VARIATIONS IN RED BLOOD CELL AND WHITE BLOOD CELL INDICES AMONG PERSONS LIVING WITH HIV ON TREATMENT AND TREATMENT NAÏVE AT NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL, NNEWI.

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ABSTRACT

Background
Human immunodeficiency virus (HIV) infection is associated with variable haematological manifestations which are more common among persons living with HIV (PLWH) than the general population.

Objectives
This cross-sectional study was designed to ascertain the variations in red blood cell and white blood cell indices in PLWH on antiretroviral treatment and those that are treatment naïve at NAUTH, Nnewi.

Methods
The study included a total of 180 adult participants which comprised eighty PLWH on antiretroviral treatment (PLWH on ART), forty PLWH not on ART (PLWH non-ART) and sixty HIV negative control subjects. Red blood cell and white blood cell indices were measured using Diatron'shaematology auto-analyser.

Results
The Red blood cell count, Haematocrit and Haemoglobin levels were significantly lower in PLWH not on ART when compared to PLWH on ART and HIV negative subjects. Mean cell haemoglobin concentration (MCHC) were significantly lower in PLWH on ART and PLWH not on ART compared to HIV negative subjects. The Mean cell volume (MCV) was significantly higher in HIV negative subjects compared to PLWH on ART and PLWH not on ART, while the total white blood cell count was significantly higher in HIV negative subjects compared to PLWH not on ART. The monocyte count was significantly higher in PLWH on ART compared to PLWH not on ART and HIV negative subjects.

Conclusion
HIV infection results in significant variations in some red cell and white cell indices. The negative variations improve among PLWH on ART compared to PLWH not on ART. This affirms the favourable prospects of antiretroviral therapy in resolving derangements in some haematological parameters.

Key words:
HIV, Red blood cell, white blood cell, antiretroviral therapy

INTRODUCTION
HIV infection is a global public health problem [1] and affects about 38.4 million individuals globally with sub-Saharan African countries including Nigeria having a high burden of the disease [2]. The impact of HIV infection extends beyond immune system dysfunction to disturbances in the hematopoietic systems which are commonly seen in HIV-infected patients. This is often marked by a reduction in blood cell counts, among which anemia, leucopenia, and thrombocytopenia are notable clinical complications that frequently emerge early and persist irrespective of disease symptoms [3][4][5]. Anaemia, thrombocytopenia and neutropenia have been found to be more common in HIV patients than in the general population. This may be caused by HIV itself, opportunistic infections or certain antiretroviral drugs (e.g. Zidovudine) [6][7]

These haematological abnormalities are known to disrupt the quality of life of people with HIV / AIDS [8] and are strong independent predictors of morbidity and mortality in HIV infection [9]. Therefore, these cytopenias serve as common indicators and crucial predictive tools for evaluating disease progression and therapeutic outcomes in individuals with human immunodeficiency virus (HIV) infection [10].

According to Bachuet al [11], in recent years, the advent of antiretroviral therapy (ART) has transformed the management of HIV infection. ART not only suppresses viral replication but also allows immune reconstitution, leading to

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improvements in CD4 counts and a potential mitigation of these hematological abnormalities. This study was therefore designed to assess the variations in the levels of red cell and white blood cell parameters in PLWH on antiretroviral therapy (ART), PLWH not on ART and HIV negative individuals in order to determine the impact of HIV infection and initiation of antiretroviral therapy on these blood cells and as well as ascertain their prognostic potentials as routine investigations carried out in the management of HIV patients.

METHODS

Study design
This study was conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State. NAUTH is a tertiary health institution owned by the Federal Government of Nigeria. It has an HIV clinic that serves patients from within and outside the state. This research was a cross-sectional study designed to assess the Variations in Red blood cell and white blood cell indices among persons living with HIV (PLWH on ART) on treatment and treatment naïve at (PLWH non-ART) NAUTH Nnewi. They were recruited by Purposive sampling technique. Sample size was calculated using G*Power software (version 3.0.10).

The calculated total sample size was 120 (40 per group) at 80% power, medium effect size and at significance level of 0.05A total of 180 adult male and female participants aged 18-65 years were recruited. This comprised 80 PLWH on ART (40 males and 40 females), 40 HIV PLWH not on ART (22 males and 18 females) and 60 HIV negative (control) participants (30 males and 30 females).This study excluded pregnant women, those who have co-morbidities such as tuberculosis, diabetes, hypertension, kidney disease, hepatic disease and other chronic illnesses as well as those who withheld their consent to participate in the study.

Ethical approval for this study was obtained from the Ethics Committee of the Nnamdi Azikiwe University Teaching Hospital Nnewi. Informed consent was sought and obtained from the participants, after a clear explanation of the objective of the study. A well-structured questionnaire was administered to each participant to obtain socio-demographic information on the age, gender and clinical details such as ART status and compliance, details of other medications that the subjects may have been on and other known disease or condition or co-morbities that the patient may have which may affect the result of the study.

Blood sample collection and analysis
A standard venepuncture technique was employed in the collection of blood samples. A soft tubing tourniquet was applied on the upper arm of the subject to enable the veins to be seen and felt. The subject was asked to make a tight fist in order to make the veins more prominent. A suitable vein (cubital vein) was selected for venepuncture. The puncture site was sterilized with 70% ethanol and allowed to dry. With the thumb of the left hand holding down the skin below the puncture site, the venepuncture was made with the bevel of the needle facing upwards in the line of the vein. The plunger of the syringe was withdrawn to allow free flow of blood into the syringe. A total of four millilitres (4mls) of blood was collected from each participant, 3ml was dispensed into a bottle containing di-potassium salt of Ethylenediamine tetra-acetic acid (K2-EDTA) at a concentration of 1.2 mg/ml of blood and was used for measurement of red cell and white cell indices. One millilitre was dispensed into plain container and allowed to clot, and serum extracted for HIV screening.

HIV rapid testing by serial testing algorithm.
The rapid HIV test kits consisting of 3 different antigens were used for the screening of HIV infection by serial testing algorithm. The first test was performed using Determine test kit (Abbott Laboratories, USA) and if positive a second test was performed using Unigold (Trinity Biotech, Ireland), if positive the participant was considered positive for HIV but if negative, the third test which acts as tie breaker was carried out using Stat pak (Chembio Diagnostic System, USA). Measurement of red blood cell and white blood cell parameters using Diatron'shaematology auto-analyser)

Procedure
Blood specimens collected in EDTA containers were mixed using mechanical haematology blood mixer for 5 minutes. The properly mixed sample was brought to the aspiration chamber of the analyser and the 'Aspirate' button pressed while the specimen container was held to ensure that the correct volume was aspirated before removing it. It was then allowed to run, and the displayed results were printed from the analyser.

Statistical analysis
The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) version 26. The

significance of differences in mean values between groups was analyzed using independent t-test, while significance of the differences in the mean values among different groups was evaluated using one-way ANOVA. $P < 0.05$ will be considered statistically significant.

RESULTS

Red blood cell counts, and Haematocrit levels were significantly lower in PLWH not on ART when compared to PLWH on ART and HIV negative subjects (P<0.05). Also, haemoglobin level and Mean cell haemoglobin concentration (MCHC) were significantly higher in HIV negative subjects compared to PLWH on ART and PLWH not on ART (P<0.05). Moreover, they were significantly higher in PLWH on ART when compared with PLWH not on ART (P<0.05). The Mean cell volume (MCV) was significantly higher in HIV negative subjects compared to PLWH on ART and PLWH not on ART (P<0.05) while the Mean cell haemoglobin (MCH) was significantly lower in PLWH on ART compared to PLWH not on ART and HIV negative control subjects (P<0.05) (Table 1).

The total white blood cell count was significantly higher in HIV negative subjects compared to PLWH not on ART (P=0.003). The mean values of the monocyte were significantly higher in PLWH on ART compared to PLWH not on ART as well as HIV negative subjects (P<0.05 respectively). Granulocyte was significantly higher in HIV negative subjects compared with PLWH on ART and PLWH not on ART (P<0.05). (Table 2).

Among the PLWH on ART the mean Monocyte count and Haematocrit was significantly higher in the males compared to the females (P= 0.016 and 0.046 respectively). However, there was no significant difference when the other parameters were compared between the males and females (P>0.05) (Table 3).

The red blood cell count, haemoglobin and Haematocrit values were significantly higher in Male PLWH not on ART compared to female PLWH not on ART (P<0.05). However, there was no significant difference when the other parameters were compared between male and female PLWH not on ART (P>0.05) (Table 4).

DISCUSSION

In our study, the levels of red blood cell count (RBC), haemoglobin (HGB) and haematocrit (HCT) decreased significantly in PLWH not on ART compared to HIV negative subjects and PLWH on ART. This implies that HIV infection results in significant decrease in these red cell parameters, but the values increase significantly in HIV subjects with treatment to values like that in HIV uninfected subjects.

This corroborates the knowledge that antiretroviral therapy plays a vital role in improving the haematological abnormalities and the general health conditions of HIV infected patients. According to Ramezaniet al [12] anaemia is a frequent complication of HIV infection with mild to moderate anaemia observed in 46% of HIV infected patients. Our finding of lower values of RBC, HGB and HCT in HIV infected treatment naïve subjects compared to negative controls agrees with many other similar studies [9][13][14].

Conversely, Anyiamet al [15] found a significantly higher levels of HCT and RBC in PLWH on ART compared to PLWH not on ART while Obiomaet al [16] found a significant higher HCT in HIV negative subjects compared to PLWH on ART and PLWH not on ART but with higher values in PLWH on ART compared with PLWH not on ART. Furthermore, our finding of a reduction in the levels of Haemoglobin between HIV infected and uninfected subjects agrees with the findings of Khareet al [17], it however disagrees with the findings of Abdollahi and Shoar [18] which showed no significant difference.

The result of our study also indicated that mean cell volume (MCV) increased significantly in PLWH on ART and PLWH not on ART compared to HIV negative subjects. This implies that whether on antiretroviral treatment or not the MCV of PLWH are increased compared to HIV uninfected subjects. This disagrees with previous studies that found a significantly higher MCV in PLWH on ART compared to treatment naïve and control subjects [14][15]. Moreover, this finding disagrees with that of Haile et al. [13] that found higher values in HIV negative controls compared to HIV infected subjects.

The mean cell haemoglobin (MCH) was significantly lower in PLWH on ART compared to PLWH not on ART and HIV negative subjects. This means that a decrease in MCH accompanies antiretroviral treatment in HIV subjects. This may imply that the antiretroviral therapy has a negative effect on MCH. This finding agrees with other studies that observed a significant decrease in MCH in PLWH on ART compared to PLWH treatment naïve subjects [15][19]. It however does not align with the findings of Ngwu and Eneh [14]

that found no significant difference in MCH among the three groups.

Mean cell haemoglobin concentration (MCHC) was significantly lower in PLWH on treatment compared to PLWH not on treatment and HIV negative controls and significantly lowers in PLWH not on treatment compared to HIV negative control. This finding implies that HIV infection is accompanied by a reduction in MCHC with a further significant reduction seen in PLWH on ART. The finding of a significant decrease in PLWH on ART compared to the controls agrees with the finding of Haile et al [13] and Ngwu and Eneh [14]. However, Anyiamet al [15] found that there was no significant difference between PLWH that are treatment naïve compared with HIV negative controls and PLWH on ART which disagrees with our own finding. Moreover, in agreement with our study, Tilahunet al. [19] found a lower MCHC in PLWH on ART compared with PLWH not on ART.

In this study, the total WBC was significantly lower in PLWH not on treatment compared to HIV negative subjects, but the value does not differ between HIV negative subjects and PLWH on ART. This may imply that with HIV infection is accompanied by a decrease in total WBC count, but with antiretroviral therapy the WBC count increases to a value that did not differ significantly from the value of normal HIV negative subjects. In consonance with our finding, Anyiamet al [15] found no significant difference in total WBC between PLWH on ART and treatment naïve subjects while Haile et al. [13] found lower values of WBC in PLWH treatment naïve compared to negative controls. The mean value of the monocyte was significantly higher in PLWH on ART compared to PLWH not on ART and HIV negative subjects. This means that

antiretroviral therapy initiates a significant increase in the value of monocytes in HIV infected subjects. Granulocyte (GRAN) was significantly reduced in PLWH (on ART and those not on ART) compared to HIV negative subjects. This means that irrespective of treatment status, granulocytes decrease with HIV infection. This finding agrees with a previous study [13].

Gender differences are known to contribute to variations in many haematological parameters. This may result from hormonal variations and other gender-based physiological differences. In our study as expected RBC, HGB and HCT were all significantly higher in males compared to the females in each category. This is in alignment to the well-established gender differences in these parameters as supported by the findings of Jacob [20]. However other similar studies found no significant differences [14][15]. In our study among PLWH on ART, we found that monocyte was significantly higher in male compared to the females. This disagrees with the findings of Anyiamet al [15] that found no significant difference in monocyte when compared between male and female PLWH on ART.

CONCLUSIONS
Red blood cell count, haemoglobin and haematocrit were decreased significantly in PLWH not on ART compared to HIV negative subjects and PLWH on ART. Thus, HIV infection results in significant decrease in red cell indices which may predispose them to anaemia, this reduction in red cell indices improves with antiretroviral therapy. Also, there were significant gender variations in red cell and white cell indices among PLWH on ART and non-ART.

Table 1: Comparison of Red cell indices among PLWH on ART, PLWH not on ART and HIV negative (control) participants (Mean ± SD)

Groups	RBC (x1012/l)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)
(A) PLWH-ART (n=80)	4.22 ± 0.59	12.12 ± 1.32	39.90 ± 4.41	95.79 ± 10.18	29.50 ± 3.72	30.89 ± 0.85	14.62 ± 1.45
(B) PLWH-NON-ART (n=40)	3.49 ± 0.57	11.22 ± 1.57	33.21 ± 4.63	95.20 ± 8.16	31.33 ± 1.46	32.83 ± 1.77	14.70 ± 0.70
(C) HIV NEGATIVE (n=60)	4.27 ± 0.47	13.18 ± 1.50	38.79 ± 4.48	91.20 ± 6.89	30.50 ± 1.70	33.92 ± 1.38	14.68 ± 0.67
F(P) values	29.006 (<0.001*)	22.877 (<0.001*)	30.976 (<0.001*)	5.027 (0.008*)	6.202 (0.002*)	139.051 (<0.001*)	0.073 (0.930)
A vs B: P-value	<0.001*	0.004*	<0.001*	0.935	<0.001*	<0.001*	0.939
A vs C: P-value	0.820	<0.001*	0.325	0.007*	<0.001*	<0.001*	0.955
B vs C: P-value	<0.001*	<0.001*	<0.001*	0.050*	0.317	<0.001*	0.997

*P<0.05 = Significant

PLWH-ART = Persons living with HIV on antiretroviral therapy
PLWH NON-ART = Persons living with HIV not on antiretroviral therapy
RBC = Red blood cell count
HGB = Haemoglobin
HCT = Haematocrit
 MCV = Mean cell volume
MCH = Mean cell haemoglobin
MCHC = Mean cell haemoglobin concentration
RDW = Red cell distribution width

Table 2: Comparison of White cell indices among PLWH on ART, PLWH not on ART and HIV negative (control) participants (Mean ± SD)

Groups	WBC (x 109/l)	LYM (x 109/l)	MONO (x 109/l)	GRAN (x 109/l)
(A)PLWH-ART (n=80)	4.56 ± 1.25	2.11 ± 0.73	0.71 ± 0.44	1.76 ± 0.97
(B)PLWH-NON-ART (n=40)	4.20 ± 1.11	1.97 ± 0.48	0.44 ± 0.13	1.81 ± 0.73
(C)HIV NEGATIVE (n=60)	4.98 ± 0.94	1.88 ± 0.35	0.50 ± 0.11	2.61 ± 0.62
F(P) values	5.923 (0.003*)	2.836 (0.061)	13.075(<0.001*)	20.513(<0.001*)
A vs B: P-value	0.225	0.418	<0.001*	0.946
A vs C: P-value	0.077	0.052	<0.001*	<0.001*
B vs C: P-value	0.003*	0.723	0.607	<0.001*

*P<0.05 = Significant
PLWH-ART = Persons living with HIV on antiretroviral therapy
PLWH NON-ART = Persons living with HIV not on antiretroviral therapy
WBC = White blood cell count
LYM = Lymphocyte count
MONO = Monocyte count
GRAN = Granulocyte count

Table 3: Comparison of White blood cell and red cell indices between male and female PLWH on ART (Mean ± SD)

Parameters	Male PLWH on ART (n=40)	Female PLWH on ART (n=40)	t-values	P-values
WBC (x 109/l)	4.74 ± 1.33	4.38 ± 1.16	1.314	0.193
LYMP (x 109/l)	2.11 ± 0.69	2.11 ± 0.78	0.002	0.999
MONO (x 109/l)	0.83 ± 0.50	0.59 ± 0.34	2.455	0.016*
GRAN (x 109/l)	1.78 ± 1.09	1.74 ± 0.86	0.156	0.876
RBC (x 1012/l)	4.27 ± 0.65	4.16 ± 0.51	0.879	0.382
HGB (g/dl)	12.38 ± 1.41	11.87 ± 1.19	1.749	0.084
HCT (%)	40.86 ± 4.65	38.91 ± 3.97	2.025	0.046*
MCV (fl)	97.14 ± 9.60	94.40 ± 10.67	1.218	0.227
MCH (pg)	29.64 ± 3.46	29.36 ± 4.01	0.339	0.736
MCHC (g/dl)	30.25 ± 0.72	30.52 ± 0.95	1.434	0.155
RDW (%)	14.76 ± 0.78	14.48 ± 1.91	0.863	0.391

*P<0.05 = Significant

Table 4: Comparison of White blood cell and red cell indices between male and female PLWH not on ART (Mean ± SD)

Parameters	Male HIV not on ART (n=22)	Female HIV not on ART (n=18)	t-values	P-values
WBC (x 109/l)	4.38 ± 1.28	3.99 ± 0.85	1.098	0.279
LYMP (x 109/l)	1.97 ± 0.56	1.97 ± 0.39	0.042	0.967
MONO (x 109/l)	0.46 ± 0.10	0.41 ± 0.16	1.176	0.247
GRAN (x 109/l)	1.98 ± 0.81	1.61 ±0.57	1.623	0.113
RBC (x 1012/l)	3.62 ± 0.69	3.34 ± 0.35	7.814	0.008*
HGB (g/dl)	11.56 ± 1.85	10.80 ± 1.05	4.926	0.033*
HCT (%)	34.19 ± 5.71	32.02 ± 2.50	5.465	0.025*
MCV (fl)	94.55 ± 9.83	96.00 ± 5.68	0.556	0.582
MCH (pg)	31.32 ± 1.55	31.33 ± 1.37	0.032	0.974
MCHC (g/dl)	32.50 ± 2.02	33.22 ± 1.35	1.297	0.202
RDW (%)	14.68 ± 0.69	14.71 ± 0.73	0.130	0.897

*P<0.05 = Significant

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