Haematological Indices of HIV Seropositive Subjects at Nnamdi Azikiwe University Teaching Hospital (Nauth), Nnewi

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Abstract

This study was carried out to provide information on the haematologic indices of HIV Patients on ART and those not on ART. Blood sample was collected from 160 subjects, 55 HIV-infected patients on antiretroviral therapy (ART), 55 HIV-infected patients not on ART and 50 apparently healthy HIV Sero-negative individuals as controls. Platelet (PLT), Total white blood cell Count (TWBC), Lymphocyte Count (LYMPH), Neutrophil Count (NEUT), Haemoglobin (HGB), Red Blood Cell Count (RBC), Mean Cell Volume (MCV), Mean cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV) and Retroviral screening (RVS) of the subjects were determined using standard methods after obtaining ethics approval and informed consent of the subjects. ANOVA and t-test were used for statistical analysis. HGB, RBC and MCHC were significantly lower in HIV patients (F=60.57; 19.26; 12.83; 9.84; P<0.05 respectively) compared with control subjects. HCT, HGB, MCV, MCH and RDW of HIV patients on ART were significantly higher (t=2.96; 3.04; 6.30; 5.10; P<0.05 respectively) compared with HIV patients not on ART.

Keywords: Haematological indices; HIV seropositive subjects; Nnamdi Azikiwe University Teaching Hospital (NAUTH); Nnewi

Introduction

Human Immunodeficiency Virus (HIV) infection represents the most important infection in the history of mankind [1]. The high rate of researches on this subject is due to the fact that HIV presents a complex knot for researchers to unravel. The human immunodeficiency virus epidemic has spawned a scientific effort unprecedented in the history of infectious disease research [2]. Despite dramatic advances in basic virology and clinical management, HIV infection has developed into a worldwide pandemic, with tens of millions of individuals infected by the virus and many millions more affected by it. In recent times, it has become one of the world's most serious health challenges and has brought about a global epidemic of massive proportions [3]. The first cases were reported in 1981 and by 1983; HIV was established as the cause of Acquired Immunodeficiency Syndrome (AIDS).

HIV attacks and destroys the CD4 cells. CD4 cells are T helper cells that lead the attack against infections and form an important component of immune response. Depletion of CD4 lymphocytes is the hall mark of HIV infection and predicts an individual's risk for infection with opportunistic pathogens as well as other complications of HIV infections [4,5]. The central feature of HIV disease is opportunistic infection and malignancy resulting from CD4 cell depletion. The remarkable reversal of this morbidity and mortality has resulted from the advent of potent antiretroviral therapy (ART) that allows the restoration of CD4 cell numbers and function. However, ART, especially those including protease inhibitors have been shown to cause, in a high proportion of HIV-infected patients, a metabolic syndrome (lipodystrophy/lipoatrophy, dyslipidemia, type 2 diabetes mellitus, insulin resistance that may be associated with an increased risk of cardiovascular disease [6].

HIV attacks blood cells especially the CD4 cells but may also have adverse effects on other blood cells such as the red cells and the platelets as well as other white cells. Haematological parameters are great indicators of health and disease [7,8].

Aim

This study is designed to evaluate the haematological indices of HIV seropositive subjects attending the out-patients HIV clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State.

Materials and Methods

Study area

The study was conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi in Anambra State of Nigeria.

Ethics consideration

Ethical approval was sought and obtained from the ethical committee of NAUTH Nnewi. The informed consent of the subjects was also obtained for the study.

Subject recruitment

One hundred and sixty (160) subjects were recruited and categorized into three groups as follows;

- Fifty-five adult HIV Sero-positive subjects on antiretroviral therapy (ART) Lamivudine (150 mg), Zidovudine (400 mg), Nevirapine (600 mg).
- Fifty-five adult HIV Sero-positive subjects not on antiretroviral therapy.
- Fifty adult Sero-negative control subjects.

Inclusion criteria

Participants included in this study were only HIV seropositive subjects between ages 18 to 65 who are assessing care at NAUTH HIV Care unit who also gave their consent.

Exclusion criteria

Those patients who were pregnant and those on other drugs aside Antiretroviral drugs were excluded from the study.

Sampling technique

Systematic random sampling method was employed in selecting the participants based on the inclusion criteria.

Sample collection

From each of the subjects, 2.5 ml of blood was collected asceptically by venepuncture and dispensed into EDTA container for FBC. Samples for FBC were analyzed within six hours of collection.

Laboratory analysis

Human Immunodeficiency Virus (HIV) by Serial Algorithm: Serial testing is the WHO standard for HIV testing. It means that when the result of the first test kit shows a non-reactive result, the tested sample was reported as HIV negative; but if the first test kit showed a reactive result, the sample was tested further by a second test kit and if the second test kit showed a reactive result, the tested sample was reported as HIV positive. But when the first two test kits gives a conflicting results (the first is reactive and the second is non-reactive), a third test using a different test kit as the tie-breaker was carried out to give the final result. For this study, Determine, Unigold and Stat-Pak HIV ½ test kits were the first, second and tie-breaker respectively.

Determine HIV 1/2 assay

Procedure: The protective foil was removed and 50 µl of the serum sample was applied to the sample pad. The result was read after 15 minutes.

Chembio Stat-Pak HIV 1/2 assay

Procedure: The test kit was removed from its pouch and placed on a flat surface. The test device was labelled with sample identification number. The 5 µl sample loop was touched on the specimen allowing the opening of the loop to be filled. The sample loop was then held vertically to touch the centre of the SAMPLE (S) well of the device to dispense approximately 5 µl of sample onto the sample pad. The Running Buffer bottle was held vertically and three drops (approximately 105 µl) of the Buffer was added slowly. The test result was read after 10 minutes of adding the Running Buffer.

Full blood count analysis using Sysmex Xp-300 analyzer (Sysmex Corporation Kobe, Japan)

Procedure: The whole blood sample in the EDTA container was mixed thoroughly by inverting the sample tube. The tube was then set to the sample probe and the start switch was pressed. The sample was removed when the status display changes from aspirating to running. The full blood count results comprising of Haematocrit, Platelet Count, Mean Platelet Volume, Erythrocyte Count, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration and Red Cell Distribution Width, Platelet Distribution width and Mean Platelet Volume was displayed about 60 seconds after starting the analysis [9].

Statistical Analysis

The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) version 20). Data were expressed as mean ± SD. The significance of differences in mean values between groups were analyzed using t-test, while significance of the differences in mean values among different groups was evaluated using one-way ANOVA. $p < 0.05$ was considered statistically significant.

Results

Table 1 shows comparison of mean ± SD of Platelets ($\times 10^9$/L), Total WBC ($\times 10^9$/L), Neutrophils ($\times 10^9$/L) and

Lymphocytes ($\times 10^9$/L) in HIV seropositive subjects on ART (group X), those not on ART (group Y) and Control subjects (HIV seronegative) (group Z).

The platelet count was significantly lower in group X (215.85 ± 60.42($\times 10^9$/L)) compared with group Y (252.51 ± 100.18 ($\times 10^9$/L) (P<0.05)). However, there was no significant difference in TWBC, LYM and NEUT among the three groups.

Table 1 Mean ± SD of PLT, TWBC, LYM and NEUT compared among HIV seropositive subjects ON ART, NOT ON ART and HIV seronegative subjects.

Groups	PLT (×10⁹/L)	TWBC (×10⁹/L)	LYM (×10⁹/L)	NEUT (×10⁹/L)
(X)HIV POS ON ART (n=55)	215.85 ± 60.42a	5.49 ± 2.04	2.34 ± 0.93	3.10 ± 1.60
(Y)HIV POS NOT ON ART (n=55)	252.51 ± 100.18	5.70 ± 1.58	2.47 ± 0.81	3.24 ± 1.09
(Z)CONTROL (n=50)	236.38 ± 61.01	6.04 ± 1.44	2.61 ± 0.85	3.40 ± 1.08
F-values	3.11	1.37	1.47	0.69
P- Values	(0.04*)	-0.26	-0.23	-0.5

A ≤ 0.05 when compared with NON ART; b ≤ 0.05 when compared with CONTROL; PLT=Platelets; TWBC=Total White Blood Cell; LYM=Lymphocyte; NEUT=Neutrophil

Table 2 shows comparisons of mean ± SD of Haemoglobin (g/dl), RBC ($\times 10^{12}$/l), MCV (fl), MCH (pg) and MCHC (%) in Seropositive on ART (group A), Seropositive not on ART (group B) and Control subjects (group C).

The mean ± SD of haemoglobin (HGB) was significantly lower in both group X (12.58 ± 1.51(g/dl)) and group Y (11.64 ± 1.70(g/dl) when compared with the corresponding values in the control (13.47 ± 1.31(g/dl)).

Table 2 Mean ± SD of HGB, RBC, MCV, MCH and MCHC compared among HIV seropositive subjects ON ART, NOT ON ART and HIV seronegative subjects.

Groups	HGB (g/dl)	RBC (×10¹²/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
(X)HIV POS ON ART (n=55)	12.58 ± 1.51b	4.15 ± 0.70b	90.48 ± 10.8a	30.78 ± 4.49a,b	33.97 ± 2.46b
(Y)HIV POS NOT ON ART (n=55)	11.64 ± 1.70b	4.34 ± 0.74b	79.90 ± 6.36b	27.11 ± 2.94	33.76 ± 2.61b
(Z)CONTROL (n=50)	13.47 ± 1.31	4.79 ± 0.50	90.49 ± 5.56	28.24 ± 2.33	35.51 ± 1.12
F-values	19.26	12.83	32.73	16.69	9.84
P- Values	0.00*	0.00*	0.00*	0.00*	0.00*

a ≤ 0.05 when compared with NON ART; b ≤ 0.05 when compared with CONTROL; HGB=Haemoglobin; RBC=Red Blood Cell; MCV =Mean Cell Volume; MCH=Mean Cell Haemoglobin; MCHC=Mean Cell Haemoglobin Concentration

Also, the mean values of Red blood cells (RBC) in group X (4.15 ± 0.70($\times 10^{12}$/l)) and group Y (4.34 ± 0.74($\times 10^{12}$/l)) were significantly lower compared with the control group Z (4.79 ± 0.50 ($\times 10^{12}$/l)). Moreover, the mean ± SD of mean cell haemoglobin concentration (MCHC) for group X (33.97 ± 2.46(g/dl)) and group Y (33.76 ± 2.61(g/dl)) were significantly lower than the control group Z (35.51 ± 1.12 (g/dl). In addition, the mean values of MCV and MCH were significantly higher in group X than group Y. However, there was no significant difference in the mean value of MCHC for group X and Y.

Table 3 shows comparison of mean ± SD of Red Cell Distribution Width RDW(fl) Platelet Distribution Width PDW(fl) and Mean Platelet Volume MPV(fl) in HIV seropositive subjects on ART (group X), those not on ART (group Y) and Control subjects (HIV seronegative) (group Z).

The mean ± SD of RDW was significantly higher in group X (48.0 ± 6.23(fl)] and group Y (42.84 ± 4.01(fl)) compared with the control [40.17 ± 3.14(fl). However, there was no significant mean difference in PDW and MPV among the three groups. In addition, the mean value of RDW was significantly higher in group X compared with group Y.

Table 3 Mean ± SD of RDW, PDW and MPV COMPARED among HIV seropositive subjects ON ART, NOT ON ART and HIV seronegative subjects.

Groups	RDW(fl)	PDW(fl)	MPV(fl)
(X)HIV POS ON ART(n=55)	48.0 ± 6.23a,b	12.59 ± 2.28	10.29 ± 1.26
(Y)HIV POS NOT On Art(n=55)	42.84 ± 4.01	12.76 ± 2.23	10.28 ± 1.02
(Z) Control (n=50)	40.17 ± 3.14	12.51 ± 1.84	10.33 ± 0.84
F-Values	35.63	0.19	0.036
P- Values	0.00*	0.83	0.97

a ≤ 0.05 when compared with NON ART; b ≤ 0.05 when compared with CONTROL; RDW=Red Cell Distribution Width; PDW=Platelet Distribution Width; MPV=Mean Platelet Volume

Discussion

Haematological findings have been found to be variable among different studies. This is as a result of factors like drugs, immune mechanisms, opportunistic infections or direct influence of the virus [10].

However, some haematological complications were observed in this study. The haemoglobin and red blood cell values were found to be reduced in HIV seropositive subjects both ART and non ART compared with the controls. This reduction does not concur with the findings of Akinbani et al. [11] and Erhabor et al. [12] but is consistence with the findings of Amegor et al. [13] and that of Dangana et al. [14]. The explanation to this is the generalized effect of HIV/AIDS on erythropoiesis through inhibition of the precursor cells from

differentiating and developing to mature red blood cells. The study also showed that the MCH and MCHC values were significantly reduced in HIV seropositive subjects compared with the controls. This finding is in consonance with that of Obirikorang and Yahoah [15]. The MCV was found to be significantly increased in HIV seropositive subjects on ART compared with those not on ART. Also the RDW of HIV seropositive subjects was significantly higher than the controls. This finding supports that of Gallego et al. [16] and could be as a result of high degree of anisocytosis observed among HIV seropositive subjects due to decreased RBC production or ineffective erythropoiesis.

Conclusion

Haematological findings have been found to be variable among different studies. Some haematological complications were observed in this study. The haemoglobin and red blood cell values were found to be reduced in HIV seropositive subjects both ART and non ART compared with the controls. The explanation to this is the generalized effect of HIV/AIDS on erythropoiesis through inhibition of the precursor cells from differentiating and developing to mature red blood cells.

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