for assessing full-haemograms. CD4 counts were enumerated using the Swelab Alfa machine. Reticulocyte counts were determined using new methylene blue staining.

Result: The overall rates of anaemia was higher in the ART-defaulters (87.9%) compared to the ART-adherent (53.7%) and -naive (55.5%) patients ($P < 0.0001$). The rates of severe and moderate anaemia were higher in ART-defaulters (40.4% and 28.3%) relative to ART-adherent (8.5% and 15.9%) and naive (8.7% and 20.3%) individuals, respectively ($P < 0.0001$). Morphologic phenotypes of the anaemia were predominantly microcytic and hypochromic anaemia dominated in the ART-defaulters (54.5% and 64.7%) compared to the ART-adherents (8.5% and 32.9%) and naive (8.7% and 17.4%) patients ($P < 0.0001$), respectively. Binary logistic regressions indicated that anaemia was associated with under-nutrition (OR, 5.634; 95% CI, 1.844-17.213; $P = 0.002$); microcytic (OR, 37.771; 95% CI, 8.604-160.581; $P < 0.0001$) and hypochromic (OR, 42.982; 95% CI, 8.622-214.267; $P < 0.0001$) cells; and severe immune suppression (OR, 168.476; 95% CI, 9.872-2875.17; $P < 0.0001$) in the ART-defaulters.

Conclusion: These results suggest that targeted interventions involving improving adherence and nutritional management can lower the burden of anaemia among ART-defaulters.

Keywords: Anaemia; Adults; Anti-Retroviral Treatment; Defaulters

List of abbreviations: 3TC: Lamivudine; AIDS: Acquired Immunodeficiency Syndrome; ART: Antiretroviral Treatment; AZT: Zidovudine; BMI: Body Mass Index; EFV: Efavirenz; Hb: Haemoglobin; HIV: Human Immunodeficiency Virus; MCH: Mean Cell Hemoglobin; MCHC: Mean Cell Haemoglobin Concentration; MCV: Mean Cell Volume; NVP: Nevirapine; RBC: Red Blood Cell; RDW: Red Cell Distribution Width; TDF: Tenofovir Disoproxil Fumarate

Introduction

Anaemia is the reduction in the erythrocytic mass and haemoglobin (Hb) levels (Hb<11.0g/dl in non-pregnant women and Hb<13.0g/dl in adolescents and adult males older than fifteen years [1]. Approximately, 24.8% of the world population suffer from anaemia of whom 30.2% are non-pregnant women and 12.7% are men [2]. Most epidemiologic and nutritional studies in Kenya reported on anaemia burden in pregnant women and children [3,4]. Although there is paucity of data on the burden of anaemia in the adult Kenyan population, previous surveys over eight years ago indicated an overall prevalence of 21.9% in non-pregnant women (15 - 49 years) and 9.2% in men (15 - 54 years) being higher in rural relative to urban communities [5]. However, the current burden of anaemia in the general Kenyan population is not known.

An estimated 4.9% of the Kenyan population is living with the human immunodeficiency virus (HIV) [6]. Anaemia is a major cause of morbidity and mortality among individuals infected by HIV [7]. The introduction and widespread use of highly active antiretroviral therapy (HAART) led to a significant reduction in HIV-associated disease burden [8-10]. However, the rates of anaemia have remained high especially in the HIV infected individuals in sub-Saharan Africa [11,12]. Evidence for these observations include cross-sectional studies indicating low rates (~11%) of anaemia among ART adherents compared to treatment-naive (~42%) [13]. The anaemia burden is further compounded by ART defaulting which leads to high rates of anaemia-related hospitalizations [14].

Despite epidemiologic studies showing that anaemia remains a health sequelae in individuals on ART and treatment naive, information regarding the levels and morphologic types of anaemia is scanty, especially in rural sub-Saharan Africa that host most of the HIV infected population [15]. Among, the few studies to examine levels of anaemia [16] reported that levels of anaemia occurring in treatment-naive individuals were mainly mild and moderate anaemia. In addition, erythrocytic morphologic studies in treatment-naive patients from Uganda indicated that normocytic normochromic anaemia was common in the anaemia cases [17]. However, previous studies in Cameroon showed that microcytic hypochromic anaemia dominated in the treatment-naive individuals as macrocytic anaemia was frequent in the ART adherent individuals [18]. Altogether, these studies suggest multifactorial aetiologies of anaemia occurring in HIV infection.

The mechanisms underlying the pathogenesis of anaemia during HIV infection are complex and multifactorial. Viral cytopathic effects and bone marrow suppression, macronutrient and micronutrient deficiencies as well as antiretroviral (ARV) drug-induced cytotoxicity and erythroid suppression are implicated in the development of HIV-associated anaemia [19]. Furthermore, the chronic nature of HIV infection is associated with production of viral proteins and opportunistic infections that stimulate the release of bone marrow suppressive factors resulting in anaemia [20]. As such, this cross-sectional study investigated the prevalence, levels and morphologic types of anaemia including haematologic and nutritional factors associated with anaemia in ART-defaulting adults attending the Siaya County Teaching and Referral Hospital, western Kenya.

Materials and Methods

Study area and population

This hospital-based cross-sectional study was conducted from June to September 2017 among 250 HIV infected adults (>18 years) attending the comprehensive care clinic (CCC) at Siaya County Teaching and Referral hospital, Western Kenya. The sample size was calculated based on the proportionality distribution formula [21]. During the sampling period, 986 patients comprising of defaulters (n = 391), -adherents (n = 323) and -naive (n = 272) individuals were attending the CCC clinic for treatment. Approximately, 25% of the individuals in each clinical group were thus recruited into the study.

The study participants were stratified based on ART-use status into: 1) ART-defaulters (non- adherents); 2) ART-adherents; and 3) ART-naive groups. ART non-adherents were individuals testing HIV-positive and reporting defaulting treatment at least on one occasion since initiating therapy. ART adherents were persons testing HIV-positive and reporting full compliance to treatment since initiating therapy. ART-naive subjects had not been initiated on ART. In addition, eligibility criteria were limited to people who at the time of recruitment were living within Siaya county, Western Kenya, and provided written informed consent. Individuals testing HIV negative and those testing HIV-positive but presenting with clinical manifestations of active HIV disease, acute or chronic illnesses or other co-existing medical conditions were excluded from the study. All the patients had CD4+ T cell counts 250 cells/μL at the time of initiating ART. The ART non-adherents and ART-adherents study participants were on first-line ART (NRTIs and NNRTIs) consisting of TDF or AZT + 3TC + NVP or EFV [22]. Demographic and ART use information including anthropometric (height and weight) measures were obtained from patients records upon enrolment. The height and weight measurements were then used in calculating the body mass index (BMI, kg/m^2). BMI was subsequently used in defining underweight (BMI18.5 kg/m^2), normal weight (BMI≥18.525.0 kg/m^2) and overweight (BMI≥25.0 kg/m^2) as previously described [23].

Laboratory procedures

About 2 milliliters of venous blood was collected into EDTA vacutainer tubes (Becton-Dickinson™ Company, New Jersey, USA), and used for performing the hematological investigations. Complete blood counts including erythrocytic measures (haemoglobin, haematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, red blood cell count and red cell distribution width were determined using an automated blood analyzer (Swelab Alfa, Stockholm, Sweden). CD4 T cells were enumerated using the FACS count (Becton-Dickinson™, Company, New Jersey, USA), and used for immunologic staging into: severe immunosuppression (CD4 T cells 200/μl); moderate immunosuppression (CD4 T cells ≥200500/μl) and normal (CD4 T≥500/μl cells) [24]. Leishman stained thin peripheral blood films were used for microscopic examination of red blood cell morphology and staining characteristics. Reticulocyte counts were determined using microscopic examination of new methylene blue-stained peripheral blood films [25]. The reticulocyte indices, reticulocyte production index (RPI) and absolute reticulocyte number (ARN) were calculated as previously described [26,27]. The RPI values were used for defining erythropoietic suppression (RPI 2.0), normal erythropoietic response (RPI 2.0-3.0), and erythroblast hyper proliferation (RPI>3.0) [27,28].

Statistical analysis

Data analysis was conducted using the IBM® SPSS software version 21.0 (SPSS Inc. Chicago, USA). Comparisons in the distribution of proportions for categorical variables (sex, anaemia levels and status, underweight, duration of ART, ART regimen, and erythropoietic suppression) amongst the study groups were performed using the Pearson's chi-square test. Continuous variables (age, erythrocytic measures, CD4 T cells, height, weight, and BMI) were compared across the study groups using the ANOVA test followed by post-hoc Tukey's correction for multiple comparisons. Mean ART duration was compared between treatment-defaulters and –adherents using the independent student t-test. Binary logistic regressions were performed in order to identify the factors associated with anaemia independently within the ART-naive, -adhering, and -defaulting individuals. All tests were two-tailed and *P* 0.05 was considered statistically significant.

Ethical considerations

This study was conducted in accordance with the revised Helsinki guidelines for human subject's research [29]. Ethical approval was obtained from the Masinde Muliro University of Science and Technology Institutional Ethics Review Committee and permission conduct the study at the comprehensive care clinic from the Siaya County Referral Hospital management. Informed written consent was obtained from the study participants prior to enrolment into the study. Information collected as part of this study was kept confidential unless required for patient care, with only access limited to the investigators.

Results

Demographic and treatment profiles

The demographic characteristics of the study participants are summarized in Table 1. A total of 250 HIV-positive adults (ART-defaulters, n = 99; -adherents, n = 82; and -naive, n = 69) were recruited into the study. The mean age (years) was similar across the study groups (*P* = 0.080). Gender distribution was also similar in the study groups (*P* = 0.287).

Characteristic	ART naive, n=69	ART adherents, n=82	ART non-adherents, n=99	*P*
Age, years	38.1 (1.5)	38.9 (1.3)	35.1 (1.2)	0.080
Gender, n (%)				
Female	46 (66.7)	48 (58.5)	54 (54.5)	0.287
Male	23 (33.3)	34 (41.5)	45 (45.5)	
Weight, kg	48.4 (0.1)	49.6 (0.8)	43.4 (0.8)a	**0.0001**
Height, cm	1.6 (0.0)	1.6 (0.0)	1.6 (0.0)	0.078
BMI, kg/m²	19.1 (0.4)	19.7 (0.3)	17.6 (0.3)a	**0.0001**
BMI, kg/m², categories, n (%)				
18.5		27 (32.9)	64 (64.6)	-
≥25.0	31 (44.9)	4 (4.9)	2 (2.0)	
≥18.525.0	5 (7.2)	51 (62.2)	33 (33.3)	
ARV regimen, n (%)	33 (47.8)			
TDF, 3TC, AZT	-	1 (1.2)	3 (3.0)	-
TDF, 3TC, EFV	-	20 (24.4)	34 (34.3)	
TDF, 3TC, NEV	-	61 (74.4)	62 (62.6)	
ART duration, months	-	77.7 (3.7)	66.9 (4.0)	**0.027**
ART duration, months, n (%)				
1-12	-	3 (3.7)	3 (3.0)	-
>12≤36	-	11 (13.4)	26 (26.3)	
>36	-	68 (82.9)	70 (70.7)	
CD3 cells/mm³	1207 (66)	1207 (67)	1089 (53)	0.224
CD4 cells/mm³	359 (310)	418 (20)	227 (14)a	**0.0001**
Immunologic stage, n (%)				
≥500 cells/mm³	23 (33.3)	27 (32.9)	7 (7.1)	**0.0001**
250-499 cells/mm³	28 (40.6)	39 (47.6)	24 (24.2)	
250 cells/mm³	18 (26.1)	16 (19.5)	68 (68.7)	

Data are presented as means (±standard error) or number (n) and proportions (%) of subjects. ART, antiretroviral therapy. BMI, body mass index. BMI18.5 kg/m², underweight; BMI≥18.525.0 kg/m², normal weight; BMI≥25.0 kg/m², over weight. TDF, tenofovir; 3TC, lamivudine; AZT, zidovudine; EFV, efavirenz; NVP, nevirapine. Age, weight, height and BMI were compared across-group using the ANOVA and post-hoc Tukey's tests. Differences in gender amongst the study groups were compared using the Pearson's chi-square. ART duration was compared between ART-non-adherents *vs*. -naive using the independent samples students' t-test. [a]*P* 0.05 *vs*. ART-adherents and ART-naive, respectively. Values in bold indicate significant *P*-values

Table 1: Demographic, treatment history and clinical characteristics of the study participants

The body weight (kg) was different amongst the groups (*P* 0.0001) and was lower in ART-defaulters versus the -adherents (*P* 0.05) and -naive (*P* 0.05) individuals. The body height (m) was not different in the study groups (*P*=0.078). Consistent with the weight, the BMI differed across the groups (P 0.0001) and was lower in the -defaulters relative to the -adherents (*P* 0.05) and -naive (*P* 0.05) subjects. Moreover, evaluation of underweight status (BMI18.5 kg/m^2) revealed that about two-thirds (~65%) of the ART-defaulters were underweight in opposition to-adherents (32.9%) and -naive (44.9%) study groups.

Evaluation of ART regimen and duration indicated that ~63% of ART-defaulters and 74% of -adherents were on first-line TDF, 3TC and NEV regimen. In addition, ~34% of the defaulters and ~24% of the -adherents were on TDF, 3TC and EFV regimen. Moreover, 3.0% of the defaulters and 1.2% of the adherents were on TDF, 3TC and AZT regimen. The ART-defaulters had been on treatment for a shorter period compared to adherent individuals (*P* = 0.027) with most of the -defaulters (~71%), and -adherents (83%) having been on ART for at least 36 months.

Erythrocytic measures, levels and morphologic types of anaemia

Erythrocytic measures of anaemia are presented in Table 2. Anaemia levels and morphologic types are presented in Figure 1. RBC count (×10^6/μl), haematocrit (%), Hb (g/dl), MCV (fl), MCHC (g/dl), RDW (%), reticulocyte counts (%), ARN (×10^9/μl), and RPI varied significantly across-groups (*P* 0.0001 for each erythrocytic measures). Post-hoc analyses showed that the RBC count, HCT, and Hb, MCV, and MCHC were lower in the-defaulters compared to–adherents (*P* 0.05) and naive (*P* 0.05) subjects, respectively. Post-hoc analyses of the ARN and RDW showed lower levels in the adherents compared to the -naive (*P* 0.05) subjects. However, the MCH levels were similar am ongst the study groups (*P* = 0.225). Consistent with lower RBC counts, reticulocytes, ARN and RPI were lower in the defaulters relative to the ART-naive individuals *(P* 0.05, respectively). The prevalence of erythropoietic suppression (RPI 2.0) was higher in the defaulters (96.0%) relative to the adherents (85.4%) and the naive (52.2%) individuals. Consistent with the lower measures of anaemia (RBC count, haematocrit and Hb) the overall rate of anaemia was higher in the defaulters (87.9%) relative to the adherent (53.7%) and naive (55.5%) groups (*P* 0.0001; Figure 1a). Likewise, rates of severe and moderate were higher in defaulters (40.4% and 28.3%) in comparison to the adherent (8.5% and 15.9%) and naive (8.7% and 20.3%) individuals, respectively (*P* 0.0001; Figure 1a).

Measure	ART naive, n=69	ART adherents, n=82	ART defaulters, n=99	*P*
RBC×10^6/μl	4.2 (0.11)	3.9 (0.11)	3.0 (0.1)[a]	0.0001
Haematocrit, %	38.4 (1.4)	35.6 (1.3)	24.9 (1.3)[a]	0.0001
Hb, g/dl	11.6 (0.3)	11.7 (0.30)	8.9 (0.3)[a]	0.0001
MCV, fl	83.1(1.5)	93.7 (1.7)[b]	71.6 (2.0)[a]	0.0001
MCH, pg	25.9 (0.4)	25.6 (0.4)	24.9 (0.3)	**0.225**
MCHC, g/dl	35.0 (0.3)	35.1 (0.3)	33.8 (0.4)[a]	0.007
RDW, %	15.4 (0.6)	13.7 (0.2)[b]	15.1 (0.2)[c]	**0.0001**
Reticulocytes, %	2.6 (0.2)	1.5 (0.1)[b]	1.7 (1.5)[b]	0.0001
ARN×109/μl	102.0 (4.8)	61.6 (4.2)[b]	52.9 (4.5)[c]	0.0001
RPI	2.0 (0.1)	1.2 (0.1)[b]	0.7 (0.1)[a]	0.0001
RPI categories, n (%)				
2.0	36 (52.2)	70 (85.4)	95 (96.0)	-
>3.0	7 (10.1)	1 (1.2)	1 (1.0)	
2.0-3.0	26 (37.7)	11 (13.4)	3 (3.0)	

Data are presented as means (±standard error) or number (n) and proportions (%) of subjects. ART, antiretroviral treatment. RBC, red blood cell; Hb, hemoglobin; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width. RPI, reticulocytes production index; RPI 2.0, suppression of erythropoiesis; RPI>3.0, reticulocytosis; RPI≥2.0≤3.0, normal erythropoietic response; ARN, absolute reticulocyte number. Differences in continuous variables across study groups were compared using the ANOVA tests followed by post-hoc Tukey's corrections for multiple comparisons. [a]*P* 0.05 *vs.* ART adherents and ART naive; [b]P 0.05 *vs.* ART naive; [c]P 0.05 *vs.* ART adherents. Values in bold are significant *P*-values

Table 2: Erythrocytic measures of anaemia

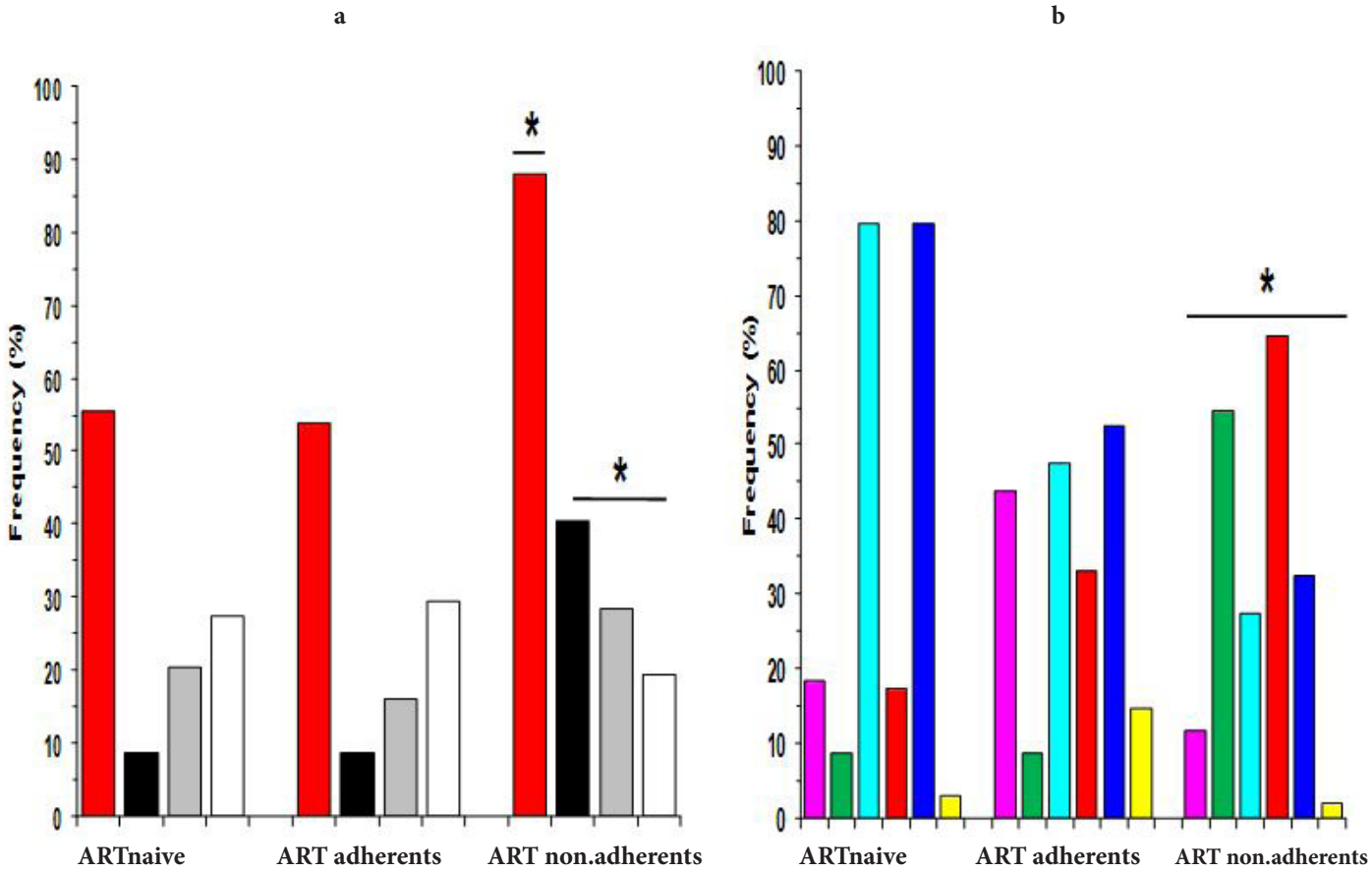

FIGURE 1: The anaemia levels and morphologic types. Anaemia status and morphologic types across study groups were determined using the Pearson's chi-square test; (**a**) Frequency of overall anaemia and anaemia levels across the study group (ART non-adherents, and ART naive). Columns shaded red indicate overall anaemia (87.9%, 53.7% and 55.5%; **P* 0.0001); black columns show severe anaemia (40.4%, 8.5% and 8.7%); gray columns represent moderate anaemia (28.3%, 15.9% and 20.3%); unshaded columns show mild anaemia (19.2%, 29.3% and 27.5%), respectively (**P* 0.0001); (**b**) Frequency of RBC morphologic types amongst the study groups (ART non-adherents, adherents and naive). Pink bars show macrocytic anaemia (11.6%, 43.9% and 18.2%); green bars represent microcytic anaemia (54.5%, 8.5% and 8.7%); light-green bars indicate normocytic anaemia (27.3%, 47.6% and 79.7%); red columns indicated hypochromic anaemia (64.9%, 32.9% and 17.4%); blue bars indicate normochromic anaemia (32.3%, 52.4%and 79.7%), while yellow bars show polychromic anaemia (2.0%, 14.6%, and 2.9%), respectively (**P* 0.0001)

Since RBC morphology can classify and predict the aetiology of anaemia [30], erythrocyte morphology was analyzed among the study participants (Figure 1b). This analysis indicated higher rates of microcytic and hypochromic anaemia in the defaulters (54.5% and 64.7%) versus the adherents (8.5% and 32.9%) and naive (8.7% and 17.4%; *P* 0.0001) subjects, respectively. In contrast, rates of macrocytic anaemia were lower in defaulters (11.6%) compared to adherents (43.9%) and naive (18.2%; P 0.0001) individuals. Conversely, the rates of normocytic and normochromic anaemia were lower in defaulters 27.3%and 32.3%) relative to adherent (79.7% and52.2%) and naive (79.7% and47.6%) individuals (*P* 0.0001).

Factors associated with anaemia

Associations of anaemia and predictor variables are presented in Table 3. Anaemia was significantly associated with underweight (*P* = 0.002), microcytic (*P* 0.0001), and hypochromic (*P* 0.0001) RBCs, as well as severe immunosuppression (*P* 0.0001) in the defaulters. Among the adherents, anaemia was associated with underweight (*P* = 0.003), macrocytic (*P* = 0.001), and polychromatic (*P* = 0.039) erythrocytes, plus moderate (*P* = 0.007) and severe (*P* = 0.003) immunosupression. In the naive subjects, anaemia was associated with normocytic (*P* = 0.041) and normochromic (*P* = 0.013) erythrocytes, as well as moderate immunosuppression (*P* 0.0001).

Measure	B	OR (95% CI)	*P*
ART non-adherents			
Underweight	1.729	5.634 (1.844-17.213)	0.002
Microcytic	3.616	37.771 (8.604-160.581)	0.0001
Hypochromic	3.761	42.982 (8.622-214.267)	0.0001

Measure	B	OR (95% CI)	P
CD4 T cells250/mm^3	5.127	168.476 (9.872-2875.17)	**0.0001**
ART adherents			
Underweight	1.592	4.912 (1.737-13.886)	**0.003**
Macrocytic	1.625	5.079 (1.867-13.820)	**0.001**
Polychromic	2.239	9.388 (1.125-78.350)	**0.039**
CD4 T cells/mm^3			
250-499	3.174	23.901 (3.929-145.385)	**0.007**
250	2.515	12.362 (2.291-66.696)	**0.003**
ART naive			
Normocytic	2.287	9.844 (1.098-88.268)	**0.041**
Normochromic	1.819	6.167 (1.474-25.803)	**0.013**
CD4 T cells 250-499/ mm^3	3.148	23.286 (4.129-131.328)	**0.0001**

Data are presented as odds ratio (OR) and 95% confidence intervals (CI). Underweight, body mass index 18.5 kg/m^2; microcytic, erythrocyte6.0μm; macrocytic, erythrocyte>8.0μm; hypochromic, enlarged erythrocyte central pallor; polychromic, no erythrocyte central pallor; normochromic, normal erythrocyte red-pink staining. Binary logistic regression was used to determine the association of anaemia and the predictor variables (underweight, microcytic, macrocytic, hypochromic and polychromic erythrocytes, and moderate (CD4 T cells 250-499/mm^3) and severe (CD4 T cells 250/mm^3) immunosuppression controlling for confounding effect of age, gender, ART duration and regimen defaulters and adherents; and age and gender in ART-naive groups. B, binary coefficient. Values in bold are significant *P*-values

Table 3: Association of anaemia with underweight, erythrocytic measures, and immunosuppression

Discussion

The prevalence of anaemia in HIV infected individuals varies considerably by region and antiretroviral treatment status, ranging from 63% to 95% [8]. However, no studies have comprehensively examined anaemia phenotypes, erythropoietic status and predictors among individuals defaulting ART. In the current study, erythrocytic measures (red blood cell counts, hematocrit, haemoglobin, mean cell volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) were lower in the ART defaulters compared to the treatment-adherents and -naive. The higher overall rates of anaemia (87.9%); including rates of severe (40.3%) and moderate (28.7%) anaemia were higher in the ART-defaulters, suggesting a high burden of anaemia in ART defaulting patients. These results are consistent with previous studies in Nigeria showing that overall anaemia is high among HIV infected individuals [31]. However, these results differ from previous studies in Uganda showing higher prevalence of anaemia among ART adherent and naive individuals [32,33]. The differences can be attributed to differences in the study population, as these studies targeted HAART experienced individuals while the current study focused on ART defaulters.

Although the morphologic type of anaemia was predominantly normocytic and normochromic in the treatment-naive individuals, it was diverse in the treatment-defaulting (microcytic, hypochromic, and normocytic) and adhering (macrocytic, normochromic, hypochromic and normochromic) individuals, signifying multiple aetiologies. While the underlying mechanisms appear multifactorial, deficiencies in iron, folate and vitamin cobalamin [10], under-nutrition, immunosuppression and ARV suppressive effects are implicated in the development of anaemia in the defaulting individuals.

Consistent with previous studies indicating reductions in the reticulocyte count and ARN in HIV patients [11], our findings showed that in addition to decreases in reticulocyte count and ARN, the RPI was decreased with higher rates of RPI 2.0 in the ART defaulting individuals, suggesting marked suppression of erythropoiesis. In contrast to the higher levels of MCV in the ART-adherents suggest direct ARV effects on the developing erythroblasts. This is consistent with previous studies in Nigeria and Uganda showing elevated MCV among adults on AZT containing regimens [34,35]. Although no studies have reported on RDW levels in ART-defaulters, the current studies showed increased RDW in treatment-defaulting individuals suggesting altered erythrocyte homeostasis at the level of erythropoiesis and survivability (that is, marked anisocytosis that is proportional to size variations). This results are consistent with previous studies showing that higher RDW in patients on HAART is associated with dyserythropoiesis and increased lysis of erythrocytes [36]. Altogether, this study has shown for the first time, profound suppression of erythropoiesis in Kenyan ART defaulting and adhering adults. In spite of ART aiming at improving the hematopoietic status [14], it appears that the rate of recovery is certainly lower in patients defaulting treatment.

Regression analysis illustrating that anaemia is associated with different factors suggests diversity in the aetiology and development of anaemia in the treatment-naive, adherents and defaulting adults. These regression analyses are similar to previous studies showing that anaemia is associated with the ART regimen in adherents and HIV virus related haemolysis in naive [37]. In addition, the results

imply susceptibility to suffering multiple morbidities. For instance, previous studies showed that ART adherents suffered high rates of adverse drug-effects [38] while naive suffer high rate of anaemia related to pathophysiology of HIV virus [39]. Therefore, anaemia management requires detailed clinical and laboratory evaluation for focused patient centered care.

It is noteworthy to mention that a prospective study design would provide additional insights into the time course of anaemia development following initiation of ART and magnitude of treatment defaulting. In addition, prospective analysis of laboratory markers of anaemia such as serum iron, folate, cobalamin, viral load and markers of inflammation [40,41], would yield additional information for classification of the morphologic phenotypes of anaemia. Likewise, bone marrow cyto- and histopathologic, and post-mortem analyses of mortality cases defaulting treatment would enhance our understanding of the nature of erythroblastic cell suppression and development of anaemia following HIV infection.

Conclusion

This study highlights high burden of anaemia in HIV infected individuals. However, the study has shown that ART non-adherents suffer high burden of anaemia relative to adherents and naive individuals. In addition, ART-defaulting persons present largely with microcytic hypochromic or microcytic normochromic anaemia, providing opportunities for improving laboratory diagnosis, treatment and care HIV patients. The implications of the results of this study include incorporation of RBC morphologic analysis in screening treatment defaulters as well as monitoring response and deleterious sequelae of HAART in HIV treatment programs.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors Contributions

PW and TW conceived and designed the study. PW conducted the laboratory experiments; and along with TW performed statistical analyses, interpretation, and co-drafted the manuscript. MB critically revised the manuscript. All authors have read and approved the manuscript.

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