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Research Article

Classification of Absolute Cd4+ T Lymphocytes Count in Immunological, Virological and Erythropoietic Growth Factor among HIV Infected Patients

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Abstract

Background: HIV-infection resulted in CD4+ T-cell depletion which is accompanied by an increase in CD8+ T-cells resulting in an inverted CD4/CD8 ratio. Low CD4/CD8 ratio has been identified as a hallmark of inmmunosenescence and a surrogate of mortality in HIV infected patients especially in ART-naïve patients

Aim: Classification of absolute CD4+ T-lymphocytes count in immunological, virological and erythropoietic growth factor among HIV infected patients

Methodology: One hundred samples each was collected from HIV positive subjects on ART and HIV positive subjects ART naïve. Six milliliters of whole blood was collected from each consented subject, 3ml was dispensed into 5ml K_2EDTA bottle for immediate analysis of absolute CD4 count, CD8 count, total white cell count and HIV screening. The remaining 3ml of blood was dispensed into plain bottle; serum was extracted for the analysis of erythropoietin and viral load

Results: Mean values of CD8, CD4/CD8, EPO and TWBC in CD4 <200 were significantly ($p<0.05$) lower compared to CD4 >500 and CD4 200-499. Mean values of VL in CD4 <200 were significantly ($p<0.05$) higher compared to CD4 >500 and CD4 200-499 among HIV subjects on ART and ART-naïve

Conclusion: Immunological and erythropoetic growth factor assessed in this study were decline while viral load was increasing as HIV infection progresses with depletion in absolute CD4 count, this study shows the efficacy of ART on the treated subjects. However, based on this study, absolute CD8 T cells count, CD4/CD8 ratio and erythropoietin can be used as surrogate makers to ascertain pathogenesis in HIV-infected subjects.

Keywords: immunological; virological; erythropoietin; hiv-infected; art/art-naïve

Abbreviations:

EPO= Erythropoietin,
VL= Viral load,
CD4= Cluster of differentiation 4,
CD8= Cluster of differentiation 8,
TWBC= Total white

Introduction

Human immunodeficiency virus (HIV) infection is characterized by gradual CD4 depletion, CD8 expansion, and immune activation. Acute HIV infection causes initial activation and robust expansion of CD8 T

cells (Mudd and Lederman, 2014). $CD4^+$ T lymphocytes are helper lymphocytes, they excrete cytokines that can activate other immune cells. CD8 lymphocytes are cytotoxic, they directly destroy virus-infected cells and remain inactivated when there is no foreign antigen. During the course of illness among patients with human immunodeficiency virus (HIV) infection, CD4+ T-cell counts and viral load are traditionally monitored in order to assess response to therapeutic intervention. However, $CD8^+$ T-cell counts may predict prognosis independently of $CD4^+$ T-cell counts, overstimulation of CD8 response and its elevated count has been associated with accelerated HIV disease progression. Following successful antiretroviral therapy (ART), CD4 counts tend to remain above 200 cells/m^3 in almost 99.2% of patients (Gale *et al.*, 2013). Despite the successful restoration of CD4 counts and HIV suppression following ART, immune activation tends to persist, and CD8 counts seldom normalize (Helleberg *et al.,* 2015). There is an emerging consensus that persistent immune activation and inflammation are due to residual HIV replication and microbial translocation that contributes to CD8 expansion (Cassol *et al.,* 2010). CD4/CD8 ratio is a characteristic feature of HIV infection. ART restore the CD4/CD8 ratio, but the ratio rarely exceeds one, particularly in the event of delayed therapeutic intervention (Serrano-Villar *et al.,* 2014). Failure to achieve normalization of the CD4/CD8 ratio has been attributed to persistence of high CD8 T-cell counts. This persistently low CD4/CD8 ratio has been demonstrated to reflect persistent innate and adaptive immune activation in HIV-infected patients. Moreover, it has recently been shown that a low CD4/CD8 ratio inversely correlates with the risk of morbidity and mortality, monitoring CD4/CD8 ratio in patients receiving ART may be useful to identify a subset of patients at much higher risk of non AIDS-defining cancer who may thus require a more intensive strategy of prevention or screening (Hema *et al.,* 2016). It has been reported there is low level of erythropoietin (EPO) response in HIV infection which is secondary to the elevation of immunosuppression and inflammation, this suggested that HIV suppresses the production of erythropoietin (Esan *et al*., 2020; Gatukui *et al*., 2014). The aim of this study was to classified absolute CD4+ T lymphocytes count in immunological, virological and erythropoietic growth factor among HIV infected patients.

Materials and Methods

Study Design

This study was carried out at Federal Teaching Hospital, Ido Ekiti, Nigeria. One hundred samples each was collected from HIV positive subjects on ART and 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 $\geq$ 500 cells/uL, Stage-2: CD4 200 – 499 cells/uL and Stage-3: CD4 < 200 cells/uL. Consented subjects were re-screened for HIV infection for the purpose of the study to confirm their HIV 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

Six milliliters (6ml) of whole blood was collected from each consented subject, 3ml was dispensed into 5ml K_2EDTA bottle for immediate analysis of absolute CD4 count, CD8 count, total white cell count and HIV screening. The remaining 3ml of blood was dispensed into plain bottle, allowed to clot and centrifuged at 2500 revolution per minute for 5minutes to extract the serum into another plain bottles, stored at -40^0C for the analysis of erythropoietin and viral load

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.

Analysis of CD4 and CD8 Count Using Flow Cytometry (Cyflow Counter)

Research samples for CD4 and CD8 count was prepared and run on the Partec cyflow counter (Partec flow cytometer, GMBH, Munster, Germany) according to the manual instructions.

Total White Blood Cell Count Haematology Analyzer

Total white blood cell count was analyzed using Haematology Analyzer (Sysmex XN 350 five parts) following Manufacture's instruction.

Viral Load Analysis

Extracted plasma from K_2EDTA sample was used to estimate HIV-RNA viral load analysis using polymerase chain reaction (PCR), the procedure was follow as describe in the manual.

Erythropoietin

Erythropoietin (EPO) was estimated using enzyme-linked immunosorbent assay (ELISA) kit, the procedure was followed as described in the manual ALPCO (2018).

Results.

TABLE 1: MEAN±SD OF CD8, VL, CD4/CD8, EPO AND TWBC ON CD4 STAGES IN HIV ART-NAÏVE SUBJECTS

CD4 STAGES	CD8 (cells/µl)	VL (copies/ml)	CD4/CD8	EPO (IU/L)	TWBC ($X10^9$/l)
≥500 N=12	1291.20 ±168.30	48799.00 ±9051.73	0.54± 0.07	4.09± 0.39	3.77± 0.49
200-499 N=57	989.91 ±132.31	98984.00 ±99574.94	0.43± 0.06	2.68 ±0.79	3.66± 0.72
<200 N=31	578.42± 122.21	177250.00 ±44183. 36	0.34± 0.07	1.54 ±0.42	3.29± 0.75
F (PVALUE)	153.38 (0.00*)	14.74 (0.00*)	42.49 (0.00*)	70.56 (0.00*)	3.25 (0.43)
≥500 VS 200-499	0.00*	0.00*	0.00*	0.00*	0.79
≥500 VS <200	0.00*	0.00*	0.00*	0.00*	0.05*
200-499 VS <200	0.00*	0.00*	0.00*	0.00*	0.08

Mean values of CD8, CD4/CD8, EPO and TWBC in CD4 <200 were lower compared to CD4 ≥500 and CD4 200-499. Mean values of VL in CD4 <200 were higher compared to CD4 ≥500 and CD4 200-499 among ART-naïve subjects. p≤0.05 was considered significant, p>0.05 was considered not significant, F-value = mean±SD of parameters was compared using ANOVA.

TABLE 2: MEAN ± SD OF CD4, VL CD4/CD8, EPO AND TWBC ON CD4 STAGES IN HIV INFECTED SUBJECTS ON ART

CD4 STAGES	CD8 (cells/µl)	VL (copies/ml)	CD4/CD8	EPO (IU/L)	TWBC ($X10^9$/l)
≥500 N=55	772.82± 65.75	79997.00 ±8290.87	0.70± 0.07	3.76± 0.51	5.72± 0.48
200-499 N=30	632.57± 83.90	80445.00± 13102.89	0.52± 0.09	3.44± 0.59	5.50± 0.67
<200 N=15	264.87± 76.75	155910.00 ±29408.91	0.33± 0.10	1.46± 0.21	2.25± 0.48
F (PVALUE)	285.51 (0.00*)	170.61 (0.00*)	145.55 (0.00*)	122.01 (0.00*)	248.64 (0.00*)
≥500 VS 200-499	0.00*	0.00*	0.00*	0.00*	0.00*
≥500 VS <200	0.00*	0.00*	0.00*	0.00*	0.00*
200-499 VS <200	0.00*	0.00*	0.00*	0.00*	0.00*

Mean values of CD8, CD4/CD8, EPO and TWBC in CD4 <200 were lower compared to CD4 ≥500 and CD4 200-499. Mean values of VL in CD4 <200 were higher compared to CD4 ≥500 and CD4 200-499 among ART subjects. p≤0.05 was considered significant, p>0.05 was considered not significant, F-value = mean±SD of parameters was compared using ANOVA

Discussion

This study has revealed a significant high CD8 T-cell count in ART-naïve compared to subjects on ART in all the CD4 stages. However, mean value of CD8 in CD4>500 was higher compared with CD4200-499 and CD4 <200 in both subjects on ART and ART-naïve. Supporting this finding, it was reported that in early HIV infection, CD8+ T-cell numbers tend to increase, reflecting expansion of memory CD8+ T cells, particularly HIV-reactive cells. Elevated $CD8^+$ T-cell counts are associated with immune activation, CD8 cell expansions persist until far advance stage of HIV disease, when all T-cell numbers tend to fall. Absolute CD8 T counts do not fall until HIV disease progresses (Krishna *et al.,* 2006). Confirming the finding in this study, Marie reported that it is possible that redistribution of lymphocytes from blood to tissues with ongoing pathological processes is part of the explanation for the observed low $CD8^+$ T-cell counts (Helleberg *et al.,* 2015). Micheal also reported that some HIV patient are able to maintain high level of CD8+ specific T cell which control viral replication but it falls after infection in most patients, which reduce immunocompetence as HIV progresses (Michael *et al.,* 2011). Marie stated that, elevation of $CD8^+$ T-cell counts during untreated HIV infection is presumably caused by increased peripheral $CD8^+$ T-cell proliferation due to antigen stimulation and immune activation (Helleberg *et al.,* 2015). As we observed in this study that CD4/CD8 ratio was less than 1 in both subjects on ART and ART-naïve. The mean value of CD4/CD8 ratio in ART was higher compared with ART-naïve. Finding in this study is similar to the report of Hema stated that CD4/CD8 ratio remains < 1 in most HIV-infected patients despite long-term CD4+ cell counts restoration on ART. A CD4/CD8 ratio < 0.5 could identify patients who require a more intensive strategy; hence, low CD4/CD8 ratio is an indicator of CD4+ T cell lymphopenia and of CD8+ T cell activation (Hema, *et al.,* 2016). Low ratio of $CD4^+$ / $CD8^+$ T cells among treated HIV-infected individuals is associated with increased morbidity and mortality as observed among CD4<200 in this study. Increase in leucopenia was observed in CD4<200 compared with CD4200-499 and CD4>500 in both ART and ART-naïve, this indicates that a higher occurrence of leucopenia with progression of HIV disease. Similar to this study Parinitha and Kulkarni reported that total white blood cell count showed significant difference between three groups with differing CD4 cell counts stages (Parinitha and Kulkarni, 2012). Supporting the findings

in this study, it was reported that elevated white blood cell count typically means that body system is actively fighting an infection and low white blood cell count suggests that some disorder, either HIV-related or non-HIV-related, is affecting the bone marrow's ability to produce white blood cells, indicating that the body system is less able to fight infection this showed that there is a correlation between CD4 count and total white blood cell. This present study revealed correlation between different stages of CD4 count and erythropoietin (EPO) among the study population. In supporting the findings in this study, Okafor reported that HIV has direct effect on the bone marrow through the expression of pro-inflammatory cytokines that suppress erythropoiesis (Okafor *et al.*, 2019). Anaemia in HIV patients is caused by depressed bone marrow function by HIV infection leading to low production of erythropoietin which resulted into ineffective production of red blood cell (Esan *et al.*, 2020), CD4>500 has higher mean value of EPO compared to CD4 200-499 and CD4<200 in HIV subjects on ART and ART-naïve confirming that the more the severity of HIV infection the lower the erythropoiesis. Confirming the findings in this study, Allen reported that the state of relative suppression of erythropoietin response by HIV has been shown to be relieved by ART. ART has been shown to block the function and expression of erythropoietin receptors. This explains why erythropoietin levels were greater in ART-treated subjects than in ART -naive subjects (Allen *et al.*, 1998). There is a correlation between CD4 classification and HIV viral load as observed in this study. The mean value of viral load CD4>500 was higher compared to CD4 200-499 and CD4<200 in HIV subjects on ART and ART-naïve. It was reported that w**hen viral load is low, CD4 counts will be high, this suggested that** HIV viral load predicts how fast the disease will progress, while CD4 count, indicate how much damage the virus has already caused to immune system (Mocroft *et al.*, 2010), The decline in CD4 count is linked to HIV viral load and is used as a measure of disease progression, the higher the HIV viral load, the faster the CD4 cell count will fall as we observed in this present study.

Conclusion

Immunological and erythropoietic growth factor assessed in this study were decline while viral load was increasing as HIV infection progresses with depletion in absolute CD4 count, this study shows the efficacy of ART on the treated subjects. However, based on this study, absolute CD8 T cells count, CD4/CD8 ratio and erythropoietin (EPO) can be used as surrogate makers to ascertain pathogenesis in HIV infected subjects because viral load cannot separately determine the rate of HIV progression to AIDS

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