

Full Length Research Paper

Microbial, biochemical and hematological indices of antiretroviral (ART) treatment naïve human immunodeficiency virus (HIV) seropositive patients

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The aim of the study was to investigate the efficacy of the use of microbial, biochemical, and hematological indices as diagnostic and prognostic parameters of antiretroviral therapy (ART) naïve human immunodeficiency virus (HIV) seropositive patients. The experimental design was a single factor completely randomized design (CRD). Twenty (n=20) healthy seronegative human subjects (control) and fifty (n=50) HIV seropositive patients were subjected to *in vitro* qualitative HIV-1/HIV-2 assay, HIV viral load (vl) test, CD4+ T lymphocyte cells count, serum albumin (A), serum aspartate aminotransferase (AST), serum total bilirubin (Tb), serum total cholesterol (C), serum triglyceride (T), Hemoglobin (Hb) tests, and blood group and genotype tests (of the HIV seropositive patients). Results on the HIV-1/HIV-2 assay: 35 (70%) HIV-2 seropositive patients, 3(6%) HIV-1/HIV-2 co-infection patients, 12(24%) HIV-1 seropositive patients. Results recorded of the control group and HIV positive patients, expressed as mean \pm standard error (S.E) (unit) were as follows : vl (0.0 \pm 0) and (400 \pm 70) (copies/ml), CD4+ (1100 \pm 100) and (350 \pm 50) (μ L), A (4.45 \pm 0.1) and (2.81 \pm 0.05) (g/dl), AST (8.3 \pm 0.7) and (40.3 \pm 0.7) (U/l), Tb (0.62 \pm 0.2) and (2.10 \pm 0.3) (mg/dl), C (165 \pm 3.4) and (100.8 \pm 0.3) (mg/dl), T (125.8 \pm 4.7) and (150.8 \pm 1.7) (mg/dl), Hb (14.25 \pm 0.1) and (8.1 \pm 0.3) (g/dl), respectively. Blood group and genotype test results (n=50): A=6(12%), B=5(10%), AB=4(8%), O=35(70%); AA=10(20%), AS=40(80%). The mean values of vl, AST, Tb and T were significantly higher (p<0.05), but those of CD4+, A, C and Hb were significantly lower (p<0.05) in the HIV seropositive patients compared with the control. The statistical regression and correlation between HIV viral copies/ml and Hb (g/dl) of HIV seropositive patients were significant (p<0.05)(r = 0.985), regression equation: \hat{Y} (copies/ml) = 160000 - 19703.704x_i (g/dl). Incidence of HIV infection correlated positively and significantly (p<0.05) with significant increase (p<0.05) in vl, AST, Tb, T and significant decrease (p<0.05) in C, CD4+, A and Hb of the ART treatment naïve HIV seropositive patients. These significant differences/alterations could be used as effective criteria/yardstick for the diagnosis and prognosis of HIV infection.

Key words: Lymphocyte, seropositive, prognosis, infection.

INTRODUCTION

The human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) is a global pandemic

(Cohen *et al.*, 2008). Approximately 36.7 million people were living with HIV globally, as at 2012 of which, approximately 17.2 million were men, 16.8 million were women and 3.4 million were less than 15 years old. There were about 1.8 million deaths from AIDS in 2010, and 1.1 million deaths from AIDS-related illnesses in 2015

(UNAIDS, 2016).

HIV is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time AIDS by causing progressive failure of the immune system, allows life-threatening opportunistic infections and cancers to thrive [Weiss (1993), Douek *et al.* (2009)].

HIV is roughly spherical, and is composed of two copies of positive single-stranded RNA each of which is tightly bound to nucleocapsid proteins, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. These single-stranded RNA code for the virus's nine genes enclosed by a conical capsid made up of 2,000 copies of the viral protein (McGovern *et al.*, 2002).

Infection with HIV present as both free virus particles and virus within infected immune cells, and occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate or breast milk (Douek *et al.*, 2009). HIV infects helper T cells (specifically CD4⁺ T cells), macrophages, microglial and dendritic cells (Cunningham *et al.*, 2010), leading to low levels of CD4⁺ T cells via pyroptosis of abortively infected T cells (Doitsh *et al.*, 2014), apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected CD4⁺ T cells by CD8 cytotoxic lymphocytes that recognize infected cells (Garg *et al.*, 2012), and subsequent loss of cell-mediated immunity (Kumar *et al.*, 2012).

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is more virulent, more infective and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 have a relative poor capacity for transmission, and are largely confined to West Africa (Reeves and Doms, 2002).

Interaction of the virion envelope glycoproteins (gp120) with the CD4 molecule on the target cells and also with chemokine co-receptors mediates HIV-1 entry to macrophages and CD4⁺ T cells. Macrophage (M-tropic) strains of HIV-1, also known as non-syncytia-inducing strains (NSI) or R5 viruses, use the β -chemokine receptor CCR5 for entry and are able to replicate in macrophages and CD4⁺ T cells [Chan *et al.* (1997), Arrildt *et al.* (2012)]. T-tropic isolates, also known as syncytia-inducing (SI) or X4 viruses strains replicate in primary CD4⁺ T cells as well as in macrophages and use the α -chemokine receptor (Berger *et al.*, 1998). People with the CCR5- Δ 32 mutation are resistant to HIV infection with R5 virus because the mutation stops HIV from binding to this co-receptor, reducing its ability to infect target cells (Tang and Kaslow, 2003).

HIV spreads between CD4⁺ T cells by two parallel routes which are hybrid spreading mechanisms viz: cell-free spread (virus particles bud from an infected T cell, enter the blood/extracellular fluid and then infect another T cell), and cell-to-cell spread (infected T cell can transmit virus directly to a target T cell via a virological synapse

and/or an antigen presenting cell (APC) can also transmit HIV to T cells by a process that involves either productive infection (in macrophages) or capture and transfer of virions in trans (in dendritic cells) [Jolly *et al.* (2004), Sattentau (2008), Zhang *et al.* (2015)].

HIV is the causative agent of acquired immune deficiency syndrome, or AIDS. HIV protease inhibitors form non-covalent complexes with the HIV protease, but bind so tightly that they could be considered, irreversible inhibitors, and include Indinavir, Nelfinavir, Lopinavir, and Saquinavir. These drugs have vastly increased the lifespan and quality of life of millions of people with HIV and AIDS (Nelson and Cox, 2008).

Other antiretroviral drugs used in treatment of HIV infection include: Nucleoside Reverse Transcriptase Inhibitors (NRTIs) e.g didanosine, which blocks reverse transcriptase, Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) e.g efavirenz, which binds to and later alters reverse transcriptase, Fusion Inhibitors e.g enfuvirtide, which blocks HIV from entering the CD4⁺ cells of the immune system; Entry Inhibitors e.g maraviroc, which blocks proteins on the CD4⁺ cells that HIV needs to enter the cells; Integrase Inhibitors e.g dolutegravir, which blocks HIV integrase, Pharmacokinetic Enhancers e.g cobicistat, which is used to increase the effectiveness of an HIV medicine included in an HIV regimen; and Combination HIV Medicines e.g abacavir and lamivudine, which contain two or more HIV medicines from one or more drug classes (FDA, 2016).

HIV has been shown to bind to erythrocytes, and candidate erythrocyte-binding molecules. Erythrocyte-HIV binding is associated with increased viral infectivity. Erythrocyte antigens may be important in the pathogenesis and epidemiology of HIV (Motswaledi *et al.*, 2013). Sayal *et al.* (1996) reported a preponderance for infection in group O Rh(D) positive men and least among groups B positive and D-negative ones in India. Group O positive individuals were thought to be highly susceptible (Ukaejiofo and Nubila, 2006). A higher prevalence of HIV-2 (71.4%) compared with HIV-1 (7.1%) in the AB blood group, and a higher susceptibility to HIV infection of Rh(D) positive (97.8%) compared with D negative (2.2%) of individuals in Adamawa State of Nigeria was reported by Abdulazeez *et al.* (2008).

Significant decrease ($p < 0.05$) in hemoglobin and hematocrit levels were observed of a 45-year-old African American man, who presented with a CD4⁺ cell count of 5/ μ L and an HIV viral load of 150,000 copies/mL, after the administration of a 4-drug regimen of abacavir, stavudine, ritonavir, and saquinavir; and trimethoprim-sulfamethoxazole for *Pneumocystis carinii* pneumonia prophylaxis and azithromycin for *Mycobacterium avium* complex infection prophylaxis (Naval-Srinivas and Preston-Church, 2003).

The mean haemoglobin concentrations of 228 consented people living with HIV/AIDS (PLWHAs) with CD4 counts

$<200 \text{ mm}^{-3}$, $200-499 \text{ mm}^{-3}$ and $\geq 500 \text{ mm}^{-3}$ were significantly lower ($p < 0.05$) in comparison with the control group (healthy HIV-seronegative individuals) (Obirikorang and Yeboah, 2009).

Viral, bacterial, and parasitic infections, sepsis, tuberculosis, coccidioidomycosis, burns, trauma, intravenous injections of foreign proteins, malnutrition, over-exercising, pregnancy, corticosteroid use, normal daily variation, psychological stress, and social isolation could lead to low CD4+ T lymphocyte counts (CD4 counts). Some of these conditions account for CD4+ counts below 200 per cubic millimeter, which is the level needed to diagnose acquired immunodeficiency syndrome (AIDS) in individuals previously positive for antibodies to the human immunodeficiency virus (HIV-positive). Some normal, healthy individual, have low CD4+ counts for no apparent reason (Irwin, 2001). It is imperative to exercise caution regarding the use of CD4+ counts to make treatment and diagnostic decisions on HIV/AIDS.

The total number of white blood cells in an average healthy adult is 4000 to 11,000/ μl . If there are insufficient numbers of white cells, especially of neutrophils (neutropenia), then the risk of bacterial and fungal infections is higher (Alcorn, 2016).

Mean AST and ALT levels observed of seventy-five (75) individuals in Specialist Hospital, Sokoto, Nigeria, listed in consecutive significant increase ($p < 0.05$) is as follows (25 individuals per group): HIV negative group (control), HIV positive non-treated group, HIV positive treated with ARVs (Abubakar *et al.*, 2014). HIV infects a wide range of non-hematopoietic cells, including hepatocytes (Megan *et al.*, 2012). Lefkowitz (1994) reported that liver enzymes elevation are frequent in HIV infected patients.

Significant increase ($p < 0.05$) in serum total bilirubin was observed of forty (40) HIV positive patients in comparison with forty (40) HIV negative, healthy subjects in Aurangabad, Maharashtra, India: the elevated level of bilirubin in the HIV positive patients was associated with impaired biliary excretion (Patil and Kamble, 2015).

Malnutrition and low concentration of serum albumin among human immunodeficiency virus (HIV)-infected individuals are cofactors for HIV disease progression (Sundaram *et al.*, 2009).

Individuals with hypoalbuminemia (serum albumin concentration $< 3.5 \text{ mg/dl}$) at antiretroviral therapy initiation were 4.52 times more susceptible/vulnerable to death than individuals with serum albumin concentrations of $\geq 3.5 \text{ mg/dl}$. Hypoalbuminemia was also independently significantly correlated with the incidence of pulmonary tuberculosis ($p < 0.001$), severe anemia ($p < 0.001$), wasting ($p = 0.002$), and $>10\%$ weight loss ($p = 0.012$) (Sudfeld *et al.*, 2013).

The mean low density lipoprotein (LDL) was significantly higher ($p < 0.01$) among the naïve HIV positive test group

compared with the control normal, healthy individuals, p value=0.000. The mean high density lipoprotein (HDL) was also significantly lower ($p < 0.01$) reaching a dyslipidemic level, in the HIV positive group in comparison with the control (Adewole *et al.*, 2010). Low levels of serum total cholesterol (TC), elevated serum triglyceride (TG), High density Lipoprotein (HDL) and Low density Lipoprotein (LDL) have been observed of HIV positive patients [Périad *et al.* (1999), Mondy *et al.* (2007)].

Elevated TG correlated positively with interferon alpha, advanced/opportunistic infection during markedly reduced immunity and delayed clearance due to reduced lipoprotein lipase activity In HIV infection (Gouni *et al.*, 1993).

The aim of the study was to investigate the efficacy of the use of microbial, biochemical, or hematological index/indices [HIV viral load (vI), CD4 count, serum albumin (A), serum aspartate amino transferase (AST), serum total bilirubin (Tb), hemoglobin concentration (Hb) (g/dl), serum triglyceride (TG), and serum total cholesterol (C)] as diagnostic parameters of HIV seropositive patients which could also be useful as prognostic parameters in managing and monitoring HIV infection.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The experimental design is a single-factor completely randomized design (CRD).

SPSS for windows (version 17.0, SPSS, Chicago, IL, USA) was used to perform the statistical analyses. The significance level was $p < 0.05$.

Selection of subjects:

Fifty ($n = 50$) clinically confirmed male HIV seropositive patients of age bracket 25-50 years and twenty normal and healthy seronegative male subjects ($n=20$) of the same age bracket, voluntarily participated in this study from Owerri municipal area. Exclusion criteria were symptoms of any other infection, history of bleeding disorders and use of any anticoagulant drug within two weeks prior to specimen collection (Omorieg *et al.*, 2009); individuals with physical sign of liver cirrhosis or any metabolic syndrome, alcoholic consumption, cigarette smokers or drug abusers, Hepatitis A, B or C positive patients (Abubakar *et al.*, 2014), sickle cell anemia patients.

The research was carried out in compliance with the Declaration on the Right of the Patient (WMA, 2000). Blood was obtained by veni-puncture carried out by a Phlebotomist nurse. The method described by Thavasu

et al. (1992) was used in obtaining the serum. Whole blood was collected in a covered test tube, and allowed to clot by leaving it undisturbed for 15-30 minutes at room temperature. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge, to obtain the blood serum. Citrate phosphate dextrose-adenine 1 (CPDA-1) stored whole blood was used for whole blood analysis.

ABO blood grouping and Rhesus D typing

The ABO blood grouping and Rhesus D typing were carried out consistent with the methods described by Jeremiah (2006). ABO blood grouping was carried out using anti-A, anti-B, and anti-AB (Biotec, Ipswich, UK), 5% red blood cell suspension in saline. After mixing proportionately, the mixture was centrifuged for 30 seconds at 1000g. The cell buttons were observed for agglutination. Agglutination of tested, resuspended red cells constituted positive results. Absence of agglutination of resuspended red cells constituted negative test results. Rhesus D typing was carried out using anti-D serum (Biotec, Ipswich, UK), control, 5% RBC suspension in saline, followed by incubation at 37°C, mixing, centrifugation for 30 seconds at 1000g. Agglutination was read macroscopically and microscopically. All negative results were confirmed using the indirect antiglobulin test (IAT) procedure (also for confirmation of weak D).

Determination of Haemoglobin Genotype

The technique for haemoglobin electrophoresis described by John and Lewis (1986) and Tidi *et al.* (2013) was employed. Fifty micro-liters of washed cells were added into khan tubes containing 50 micro-liter of 0.1% white saponin and were mixed thoroughly (haemolysate). The haemolysate was centrifuged to remove any debris. The supernatant was used for the test. Cellulose acetate papers were soaked and blotted. Haemoglobin genotype controls used include: HbA, HbF, HbS and HbC. One hundred ml of the Tris-EDTA and boric acid buffer was introduced into each of the outer section of the electrophoresis chamber. One micro-liter of each haemolysate sample (tests and controls) was transferred into the well plate. Using an applicator, 0.5 micro-liter of the haemolysate (samples and controls) was applied onto the cellulose acetate paper leaving about 0.5 cm gap for each sample. The cellulose paper was placed on a cathode bridge of the electrophoresis chamber containing Tris-EDTA and boric acid buffer. Two hundred voltages were applied for 15 minutes, and the results recorded.

Hemoglobin concentration assay

The method described by ICSH (1996) which is based on the photocolometric detection of cyanmetahemoglobin

was used for the quantitative assay of hemoglobin concentration (g/dl).

***In vitro* qualitative HIV-1/HIV-2 assay**

The highly sensitive HIV-1/HIV-2 enzyme immunoassay (EIA) was carried out, and the Western blot, a highly specific immunoblot that allows for the visualization of antibodies to the structural polypeptides of HIV was employed as a confirmatory test using the sera of all patients who tested HIV seropositive by the EIA screen tests [CDC (2001), Zhang and Versalovic (2002)].

The HIV viral load test is used along with a CD4+ count to determine the status of HIV in a person diagnosed with the infection, and also to monitor the effectiveness of antiretroviral treatment (ART) over time.

HIV viral load test

The Bayer Versant Human Immunodeficiency Virus Type 1 Branched-DNA Viral Load Assay was employed in the determination of the HIV viral load of the HIV seropositive patients, using Bayer Quantiplex HIV-1 bDNA version 3.0 (bDNA 3.0) assay as described by Elbeik *et al.* (2000).

CD4+ T lymphocyte cells count

The CD4 T- cells were enumerated using flow cytometry (FCM) method (Cassens *et al.*, 2004), using Cyflow Counter (Partec, Munster, Germany).

***In vitro* quantitative analysis of aspartate amino transferase (AST) activity**

In vitro quantitative determination of serum aspartate amino transferase (AST) activity was carried out using the method employed by Reitman and Frankel (1957). The test based on the reaction in which L-aspartate and α -ketoglutarate are converted to L-glutamate and oxaloacetate by the catalytic activity of AST. The oxaloacetate so formed, forms a complex known as oxaloacetate hydrazone with 2,4-dinitrophenyl hydrazine. The intensity of the colour of the hydrazone, which is measurable with a spectrophotometer (Thermo scientific model G10S UV-Vis) at 546nm is directly proportional to the AST enzyme activity.

Quantitative *in Vitro* Determination of Serum Albumin

Quantitative *in vitro* determination of serum albumin was carried out using the method described by Qureshi and Qureshi (2001) and Huang and Fraker (2003). Serum albumin was determined using human albumin standards and sigma diagnostics albumin reagent (Sigma, St. Louis, MO) containing bromocresol green. The absorbance of the mixture of the reagent and serum albumin was measured

at 578 nm against a reagent blank.

Serum total bilirubin assay

Serum total bilirubin assays was carried out consistent with the method described by Simmons (1968). In the determination of total bilirubin, the serum was added to a caffeine reagent, which acted as an accelerator, and then mixed with combined diazo reagent. The diazo reaction was terminated by the addition of ascorbic acid which destroyed the excess diazo reagent, the azo bilirubin was made alkaline by the addition of a tartrate buffer, and the intensity of the colour (absorbance) was read at 600 nm.

Serum lipid assays

Serum total cholesterol (C), and serum triacylglycerol (TG) were determined using commercial kits (Randox Laboratory Ltd., UK), in conformity with the methods employed by Ibegbulem and Chikezie (2012); Chikezie and Okpara (2013).

RESULTS

Results on the *in vitro* qualitative HIV-1/HIV-2 assay reveal that 35 (70%) of the patients were HIV-2 seropositive, 3(6%) of the patients suffered HIV-1/HIV-2 co-infection, 12(24%) of the patients were HIV-1 seropositive. Twenty (20) healthy subjects were HIV seronegative.

Shown in Table 1 are results on blood genotype and blood group/typing of the HIV seropositive patients. The number/percentage of the different types of blood groups of the HIV seropositive patients listed in order of sequential significant decrease ($p < 0.05$) were: 35(70%)O, 6(12%)A/5(10%)B/4(8%)AB. The number/ percentage of the different blood typing of the HIV seropositive patients listed in order of sequential significant decrease ($p < 0.05$) were: 38(76%)Rh D+, 12(24%)Rh D-. The number/percentage of the different blood genotypes of the HIV seropositive patients listed in order of consecutive significant decrease ($p < 0.05$) were: 40(80%)AS, 10(20%)AA.

The CD4+ T lymphocyte (white blood) cell count of the HIV seropositive patients was significantly lower ($p < 0.05$) than that index of the healthy seronegative subjects (control) (Table 2).

Table 2 also shows results on the viral load of the HIV seropositive patients, and healthy seronegative subjects. The HIV seropositive patients had a mean viral load of 400 ± 70 copies/mL. The healthy HIV seronegative subjects had no detectable viral load, and actually were not HIV infected as confirmed by the highly sensitive HIV-1/HIV-2 enzyme immunoassay (EIA) and Western blot assay.

The mean value of serum albumin (g/dl) of the HIV seropositive patients was significantly lower ($p < 0.05$) than that index of the healthy HIV seronegative subjects (Table 2). The mean value of aspartate amino transferase activity (U/l) of the HIV seropositive patients was significantly higher ($p < 0.05$) than the corresponding index of the healthy HIV seronegative subjects (Table 2). Table 3 shows the results on the serum total bilirubin, serum triglyceride, and serum total cholesterol of the HIV seropositive patients and the healthy HIV seronegative subjects. The mean values of serum total bilirubin, and serum triglyceride of the HIV seropositive patients were significantly higher ($p < 0.05$), but the mean values of serum total cholesterol was significantly lower ($p < 0.05$), in comparison with the corresponding indices of the healthy HIV seronegative subjects.

The mean value of hemoglobin (Hb) concentration of the HIV seropositive patients was significantly lower ($p < 0.05$) compared with the corresponding index of healthy HIV seronegative subjects (Figure 1).

DISCUSSION

In the present study, it was observed that 35 (70%) of the patients were HIV-2 seropositive, 3(6%) of the patients were HIV-1/HIV-2 co-infected, 12(24%) of the patients were HIV-1 seropositive, consistent with the findings of Abdulazeez *et al.* (2008), and Reeves and Doms (2002) who postulated that fewer of those exposed to HIV-2 have a relative poor capacity for transmission, and are largely confined to West Africa.

The results on blood genotype and blood group/typing of the HIV seropositive patients (Table 1) show a preponderance for HIV infection in group O Rh(D)+ male patients and least among group AB patients. The infection is prevalent among Rh D+ patients compared with Rh D- ones and corroborates the findings of Sayal *et al.* (1996), Ukaejiofo and Nubila (2006), and Abdulazeez *et al.* (2008), who separately, reported high susceptibility to HIV infection of blood group O Rh D + individuals.

CD4+ T lymphocyte (CD4) cell count and HIV RNA (viral load) are the two surrogate markers of antiretroviral treatment (ART) and highly active antiretroviral therapy (HAART) responses and HIV disease progression, useful in managing and monitoring HIV infection (AIDS Info, 2016).

The CD4+ T lymphocyte cell count of the HIV seropositive patients was significantly lower ($p < 0.05$) than that index of the healthy seronegative subjects (control) by a margin of 750/ μ L difference (Table 2). This corroborates the findings of Omoregie *et al.* (2009) who observed significant reduction ($p < 0.001$) in CD4+ count of HIV seropositive patients compared with the seronegative controls. The normal CD4+ count range is

Table 1. Results on the blood genotype, and blood group/typing tests of the HIV seropositive patients.

Blood genotype	Blood group/typing			
	A	B	AB	O
AS	1Rh D+, 2Rh D-	2Rh D-	3Rh D+	30Rh D+, 2Rh D-
AA	2Rh D+, 1Rh D-	2Rh D-, 1Rh D+	1Rh D+	3Rh D-

(n = 50).

Values indicate no. of HIV seropositive patients that fall under intersecting classifications of: blood group/typing and blood genotype.

Table 2. Results on the biochemical indices: CD4+ T lymphocyte (white blood) cell count, HIV viral load, serum albumin, aspartate amino transferase activity of the HIV seropositive patients, and healthy seronegative subjects.

	CD4+ T lymphocyte (white blood) cell count / (μL)	HIV viral load (vl) (copies/mL)	Serum albumin (A) (g/dl)	Aspartate amino transferase activity (AST) (U/l)
Healthy seronegative subjects	1100 ± 100 ^a	0.0±0 ^a	4.45±0.1 ^a	8.3±0.7 ^a
HIV seropositive patients	350 ± 50 ^b	400 ± 70 ^b	2.81 ± 0.05 ^b	40.3±0.7 ^b

Results are expressed as mean ± standard error (S.E) (unit) (n1 =sample size of healthy seronegative subjects =20, n2 sample size of HIV seropositive patients = 50).

Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

400 – 1600 cells/mm³. ART treatment naïve HIV seropositive patients who have CD4+ count of much less than 600 could still transmit HIV. An adequate response for patients on HIV therapy is defined as an increase in CD4+ count in the range of 50 to 150 cells/mm³ during the first year of ART, generally with an accelerated response in the first 3 months of treatment, with subsequent average increases of approximately 50 to 100 cells/mm³ per year until a steady state level is reached (Kaufmann *et al.*, 2003).

Devices used to determine the viral load cannot detect HIV if there are fewer than 40 to 50 copies of HIV/mL (undetectable viral load). Optimal viral suppression describes a viral load persistently below the level of detection (HIV RNA <20 to 75 copies/mL, depending on the assay used). The AIDS Clinical Trials Group (ACTG) define virologic failure as a confirmed viral load >200 copies/mL. The determination of pre-treatment viral load level is of some importance in the selection of an initial ARV regimen because most approved ARV drugs or regimens have been associated with poorer responses in patients with high baseline viral load. The HIV seropositive patients had a mean viral load of 400 ± 70 copies/mL (each had a confirmed viral load >200 copies/mL) (Table 2), and therefore could be described as suffering from virologic failure (AIDS Info, 2016).

The mean value of serum albumin (g/dl) of the HIV seropositive patients was significantly lower (p<0.05) than the corresponding index of the healthy HIV seronegative subjects (Table 2), and is in keeping with the finding that serum albumin was lower in non-ART HIV seropositive individuals compared with the control (HIV seronegative individuals) (Ezugwu *et al.*, 2015). Serum albumin levels are a good predictor of the severity of HIV infection in antiretroviral therapy treatment naïve patients and can also indicate the extent of a patient's response to HIV treatment. Pretreatment albumin correlated significantly and positively (p=0.006) with pretreatment CD4+ cell count (Olawumi and Olatunji, 2006). Serum albumin would be a useful biochemical test for HIV disease in resource-limited settings (Sundaram *et al.*, 2009). Production of free radical is increased in HIV infection. Serum albumin is a major antioxidant agent which becomes depleted significantly as it combats oxidative stress by bio-transforming the free radicals to less/non-toxic compounds that are easily excretable.

The mean value of aspartate amino transferase activity (U/l) of the HIV seropositive patients was significantly higher (p<0.05) than the corresponding index of the healthy HIV seronegative subjects (Table 2). This finding is lent credence by the observations that the activities of serum alanine aminotransferase (ALT) and aspartate amino-

Table 3. Results on the biochemical indices: serum total bilirubin (Tb), serum triglyceride (T), and serum total cholesterol (C) of the HIV seropositive patients, and healthy seronegative subjects.

	Serum total bilirubin (Tb) (mg/dl)	Serum triglyceride (T) (mg/dl)	Serum total cholesterol (C) (mg/dl)
Healthy seronegative subjects	0.62±0.2 ^a	125.8±4.7 ^a	165 ± 3.4 ^a
HIV seropositive patients	2.10±0.3 ^b	150.8±1.7 ^b	100.8 ± 0.3 ^b

Results are expressed as mean ± standard error (S.E) (unit) (n1 = 20, n2 = 50). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

transferase (AST) of HIV infected asymptomatic patients were significantly higher (p<0.05) than those of apparently healthy subjects who tested negative for antibodies for HIV 1 and 2 (control) (Ignatius *et al.*, 2009). HIV infection is associated with endothelial dysfunction and liver damage, both of which can result in blood coagulation defects (Andrade and Cotter, 2006), leading to the leakage of the parenchymal enzymes e.g aspartate amino transferase (AST) and membranous enzymes e.g alkaline phosphatase, into the blood circulation. Oxidative stress engendered by HIV infection is implicated as a causative agent of elevated AST enzyme activity. However, associated disease conditions of HIV infection such as hepatitis, cirrhosis, hepatic cholestasis, hepatobiliary disease could be secondary in causing an increase in the activities of AST enzyme (Lawn, 2004). The International Federation of Clinical Chemistry (IFCC) estimated normal reference range for AST is 8-20U/l (Schneider *et al.*, 2005).

The mean value of serum total bilirubin (Tb) of the HIV seropositive patients was significantly higher (p<0.05) compared with the healthy HIV seronegative subjects (Table 3). The results conform with the postulates of Analike *et al.* (2006), who posited that total bilirubin (Tb) of HIV positive patients (not on ART) was significantly increased (p<0.01) compared with the control HIV seronegative subjects in an evaluation of liver function tests of HIV positive patients in Nnewi, Nigeria.

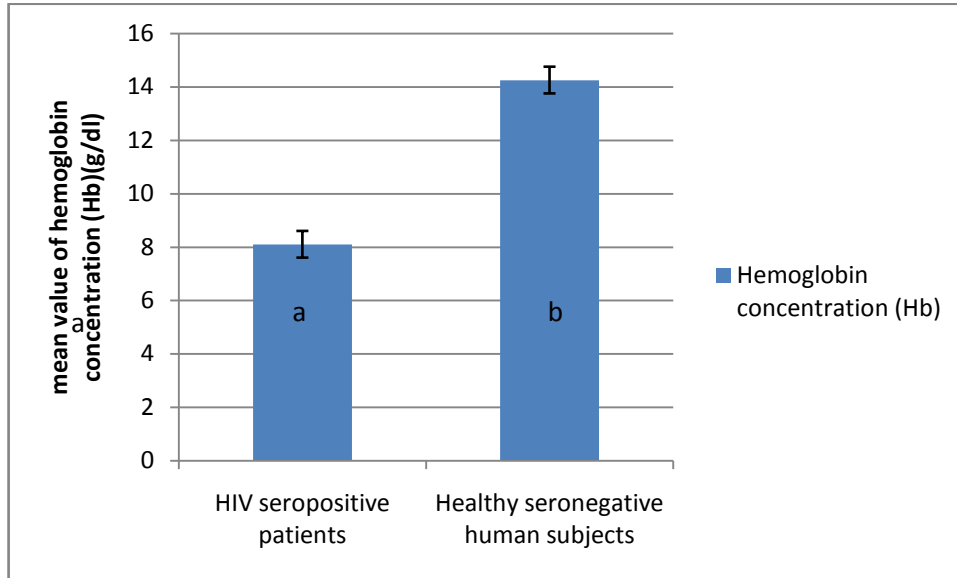
Abnormalities of the biliary tract including intra and extra hepatic cholangitis sclerosis, papillary stenosis and acalculous cholecystitis have been implicated as major causative factors of elevated serum bilirubin levels in HIV seropositive patients. The infecting organisms are usually *Cryptosporidia*, *Cytomegalovirus*, or *Microsporidia* that infect vascular endothelium causing ischemic vasculitis and bile duct damage (Patil and Borgaonkar, 2015).

The mean values of serum total bilirubin, and serum triglyceride of the HIV seropositive patients were significantly higher (p<0.05), but the mean values of serum total cholesterol was significantly lower (p<0.05),

in comparison with the corresponding indices of the healthy HIV seronegative subjects: a finding consistent with the report that HIV-positive patients had significantly lower mean value (p=0.01) of serum total cholesterol, and significantly higher mean value (p=0.01) of serum triglyceride compared with the controls in a study of lipid profile of anti-retroviral treatment-naïve HIV-Infected Patients in Jos, Nigeria (Daniyam and Iroezindu, 2013).

Changes in lipid profile of HIV seronegative patients has been found to correlate with the degree of immunosuppression. The mechanism of lipid disorders in ART-naïve HIV-infected patients is cytokine-mediated. Interferon- α is believed to facilitate increase in TG levels through a decrease in TG clearance as well as an increase in *de novo* hepatic lipogenesis and VLDL synthesis. Low levels of serum total cholesterol have been associated with elevated levels of β -2 microglobulin [Grunfeld *et al.* (1992), Khiangte *et al.* (2007)].

The mean value of hemoglobin (Hb) concentration of the HIV seropositive patients was significantly lower (p<0.05) compared with the corresponding index of healthy HIV seronegative subjects (Figure 1), and is in agreement with the findings that: prevalence of anemia was significantly higher among HIV-positive women (29%) compared with HIV-negative women (8%) (p < 0.001), At highly active antiretroviral treatment (HAART) initiation, hemoglobin level significantly increased (p < 0.001) by 1.4 ± 0.1 g/dl in 8 months: an indication that the hemoglobin concentration is an efficient prognostic index of HIV treatment. HAART is associated with a significant improvement in hemoglobin levels (Masaisa *et al.*, 2011). Bone marrow suppression by various cytokines, toxic depletion by the virus, immune destruction following sensitization with viral proteins and vitamin B₁₂ deficiency are some of the mechanisms responsible for anemia and are considered important as a prognostic indicator in AIDS patients [Olayemi *et al.* (2008), Volberding *et al.* (2003)]. Autoimmune haemolytic anaemia commonly occurs in HIV-infected individuals (Motswaledi *et al.*, 2013).



Statistical graphical results are expressed as mean ± standard error (mg/dl) ($n_1 = 20, n_2 = 50$). Error bars represent values of standard error (0.1 – 0.3 g/dl). Bars labeled with the same letters represent mean values of hemoglobin concentration (g/dl) which are not significantly different ($p < 0.05$).

Figure 1. Results (graphical) on the hematological index : hemoglobin concentration (g/dl) of the HIV seropositive patients, and healthy seronegative subjects.

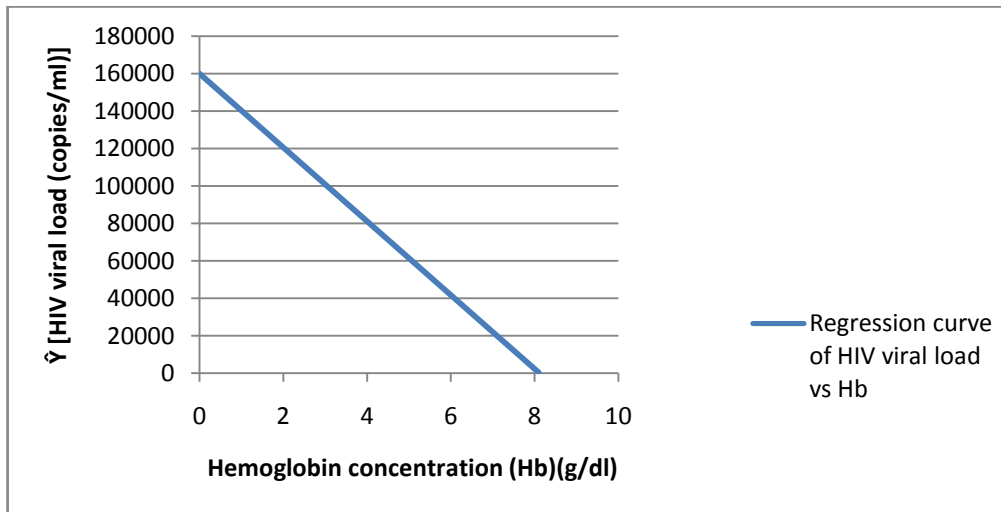


Figure 2. Regression curve of HIV viral load (copies/ml) vs Hemoglobin concentration (Hb)(g/dl) of the HIV seropositive patients: \hat{Y} (copies/ml) = $160000 - 19703.704x_i$ (g/dl).

Multiple regression studies revealed that The CD4+ cell count regressed significantly ($p < 0.05$) with serum total cholesterol, HIV viral load, serum albumin, serum total bilirubin, serum aspartate aminotransferase of the HIV seropositive patients. Incidence of HIV infection correlated positively and significantly ($p < 0.05$) with

significant increase ($p < 0.05$) in HIV viral load, serum aspartate aminotransferase (AST), serum total bilirubin, and serum triglyceride and significant decrease ($p < 0.05$) in serum total cholesterol, serum albumin CD4+ cell count, and hemoglobin concentration of the HIV seropositive patients. Observed values of hemoglobin

concentration could be used with high precision to predict HIV viral load of the HIV seropositive patients from the regression curve [\hat{Y} (predicted value of HIV viral load) = \hat{Y} (copies/ml) = 160000 – 19703.704 x_i (g/dl)], x_i is observed value of hemoglobin concentration (g/dl) (figure 2). The gradient (slope) of the regression curve is negative: an indication that HIV viral load increase as hemoglobin concentration decrease (an inverse relationship) thus confirming the postulate by Motswaledi *et al.* (2013), that HIV binds to erythrocytes, and is associated with increased viral infectivity. The correlation statistical analysis of hemoglobin concentration and HIV viral load of the HIV seropositive patients was significant ($p < 0.05$), and positive with a Pearson's product moment correlation coefficient (r) of 0.985.

CONCLUSION

Incidence of HIV infection correlated positively and significantly ($p < 0.05$) with significant increase ($p < 0.05$) in HIV viral load, serum aspartate aminotransferase (AST), serum total bilirubin, and serum triglyceride and significant decrease ($p < 0.05$) in serum total cholesterol, CD4+ T lymphocyte cell count, serum albumin and hemoglobin concentration of the ART treatment naïve HIV seropositive patients. There was a significant ($p < 0.05$), positive, association between HIV viral load and hemoglobin concentration of the ART treatment naïve HIV seropositive patients. Observed values of hemoglobin concentration could be used with high precision (98.5%) to predict HIV viral load of ART treatment naïve HIV seropositive patients. The HIV seropositive patients should be placed on ART/HAART.

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