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Hematological Profile of HIV ART Naïve and HIV ART Experienced Individuals at the Aminu Kano Teaching Hospital North Western Nigeria

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Abstract
Nigeria has the second highest number of people living with HIV in the world after South Africa, with many factors contributing to increased rates of the infection in the country such as large population and poverty which prevails in Northern Nigeria. In Nigeria as in other parts of the world, the corner stone for HIV treatment is antiretroviral therapy (ART) and prophylaxis for the prevention of opportunistic infections (OIs). Haematologic complications like anaemia which is already a health burden in poverty endemic areas often lead to disease progression and eventually morbidity and mortality. Determining the hematological profile of HIV infected populace at different treatment stages of the infection could perhaps throw more light on the etiology of the hematological complications seen during the course of the disease in our populace. Blood samples collected from 200 subjects were used for Complete Blood Count and CD T lymphocyte determinations. The mean hemoglobin, Packed Cell Volume and Red Blood Cells concentrations were significantly lower (P≤0.05) in the HIV groups particularly the ART naïve group when compared with the apparently healthy groups. Anemia and neutropenia were the most common hematological abnormalities observed in the study with highest prevalence of anemia found in the ART naive group.
Keywords: Haematological, HIV, antiretroviral therapy, anemia

Introduction
Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) is a major global public health problem and constitutes one of the most serious socioeconomic and health issues facing Nigeria. Worldwide, Nigeria has the second highest number of new infections reported each year, and an estimated 3.70 percent of the population are living with HIV. Although HIV prevalence is much lower in Nigeria than in other African countries, such as South Africa and Zambia, the size of Nigeria's population (around 166.6 million) means that the disease burden is much higher (1). Nigeria recorded 300,000 new cases in 2012, even as the country was ranked as number eight among 12 countries in the world that have recorded a decline in the new HIV infection rate. The high HIV/AIDS related morbidity and mortality in developed countries has been drastically reduced by the advent of effective combination of antiretroviral therapy (2). The antiretroviral therapies hamper the growth of the virus, thereby causing the suppression of viral particle multiplication and eventually leads to a decreased viral load, thereby prolonging the patient's life span. The Impediments to effective use of triple antiretroviral therapy or highly active antiretroviral therapy (HAART) in public health systems in Sub-Saharan Africa include the cost of the drugs, the difficulties in administering and monitoring the drugs, fragile health infrastructures, weak drug procurement and distribution systems, as well as the side effects associated with the drugs and the development of drug resistance by the virus (3).
The Hematologic manifestations of the human immunodeficiency virus (HIV) infection are a well-recognized complication of the disease and may be clinically important in many patients especially in impoverished communities. Although HIV associated anemia is multi factorial, the principal factors are infiltration of the bone marrow by neoplasm or infection, use of myelosuppressive medications such as zidovudine, HIV infection itself, a decreased production of endogenous erythropoietin, hemolytic anemia that may result from RBC autoantibodies, or may also develop as a consequence of the use of various medications (4). Anemia has a profound effect on the quality of life of people by inducing such symptoms as loss of stamina, rapid heart rate and shortness of breath. It has also been identified as a risk factor for early death in patients with AIDS. Oladiende (5) reported that ignorance, poverty, malnutrition, low educational background and gender bias significantly contribute to high prevalence of anemia, and that these factors are rife in rural communities in Nigeria. Furthermore access to information and health services is much poorer in rural settlements than cities and rural dwellers are less likely to be informed with protective measures and practices against HIV infection.

Materials and Methods
Study population: A total of 200 consecutive individuals were enrolled for the study comprising 150 HIV infected patients ages 18 to 55 years seeking treatment at the S.S Wali Center for HIV, Aminu Kano Teaching Hospital (AKTH), Kano and 50 apparently healthy individuals (controls) recruited from the population of blood donors from the blood donor unit of the AKTH, Kano. Informed consent was sought from the subjects and

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baseline clinical details obtained from hospital records including basic demographic information. Ethical approval for the study was obtained from the ethical committee of the Hospital.

Sampling Techniques/ Data Collection: At the respective clinics, demographic data and necessary medical history of subjects was obtained from hospital records following consent given by subjects. Subjects were then divided into four groups of fifty individuals as follows: Group I:ART Naive HIV positive individuals- (have not commenced any treatment); Group II: Stable HIV individuals – (on Anti Retrovirals Therapy (ARTs) and prophylactics for at least 3 months without any clinical episodes); Group III: HIV infected individuals with opportunistic infections specifically pulmonary tuberculosis and pneumonia (also on ARTs) and Group IV: Control group (Apparently Healthy individuals).

Sample Collection and Processing: Exactly 2.00 ml of blood sample was collected from peripheral vein (antecubital vein puncture) of each subject using a 5ml syringe and then dispensed into Disodium Ethylene Diaminotetra-Acetate anticoagulant (EDTA) sample tubes. The whole blood was processed immediately for determination of CD4 T lymphocyte counts by flow cytometric analysis using CD4 easy count test kit (Partec GmbH, Germany) and the flow cytometer. Complete Blood Count was carried out on all samples collected using a fully automated analyzer (Sysmex KX 21 N haematology analyser) for the determination of White Blood Cells: WBC and differentials; Red Blood Cells (RBC) and their indices (MCV; MCH and MCHC), Haemoglobin, Packed Cell Volume (PCV).

Statistical Analysis : Data recording was done on Microsoft excel before being exported to Statistical Package for Social Sciences (SPSS) programme version 16 (Chicago, IL, USA). Analyses were carried out using both inferential and descriptive statistics with mean and standard deviations (SD) range and percentages. Microsoft Excel and Word in Windows 2007 were used for graphics and tables. The student t test and ANOVA were used to test for significant differences in means of various groups. All reported p-values <0.05 were considered statistically significant.

Results

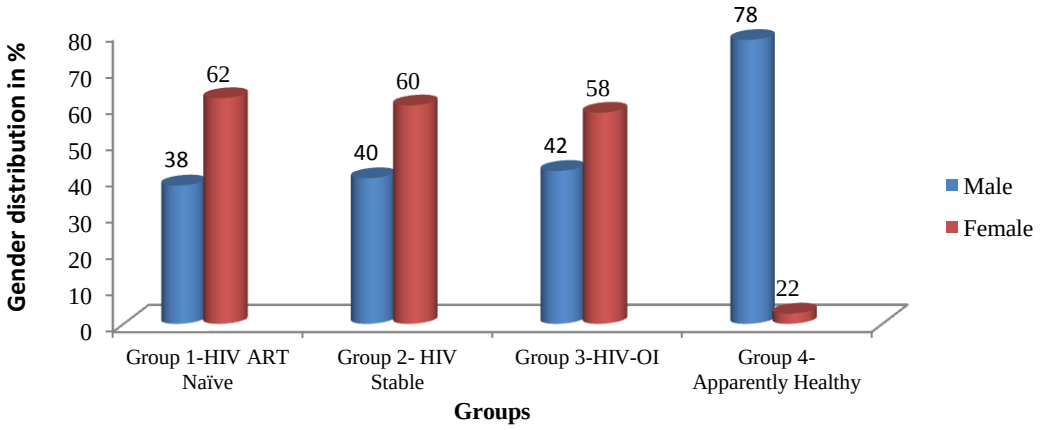

Figure 1: Gender Distribution of HIV Infected and Apparently Healthy Individuals (Control) groups attending Aminu Kano Teaching Hospital, Kano.

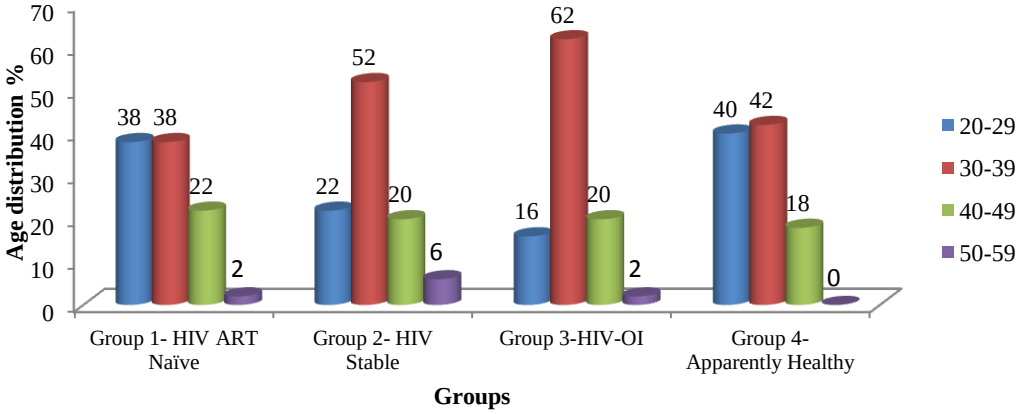

Figure 2: Age Distribution of study population based on decades across groups of HIV patients attending Aminu Kano Teaching Hospital, Kano.

Gender distribution between groups showed a higher percentage of females among the HIV groups than males while the apparently healthy group had a higher percentage of males than females (Figure 1). The mean age of study population was 34.29 ± 7.19 years, with males having a mean age of 35.41 ± 6.79 years and females 33.18±7.43years. There was a significant difference ($p \leq 0.05$) in age of subjects between groups. The highest number of individuals was observed in the third decade age range followed by the second decade range across the study population (Figure 2).

There was significant difference ($p \leq 0.05$) in CD_4 count between the apparently healthy group and the HIV infected groups with the HIV ART naïve group also significantly different ($p \leq 0.05$) from the HIV stable and HIV-OI groups (Figure 3). There was no significant ($p \geq 0.05$) relationship between CD_4 count and RBC in the HIV stable and HIV/OIs groups while a significant (≤ 0.05) relationship was found with the HIV ART naïve and apparently healthy groups. However, a significant ($p \leq 0.05$) relationship between CD_4 count and hemoglobin was found in the HIV ART naïve group (Table 1).

Table1: Correlation Coefficients (r) of CD_4 Count with Haematological Parameters of HIV Infected and Apparently Healthy Individuals attending Aminu Kano Teaching Hospital, Kano.

Group	**HIV ART Naive**	**HIV Stable**	**HIV-OI**	**Apparently Healthy**
Parameter	**r- value**	**r- value**	**r-value**	**r-value**
RBC	0.45**	-0.15	0.01	-0.30*
MCH	0.08	0.02	0.24	0.21
MCHC	0.20	-0.03	0.33*	0.13
MCV	-0.07	0.08	0.16	0.19
PLT	0.07	0.12	-0.11	0.28
HGB	0.31*	-0.16	0.19	-0.18
PCV	0.32*	-0.11	0.08	-0.27
WBC	-0.10	0.24	0.09	0.30*
LYM	0.22	0.29*	0.41**	0.30*
NEUT	-0.10	-0.18	-0.20	-0.32*

**correlation is significant at $P \leq 0.01$
*Correlation is significant at $p \leq 0.05$

Table 2: Haematological Parameters in HIV Infected and Apparently Healthy Individuals attending Aminu Kano Teaching Hospital, Kano.

Parameter	**HIV ART Naïve n=50**	**HIV Stable n=50**	**HIV-OIs n=50**	**Apparently healthy n=50**
RBC×10^6/µL	4.3±0.6^b	4.1±0.7^b	4.2±0.8^b	5.1±0.5^a
MCH (pg)	26.6±2.6^b	30.2±3.9^a	30.4±4.6^a	27.6±1.9^b
MCHC(g/dL)	32.5±1.5^b	33.9±1.5^a	33.5±1.7^a	33.6±1.1^a
MCV (fL)	82.1±6.7^b	91.1±13.8^a	90.8±10.9^a	81.9±3.9^b
PLT×10^3/µL	277.1±78.9^a	267.0±69.8^a	262.5±95.8^a	236.5±71.6^a
HGB (g/dL)	11.8±3.7^b	12.1±1.2^b	12.6±1.8^b	14.0±1.1^a
PCV (%)	34.3±5.5^c	36.1±4.8^c	37.6±5.1^b	41.7±2.9^a
WBC×10^3/µL	5.3±1.9^a	4.7±1.1^a	5.0±1.5^a	5.5±1.3^a
LYM (%)	35.8±12.2^c	42.6±11.6^b	40.0±10.9^d	46.4±10.2^a
NEUT (%)	51.6±14.5^a	47.1±12.6^c	48.0±15.2^a	42.2±10.7^b

Mean ± SD Values of Haematological Parameters in HIV Infected and Apparently Healthy Individuals.
a, b, c,d =Data along the same row with different superscript alphabets are statistically significant ($p \leq 0.05$)

The mean values of hematological parameters tested are shown in Table 2. A significant difference ($p \leq 0.05$) was observed between all the HIV groups and the apparently healthy group in most hematological indices with 30.80 % of individuals in the HIV ART naïve group having low levels of RBC count, 36.50 % in the HIV stable group, 32.70 % in the HIV-OI group and 0.00 % in the apparently healthy group. A significant difference ($p \leq 0.05$) was found between groups in hemoglobin levels. Low hemoglobin values (an index of anemia) was observed in the study population. Within the ART naïve group, mild anemia (HGB≤ 12g/dL) was observed in 44.00 % of individuals, moderate anemia (HGB≤10g/dL) in 22.00 % and severe anemia (HGB≤ 7g/dL) in 2.00

% of individuals. Severe anemia was also observed in the HIV-OI group though minimal with about 2.00 % of individuals. When compared with the apparently healthy group, the HIV groups had a larger percentage of individuals with either mild, moderate or severe anemia. Only mild anemia was observed in the apparently healthy group with 4.00 % of individuals (Figure 4). There was also a significant difference ($p \leq 0.05$) in terms of gender within the different groups. Females were predominantly more anaemic than males in the HIV groups. The lowest PCV level was recorded in the HIV ART naïve group (36.30 %). A significant difference was found between the apparently healthy group and the HIV groups with the HIV ART naïve and HIV-OI group significantly different ($p \leq 0.05$) from the HIV stable group. The highest level of neutrophil count was observed in the HIV ART naïve group with 57.10 % of individuals in the group having high neutrophil counts followed by the HIV-OI group with 28.60 %.

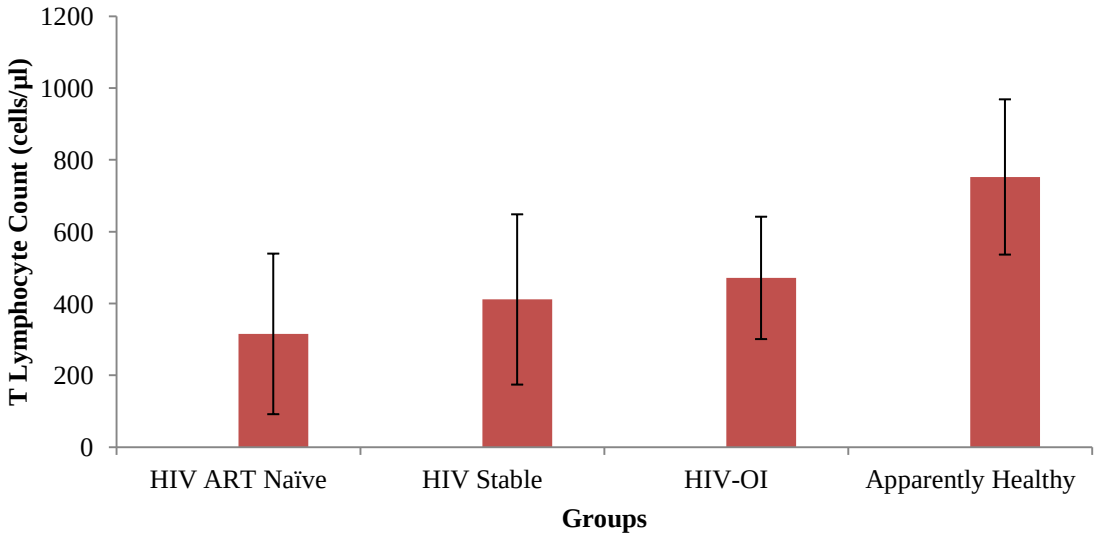

Figure 3: Variation in CD_4 Count in HIV Infected and Apparently Healthy Individuals attending Aminu Kano Teaching Hospital, Kano

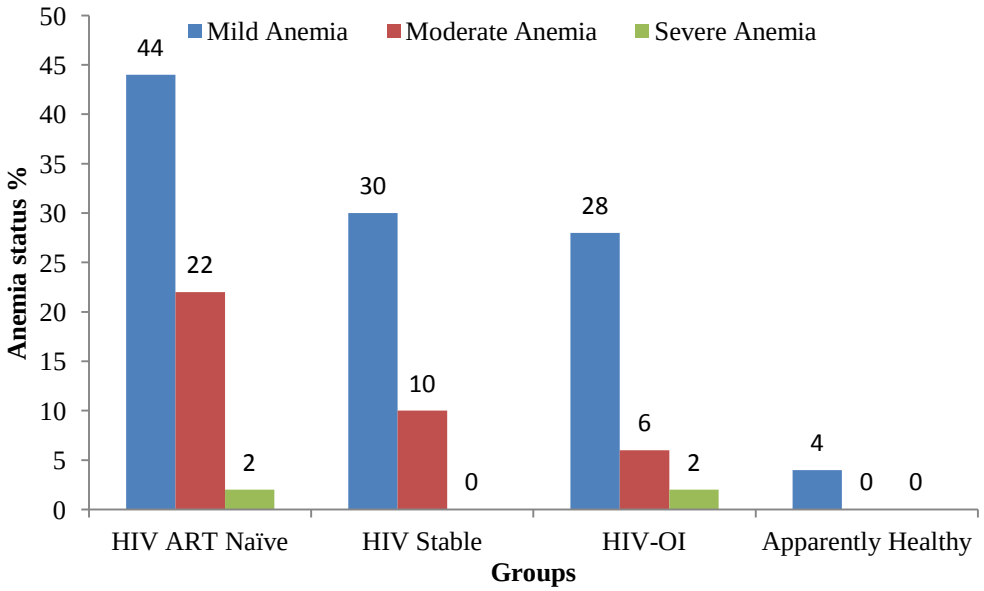

Figure 4: Distribution of Anemia in HIV Infected and Apparently Healthy Individuals attending Aminu Kano Teaching Hospital, Kano.

Discussion

The higher number of females seen in the HIV groups could be due to the fact that in some communities/societies females are more likely to seek treatment than males when encountered with medical

issues. In the apparently healthy group, the higher number of males than females found could be attributed to the fact that the apparently healthy group comprised a high number of blood donors from the donor clinic. The higher rate of male donors than females may be a result of screening criteria of the donors usually excluding females from blood donation. This study also stresses the preponderance of females in the prevalence of HIV infection and supports the established fact that women are biologically more vulnerable to HIV/AIDS and more likely to contact infection from their male partners as their sexuality and gender disadvantage in terms of culture, economic and social factors place them more at risk of infection than men. The World Health Organization (WHO) reported that HIV/AIDS affects females most severely in sub-Saharan Africa and women of reproductive age make up almost 57% of adults living with HIV, accounting for up to 80.00 % of HIV infected women in the world (6).

The highest number of individuals was found in the third decade age range (34.8 ± 7.6 years) in the HIV groups while the fifth and fourth decades recorded the lowest number of individuals both in the HIV groups and the apparently healthy group. This agrees with reports by several authors who recorded mean age of HIV patients in the third decade range. A study in Kano by Tamuno and Babashani (7), showed that about 86.00 % (88.00 % of HAART-naïve patients and 83.00 % of patients on HAART) of the study population fell within the 20 – 49 years age brackets which is known to be the sexually active age group with highest peak percentage observed within the 30 – 39 age groups for both sexes.

Within the HIV groups, 62.00 % (32/50) had CD_4 count of ≤350 cells/µl (the WHO recommended criterion for instituting ART) in the HIV ART naïve group, 46.00 % (23/50) in the HIV stable group and 26.00 % (13/50) in the HIV/OI group. This agrees with a study by Nwokedi *et al*., (8) who reported a mean CD_4 count of 302 cells/µl for HIV patients. Also, the lowest CD_4 count value was found in the HIVART naïve group (12 cells/µl). This result was expected as the ART naïve patients were those that had not initiated treatment with ARTs/prophylactics in contrast to other HIV groups that were on ART regimen and prophylactics. The HIV viral burden directly and indirectly mediates CD_4+T-cell destruction. The result of this destruction is failure of T-cell production and eventual immune suppression (9).

The most common hematological abnormalities were neutropenia and anemia, seen in 56% (112/200) and 37.00 % (74/200) respectively. Ogba *et al.,* (10) reported that hematological complications among HIV patients are generally marked with cytopaenias such as anemia, neutropenia, lymphopaenia and thrombocytopaenia and that the incidence and severity of the cytopaenia generally correlate with the stage of the disease, with anemia being the most commonly encountered hematological abnormality and a significant predictor of progression to AIDS or death.

Within the HIV groups the ART naïve had the highest prevalence of anemia (68.00 %), 40.00 % for stable group and 36.00 % for the OI group. A study by Dikshit *e. al.,* (11) reported that the most common hematological abnormality seen in HIV patients in their study was anemia (65.50 %). The HIV patients on ART/prophylactics and TB treatment had a lower prevalence of anemia than the ART naïve. The observed prevalence of anaemia in the HIV stable group could be attributed to ARTs. Omoregie *et al.,* (12) observed that HAART did not improve PCV of HIV patients and Mildvan (2003) reported that HIV patients on HAART still develop mild to moderate anemia. Zidovudine (AZT) has been reported by several authors to cause anemia by inhibition of hemoglobin synthesis and toxicity to bone marrow cells, particularly, erythroid lines (13).

The difference in the anaemic pattern in the HIV groups with the ART naïve recording highest prevalence could be linked to the effectiveness of ART treatment in the other HIV groups. Recent studies have confirmed the ability of HAART to correct or improve the anemia of HIV infection in contrast with other studies. As indicated in many studies, low CD_4 count has been found in most HIV patients with anemia, particularly ART naïve patients. It may be possible to postulate that ARTs while increasing CD_4 count levels may also be improving hemoglobin levels although some ARTs like zidovudine have been implicated in anemia among HIV patients on this therapy. Also, the possibility of other factors that may interfere with RBCs and hemoglobin along with PCV levels thereby leading to anemia should be considered. Neutropenia is frequently observed in advanced stages of HIV infection after development of AIDS, and has been associated with certain types of antiretroviral medications used to treat HIV infection. The reduced incidence of anemia, the overall improvement in PCV and hemoglobin concentration in the HIV Stable group who are on ART in this study confirms the effectiveness of ART in improving the quality of life of HIV patients. Neutropaenia was common in this study (48.60 %) in the HIV groups. Neutropaenia is a common manifestation of HIV infection itself, Hepatitis B infection and Tuberculosis. Munyazesa *et al*., (14) in a Rwandan study reported that neutropenia was independently associated with low CD_4 lymphocyte count, and that this suggests that the stage of HIV-infection is an important determinant to pretreatment neutropaenia.

Conclusion: Anaemia and neutropaenia were the most common haematological abnormalities found in this study within all HIV groups. The finding that the ART naive HIV patients had significantly higher prevalence of haematological abnormalities when compared with the ART experienced groups indicates the need for large scale and longitudinal study for further characterization of HIV related hematological abnormalities. Within the HIV groups, the HIV ART naïve group had lower levels of RBC and its indices, haemoglobin and PCV with

highest neutrophil counts. The use of differential diagnosis of HIV-related haematological abnormalities like anaemia and neutropaenia permits selection of appropriate treatment regimens by determining the etiology of the disorder. Treatment regimens for HIV-related anaemia should address the underlying cause and may include dietary iron supplementation, modulation of medications, and specific treatment of underlying causes.

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