Original Research Article

Changes in Some Red Cell Enzymes, Coagulation and White Cell Parameters in Relation to CD4 Count Values in HIV Subjects

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ABSTRACT

Aim: To assess the effect of CD4 count stage of HIV positive patients on antiretroviral treatment (ART) and those not on antiretroviral treatments on G-6-PD, Pyruvate kinase enzyme activity, some coagulation and white cell parameters.
Study Design: Case-control study
Place of Study: Nnamdi Azikiwe University Teaching Hospital Nnewi, Nigeria.
Methodology: We included 181 subjects; Sixty HIV patients on ART; Sixty HIV patients not on ART and Sixty-one apparently healthy individual control. Glucose-6-Phosphate Dehydrogenase (G-6-PD) activity, Pyruvate kinase (PK) activity, Activated Partial Thromboplastin time (APTT), Prothrombin time (PT), Platelet count (PLT), CD4 count, total white cell count (TWBC), differential WBC count (NEUT, LYM and MXD) and Human Immunodeficiency virus (HIV) status were determined.
Results: In ART subjects, APTT and PT were significantly prolonged in subjects with CD4 count < 200 and 200 – 499, compared to ≥ 500 (P <0.05), APTT was significantly higher in < 200 compared to 200 – 499. TWBC was lower in < 200 compared to ≥ 500 (P < 0.05) and LYM was reduced in < 200 compared to 200 – 499 and ≥ 500 (P < 0.05). However, G-6-PD, PK, PLT, NEUT and MXD showed no significant different in the three groups (P > 0.05). In non-ART, G-6-PD activity was significantly lower in subjects with CD4 count < 200 and 200 – 499 compared to ≥ 500 (P < 0.05) while APTT was lower in < 200 compared to 200 – 499 and ≥ 500 9P < 0.050. However, PK, PT, PLT, TWBC, NEUT, LYM and MXD showed no significant difference (P > 0.05).
Conclusion: This study showed that in ART subjects, APTT, PT, TWBC and LYM were prolonged with progression of HIV infection, while in non-ART, G-6-PD and APTT were decreased with HIV progression as depicted by decreased CD4 count.
Keywords: Pyruvate kinase; G-6-PD; HIV/AIDS; prothrombin time; activated partial thromboplastin time.

INTRODUCTION

Human Immunodeficiency Virus (HIV) has emerged a global disaster ever since its discovery. [1] Nigeria's HIV/AIDS prevalence rate is now 3.4 per cent, according to the report of a new national

survey conducted by the federal ministry of Health (FMOH) for the 2012 National HIV/AIDS and Reproductive Health Survey-Plus (NARHS Plus). Meanwhile, HIV/AIDS continues to spread globally and remains a worldwide pandemic affecting over 40 million people. [2,3] It is now the leading cause of death in sub-Saharan Africa and the fourth leading cause of mortality worldwide. Highly active antiretroviral therapy (HAART) has generally been taken as the gold standard in the management of HIV patients. [4] With its introduction in 1996, HAART appears to have effectively controlled viral replication in HIV/AIDS patients and has successfully improved their quality of life and prolonged their life-expectancy, [5] with a near normal turnover of both CD4 and CD8 T-cell populations. [6] HIV infection leads to a progressive reduction in the number of T cells expressing CD4, thus Medical professionals refer to the CD4 count to decide when to begin treatment during HIV infection as well as to determine efficacy of treatment. In molecular biology, CD4 (cluster of differentiation 4) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells. They are white blood cells that are an essential part of the human immune system and play an important role in monitoring HIV disease progression. The United States Centre for Disease Control and Prevention created a classification system for HIV, and updated it in 2008. [7] This system classifies HIV infections based on CD4 count and clinical symptoms, and describes the infection in three stages: Stage 1: CD4 count $\geq$ 500 cells/µl and no AIDS defining conditions; Stage 2: CD4 count 200 to 500 cells/µl and no AIDS defining conditions; Stage 3: CD4 count $\leq$ 200 cells/µl or AIDS defining conditions; Unknown: if insufficient information is available to make any of the above classifications

Disorders of the haematopoietic system including anaemia, leucopenia and thrombocytopenia are common throughout the course of HIV infection. These could be due to direct effects of HIV infection, secondary infections, neoplasms or side effects of therapy. [8] Prothrombin time (PT) and activated partial thromboplastin time (APTT) are screening tests for the extrinsic and intrinsic clotting systems respectively. They detect deficiency or inhibition of clotting factors in either system, and are the first tests in screening for coagulation disorders. In HIV infection, the liver is affected. The liver is the major organ responsible for the synthesis of most coagulation factors and infection of the liver by HIV can lead to abnormal production of coagulation factors. [9]

There are studies on haematological changes in HIV infection; however only a few have related these changes with CD4 count. However, in the present study some red cell enzymes, coagulation and white cell parameters have been compared based on CD4 cell counts to highlight the pattern of these haematological changes with disease progression.

MATERIALS AND METHODS

This study was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State, a tertiary health institution serving patients of high, middle and lower socioeconomic status. It houses a major HIV/AIDS Centre (IHVN Clinic) serving patients from all parts of the state and beyond, with a registered HIV Sero-positive patient population of over 6000.

One hundred and eighty one (181) subjects aged between 18-60 years were recruited and categorized into three groups comprising; Sixty adult HIV Seropositive

subjects on antiretroviral therapy (ART) - Lamivudine, Zidovudine, Nevirapine (30 males and 30 females), Sixty adult HIV Sero-positive subjects not on antiretroviral therapy (25 males and 35 females) and Sixty-one adult Sero-negative control subjects (31 males and 30 females) respectively.

Seven millilitres (7mls) of blood sample was collected aseptically from the veins by venepuncture and aliqoted as follows; Two millilitres (2mls) into a plastic bottle containing 0.22mls (220µl) of 3.2% Sodium citrate to give a final blood: citrate ratio of 9:1. The sample was mixed properly by reverse uniform inversion and centrifuged within one hour at 3000rpm for 10 minutes at room temperature. The clear plasma was separated into a clean dry plastic container and used for Prothrombin time (PT) and Activated Partial Thromboplastin time (APTT) determination. Three millilitres (3mls) was drawn into bottles containing di-potassium salt of Ethylenediamine tetra-acetic acid (K2-EDTA) at a concentration of 1.5mg/ml of blood and used for Glucose-6-Phosphate Dehydrogenase enzyme activity assay, Full blood count and CD4 count while three millilitres (2mls) was drawn into plain sample bottles. Serum was obtained after clotting by spinning at 3000rpm for 10 minutes and used for Pyruvate kinase enzyme assay and HIV Screening. All the tests were carried out according to manufacturer's instructions.

Ethical Consideration

Ethical approval was obtained from Nnamdi Azikiwe University Teaching Hospital ethics committee (NAUTHEC) before the commencement of the study and the informed consent of the subjects were obtained before the commencement of the study.

Laboratory Methods

Determination of HIV status
Determine HIV 1/2 assay ((Alere Medical Co Ltd, Japan, 2012. Lot No: 52593K100)

The Determine^{TM} HIV ½ is an in vitro qualitative immunochromatographic test kit for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. The protective foil was removed and 50µl of the serum sample was applied to the sample pad. The result was read after 15 minutes. To ensure assay validity, a procedural control bar is incorporated in the assay device. The test is positive when red bars appear in both the control window and the patient window of the strip. The test is negative if one red bar appears in the control window of the strip and no red bar appears on the patient window of the strip. The test is invalid if there is no red bar in the control window of the strip and even if a red bar appears in the patient window, the result is invalid and should be repeated.

Chembio HIV ½ Stat-pak^{TM} assay (Chembio diagnostic System Inc. 2012, USA. Lot No. HIV070612)

The test kit was removed from its pouch and placed on a flat surface. The test device was labelled with sample identification number. The 5µl 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 5µl of sample onto the sample pad. The Running Buffer bottle was held vertically and three drops (approximately 105µl) of the Buffer was added slowly. The test result was read after 10 minutes of adding the Running Buffer.

Glucose-6-phosphate dehydrogenase (G-6-PD) enzyme assay (as described by the manufacturers of the kit; Randox laboratories LTD UK 2013. Lot No. PD410)

The enzyme activity is determined by measurement of the rate of absorbance change at 340nm due to the reduction of NADP+. Two millilitres (2mls) aliquot of 0.9% NaCl solution (Normal saline) was used to wash 0.2ml of the blood sample. It was centrifuged after each wash for 10 minutes at 3000 rpm. The washing process was repeated three times. The washed centrifuged erythrocytes was suspended in 0.5ml of Digitonin (Solution 4) and allowed to stand for 15 minutes at $40^{o}C$ and then centrifuged again. The supernatant (haemolysate) was used within two hours for the assay as follows;

One millilitre (1000μl) of the Buffer (R1), 0.03ml (30μl) of NADP (R2) and 0.015ml (15μl) of haemolysate were added into a clean test tube. It was mixed and incubated for five minutes at $37^{0}C$; 0.015mL (15μL) of substrate (R3) was then added and mixed properly. The initial absorbance was taken at 340nm, against air, with a cuvette of 1cm light path at room temperature and the Timer was started simultaneously. Readings were taken again after 1, 2 and 3 minutes.

Glucose-6-Phosphate Dehydrogenase (G-6-PDH) activity was expressed as $mU/10^9$ erythrocytes.

$mU/10^9$ erythrocytes = mU/erythrocytes per ml blood/RBC count per ml

mU/ erythrocytes per ml blood = 33650 x Change in Absorbance (340nm) x TCF (Temperature Correction Factor)

Where TCF at $25^{0}C$ = 2.076

Pyruvate kinase enzyme assay (as described by the manufacturers of the kit; Sigma-Aldrich Co LTD US, 2012Lot No: BV80109V)

Pyruvate kinase activity determines the pyruvate concentration by a coupled enzyme assay, which results in a colorimetric (570nm) product that is proportional to the pyruvate present. One unit of Pyruvate kinase is the amount of enzyme that will transfer a phosphate group from Phospho-enolpyruvate (PEP) to ADP to generate 1.0 μmole of pyruvate per minute at 25°C.

Sample preparation: Fifty microliter (50μl) of the serum sample was added directly to the wells. For the positive control, 5μl of the pyruvate kinase positive control was added to the wells and the volume was adjusted to 50μl with pyruvate kinase assay buffer.

Pyruvate standard preparation: Ten microliter (10μl) of the 100nmole/μl pyruvate standard was diluted with 990μl of the pyruvate kinase assay buffer to prepare a 1nmole/μl standard solution. 0, 2, 4, 6, 8 and 10μl of the 1nmole/μl standard solution was added into a 96 well plate generating 0 (blank), 2, 4, 6, 8 and 10 nmole/well standards. Pyruvate kinase assay buffer was added to each well to bring the volume to 50μl.

Assay reaction: The reaction mix was set up by pipetting 44μl of pyruvate kinase assay buffer, 2μl of the pyruvate kinase substrate mix, 2μl of the pyruvate kinase enzyme mix and 2μl of the fluorescent peroxidase substrate into a tube. The reaction mix for the sample blank also followed the same protocol with the omission of the pyruvate kinase substrate mix. Fifty microliter (50μl) of the appropriate reaction mix was added to each well and mixed properly. After two minutes, the initial measurement (A570) initial) was taken with a microplate reader at 570nm and the subsequent reading ((A570) final) was taken after five (5) minutes of incubation at $25^{0}C$. The pyruvate kinase standard curve was plotted after correcting for the background by subtracting the final measurement obtained for the 0 (blank) standard from the final measurement of the standards.

Calculations: ΔA570 = (A570) final – (A570) initial

The sample blank Δ measurement (ΔA570) value was subtracted from the sample Δ

measurement values (ΔA570) and the values obtained for each sample was compared to the standard curve to determine the amount of pyruvate generated by the kinase assay (B).

The pyruvate activity of each sample was then calculated as follows;

PK Activity = B × Sample dilution factor/ Reaction time × V

Where: B = Amount (nmole) of pyruvate generated between (T)initial and (T)final.

Reaction time = (T) final − (T) initial (minutes).

V = Sample volume (ml) added to the well.

The results were reported as nmole/min/ml = milliunit/ml; where one milliunit (mU) of the pyruvate kinase is defined as the amount of enzyme that will transfer a phosphate group from Phosphoenolpyruvate to ADP to generate 1.0 nmole of pyruvate per minute at 25^0C.

Prothrombin time (PT) [10]

Two hundred microliter (200μl) of Calcium-rabbit brain Thromboplastin mixture was placed in a clotting tube within a water bath at 37^0C, and incubated for 2 minutes at that temperature. Hundred microliter (100μl) of test (or control) plasma was then added and a Stop watch was started. The tube was gently tilted at regular intervals (returning to the water bath between tilting) and the time for the formation of a clot was recorded to give the Prothrombin Time (PT).

Activated partial thromboplastin time (APTT) [11]

It is a measure of the combined effect of the clotting factors of the Intrinsic and common coagulation pathways. Two hundred microliter (200μl) of Kaolin platelet substitute mixture was placed in a clotting tube in a 37^0C water bath and incubated for 2 minutes to attain temperature. Hundred microliters (100μl) of test plasma (or control) was added and the tube gently tilted at intervals for exactly 2 minutes. One

hundred microliter (100μl) of 0.025M Calcium Chloride (pre-incubated at 37^0C) was then added and a stopwatch started. The tube was tilted at regular intervals and the time for clot formation was recorded as the Activated Partial Thromboplastin Time (APTT).

Full blood count (FBC) estimation

By automation using Sysmex automated haematology analyser KX 21N model manufactured by Sysmex Corporation Kobe, Japan.The sample in EDTA bottle was placed in the Spiral Mixer and allowed to mix very well. Whole blood mode was activated in the LCD screen, the sample no (code) was inputted via the key board and then the enter key is entered. Then the sample was mixed very well again, the cap was removed and inserted into the probe and the START button was pressed. The LCD screen displayed ANALYSING; the sample was removed and re-capped. The unit executed automatic analysis and displayed the result on the LCD screen.

CD4 CELL COUNT

This was carried out using the Partec new model Cyflow Counter 2 which is an automated flow cytometer for the enumeration of CD4 and CD8- T lymphocyte cells in whole blood. Twenty microliter (20μl) of CD4-PE antibody and twenty microliter (20μl) of well mixed whole blood (EDTA) were added into Partec test tubes (Rohren tubes). The tubes were mixed gently and incubated in the dark for 15 minutes at room temperature. Eight hundred microliter (800μl) of CD4 buffer was added to the tubes and mixed gently. The cells were then counted on the Cyflow counter.

Statistical Methods

The data obtained was analysed 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

analysed using t-test, while significance of the differences in mean values among different groups was evaluated using one-way ANOVA. $P<.05$ was considered statistically significant.

RESULTS

Table 1 showed that for ART subjects, APTT was significantly higher in both subjects with CD4 <200 and 200 - 499 when compared with the corresponding values in subjects with CD4 ≥500 (P<0.05). Similarly, PT was significantly higher in CD4 <200 group and 200 – 499 group compared to ≥500 (P<0.05). Moreover, APTT for <200 was significantly higher than that of 200 – 499 group. However, no significant difference was observed in the mean values of G-6-PD and PK between subjects with CD4 count <200, 200 - 499 and ≥500 respectively (P>0.05).

Table 1: Mean±SD of red cell enzymes and coagulation parameters compared among HIV seropositive on art based on the cd4 count grouping.

CD4 COUNT (cells/µl)	G-6-PD (mU/10^9 RBC)	PK (mU/ml)	APTT (sec)	PT (sec)	PLT (X 10^9/L)
(A)< 200 (n = 13)	384.00 ± 325.55	33.46 ± 16.09	45.46 ± 2.73	21.23 ± 2.86	226.23 ± 74.63
(B)200 – 499 (n = 22)	468.00 ± 459.59	38.77 ± 17.53	42.36 ± 1.53	20.05 ± 1.62	285.05 ± 160.27
(C)≥ 500 (n = 25)	421.16 ± 383.42	41.60 ± 15.86	40.76 ± 1.71	18.00 ± 1.50	281.36 ± 154.70
F (P) – value	0.19 (0.83)	1.04 (0.36)	25.79 (0.00*)	13.97 (0.00*)	0.80 (0.46)
A vs B: P-value	0.81	0.64	0.00*	0.38	0.32
A vs C: P-value	0.95	0.32	0.00*	0.00*	0.31
B vs C: P-value	0.93	0.83	0.00*	0.00*	1.00

Significant at P < 0.05

Key: F(P) value = Mean ± SD of parameters compared within the HIV seropositive on ART based on CD4 Count grouping using ANOVA.

A vs B: (P) value = Mean ± SD of parameters compared between HIV seropositive on ART with CD4 count < 200cells/µl and 200 – 499cells/µl using t-test.

A vs C: (P) value = Mean ± SD of parameters compared between HIV seropositive on ART with CD4 count <200cells/µl and ≥ 500 cells/µl using t-test.

B vs C: (P) value = Mean ± SD of parameters compared between HIV seropositive on ART with CD4 count 200 – 499cells/µl and ≥ 500 using t-test.

Table 2 showed that for non-ART subjects, G-6-PD was significantly lower in subjects with CD4 <200 and 200 - 499 compared to those with CD4 count ≥500 (P<0.05). Similarly, APTT were significantly lower in <200 group compared to 200 - 499 and ≥500 (P<0.05). However, no significant difference was observed in the mean values of PK and PT for 200 – 499 group and ≥500 group when compared (P > 0.05).

Table 2: Mean ± SD Of Red Cell Enzymes And Coagulation Parameters Compared Among HIV Seropositive Not On Art Based On The Cd4 Count Grouping

CD4 COUNT (cells/µl	G-6-PD (mU/10^9 RBC)	PK (mU/ml)	APTT (sec)	PT (sec)	PLT (x10^9/L)
< 200 (n = 13)	74.00 ± 6.25	32.00 ± 13.75	38.00 ± 0.00	± 1.15	294.67 ± 135.86
200 – 499 (n = 22)	218.31 ± 155.90	43.34 ± 12.03	41.03 ± 3.24	18.16 ± 2.03	290.53 ± 125.37
≥ 500 (n = 25)	390.00 ± 163.07	39.40 ± 14.34	41.68 ± 4.27	18.16 ± 1.40	273.80 ± 105.97
F (P) – value	11.29 (0.00*)	1.40 (0.26)	1.39 (0.26)	0.63 (0.54)	0.16 (0.86)
A vs B: P-value	0.00*	0.48	0.00*	0.38	1.00
A vs C: P-value	0.00*	0.69	0.00*	0.37	0.97
B vs C: P-value	0.00*	0.52	0.80 1.00		0.85

Significant at P< 0.05

Key: F(P) value = Mean ± SD of parameters compared within the HIV seropositive not on ART based on CD4 count grouping using ANOVA.

A vs B: (P) value = Mean ± SD of parameters compared between HIV seropositive not on ART with CD4 count < 200cells/µl and 200 – 499 cells/µl using t-test.

A vs C: (P) value = Mean ± SD of parameters compared between HIV seropositive not on ART with CD4 count 200 cells/µl and ≥ 500 cells/µl using t-test.

B vs C: (P) value = Mean ± SD of parameters compared between HIV seropositive not on ART with CD4 count 200 – 499 cells/µl and ≥ 500 using t-test.

Table 3 showed that TWBC for seropositive on ART was significantly higher in those with CD4 count ≥ 500 compared to < 200 (P < 0.05). Moreover LYM was lower in those with CD4 < 200 compared to 200 – 499 and ≥ 500 (P < 0.05). However there was no significant difference in NEUT and MXD for the three CD4 groups (P > 0.05).

Table 3: Mean ± SD Of Parameters Compared For The Seropositive On Art Based On The CDC HIV Classification

CD4 COUNT	TWBC (X10⁹/L)	NEUT (X10⁹/L)	LYM (X10⁹/L)	MXD (X10⁹/L)
< 200 (n = 13)	3.75 ± 1.35	2.12 ± 1.03	1.26 ± 0.52	0.38 ± 0.24
(B) 200 – 499 (n = 22)	4.99 ± 1.67	2.77 ± 2.31	1.85 ± 0.48	0.66 ± 0.50
≥ 500 (n = 25)	5.66 ± 1.58	2.86 ± 1.14	2.30 ± 0.86	0.57 ± 0.27
F (P) Values	6.35 (0.00*)	0.94 (0.40)	10.35 (0.00*)	2.26 (0.11)
A vs B: P-values	0.06	0.49	0.01*	0.09
A vs C: P-values	0.00*	0.12	0.00*	0.09
B vs C: P-values	0.35	0.98	0.08	0.75

Significant at P < 0.05

F(P) value = Mean ± SD of parameters compared within the Seropositive on ART based on CDC HIV classification using ANOVA.

A vs B: (P) value = Mean ± SD of parameters compared between Seropositive on ART with CD4 count < 200 cells/µl and 200 – 499 cells/ µl using t-test.

A vs C: (P) value = Mean ± SD of parameters compared between Seropositive on ART with CD4 count < 200 cells/µl and ≥ 500 cells/ µl using t-test.

B vs C: (P) value = Mean ± SD of parameters compared between Seropositive on ART with CD4 count of 200 – 499 cells/ µl and ≥ 500 cells/ µl using t-test

Table 4 showed that for HIV seropositive subjects not on ART, there was no significant difference in TWBC, NEUT, LYM and MXD when compared among CD4 count groups of < 200, 200 – 499 and ≥ 500 (P >0.05).

Table 4: Mean ± SD Of Parameters Compared For The Seropositive Not On Art Based On The CDC HIV Classification

CD4 COUNT (cells/µl)	TWBC (X10⁹/L)	NEUT (X10⁹/L)	LYM (X10⁹/L)	MXD (X10⁹/L)
< 200 (n = 13)	5.97 ± 3.71	3.10 ± 1.80	2.50 ± 1.78	0.37 ± 0.31
(B) 200 – 499 (n = 22)	5.69 ± 1.78	2.96 ± 1.76	2.18 ± 0.94	0.56 ± 0.25
≥ 500 (n = 25)	6.46 ± 1.61	3.07 ± 1.10	2.63 ± 1.00	0.65 ± 0.37
F (P) Values	1.28 (0.29)	0.05 (0.96)	1.42 (0.25)	1.35 (0.27)
A vs B: P-values	0.99	0.99	0.95	0.60
A vs C: P-values	0.97	1.00	0.99	0.42
B vs C: P-values	0.21	0.95	0.21	0.59

Significant at P< 0.05

Key:

F(P) value = Mean ± SD of parameters compared within the Seropositive not on ART based on CDC HIV classification using ANOVA.

A vs B: (P) value = Mean ± SD of parameters compared between Seropositive not ART with CD4 count < 200cells/µl and 200 – 499 cells/ µl using t-test.

A vs C: (P) value = Mean ± SD of parameters compared between Seropositive not ART both with CD4 count 200 cells/µl and 500 cells /µl using t-test.

B vs C: (P) value = Mean ± SD of parameters compared between Seropositive not ART with CD4 count of 200 – 499 cells/ µl and ≥ 500cells/ µl using t-test.

DISCUSSION

Haematological abnormalities frequently encountered in HIV-infected individuals are anaemia, granulocyte disorders, thrombocytopenia, lymphomas, coagulopathies and vascular malignancies like Kaposi sarcoma. [12] It is well documented that CD4 cells are the primary target of HIV and is the most commonly used marker to determine HIV progression and also helps to obtain information on immune responses, staging of HIV disease, risk of mother to child transmission, use and response to antiretroviral treatment. [13] Therefore, reduction in absolute number of CD4+ T cells occurs as one of the earliest

immunologic abnormalities of HIV infection and is the most important prognostic indicator for risk of developing opportunistic infections. [14] Similarly, CD4 Lymphocyte count is essential for assessment of immune status in HIV-infected persons as the pathogenesis of AIDS is largely attributed to a decrease in absolute CD4 cell counts. CD4 cell counts are the criterion for categorising HIV-related clinical conditions by CDC classification system for HIV infection. [15] In line with this, patients in the present study were divided into three groups based on CD4 Lymphocyte count while G-6-PD, PK, coagulation and white cell parameters were compared in these three groups.

From our study, APTT and PT were prolonged in ART patients with CD4 count of <200 and 200 – 499 compared to ≥500; this shows a derangement of both the Extrinsic and intrinsic haemostatic pathway with progression of HIV infection as depicted by a lowered CD4 count. This could be as a result of liver damage. Because the liver is the major organ responsible for the synthesis of most coagulation factors and there is documented evidence that infection of the liver by HIV can lead to abnormal production of coagulation factors. [9]

APTT was also more prolonged in <200 compared to 200 – 499; this implies that the derangement in the extrinsic haemostatic pathway was directly related to the reduction in CD4 count in HIV infected patients on antiretroviral therapy. Similarly, TWBC in ART patients was reduced in <200 than ≥500, this indicates a higher occurrence of Leucopenia with progression of disease; moreover LYM in ART was reduced in<200 than 200 – 499 and ≥500 also indicating higher possibility of lymphopenia with HIV disease progression. These findings were supported by previous studies [16] which discovered that

Haemoglobin, mean total Leukocyte count, mean absolute Lymphocyte count was lower with reducing CD4 cell count, indicating a higher occurrence of anaemia, leucopenia and lymphopenia with reducing CD4 count or disease progression.

Moreover, in non-ART subjects G-6-PD activity was lower in subjects with CD4 count of <200 and 200 – 499 compared to ≥500; this indicates that unlike in ART subjects, G-6-PD activity decreased with HIV disease progression. The reason for the decrease in G-6-PD activity is not clear but could be attributed to the generalised impact of HIV infection on the body system which sometimes leads to suppression and derangement of some enzyme functions. APTT was significantly reduced in CD4 count<200 compared to 200 – 499 and ≥500, this shows that derangement of the extrinsic haemostatic pathway in patients not on ART is not proportionate to disease progression.

Also, normal platelet count was observed in all the groups and contradicts the general notion that thrombocytopenia due to increased platelet destruction and decreased platelet production predominates in HIV subjects, [17] with concomitant negative effect on normal haemostasis which predisposes the individual to bleeding tendencies. This also implies that platelet count is not affected with HIV disease progression which agrees with a previous study. [18]

CONCLUSION
This study shows that in ART subjects, both the extrinsic (APTT) and intrinsic (PT) haemostatic pathways were deranged and Leucopenia and lymphopenia were also observed with progression of HIV infection as depicted by lowered CD4 count. While in non-ART, G-6-PD activity and APTT were decreased with HIV progression as also depicted by decreased CD4 count.

Recommendation

Coagulation tests like PT and APTT should be included in the routine tests for management of HIV patients. Liver function test and viral load assay should be conducted in future studies to ascertain the contribution of liver damage and viraemia to abnormal coagulation parameters in HIV patients.

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