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Prevalence and Associated Factors of Anemia in Treatment-naïve HIV-positive Subjects in Southeast Nigeria

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Abstract **Background and Objectives:** Anemia is an issue in human immunodeficiency virus (HIV) infection. This study was to determine the prevalence and associated risk factors of anemia in highly active anti-retroviral therapy (HAART)-naïve, HIV-positive subjects in Owerri, Southeast Nigeria. **Methodology:** This was a cross-sectional study of HAART-naïve HIV-positive subjects. Anthropometric and demographic data were collected. Hemoglobin (Hb) and other relevant investigations were performed. Anemia was defined, according to World Health Organization criteria, as Hb <13.0g/dl in males and Hb <12.0g/dl in females. Association of variables with anemia and the strength of variables to predict anemia were determined. **Results:** Mean Hb was 11.3±1.7g/dl in males and 11.1±1.9g/dl in females. Hb<13.0g/dl was present in 66.4% of males, while Hb<12.0g/dl was present in 67.8% of females. Overall, Hb≥12.0g/dl was present in 128(32.6%), Hb<12.0g/dl in 252(67.4%), Hb 11.0-12.0g/dl in 177(45.0%), Hb 8.0-10.9g/dl in 82(20.9%) and Hb<8.0g/dl in 6(1.5%) of the HIV subjects. Body mass index (BMI) and cluster of differentiation 4 (CD4) cells count predicted hemoglobin, while underweight, BMI, CD4 cells count <200/ml, spot urine protein (SUP) and 24-hour urine protein (24HUP) predicted anemia in HIV-positive subjects. **Conclusion:** The prevalence of anemia was high (67.4%) in HAART-naïve HIV-positive subjects. BMI and CD4 cells count were predictors of hemoglobin in HIV-positive subjects. CD4 cells count <200, underweight, BMI, 24HUP and SUP were predictors of anemia in HAART-naïve HIV-positive subjects. Abnormalities of weight changes and renal function were common in HAART-naïve HIV-positive subjects who were anemic in Owerri, Southeast Nigeria. Anemic HAART-naïve HIV-positive subjects should be evaluated at the early stages of the infection for underweight and renal damage.

Keywords: *HIV subjects, anemia, prevalence, risk factors, Nigeria*

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1. Introduction

Across the globe, human immunodeficiency virus (HIV) infection is a healthcare problem. Sub-Saharan Africa accounts for about 70% of world HIV subjects population. [1] Nigeria has an HIV infection prevalence of 3.7%. [2] The world prevalence of anemia in HIV subjects is not well-defined. However, studies have shown variable prevalence of anemia in HIV patients, with values ranging from 20% to 95%.[3,4] Two studies in Nigeria showed 60.61% and 64.0%. [5,6]

In two studies, anemia was noted to be commonly associated with HIV infection. [3,7] Studies have shown that among the hematological abnormalities seen in HIV infection, anemia topped the list, compared to leucopenia and thrombocytopenia.[3,4]

Factors associated with anemia in HIV infection included: accelerated disease progression and mortality, low CD4 cells count, high viral load, female gender, African American individuals, and Zidovudine use [3,8-14].

The impact of anemia in HIV-positive subjects has been enormous. This included reduced quality of life, tiredness and increased tendency for HIV dementia. [10] Many factors have been shown to contribute to anemia in HIV infection. They included low red blood cells (RBC) synthesis, destruction of RBCs, docile RBC synthesis, autoimmune destruction of RBCs and vitamin B12 deficiency [15,16].

Normocytic, normochromic RBCs, diminished cytokine-controlled erythropoietin response and normal iron stores, have been documented as features of anemia of chronic disease associated with HIV infection [3,17,18].

Studies have shown that the use of highly active antiretroviral therapy (HAART) in HIV infection resulted in appreciation in hemoglobin (Hb) levels. [15,19] There has been remarkable improvement in availability of, and access to, HAART. In addition, interventions in anemia in HIV infection have been of immense beneficial outcomes in subjects with HIV infection. [20] However, there is a paucity of reports on anemia and its potential risk factors in HAART-naïve, HIV-positive subjects in Nigeria and almost none on the risk factors of anemia in HIV-positive

subjects emanating from Southeast Nigeria. This prompted us to embark on this study to determine the prevalence and associated risk factors of anemia in HAART-naïve HIV-positive subjects in Southeast Nigeria.

2. Materials and Methods

This was a cross-sectional study carried out in Federal Medical Centre (FMC), Owerri, Nigeria, between March and June 2011. FMC Owerri is a tertiary hospital. There is only one other tertiary health institution in the state. FMC Owerri receives referrals from the state as well as from neighboring states. The population of Imo State is about 3,927,563. Owerri Municipal, where the hospital situates, has a population of about 125,337. [21]

The study subjects comprised of 393, 18-65 year-old HAART-naïve HIV-positive subjects, and 136 age- and sex-matched HIV-negative subjects as Control, consecutively drawn from an HIV clinic and an Out-patient clinic of the hospital, respectively.

The Ethical Research Committee of the hospital approved the study. All the subjects that participated in this study gave informed consent.

Demographic and anthropometric data were obtained. Investigations done included HIV screening and confirmatory tests, Hb, serum creatinine (SCr), spot urine protein (SUP), spot urine creatinine (SUCr), spot urine osmolality (SUOsm), 24-hour urine protein (24HUP), 24-hour urine creatinine (24HUCr), 24-hour urine osmolality (24HUOsm), CD4 cells count, fasting serum lipid profile (FSLP) (total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL). Subjects aged below 18 or above 65 years, those who received recent blood transfusion, those who have known hematological diseases such as sickle cell anemia, those who have terminal illness and those who were pregnant were excluded from the study.

Osmolality was determined by freezing point depression using Precision System Osmette 5002 osmometer, protein by photometric method and creatinine by modified Jeff's method. Spot urine creatinine/osmolality ratio (SUCOR), and creatinine clearance (ClCr) were determined.

2.1. Statistical Analysis

The data were analyzed using SPSS version 17.0 (SPSS Int. Chicago, II, USA). The distribution and characterization of clinical and laboratory features among HIV-positive subjects with anemia and those without anemia were analyzed using cross-tabulation, while statistical significance of association of these variables with anemia was determined using chi square. Correlation statistics were used to determine association of variables with Hb, (defined here as continuous Hb values) and with anemia. Multivariate linear regression analyses were used to determine the strength of variables to predict Hb and anemia. $P \leq 0.05$ was taken as statistically significant.

2.2. Definition of Terms

Anemia was defined according to World Health Organization (WHO) criteria [22]:

No anemia: Hb≥13.0g/dl in males and Hb≥12.0g/dl in females

Mild anemia: Hb 11-13g/dl in males and Hb 11-12g/dl in females

Moderate anemia: Hb 8-10.9g/dl in males and Hb 8-10.9g/dl in females

Severe anemia: Hb <8g/dl in males and Hb<8g/dl in females

However, in this study, anemia was defined as Hb<13.0g/dl in males and Hb<12.0g/dl in females.

Hemoglobin: defined as continuous hemoglobin values

3. Results

Out of 393 subjects recruited in this study, one was excluded from the study on account of incomplete data and errors in sample collection. The mean age of the HIV-positive subjects was 39±11 years. Females were 282(72.0%) and males 110(28.0%).

Table 1 shows the mean values of variables. The mean Hb of the HIV-positive subjects was 11.2±1.8g/dl. Furthermore, mean Hb was 11.3±1.7g/dl in males and 11.1±1.9g/dl in females. Seventy-three (66.4%) of the 110 male HIV subjects have Hb<13.0g/dl, while 192(67.8%) of the 282 female HIV-positive subjects have Hb<12.0g/dl. There was no significant difference in the prevalence of anemia between male and female HIV-positive subjects, p=0.779. Overall, out of 392 HIV subjects, 128(32.6%) have Hb≥12.0g/dl, 252(67.4%) have Hb<12.0g/dl, 177(45.0%) have Hb 10.0-12.0g/dl, 82(20.9%) have Hb 8.0-9.9g/dl and 6(1.5%) have Hb <8.0g/dl.

Table 1. Clinical and laboratory characteristics of variables in HAART-naïve HIV-positive subjects (n=392)

Variables (mean±SD)	HIV Subjects
Body Mass Index (kg/m^2)	26.2± 5.4
Waist circumference	85.3±13.4
Hemoglobin (g/dl)	11.2±1.8
CD4 cells	416±209
Spot Urine Protein(mg/dl)	11.89±19.13
Spot Urine Creatinine (mg/dl)	137.21± 98.47
SUOsm (mOsm/kgH_2O)	464±271
24-Hour Urine Protein (g)	0.187± 0.290
24-Hour Urine Creatinine (mg)	1507±781
24HUOsm(mOsm)	564 ± 501
SUCOR (mg/dl/mOsm/kgH_2O)	0.422± 0.486
Cholesterol (mmol/l)	4.26± 0.90
Triglyceride (mmol/l)	1.23± 0.37
HDL (mmol/l)	1.18± 0.39
LDL (mmol/l)	2.05 ±0.58
Creatinine Clearance (mls/min)	91.42±22.98

SD=standard deviation, SUOsm=spot urine osmolality, 24UOsm=24-hour urine osmolality, SUCOR=spot urine creatinine/osmolality ratio, HDL=high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, HAART=highly active antiretroviral therapy.

Table 2 shows the relationship between anemia and selected risk factors in HAART-naïve HIV-positive subjects. There was significant association between anemia and body mass index (BMI), p=0.001. Out of 24 subjects with BMI <18.5, 20(83.3%) have anemia and out

of 134 subjects with BMI 18.5-24.9, 103(76.9%) have anemia. Out of 154 subjects with BMI 25-29.9, 96(64.0%) have anemia. Similarly, out of 85 subjects with BMI ≥30, 46(54.1%) have anemia. The prevalence of anemia was highest among subjects with lowest BMI and lowest among subjects with highest BMI. This showed that the prevalence of anemia in HAART-naïve HIV-positive subjects increased as BMI decreased.

Table 2. Relationship between Anemia and Selected Risk Factors in HAART-naïve HIV-positive Subjects (n=392)

VARIABLES	ANEMIA Absent (n/%) N=127	ANEMIA Present (n/%) N=266	Chi Square	LHR	P value
BMI <18.5	4(16.7%)	20(83.3%)	15.859	0.001	0.001
18.5-24.9	31(23.1%)	103(76.9%)			
25.0-29.9	54(36.0%)	96(64.0%)			
≥30	39(45.9%)	46(54.1%)			
Waist Circ (cm)					
Male <102	28(29.8%)	66(70.2%)	4.289	0.044	0.038
≥102	9(56.2%)	7(43.8)			
Female <88	34(22.4%)	118(77.6%)	14.418	<0.001	<0.001
≥>88	57(43.5%)	74(56.5%)			
CD4 cells count					
<200	9(18.4%)	40(81.6%)	5.033	0.019	0.025
≥200	118(34.4%)	225(65.6%)			
ClCr≥90mls/min	71(35.7%)	128(64.3%	2.112	0.339	0.349
60-89	44(30.8%)	99(69.2%)			
30-59	8(24.2%)	25(75.8%)			
24HUP<0.300g	113(35.2%)	208(64.8%)	6.158	0.083	0.048
≥0.300g	10(18.5%)	44(81.5%)			
FSLP (mmol/l)					
Chol T Des (<5.2	117(33.6%)	231(66.4%)	1.777	0.391	0.441
BorderL(5.2-6.2)	9)23.1%)	30(76.9%)			
High (>6.2)	2(33.3%)	4(66.7%)			
LDL Des (<2.6)	110(34.1%)	213(65.9%)	2.303	0.169	0.129
BorderL (2.6- 4.1)	17(24.6%)	52(75.4%)			
High (>4.1)	-	-			
HDL Low (<1)	41(30.4%)	94(69.6%)	0.453	0.500	0.501
High (≥1)	87(33.7%)	171(66.3%)			
TG Des <1.7)	115(32.5%)	239(67.5%)	10.788	0.014	0.013
BorderL (1.7-2.2)	6(20.0%)	24(80.0%)			
High >2.2)	6(75.0%)	2(25.0%)			

LHR=Likelihood ratio, BMI=body mass index, Waist Circ=waist circumference, ClCr=creatinine clearance, 24HUP=24-hour urine protein, FSLP=fasting serum lipid profile, CholT=total cholesterol, Des=desirable BorderL=borderline, LDL=low density lipoprotein cholesterol, HDL=high density lipoprotein cholesterol, TG=triglyceride. HAART=highly active antiretroviral therapy.

There was significant association between anemia and waist circumference in male, p=0.038, and female, p<0.001, HIV-positive subjects. Among male subjects with Wc <102cm, 66(70.2%) have anemia and among those with ≥102cm, 7(43.8%) have anemia. This indicated that the prevalence of anemia in male HIV-positive subjects increased as Wc decreased. Similarly, among female subjects with Wc <88cm. 118(77.6%) have anemia, while among those with ≥88cm, 74(56.5%) have anemia. This also showed that the prevalence of anemia increased as Wc decreased in female HAART-naïve HIV-positive subjects.

There was significant association between anemia and CD4 cells count in HIV-positive subjects, p=0.025. Out of 49 subjects with CD4 <200, 40(81.6%) have anemia, while out of 343 subjects with CD4 ≥200, 225(65.6%) have anemia. This demonstrated that the prevalence of anemia increased as CD4 declined.

There was significant association between anemia and 24HUP, p=0.048. Out of 321 subjects with 24HUP<0.300g, 208(64.8%) have anemia, while out of 54 subjects with 24HUP≥0.300g, 44(81.5%) have anemia. This showed that the prevalence of anemia increased as proteinuria increased in HAART-naïve HIV-positive subjects.

There was significant association between anemia and serum triglyceride in HIV-positive subjects, p=0.013. The prevalence of anemia was highest (80.0%) among those with serum triglyceride border line (1.7-2.2mmol/l), and lowest among those with high >2.2mmol/l.

There was no significant association between anemia and ClCr, p=0.349, serum cholesterol, p=0.441, LDL, p=0.121, HDL, p=501 in HAART-naïve HIV-positive subjects.

Correlation of variables with Hb is shown in Table 3. There was significant correlation between Hb and BMI (r=0.334, p<0.001), Wc (r=0.303, p<0.001), CD4 cells count (r=0.224, p<0.001), SUP (r=-0.178, p<0.001), 24HUP (r=-0.143, p<0.001, serum triglyceride (r=0.156, p=0.044) in HAART-naïve HIV-positive subjects. Conversely, there was no significant correlation between Hb and SUCr, p=0.547, SUOsm, p=0.972, 24HUCr, p=0.111, 24HUOsm, p=0.653, SUCOR, p=0.320, SCr, p=0.513, serum cholesterol, p=0.671, HDL, p=0.635, LDL, p=0.883, ClCr, p=0.395.

Table 3. Correlation of Anemia with selected variables in HAART-naïve HIV-positive subjects (n=392)

Variables	Correlation coefficient(r)	P value
Body mass index	0.344	<0.001
Waist circumference	0.303	<0.001
CD4 cells count	0.224	<0.001
Spot urine protein	-0.178	<0.001
Spot urine creatinine	-0.031	0.547
Spot urine osmolality	-0.002	0.972
24-hour urine protein	--0.143	<0.001
24-hour urine creatinine	0.082	0.111
24-hour urine osmolality	0.023	0.653
SUCOR	0.050	0.320
Serum creatinine	-0.033	0.513
Serum cholesterol (total)	-0.022	0.671
Serum Triglyceride	0.156	0.044
Serum HDL	0.024	0.635
Serum LDL	-0.021	0.883
Creatinine clearance	-0.043	0.395

SUCOR=spot urine creatinine osmolality ratio, HDL=high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol.

There was significant correlation between BMI and Wc, (r=0.731, p<0.001), and between serum cholesterol and serum triglyceride, (r=0.592, p<0.001) in HAART-naïve HIV-positive subjects.

Table 4 shows a multivariate linear regression analysis of the potential risk factors with Hb in HAART-naïve HIV-positive subjects. BMI and CD4 cells count predicted Hb in HIV-positive subjects. However, further multivariate linear regression of variables with anemia (Hb<12.0g/dl) showed that CD4 cells count <200, underweight, BMI, SUP and 24HUP predicted anemia in HAART-naive HIV-positive subjects (Table 5).

Table 4. Multivariate linear regression of variables with Hemoglobin (continuous hemoglobin values) in HAART-naïve HIV-positive Subjects (n=392)

Variables	Beta	T	P value	95% CI
Body mass index	0.215	2.950	0.003	0.024—0.122
Waist circumference	0.126	1.680	0.094	-0.003-0.038
Spot urine protein	-0.143	10886	0.060	-0.029-0.001
24-hour urine protein	0.019	0.151	0.880	-1.462-1.704
Triglyceride	0.054	0.688	0.523	-0.002-0.009
CD4 cells count	0.179	3.703	<0.001	0.001-0.002

CI=Confidence Interval. HAART=highly active antiretroviral therapy.

Table 5. Multivariate linear regression of variables with Anemia (hemoglobin <12.0g/dl in females and <13.0g/dl in males) in HAART-naïve HIV-positive Subjects (n=252)

Variables	Beta	t	P value	95% CI
Body mass index	0.243	2.786	0.006	-0.43-0.003
Underweight	-0.371	-1.471	0.046	-0.014-0.003
Waist circumference	0.094	1.083	0.280	0.012-0.034
Spot urine protein	-0.288	-3.137	0.002	-0.001-0.011
24-hour urine protein	0.232	2.528	0.012	-0.283-0.472
Triglyceride	0.042	0.695	0.530	-0.003-0.007
CD4 cells count	0.038	0.629	0.530	-0.001-0.000
CD4 cells count <200	0.427	2.915	0.006	-0.001-0.003

Underweight=body mass index <18.5. HAART=highly active antiretroviral therapy. CI=Confidence Interval.

4. Discussion

In this study, the prevalence of anemia was 67.4% in HAART-naïve HIV-positive subjects. Anemia has significant association with BMI, p=0.001, waist circumference, p=0.038, CD4 cells count, p=0.025, 24HUP, p=0.048, serum triglyceride, p=0.013. BMI and CD4 cells count were predictors of Hb in HAART-naïve HIV-positive subjects. BMI, 24HUP and SUP were predictors of anemia in HAART-naïve HIV-positive subjects.

The prevalence of anemia of 67.4% in HAART-naïve HIV-positive subjects found in this study is similar to those reported in some studies. [5,6,23,24,25] In contrast, some studies revealed a higher prevalence of anemia in HIV subjects. [26,27] Still, some studies demonstrated lower values. [3,28,29,30] These observed differences in prevalence might be explained in part by differences in study design and variability in the stages of HIV infection at the time the studies were conducted. Early reporting by subjects in response to campaigns for voluntary testing and counselling might account for low prevalence of anemia in our study. In addition, some of the studies were carried out in HIV-positive populations on HAART, in sharp contrast to ours that was done in HAART-naïve subjects.

In this study, there was significant association between anemia and BMI, p=0.001, in HAART-naïve HIV-positive subjects. This is in agreement with studies that demonstrated a similar association. [9,12,15,31] Underweight has been shown to be associated with anemia in HIV-positive subjects. [31] Furthermore, our study also showed that both underweight and BMI were predictors of anemia. Anyabolu et al documented that both BMI and anemia were associated with HIV infection.[32] However, that study did not evaluate the relationship between BMI and anemia. Literature search did not reveal any study that evaluated the strength of BMI, underweight or obesity in predicting anemia in HIV-positive subjects.

Our study showed that there was significant association between waist circumference, an index of weight changes, and anemia. This agrees with related studies that showed a similar association between BMI and anemia.[9,12,15,31] However, waist circumference was not a predictor of anemia in this study, despite the high correlation demonstrated here between BMI and waist circumference. However, we did not find, from literature search, any study that examined the relationship between waist circumference as an index of weight changes and anemia in HIV-positive subjects.

This study found that CD4 cells count has significant association with anemia, and further regression demonstrated that CD4 cells count <200 predicted anemia in HAART-naïve HIV-positive subjects. This finding is similar to that reported in two studies. [4,9,15,33] Expectedly, low CD4 cells count is commonly found in HIV-positive subjects who have anemia. [4,9] In contrast, it disagrees with another report that did not find any association between anemia and CD4 cells count in HIV-positive subjects, [6] probably, because that study was conducted in an HIV-positive population on HAART.

We observed that 24HUP was a predictor of anemia in HAART-naïve HIV-positive subjects in this study. From literature search, we could not find any study that

evaluated the association between 24HUP and anemia in HIV-positive subjects. However, it has been shown that proteinuria is a marker of renal damage and that anemia is also associated with kidney damage in HIV patient population. [32]

It was demonstrated, in this study, that serum triglyceride was significantly associated with anemia, but did not predict anemia in HAART-naïve HIV-positive subjects. We could not find any study that evaluated the association between anemia and lipid abnormalities in HIV-positive subjects. Nonetheless, It has been documented that dyslipidemia occurred in HIV-positive subjects on HAART. [13] The demonstration, in this study, that underweight predicted anemia might perhaps, also explain, here, the association between serum triglyceride and anemia. Underweight or weight loss is commonly associated with dyslipidemia.

SUP was a predictor of anemia in this study. Spot urine protein may vary over the day and may not reflect real time 24HU., [34] Nevertheless, a high value of SUP may point towards renal damage. We could not find any study that examined the association between SUP and anemia in HIV-positive subjects, from literature search.

This study did not find any significant association between anemia and ClCr, p=0.349, serum cholesterol, p=0.441, LDL, p=0.121, HDL, p=501 in HAART-naïve HIV-positive subjects.

5. Conclusion

The prevalence of anemia was high (67.4%) in HAART-naïve HIV-positive subjects. BMI and CD4 cells count were predictors of hemoglobin in HAART-naïve HIV-positive subjects. CD4 cells count <200, underweight, BMI, 24HUP and SUP were predictors of anemia in HAART-naïve HIV-positive subjects. Abnormalities of weight changes and renal function were common in HAART-naïve HIV-positive subjects who were anemic. Anemic HAART-naïve HIV-positive subjects should be evaluated at the early stages of the infection for underweight and renal damage.

Strength of the study: The study was conducted in a Sub-Saharan African population that was 100% black people. We have a lot of data including a number of laboratory tests for the population being studied.

Limitations of the study: We did not evaluate mean cell volume, leucocytes count, platelets count and blood film. These would have further helped in categorizing the anemia in the study population.

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