Correlation of HIV progression with absolute CD4 T-Lymphocyte count and red blood cell indices among HIV-Positive patients on art and art-naive problem.

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Abstract

Background: Human immunodeficiency virus (HIV) is characterized by progressive damage to the body's immune system which results in a number of opportunistic infections, immunological and haematological complications.The most important biomarkers of disease stage and progression in patients with an HIV infection are the CD4 count and viral load. However, red blood cell indices and some other haematological parameters reflect the degree of HIV disease progression.

Aim: To Correlate HIV progression with CD4 T-Lymphocyte Count and Red Blood Cell Indices among HIV-Positive Patients on ART and ART-Naïve.

Methodology: The study was a cross sectional study conducted at Federal Teaching Hospital (FETHI), IdoEkiti. Two hundred samples were collected from consented HIV patients, grouped as one hundred (100) HIV positive subjects on ART, one hundred (100) HIV positive subjects ART naive. Each group was classified into three stages of HIV infection using their CD4 values according to Centers for Disease Control as follows: Stage-1 CD4 ≥ 500 cells/uL, Stage-2: CD4 200–499 cells/uL and Stage-3: CD4<200 cells/uL. Three milliliters (3ml) of whole blood was collected and dispensed into 5ml K2EDTA bottle for immediate analysis of haematological parameters using haematology analyzer, CD4 count was analyzed using flow cytometer and subjects sample were re-screening for HIV using serial algorithm method.

Results: Mean values of RBC indices in CD4 stages<200 were lower compared to CD4 stages 200-499 and CD4 stages>500 among ART and ART-naive. There is significant difference (p<0.05) in red cell indices of ART and ART-naïve.

Conclusion: This study established correlation between absolute CD4 T-Lymphocyte count and red blood cell indices among HIV-positive patients on ART and ART-Naive as HIV-infection progresses.

Introduction

The identification of laboratory tests that help the clinician to predict progression is useful not only to monitor the patients' disease evolution but also to define the right time to initiate treatment in HIV infected patients [1]. Absolute CD4+ count, viral load and haematological parameters are the most common indicator for monitoring the HIV disease progression [2]. HIV virus invades the human host cells and replicates preferentially in CD4+ T cells, leading to increasing viral numbers and a failing immune system [3]. Human immunodeficiency virus (HIV) infection is commonly associated with haematopoietic system causing decreased or ineffective production of blood cells. HIV attacks and destroys blood cells especially the CD4 cells [4], 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 infection [5]. CD4 count falls below about 200 cells per microliter of blood make the immune system becomes less able to fight certain infections [6]. The most important biomarkers of disease stage and progression in patients with an HIV infection are the CD4 count and viral load [7]. However, haematological abnormalities can influence or predict the

prognosis of peoples with HIV infection. Red blood cell indices and some other haematological parameters reflect the degree of HIV disease progression [8]. Decrease rates of haemoglobin, haematocrit and red cell indices values have been correlated with falling rate of CD4 counts [9]. In ARTtreatment,monitoringofhaemoglobin and haematocrit value are very essential, HIV-patientswhodeveloped severeanaemiabeforetheinitiationofARTwereatanincreasedrisko fdeath [10]. It has been reported that increases in haemoglobin value is predictive of both disease progression and treatment success. The aim of this study was to correlate HIV progression with absolute CD4 T-Lymphocyte count and red blood cell indices among HIV-positive patients on ART and ART-Naive.

Materials and Methods

Study design

This study was a cross sectional study conducted at Federal Teaching Hospital (FETHI), IdoEkiti, Nigeria. Study population was grouped into two, one hundred (100) HIV positive subjects on ART, one hundred (100) HIV positive subjects ART naïve. Each of these groups was classified into

three stages of HIV infection using their CD4 values according to Centers for Disease Control as follows: Stage-1 CD4 ≥ 500 cells/uL, Stage-2: CD4 200 – 499 cells/uL and Stage-3: CD4<200 cells/uL. Consented HIV subjects were re-screened for HIV infection for the purpose of the study to confirm their HIV positive status using serial algorithm method. Patient's consent was sort for through an informed consent form and ethical approval was obtained from Federal Teaching Hospital, Ido-Ekiti.

Sample collection and sample preparation

Three milliliters (3ml) of whole blood was collected from each consented subject was dispensed into 5ml K2EDTA bottle for immediate analysis of haematological parameters, CD4 count and HIV screening.

Methodology

HIV screening test: Human immunodeficiency virus was diagnosed using serial algorithm method. Determine HIV-1/2 (Abbott Diagnostic Division, Belgium/Luxemburg), Uni-Gold HIV Kit (Trinity Biotech, Wicklow Bay, Ireland) and Chembio HIV ½ Stat-PakTM Assay. Patients reactive to antibody screening tests were considered positive and recruited into the study; the test was carried out according to the manufacturer's instruction.

Haematological parameters: Haematologicalparameters was analyzed using Haematology Analyzer (Sysmex XN 350 five parts) following Manufacture's instruction.

Analysis of CD4 count using flow cytometry

Research samples for CD4 count was prepared and run on the Parteccyflow counter (Partec flow cytometer, GMBH, Munster, Germany) according to the manual instructions

Results

Table 1 showed comparison CD4 stages with red cell indices in HIV-infected ART naïve. The parameters include RBC, Hb, HCT, MCV, MCH, MCHC and RDW. The mean ± SD of RBC 2.66 ± 0.53 in CD4<00 was significantly ($p<0.05$) lower compared to 4.98 ± 0.29 and 3.78 ± 0.61 in CD4>500 and CD4

200-499 respectively (F-value 83.37; p-value 0.00).Mean ± SD of Hb 5.98 ± 0.84 in CD4<200 was significantly ($p<0.05$) lower compared to 14.18 ± 0.91 and 10.19 ± 2.22 in CD4>500 and CD4 200-499 respectively (F-value 106.01; P-value is 0.00).The mean ± SD of HCT 17.17 ± 1.75 in CD4<200 was significantly ($p<0.05$) lower compared to 37.53 ± 0.69 and 29.65 ± 5.40 in CD4>500 and CD4 200-499 respectively (F-value 132.12; P-value 0.00). The mean ± SD of MCV 80.31 ± 10.53 in CD4>200 was significantly ($p<0.05$) lower compared to 81.78 ± 5.98 and 86.55 ± 9.68 in CD4>500 and CD4 200-499 respectively (F-value 4.32; p-value 0.01). Mean ± SD of MCH 27.67 ± 1.54 in CD4<200 was lower compared to 28.43 ± 1.65 and 28.31 ± 2.64 in CD4 200-499 respectively although the difference is not significant ($p>0.05$), (F value 0.93; P- value 0.40). Mean ± SD of MCHC 31.42 ± 1.34 in CD4<200 was significantly ($P<0.05$) lower compared to 34.25 ± 1.14 and 33.09 ± 1.52 in CD4>500 and CD4 200-499 respectively (F-value 21.57; p-value 0.00). Mean + SD of RDW 20.18 ± 4.53 in CD4<200 was significantly higher compared to 19.13 ± 5.10 and 16.19 ± 3.98 in CD4>500 and CD4 200-499 respectively (F-value 6.18; p-value 0.00). Multiple comparison between CD4>500 and CD4 200- 499 showed that, mean ± SD of RBC, Hb, HCT, MCHC and RDW in CD4>500 were significantly ($p<0.05$) higher compared to CD4 200-499. Mean ± SD of MCV in CD4>500 was significantly ($P<0.05$) lower compared to CD4 200-499. Mean ± SD of MCH in CD4>500 was higher compared to CD4 200-499, although the difference was not significant ($p>0.05$). Multiple comparison between CD4>500 and CD4<200 showed that, mean ± SD of RBC, Hb, HCT and MCHC in CD4<200 were significantly ($p<0.05$) lower compared to CD4>500. The mean ± SD of MCV and MCH in CD4<200 were lower compared to CD4>500 although the difference was not significant ($p>0.05$). Mean ± SD of RDW in CD4<200 was higher compared to CD4>500, although the difference was not significant ($p>0.05$). Multiple comparison between CD4 200-499 and CD4<200 showed that, mean ± SD of RBC, Hb, HCT, MCV and MCHC in CD4<200 were significantly ($p<0.05$) lower compared to CD4 200-499. Mean ± SD of MCH in CD4<200 was lower compared to CD4 200- 499, although the difference was not significant ($p>0.05$). The mean ± SD of RDW in CD4<200 was significantly ($p<0.05$) higher compared to CD4 200-499.

Table 1. *Mean values of RBC indices in CD4 stages<200 were lower compared to CD4 stages 200-499 and CD4 stages>500 among ART-naïve. *p<0.05 was considered significant, p>0.05 was considered not significant, F-value=mean ± SD of parameters was compared using ANOVA *=<0.05.*

CD4 Stages	RBC(X109/l)	HB(g/dl)	HCT(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	ROW(%)
>500N=12	4.98 ± 0.29	14.18 ± 0.91	37.53 ± 0.69	81.78 ± 5.98	28.43 ± 1.65	34.25 ± 1.14	19.13 ± 5.1
200-499 N=57	3.78 ± 0.61	10.19 ± 2.22	29.65 ± 5.40	86.55 ± 9.68	28.31 ± 2.64.76	33.09 ± 1.52	16.91 ± 3.98
<200 N=31	2.66 ± 0.53	5.98 ± 0.84	17.17 ± 1.75	80.31 ± 10.53	27.67 ± 1.54	31.42 ± 1.34	20.18 ± 4.53
F (P•VAWE)	83.37 (0.00*)	106.01 (0.00*)	132.12 (0.00*)	4.62 (0.01*)	0.93 (0.40)	21.57 (0.00*)	6.18 (0.00*)
>500 VS 200-499	0.00*	0.00*	0.00*	0.05*	0.98	0.02*	0.04*
>500 VS<200	0.00*	0.00*	0.00*	0.84	0.37	0.00*	0.81

200-499 VS<200	0.00*	0.00*	0.00*	0.02*	0.33	0.00*	0.00*

Table 2 showed comparison CD4 stages with red cell indices in HIV infected subjects on ART. Parameters include RBC, Hb, HCT, MCV, MCH, MCHC and RDW. The mean ± SD of RBC 1.86 ± 0.62 in CD4<200 was significantly (p<0.05) lower compared to 5.10 0 ± 0.48 and 4.91 ± 0.62 in CD4>500 and CD4 200- 499 respectively (F-value 209.48; p-value 0.00). Mean ± SD of Hb 2.71 ± 0.47 in CD4<200 was significantly (P<0.05) lower compared to 14.39 ± 1.80 and 12.43 ± 1.30 in CD4>500 and CD4 200-499 respectively (F-value 344.13; p-value 0.00). The mean ± SD of HCT 9.05 ± 1.13 in CD4<200 was significantly (P<0.05) lower compared to 43.56 ± 2.39 and 46.88 ± 54.35 in CD4>500 and CD4 200-499 respectively (F-value 9.28; p-value 0.00). The mean ± SD of MCV 76.65 ± 1.32 in CD4<200 was significantly (P<0.05) lower compared to 109.66 ± 6.64 and 87.01 ± 52.44 in CD4>500 and CD4 200-499 respectively (F-value 271.52; p-value 0.00). The Mean ± SD of MCH 22.03 ± 1.23 in CD4<200 was significantly (p<0.05) lower compared to32.10 ± 1.19 and 30.77 ± 1.66 in CD4>500 and CD4 200-499 respectively (F-value 329.38; p-value 0.00).The Mean ± SD of MCHC 28.00 ± 0.66 in CD4<200 was significantly (p<0.05) lower compared to 35.06 ± 0.71 and 34.36 ± 1.97 in CD4>500 and CD4 200-499 respectively (F-value 199.10; p-value 0.00). The Mean ± SD of RDW 22.06 ± 1.08 in CD4<200 was significantly (p<0.05) higher compared to 14.52 ± 0.62 and 14.72 ± 2.65 in CD4>500 and CD4 200-499 respectively (F-value 143.33; p-value 0.00). Multiple comparison between CD4>500 and CD4 200-499 showed that, mean ± SD of RBC, Hb, MCV and MCH in CD4>500 were significantly (p<0.05) higher compared to CD4 200-499. Mean ± SD of HCT and RDW in CD4>500 were lower compared to CD4 200-499, although the difference was not significant (p>0.05). The mean ± SD of MCHC in CD4>500 was significantly (P<0.05) higher compared to CD4 200-499, the difference was not significant (p>0.05). Multiple comparison between CD4>500 and CD4<200 showed that, mean ± SD of RBC, Hb, HCT, MCV, MCH and MCHC in CD4<200 were significantly (p<0.05), lower compared to CD4>500. Mean ± SD of RDW in CD4<200 was significantly (p>0.05) higher compared to CD4>500. Multiple comparison between CD4 200-499 and CD4<200 showed that, mean ± SD of RBC, Hb, HCT, MCV, MCH and MCHC in CD4<200 were significantly (p<0.05), lower compared to CD4 200-499. Mean + SD of RDW in CD4<200 was significantly (p<0.05) higher compared to CD4 200-499.

Table 2. *Mean values of RBC indices in CD4 stages<200 were lower compared to CD4 stages 200-499 and CD4 stages>500 among ART. p<0.05 was considered significant, p>0.05 was considered not significant, F-value=mean ± SD of parameters was compared using ANOVA.*

CD4 Stages	RBC(X109/l)	HB(g/dl)	HCT(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	ROW(%)
>500 N=55	5.01 ± 0.48	14.18 ± 1.80	43.56 ± 2.39	109.66 ± 6.64	32.10 ± 1.19	14.52 ± 0.62	19.13 ± 5.1
200.499 N=30	4.19t ± 0.62	12.34 ± 1.30	46.88 ± 54. 35	87.01:1 ± 52.44	30.77t ± 1.66	34.36 ± 1.97	14.73 ± 2.65
<200 N=15	1.86 ± 0.62	2.71 ± 0.47	9.05 ± 1.13	76.65 ± 1.32	22.03 ± 1.23	28.00 ± 0.66	22.06 ± 1.08
F (P•VAWE)	209.48 (0.01*)	344.13 (0.00*)	9.28 (0.00*)	271.52 (0.00*)	329.38 (0.40)	199.10 (0.00*)	143.33 (0.01*)
>500 VS 200-499	0.00*	0.00*	0.94	0.00*	0.00*	0.16	0.91
>500 VS<200	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
200-499 VS<200	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

Discussion

In this study, there is correlation between red cell indices and CD4 count in both ART and ART-naïve. Findings in this study show that, the severity of HIV infection in ART-naïve was higher compared to HIV-infected on ART, as HIV disease progresses there is increase destruction of red blood cells, it was observed in this study that progressive CD4 count depletion was associated with lower haemoglobin concentrations. Supporting the findings in this study, HIV infection is characterized by alteration in enzyme activities and in haematological parameters in both HIV-infected patients on ART and ART-naive. HIV disease progression was higher in CD4<200 cells/mm³ compared to CD4200-499 cells/mm³ and CD4>500 cells/mm³ in both ART and ART-naïve. The risk of having CD4 count<200 cells mm⁻³ is twice in ART-naïve patients than those on ART and the chances of having CD4 count between 200−499 cells mm⁻³ is twice times more in patients on ART than those who are ART-naïve. CD4 counts<200 cells mm⁻³ was strongly associated with severe anaemia in ART-naïve patients and could lead to rapid disease progression and decreased survival causing the immune system less able to fight certain infections [2]. In the advanced stage of HIV disease, the blood cell counts were lower than the early stage of the diseases. Supporting this study, it was reported that HIV-infection cause bone marrow abnormalities with progressive immunologic deterioration especially in advanced HIV disease [11]. It was reported that the generalized effect of HIV/AIDS infection on erythropoiesis in the bone marrow is the inhibition of precursor cells from differentiating and developing to mature red blood cells. Alem reported that soluble factors like HIV proteins and cytokines inhibit the

growth of hematopoietic cells in the bone marrow [12]. Red cell indices was found to be significantly increased in HIV sero-positive subjects on ART compared with ART-naïve according to the CD4 stages as HIV infection progresses which was observed in this study which shows the impact of antiretroviral therapy in the management of HIV infected patients. Variation in red cell indices in this study could be as a result of high degree of anisocytosis commonly observed among HIVsero-positive subjects due to decreased red blood cell production or ineffective erythropoiesis [3]. This study showed a strong and consistent association with the progression of HIV disease as measured by CD4 count and red cell indices. This association is as a result of increase viral burden as HIV disease progresses. Findings in this study are consistent with previous studies which showed that low haemoglobin levels increase the risk of AIDS and increase the risk of death among HIV patients with advanced immunodeficiency [13]. Significant and positive correlation was found when blood haemoglobin level was compared with their respective CD4 count of the subjects. This study showed that haemoglobin levels provide prognostic information independent of that provided by the CD4 lymphocyte count, low hemoglobin is independently associated with decline in CD4 lymphocyte count in HIV infected individuals. Our study showed that low hemoglobin (Hb) is strongly associated with decline in CD4 –lymphocyte count in HIV infected patients. As disease progression occurs in HIV infection/AIDS there is increase in HIV viral load and subsequent death of CD4 lymphocytes.

Conclusion

This study established correlation between absolute CD4 T-Lymphocyte count and red blood cell indices among HIV-positive patients on ART and ART-Naïve as HIV disease progresses. ART-naïve patients have a high risk of developing reduced red cell indices and absolute CD4 counts compared to their counterparts on ART.

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