Evaluation of Iron Status in Anemia of Chronic Disease Among Patients With HIV Infection

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OBJECTIVE: In HIV-infected populations from developing countries, it is unclear what proportion of anemia is attributable to iron deficiency (ID). The objective of this study was to evaluate the iron status in anemia of chronic disease of patients with Human Immunodeficiency Virus (HIV) infection attending Federal Medical Centre, Makurdi.

DESIGN AND SETTING: A total of 312 subjects comprising of 207 confirmed HIV positive patients and 105 apparently healthy subjects as control were evaluated for indices of anemia using SYSMEX KX 21N hematology analyzer machine (Kobe, Japan), CD4 count using CYFLOW SL machine (Artec, Germany), and total iron binding capacity and serum iron using colorimetric method.

RESULTS: While results showed that Serum Iron, Transferrin Saturation, PCV, RBC, MCV, MCH, MCHC and RDWCV are within normal reference range but statistically different ($p<0.05$) compared to the controls. Stratifying them on the basis of CD4 count showed that in AIDS patients the indicators are generally lower with Hb, PCV, MCH and RDWCV showing statistical significance ($p<0.05$) compared with patients with CD4 >200cells/mm^3. Serum iron (50%) and transferring saturation (47.9%) contributed highest to anemia prevalence especially in males while Hb concentration (47.2%) is the major contributor to anemia in females.

CONCLUSION: It was concluded therefore that albeit, on average, the parameters of iron status did not indicate iron deficiency or iron overload in the HIV-status groups and AIDS patients, a large percentage of patients did have anemia of chronic disease with HIV-infected women afflicted more often. The anemia is generally normocytic hypochromic in AIDS patients.

INDEX TERMS: Serum Iron (SI), Total Iron Binding Capacity (TIBC), Transferrin Saturation (TS), Hemoglobin, Human Immunodeficiency Virus, Makurdi

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INTRODUCTION

Anemia is identified as the most common complication seen in patients with HIV infection because it has been documented that up to 95% of HIV/AIDS patients are anemic.[1] Sullivan *et al.*[2] also reported that up to 30% of asymptomatic HIV patients have anemia while up to 90% of them that have AIDS also have anemia. The most common anemias encountered in patients with HIV/AIDS is iron deficiency anemia and anemia of chronic disease. Iron deficiency (ID) is one of the major underlying nutritional factors of HIV anemia. It is worthy of note that it is difficult to quantify the contribution of ID to the anemia of HIV infection because in inflammatory condition of HIV disease, the

proinflammatory cytokines induce an anemia of inflammation (anemia of chronic disease) and alter biochemical indicators of iron status that are commonly used to diagnose ID.[3]

Although the reasons are not fully understood, anemia of chronic disease occurs widely among people living with HIV/AIDS.[2] Some researchers associate the anemia with HIV-disease progression and an increased risk of death,[2] even independent of CD4 count and viral load.[4] Apart from elevated proinflammatory cytokines, other risk factors for HIV-related anemia of chronic disease have been identified, including clinical AIDS, CD4+ cell counts <200 cells/mL, high plasma viral load, female sex, African American ethnicity, and zidovudine use.[5]

Nutrition is also an important factor in the course of HIV infection and is generally accepted as a major determinant of immune functioning. Nutritional factors, although not the most important etiological determinants may change immune function to facilitate disease progression, influence viral expression, and play a significant role in disease processes and related morbidity and mortality.[6] Among all the nutrients, iron stands out as having a particularly crucial role in mediating host–pathogen interactions. Iron also plays a role in immune function and cognitive status and many other reactions including the synthesis of DNA, collagen, and bile acids.[7]

The paucity of studies on iron status in anemia of chronic disease among HIV-infected persons in sub-Saharan Africa is an important research gap, as there is evidence that decreased iron status,[8] as well as elevated storage iron[8,9] in anemia of chronic disease may lead to adverse HIV-related outcomes. The potential risks of altered iron status in anemia of chronic disease are of special relevance for treatment programs recommending supplemental iron for patients with HIV infection.[10]

To expand the knowledge on indicators of iron status in anemia of chronic disease with its relation to CD4 count in Makurdi metropolis, we conducted a study in HIV-infected persons attending Federal Medical Center, Makurdi in Benue state, North-Central Nigeria.

MATERIALS AND METHOD

Subjects

A total of 312 subjects, comprising of 207 confirmed HIV positive patients attending Federal Medical Center, Makurdi, Benue state, Nigeria and 105 confirmed HIV negative as controls drawn from volunteers living within Makurdi metropolis were recruited in the study. The selection of the volunteers who were apparently healthy individuals was based on the following criteria: age range between 25-60 years, no history of drug usage (including vitamins/iron, antibiotics), no recent history of blood loss, and must not have received any blood transfusion in the previous 12 months. Additional criteria for females included not being pregnant, and not lactating or menstruating at the time of blood collection. Ethical approval was sought for and obtained from the ethical standard committee of the Federal Medical Centre, Makurdi, and informed consent was obtained from the subjects before samples were collected.

Sample Collection

A total of 5ml non-hemolysed blood was drawn from each confirmed HIV positive and negative volunteers in the morning after an overnight fast of between 9pm and 8am. While 2ml was transferred into a clean, screw-capped glass tube without anticoagulant and allowed to stand at room temperature until separation of the serum was started by centrifugation speed of 3000 rev/min for 5 minutes. The separated sera were kept at below 20°C until analyses were done. Analyses conducted on the sera were the determination of serum iron and unsaturated iron binding capacity, and calculation of total iron-binding capacity and iron saturation. The remaining 3ml of blood was transferred into an EDTA anticoagulated bottles for immediate analyses of Indices of anemia.

Sample Analysis

The analyses were done at the Laboratory services building, Hematology and Chemical Pathology Sections, at Federal Medical Center, Makurdi. Before the analysis, the separated frozen sera were placed at room temperature to allow thawing, and were then mixed.

HIV Screening and Confirmatory Tests

HIV rapid screening was carried out using Uni-Gold HIV1/2 and Determine HIV1/2 assay kits (Abbott, Germany). While the confirmatory tests were conducted using Western Blot kits (Immunetics, Boston USA)

CD4 Count Analysis
Enumeration of CD4 lymphocyte count was performed using SL Cyflow machine (Artec, Germany).

Indices of Anemia
Indices of anemia including Hb, PCV, RBC, MCV, MCH, MCHC and RDWCV was carried out using the SYSMEX KX-21N hematology analyzer method (Kobe, Japan).

Measurement of Serum Iron and Unsaturated Iron Binding Capacity (UIBC)
Serum Iron concentration and UIBC was measured by the Forrozine method[11] and the assay was carried out using the Iron/TIBC reagent set (TECO Diagnostics, Anaheim, Germany) by colometric method. Before running the assay, the analyzer was calibrated by iron standard (500µg/dl) included in each kit. To assess the accuracy of the analyzer, Precinorm U and Precipath U controls were run with the assay in addition to Precinorm UPX control, which was used to assess precision.

Total Iron Binding Capacity (TIBC) and Iron Saturation (IS) Fraction
The TIBC and IS[11] were calculated according to the following formulae:

$$\text{TIBC} = \text{Iron level} \times \text{UIBC}$$

$$\text{IS\%} = \frac{\text{Serum Iron Concentration}}{\text{TIBC}} \times 100$$

Hypothesis Testing
H_0: All HIV/AIDS patients have anemia of chronic disease
H_1: Not all HIV/AIDS patients have anemia of chronic disease

Statistical Analysis
Results for the pooled data were expressed as mean ± SD and student "t" test was used to calculate the level of significance at $p < 0.05$ values using the Epi Info 3.3.1 statistical software. Prevalence was determined as percentage of exact value to the total value.

RESULTS
Values of iron status as well as hematological indices for confirmed HIV positives and negatives are shown in Table 1. From the table, only the TIBC showed no significant difference ($p>0.05$) between HIV patients and the controls. Although other indicators of iron status and anemia (with the exception of Hb) are within normal range, they showed statistically significant difference ($p<0.05$) between test subjects and controls. On the other hand, Table 2 depicted that only Hb, PCV, MCH and RDWCV showed statistical significance ($p<0.05$) when the variables are stratified at CD4 below and above 200 cells/mm^3.

The p-value is the probability of obtaining a test statistics. It is used in statistics for hypothesis testing; it is the significance of the test. P-values are generally considered significant if they are less than 0.05. When the null hypothesis is rejected, the result is said to be statistically significant. So calculation of exact p-values simply requires integration of the distribution under study.

Table 3 reflected the prevalence of anemia of chronic disease. The indicators of iron status (serum iron [50.0%] and percentage of iron saturation [47.9%]) were the major contributors of anemia especially in the males while anemia in the females was largely contributed by hemoglobin concentration. The prevalence of HIV/AIDS among males and females as well as at different age groups is shown at Figure 1 and II. The female group showed higher prevalence (77%) compared with male (23%). While within the different age groups the highest prevalence was found within the 25-34 age groups (54%).

DISCUSSION
The moderate reduction in Hb concentration, PCV, and the total RBC count of test subjects is statistically significant ($P<0.05$) compared with the controls (Table 1). This general reduction suggests anemia. In this study Hb concentration was chosen to define the anemic status of the test subjects since the degree or severity of the anemia is directly proportional to the concentration of the haemoglobin as suggested by Abram *et al.*[11] Anemia was defined as Hb <11.0 g/dl, according to the WHO-recommended cutoff of 12.0 g/dl minus a 10 g/L adjustment to account for lower Hb reported in blacks.[12] It is important to note that in the presence of anemia, the iron status of this group of subjects lie within the reference range albeit they (except TIBC) showed statistical significance ($p<0.05$) to controls. This is not consistent with studies by Fuchs *et al.*[13] and Gordeuk *et al.,*[9] which showed relatively lower values of

Table 1. Comparison of Mean ± SD HIV positive and HIV negative groups

Parameters	HIV positives	HIV negatives	Z-score	p-Value
TSI (µg/dl)	76.00±38.21	98.21±14.13	5.511	0.000*
TIBC (µg/dl)	384.11±73.34	400.19±47.91	1.646	0.1
TS (%)	20.27±10.56	24.81±4.42	3.900	0.0011*
Hb (g/dl)	10.95±1.69	13.84±1.01	13.462	0.000*
PCV (%)	35.43±4.55	42.69±3.42	11.234	0.000*
RBC ($x10^{12}$/l)	3.89±0.63	5.07±0.43	14.750	0.000*
RDWCV	15.70±2.73	14.01±0.97	6.041	0.000*
MCV (fl)	91.42±14.49	84.65±4.16	4.801	0.0015*
MCH (pg)	28.02±4.78	27.01±1.59	2.094	0.026*
MCHC (g/dl)	30.74±2.15	32.17±1.51	4.767	0.0015*
CD4	342.32±250.06	787.57±253.83	10.563	0.000*

TSI: Total Serum Iron; TIBC: Total Iron Binding Capacity; TS: Transferrin Saturation; Hb: Hemoglobin; PCV: Packed Cell Volume; RBC: Red Blood Cell; MCV: Mean Cell Volume; MCH: MCHC: RDWCV: CD4: Cluster of Differentiation 4; * Significance at p<0.05

Table 2. Mean ± SD of Indicator of Iron status and Anemia stratified by CD4 count

Parameters	<200 (87)	≥200 (120)	Z-score	p-Value
TSI (µg/dl)	70.70±32.57	79.84±41.54	1.255	0.270
TIBC (µg/dl)	390.28±76.93	379.64±70.61	0.724	0.460
TS (%)	18.55±9.03	21.52±11.41	1.478	0.151
Hb (g/dl)	10.41±1.81	11.34±1.50	2.818	0.004*
PCV (%)	34.04±4.99	36.44±3.93	2.697	0.007*
RBC ($x10^{12}$/l)	3.89±0.73	3.89±0.56	0.000	>0.05
MCV (fl)	88.85±12.93	93.28±15.30	1.593	0.27
MCH (pg)	26.84±5.24	28.88±4.23	2.152	0.026*
RDWCV	16.46±2.35	15.14±2.87	2.588	0.019*

TSI: Total Serum Iron; TIBC: Total Iron Binding Capacity; TS: Transferrin Saturation; Hb: Hemoglobin; PCV: Packed Cell Volume; RBC: Red Blood Cell; MCV: Mean Cell Volume; MCH: Mean Cell Hemoglobin; RDWCV: * Significance at p<0.05.

Table 3. Prevalence of Anemia of Chronic Disease in the study by selected variables

Variables	No: Anemic (%)	No: No-anemic (%)	Value of Anemia
TSI (µg/dl)	65 (31.4)	142 (68.6)	<60 µg/dl
Male (48)	24 (50)	24 (50)	<65 µg/dl
Female (159)	45 (28.3)	114 (71.7)	<60 µg/dl
TIBC (µg/dl)	6 (2.8)	201 (97.1)	<250 µg/dl
TS (%)	85 (41.1)	122 (59.9)	<15 %
Male	23 (47.9)	25 (52.1)	<20 %
Female	64 (40.2)	95 (59.7)	<15 %
Hb (g/dl)	87 (42.0)	120 (58.0)	<11 g/dl
Male	17 (35.4)	31 (64.6)	<12.4 g/dl
Female	75 (47.2)	84 (52.8)	<11.4 g/dl
MCV	41 (19.8)	166 (80.2)	<81 fl
MCH	93 (44.9)	114 (55.1)	<27 pg
Total % Average	(35.9)	(60.7)	

TSI: Total Serum Iron; TIBC: Total Iron Binding Capacity; TS: Transferrin Saturation; Hb: Hemoglobin; MCV: Mean Cell Volume; MCH: Mean Cell Hemoglobin * Significance at p<0.05.

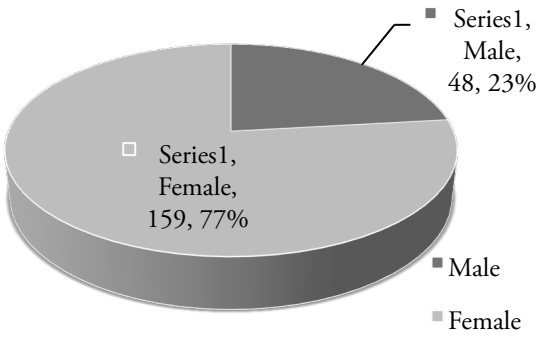

Figure 1. Prevalence of HIV/AIDS among male and female groups

iron status in HIV patients. These higher values of iron status may be a reflection of adequate iron stores, probably due to high levels of meat consumption among Makurdi residents. The significant influence of meat intake on iron stores among adults has been reported and serum iron levels decrease once body iron stores are depleted, and increase with iron overload.[9]

In an attempt to further evaluate the indicators of iron status in anemia of chronic disease, we stratified the test subjects on the basis of CD4 value (Table 2). Patients are adjudged to have AIDS with the CD4 count <200 cellsμ/L. In this study therefore, 55.2% of patients with CD <200 cells/mm^3 are anemic with iron status which did not differ significantly ($p>0.05$) from those patients with CD4 >200 cells/mm^3, of which 32.5% are anemic. However, close observation revealed that the indicators of iron status and anemia were relatively lower than that of the Table 1 above, which further revealed the impact of AIDS.

Among the indices of anemia, Hb, PCV, and MCH showed significant difference ($p<0.05$) compared with the patients with CD4 >200cells/mm^3 (Table 2). An MCV value below 81 fL indicates that the erythrocytes are microcytic (small), while a MCH below 27pg indicates that the erythrocytes are hypochromic (pale). A microcytic, hypochromic anemia occurs when body stores are depleted and the iron deficiency is severe, but both the MCV and MCH remain normal in early iron deficiency. In contrast to iron deficiency anemia, the anemia of chronic disease is usually, but not always, characterized by a normocytic, hypochromic blood picture, which could explain the relatively high percentage of especially HIV-infected patients with CD4 <200cells/mm^3. Furthermore, serum iron levels and transferrin saturation are low in chronic disease,[9] as was found in a low percentage of patients included in this study. The high levels of meat consumption among Makurdi residents have positively improved the iron status and anemia impacted by the disease similar to the report by Walsh *et al.*[14]

The prevalence of anemia described in this study (Table 3) showed that 44.9%, 42.0% and 41.1% of anemia described in this study was attributed by MCH, Hb and TS at values <27pg, <11g/dl and <15% respectively. This further confirmed the previous studies that reported that the anemia of chronic disease exhibited by HIV positive patients is normocytic hypochromic, and that used Hb concentration as an indicator of anemia at value less than 11g/dl.[15] Categorizing the prevalence of anemia by gender also showed that parameters of iron status contributed more to anemia (50% SI and 47.9% TS) in males while decreased Hb concentration is the major cause of anemia in females (47.2% Hb). While the lowest prevalence rates of anemia are contributed by TIBC (2.8%) and MCV (19.8) at values less than 250 μg/dl and <81fl respectively.

Figure 1 of this study showed that females have higher prevalence rate of HIV infection (77%) compared with the males. Anemia defined by Hemoglobin concentration showed that females are generally more anemic (47.2%) than males (35.4%). The higher prevalence of anemia in the female group is as a result of increased prevalence rate of HIV infection observed in the study. This can be explained by the fact that the females are generally more vulnerable than the males and generally share the same sex partner with other females. This is a typical phenomenon common among residents in Makurdi particularly among the youth. This observation is further proved by the highest prevalence rate of HIV infection among 25-34 age groups (Figure 2). This is in line with the study by Walsh *et al.*[14] who reported high prevalence of HIV infection in Mangaung, especially among women between 25 and 34 years of age.

CONCLUSION

The results of the study indicated that prevalence of HIV infection is higher in females attending FMC Makurdi, especially among patients between 25 and 34 years of age. Although, on average, the parameters of iron status did not indicate iron deficiency in the HIV-

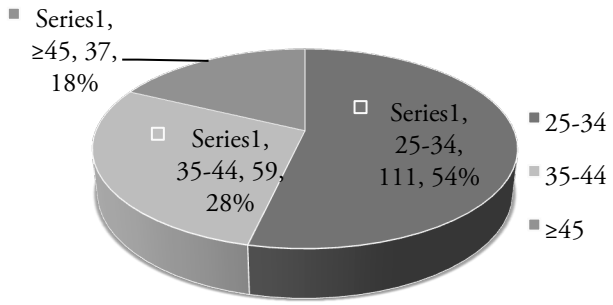

Figure 2. Prevalence of HIV/AIDS at different age groups

status groups and AIDS patients (defined by CD4 <200cells/mm^3), an average percentage of patients did have anemia of chronic disease (defined by Hb concentration <11g/dl), with HIV-infected women afflicted more often. The anemia is generally normocytic hypochromic. The iron status indicators contributed more to anemia especially in males while hemoglobin concentration impacted anemia more in females with the average prevalence of 36.9%.

We recommended that complete haemogram and serum ferritin of HIV/AIDS patients be investigated for differential diagnosis of anemia and effective management of their health conditions.

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