ORIGINAL ARTICLE

Haematological Changes in HIV Subjects Receiving Antiretroviral Treatment in Enugu State University of Science and Technology Teaching Hospital, Enugu, Nigeria

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Summary

BACKGROUND

Human immunodeficiency virus (HIV) infection is connected with a wide range of haematological abnormalities. The use of antiretroviral drugs can have positive or negative changes in the haematological parameters. This study aimed to determine red cell indices and a reticulocyte count of HIV- patients taking antiretroviral treatment and those not yet been placed under antiretroviral treatment.

MATERIALS AND METHODS

The study was conducted at Enugu state university of Science and Technology teaching hospital (ESUTH) in Enugu, Nigeria from June to November 2020. It was a case-control study. The study population was 60 subjects consisting of 40 (18 males and 22 females) HIV subjects under antiretroviral treatment (ART) and twenty (8 males and 12 females) HIV patients who are not under ART with an age range of 15-60 years respectively. Also, 28 healthy subjects were used as control. The Haemoglobin (HGB), Packed cell volume (PCV), Red cell count (RBC), Mean cell haemoglobin (MCH), Mean cell volume (MCV), Mean cell haemoglobin concentration (MCHC), Reticulocytes count (RC) Red cell distribution width- standard deviation (RDW-SD), Red cell distribution width-coefficient of variation (RDW-CV) and Human Immunodeficiency Virus (HIV) status of the study subjects were determined.

RESULTS

The results show a significantly increased level of PCV, HGB, MCV and RBC in HIV- subjects under antiretroviral treatment compared to those not under antiretroviral treatment ($p<0.001$). There was a significant decrease in PCV, HGB, RBC and RDW-CV of HIV subjects not under antiretroviral treatment compared to control subjects ($p<0.001$). Moreover, there was a significant decrease in MCHC ($p=0.004$) and RDW-CV ($p<0.001$) of HIV- subjects under antiretroviral treatment compared to control subjects.

CONCLUSION

There was a statistically significant decrease in MCHC, RDW-CV, PCV, HGB and RBC of HIV- subjects under antiretroviral treatment and HIV subjects not under antiretroviral treatment compared to control subjects. Packed cell volume HGB, MCV and RBC were also found to be higher in HIV- subjects under antiretroviral treatment compared to those not under antiretroviral treatment.

***Keywords**: HIV, Antiretroviral Treatment, Hemoglobin, Mean Cell haemoglobin Concentration, Red Cell Count*

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Introduction

Anaemia is one of the haematological complications that are commonly present in human immunodeficiency virus (HIV) infection. Anaemia becomes more noticeable as the disease progresses [1]. Morbidity and mortality in HIV patients are most times caused by anaemia [2]. Anaemia in HIV-infected patients is a sign of transition to acquired immune deficiency syndrome (AIDS) or death. Anaemia occurs in more than 70% of individuals living with HIV [3]. Anaemia is associated with a high risk of disease progression and death in both antiretroviral-treated and untreated individuals [4]. Changes in cytokine expression, chemotherapeutic treatment side effects, HIV-related metabolic disorders and micronutrient deficiencies are some of the factors that can cause HIV- related anaemia [5]. Microcytic hypochromic anaemia followed by macrocytic anaemia has been found in variable percentages in HIV patients, but normocytic normochromic is the most common morphological type of anaemia in HIV patients [6]. The normal healthy range of reticulocytes is 0.5% to 1.5% in adults. A reticulocyte count that is higher than normal can be a sign of anaemia [7]. In low-income countries like Nigeria, anaemia in HIV patients continues to be a source of concern. In Nigeria, different studies have been conducted to detect anaemia in HIV-infected individuals but data are still scarce in some areas. The purpose of this study was to determine red cell indices and a reticulocyte count of HIV- patients taking antiretroviral treatment.

Materials and Methods

Study site and design

This study was a case-control study. It was carried out at Enugu State University of Science and Technology Teaching Hospital (ESUTTH), Enugu, Nigeria. It is the second largest health care facility in Enugu which is the capital of Enugu state. HIV was one of the commonest causes of medical ward admissions in ESUTTH. A previous study at ESUTTH showed that out of 3,865 cases recorded that 503 (13.01%) were HIV with a case fatality rate of 127/503 (25.2%) [8].

Period of data collection

The study was conducted at Enugu state university of Science and Technology teaching hospital (ESUTH) in Enugu, Nigeria from June to November 2020.

Sample size

A total number of 88 subjects which were divided into three strata were randomly selected; the first strata included HIV-positive patients under ART, the second strata were HIV-positive patients not yet under ART and the third and last strata were the adult seronegative patients.

Subject recruitment

Eighty-eight (88) subjects were recruited by simple random sampling and categorized into three groups comprising; Forty adult HIV-positive subjects under antiretroviral therapy (ART)-Tenofovir, Lamivudine, Dolutegravir (18 males and 22 females), Twenty adult HIV-positive subjects not under antiretroviral therapy (8 males and 12 females) and Twenty-eight adult Sero-negative control subjects (13 males and 15 females) respectively.

Ethical considerations

The study protocol was approved by the Research Ethics committee of Enugu State University of Science and Technology Teaching Hospital (ESUTTH) Enugu. Each participant in

this study signed an informed consent form before blood samples were collected from them.

Sample collection

Five millimetres (5mls) of blood was aseptically drawn by venipuncture. Three millimetres (3mls) of the 5mls of the blood were aseptically dispensed into dipotassium salt of ethylene diamine tetra-acetic acid and were used for red cell indices and reticulocyte count. Two millimetres (2mls) of the 5mls of blood were dispensed into a plain sample bottle. It was allowed to clot and spun in a centrifuge at 3000 revolutions per minute (rpm) for 10 minutes to extract the serum which was used for HIV testing.

Statistical analyses

The data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 21.0) and evaluated using one-way analysis of variance (ANOVA). ANOVA ($P<0.05$) was considered statistically significant.

Rapid HIV antibody testing

Highly specific and sensitive rapid HIV antibody tests can be measured as screening or confirmatory tests. These kits are commercially available and can be used in remote areas because they don't require refrigeration. Whole blood, serum or plasma samples can be used here. These kits have inbuilt control making them easily performed and interpreted and quick to use (10 minutes).

Determine HIV 1/2 test

Principle

It is based on the immunochromatographic antibody test method. The antibody in the specimen (serum) with specific antigen bound to colloidal gold particles. The antibody-antigen colloidal gold complex then migrates along the membrane where it becomes captured by a line of a specific antigen to produce a colour change.

Procedure

Fifty microlitres (50µl) of the sample was placed on the sample application pad and read after 15 minutes. The positive result had a pink line in the test (T) area and another in the control (C) area while the negative test results had only a pink line in the control (C) area.

Reticulocyte count estimation

Principle

The reticulocytes are immature red cells containing ribosomal RNA. This ribosomal RNA is stained supravitally using brilliant cresyl blue which is an isotonic solution. It is incubated and a thin film is prepared afterwards, the film is examined microscopically. The reticulocyte count is expressed as a percentage or preferably in absolute number with the availability of RBC count.

Procedure

Two drops of brilliant cresyl blue and 4 drops of EDTA anticoagulated blood were added to a test tube mixed and incubated for 20 minutes at room temperature. The bottom of the tube was tapped to resuspend the cells. A thin film was made and allowed to air dry. The film was examined microscopically with a 10× objective lens to select the area where the RBC did not overlap and then examined using a 100×objective lens (oil immersion). 1000 cells were counted systematically noting the number of the reticulocyte.

Calculation

$$\text{Retics\%} = \frac{\text{number of reticulocytes counted} \times 100}{\text{no of red cells} + \text{reticulocyte counted}}$$

Absolute reticulocyte count = % retics count × RBC count

Estimation of red cell indices and red cell distribution width using an automated haematology analyzer

Principle

Whole blood is drawn through an aperture and a low-frequency electric current is applied between the two electrodes (internal and

external electrodes). Electrical resistance (impedance current) between the two electrodes occurs as the cells pass through the sensing aperture causing a voltage pulse which is proportional to the cell volume and the number of the pulse is proportional to the number of cells using a threshold circuit to discriminate and count cells of specific volume.

Procedure

Ethylene diamine tetra-acetic acid anticoagulated blood sample was placed on a haematology analyzer mixer to get a homogenous mixture. The switch on the analyzer was on after the sample has been placed under the tube holder. The tube holder slides into the sample and the sample was aspirated. The sample was placed back in the sample rack while the result on the display was recorded.

Results

Table 1 shows mean ± SD of PCV (28.200±5.094 %), HGB (9.430±1.763 g/dl) and RBC (1.376±0.394 x 10^{12}/l) of those not on ART were significantly lower compared with PCV (38.425± 4.284 %; 40.000± 5.228 %), HGB (12.675±1.409 g/dl; 13.400±1.782 g/dl) and RBC (4.305±0.579 x 10^{12}/l; 4.689±0.717 x 10^{12}/l respectively) of those on ART and control subjects ($p<0.05$). In addition, the mean ± SD of MCV (83.215±9.846 fl) of those not on ART were significantly lower compared with MCV (89.745±7.128 fl) of those on ART (p=0.034). Moreover, the RDW-CV of those on ART (10.260±0.867 fl) and those not on ART (11.025 ± 0.616 fl) were significantly lower compared with RDW-CV (15.168 ±0.689 fl) of control subjects ($p= < 0.001$). Table 2 shows the mean ± SD of HGB, PCV, RBC, MCH, MCV, MCHC, RC, RDW-SD, and RDW-CV of male and female HIV subjects on ART and those not on ART. The result shows that PCV of female HIV subjects not on ART was significantly lower compared with PCV of female HIV subjects on ART ($p=<0.001$), male HIV subjects on ART and male HIV subjects not on ART (p=0.011) respectively

Table 1

Comparison of Mean Values of Haemoglobin (HGB), Packed cell volume (PCV), Red cell count (RBC), Mean cell haemoglobin (MCH), Mean cell volume (MCV), Mean cell haemoglobin concentration (MCHC), Reticulocytes count (RC) Red cell distribution width- standard deviation (RDW-SD), Red cell distribution width-coefficient of variation (RDW-CV) of HIV subjects on ART, HIV treatment naïve subjects and control subjects

	PCV	HGB	RBC	MCV	MCH	MCHC	RDW-SD	RDW-CV	RC
HSA N=40	38.425 ±4.284	12.675 ±1.409	4.305 ±0.579	89.745 ±7.128	29.655 ±2.387	32.960 ±0.739	45.563 ±4.621	10.260 ±0.867	0.340 ±0.141
HTN N=20	28.200 ±5.094	9.430 ±1.763	1.376 ±0.394	83.215 ±9.846	28.365 ±3.716	33.225 0.125	46.735 ±3.834	11.025 ±0.616	0.270 ±0.113
C N=28	40.000 ±5.228	13.400 ±1.782	4.689 ±0.717	86.314 ±5.509	29.014 ±1.996	33.500 ±0.593	46.546 ±3.444	15.168 ±0.689	0.289 ±0.152
F(p-value)	40.778 (<0.001)*	38.773 (<0.001)*	29.355 (<0.001)*	5.479 (0.006)*	1.653 (0.198)	6.607 (0.002)*	0.743 (0.476)	16.623 (<0.001)*	2.058 (0.134)
HSA VS HTN	<0.001**	<0.001**	<0.001**	0.034*	0.348	0.081	0.555	0.453	0.105
HSA VS C	0.394	0.182	0.059	0.073	0.457	0.004*	0.576	<0.001**	0.352
HTN VS C	<0.001**	<0.001**	<0.001**	0.422	0.759	0.060	0.983	<0.001**	0.870

Key=p<0.05

Abbreviations: HSA= HIV subjects on ART; HTN= HIV treatment naïve subjects; C= control

Similarly, the HGB values of female HIV subjects not on ART were significantly lower compared with HGB values of female HIV subjects on ART (p=<0.001), male HIV subjects on ART (p=<0.001) and male HIV subjects not on ART (p=0.008) respectively. In addition, the MCV of female HIV subjects not on ART were significantly lower compared with MCV values of female HIV subjects on ART (p=<0.001), male HIV subjects on ART (p= <0.001) and male HIV subjects not on ART (p=<0.001) respectively. Moreover, the MCH of female HIV subjects not on ART was significantly lower compared with the MCH of male HIV subjects, not on ART (p=0.008).

Table 3 shows that there was no significant difference in the mean values of HGB, PCV, RBC, MCH, MCV, MCHC, RC, RDW-SD, RDW-CV of HIV subjects that are on ART and are within age ranges of 15-30 years, 31-45 years and 46-60 years (P>0.05).

Table 2

Comparison of Mean Values of Haemoglobin (HGB), Packed cell volume (PCV), Red cell count (RBC), Mean cell haemoglobin (MCH), Mean cell volume (MCV), Mean cell haemoglobin concentration (MCHC), Reticulocytes count (RC) Red cell distribution width- standard deviation (RDW-SD), Red cell distribution width-coefficient of variation (RDW-CV) of male and female HIV subjects on ART and HIV treatment naïve subjects.

	PCV	**HGB**	**RBC**	**MCV**	**MCH**	**MCHC**	**RDW-SD**	**RDW-CV**	**RC**
FHSA N=22	37.136 ±3.796	12.332 ±1.264	4.164 ±0.587	89.409 ±6.527	29.732 ±2.069	33.191 ±0.534	44.541 ±4.936	14.309 ±0.886	0.314 ±0.175
MHSA N=18	40.000 ±4.419	3.094 ±1.497	4.478 ±0.537	90.156 ±7.975	29.561 ±2.787	32.678 ±0.865	46.811 ±3.987	14.200 ±0.864	0.372 ±0.123
FHTN N=12	25.750 ±4.864	8.558 ±1.623	3.314 ±0.473	77.450 ±7.826	26.558 ±3.686	33.208 ±0.131	47.125 ±4.156	13.942 ±0.697	0.267 ±0.123
MHTN N=8	31.875 ±2.748	10.738 ±1.018	3.468 ±0.234	91.863 ±4.947	31.075 ±1.463	33.250 ±0.119	46.150 ±3.480	14.150 ±0.487	0.275 ±0.104
F(p-value)	32.603 (<0.001)*	29.227 (<0.001)*	4.193 (0.002)*	10.397 (<0.001)**	5.816 (0.002)*	3.493 (0.021)*	1.306 (0.282)	0.548 (0.652)	1.626 (0.194)
FHSA VS MHSA	0.152	0.331	0.306	0.989	0.996	0.149	0.386	0.979	0.606
FHSA VS FHTN	<0.001**	<0.001**	0.386	< 0.001**	0.064	0.999	0.386	0.552	0.799
FHSA VS HTN	0.003*	0.013*	0.605	0.696	0.234	0.961	0.755	0.924	0.880
MHSA VS FHTN	<0.001**	<0.001**	0.008*	< 0.001**	0.111	0.084	0.997	0.804	0.126
MHSA VS MHTN	<0.001**	< 0.001**	0.606	0.909	0.294	0.008*	0.973	0.998	0.200
FHTN VS MHTN	0.011	0.008*	0.422	<0.001**	0.008*	0.882	0.940	0.860	0.998

Key= p<0.05

Abbreviations: FHSA= Female HIV subjects on ART; MHSA= Male HIV subjects on ART; FHTN= Female HIV treatment naïve; MHTN= Male HIV treatment naïve

Table 3

Comparison of Mean Values of Haemoglobin (HGB), Packed cell volume (PCV), Red cell count (RBC), Mean cell haemoglobin (MCH), Mean cell volume (MCV), Mean cell haemoglobin concentration (MCHC), Reticulocytes count (RC) Red cell distribution width- standard deviation (RDW-SD), Red cell distribution width-coefficient of variation (RDW-CV) of HIV subjects on ART across the different age range (15-30 years, 31-45 years and 46-60 years).

	PCV	HGB	RBC SD	MCV	MCH	MCHC	RDW-	RDW-CV	RC
HA1 N=16	38.313 ±4.586	12. 681 ±1.507	4.243 ±0.651	90.506 ±6.345	30.238 ±2..289	33.244 ±0.339	44.531 ±4.739	14.244 ±0.914	0.306 ±0.124
HA2 N=14	37.000 ±3.883	12.221 ±1.236	4.176 ±0.492	89.236 ±7.405	29.271 ±2.189	32.900 ±0.671	45.571 ±4.189	14.321 ±0.886	0.336 ±0.164
HA3 N=10	40.600 ±3.777	13.300 ±1.361	4.584 ±0.531	89.240 ±8.493	29.260 ±2.834	32.590 ±1.106	47.200 ±4.988	14.200 ±0.849	0.400 ±0.125
F(p-value)	2.196 (0.126)	1.778 (0.183)	1.645 (0.207)	0.145 (0.865)	0.785 (0.463)	2.695 (0.081)	1.028 (0.368)	0.059 (0.943)	1.397 (0.260)
HA1 VS HA2	0.676	0.634	0.946	0.871	0.474	0.220	0.801	0.970	0.848
HA1 vs HA3	0.367	0.536	0.330	0.914	0.636	0.213	0.385	0.992	0.174
HA2vs HA3	0.083	0.143	0.163	1.000	1.000	0.716	0.683	0.939	0.531

Key=p<0.05

Abbreviations: HA1= HIV subjects on ART and between 15-30 years; HA2= HIV subjects on ART and between 31-45 years; HA3= HIV subjects on ART and between 46-60 years

Table 4

Comparison of Mean Values of Haemoglobin (HGB), Packed cell volume (PCV), Red cell count (RBC), Mean cell haemoglobin (MCH), Mean cell volume (MCV), Mean cell haemoglobin concentration (MCHC), Reticulocytes count (RC) Red cell distribution width- standard deviation (RDW-SD), Red cell distribution width-coefficient of variation (RDW-CV) of HIV treatment naïve subjects across the different age range (15-30 years, 31-45 years and 46-60 years).

	PCV	HGB	RBC	MCV	MCH	MCHC	RDW-SD	RDW-CV	RC
HTN 1 N=6	28.400 ±6.348	9.660 ±2.317	3.490 ±0.431	80.660 ±12.148	29.380 ±5.389	33.240 ±0.089	46.020 ±2.794	13.920 ±0.602	0.300 ±0.158
HTN 2 N=9	31.000 ±7.064	8.817 ±2.377	3.173 ±0.574	82.600 ±10.807	27.617 ±3.750	33.250 ±0.122	46.617 ±4.582	14.200 ±0.723	0.367 ±0.052
HTN 3 N=5	31.000 ±0.707	10.300 ±0.212	3.420 ±0.109	90.640 ±1.625	30.140 ±0.545	33.180 ±0.084	49.660 ±1.038	14.000 ±0.748	0.300 ±0.071
F(p-value)	0.871 (0.442)	0.794 (0.473)	0.855 (0.448)	1.557 (0.248)	0.646 (0.540)	1.722 (0.504)	1.792 (0.205)	0.239 (0.791)	0.813 (0.465)
HTN1vs HTN2	0.887	0.827	0.555	0.959	0.816	0.987	0.962	0.769	0.663
HTN1vs HTN3	0.663	0.820	0.920	0.271	0.948	0.543	0.088	0.981	1.000
HTN vs HTN3	0.345	0.357	0.590	0.258	0.315	0.527	0.328	0.897	0.251

Key= p<0.05

Abbreviations: HTN 1= HIV treatment naïve subjects, between ages of 15-30 years; HTN 2= HIV treatment naïve subjects, between ages of 31-45 years; HTN 3= HIV treatment naïve subjects, between ages of 46-60 years

Table 4 shows that there was no significant difference in the mean values of HGB, PCV, RBC, MCH, MCV, MCHC, RC, RDW-SD, or RDW-CV of HIV subjects that are not on ART and are within age ranges of 15-30 years, 31-45 years and 46-60 years (P>0.05).

Discussion

This is a study on HIV patients receiving ART treatment at ESUTTH to determine if there is a statistically significant difference in some haematological parameters between those on ART and those not yet on ART. One of the major purposes of the haematological test is to detect anaemia. Packed cell volume is the percentage of red blood cells in peripheral blood. A decreased PCV means red blood cell loss due to any of the following reasons such as cell destruction, blood loss, and failure of bone marrow production. An increased PCV can be due to dehydration or an abnormal increase in red blood cell production [9]. The present study result showed decreased PCV in HIV patients, not on ART which is in line with the observation made by Ballah *et al*., (2013) on HIV subjects on HAART in Northern Nigeria [10]. Haemoglobin plays a crucial role in maintaining the shape of red blood cells. Normal red blood cells are round with narrow centres that resemble a doughnut without a hole in the middle. A decreased haemoglobin level is referred to as anaemia A lower than normal number of red blood cells is also anaemia and haemoglobin levels reflect this number [11]. The decrease in haemoglobin level of those not yet ART treatment observed in this study is in agreement with previously published reports [12-15]. Red blood cells carry oxygen from the lungs to the tissues. The mature human red blood cell is covered with a membrane composed of lipids and proteins, lacks a nucleus, and contains haemoglobin [16]. Some of the ART drugs are associated with neutropenia and macrocytic anaemia [17]. The red blood cell count of HIV subjects not on ART was found to be significantly lower compared with control subjects. This observation is in agreement with the finding of Malapati *et al*., (2020) [18]. The significant decrease in MCV and RDW-CV compared to control subjects indicates that HIV-positive individuals experience microcytic hypochromic anaemic conditions. Heterosexual sex and marriage have been the major source by which women got infected with HIV. However, the impact of HIV is often higher in women compared with men This may be due to a combination of social, cultural, economic and biological factors that makes women more vulnerable to HIV infection [19]. In this study PCV, HGB, MCV and MCH of female HIV-positive subjects not on ART were found to be significantly lower compared with their male counterpart. The finding is in line with a study carried out by Jacob, (2017) in a study done at Ido-Ekiti, Nigeria [20]. In this study, HIV subjects on ART treatment shows better improvement in haematological parameters compared to those, not on ART treatment of both sexes, which is in line with the earlier report by Kumarasamy *et al*., (2008)' that reported that both men and women positive with HIV showed consistent improvement during treatment [21].

Study limitations

This study did not include the duration of drugs to which the patients were exposed. Several antiretroviral (ARV) drugs most importantly nucleoside reverse transcriptase inhibitors, are known to cause anaemia in adults.

Conclusion

This study indicated that PCV, HGB, RBC, MCV and RDW-CV were found to be statistically significantly lower in HIV treatment naïve subjects. Similarly, PCV, HGB, MCV and MCH were also significantly lower in female HIV treatment naïve subjects. These

observations suggest the presence of microcytic hypochromic anaemia in HIV subjects that are not receiving ART treatment.

Recommendations

Based on the finding of reduced levels of red cell indices and reticulocyte count in HIV treatment naïve subjects, intake of ART drugs is recommended for all patients with HIV to prevent anaemia in HIV patients. Future studies should be done with larger sample size.

Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

Author A' designed the study, wrote the protocol and wrote the first draft of the manuscript, performed the statistical analysis and managed the analyses of the study. Author B' managed the literature searches. All authors read and approved the final manuscript.

Short Running Title

Haematological changes in HIV subjects receiving antiretroviral treatment

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