EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 5 (May-2015) pp. 395-402

www.ijcrar.com

Changes in some biochemical parameters of HIV seropositive patients on antiretroviral therapy in Dalhatu Araf specialist hospital, Lafia, Nasarawa state

Nwosu, D.C.[1], Obeagu, Emmanuel Ifeanyi[2], Ezeama, M.C.[3], Agu, G.C.[4] and Amajioyi, O.[5]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria
[2]Diagnostic Laboratory Unit, University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
[3]Department of Nursing Science, Faculty of Health Sciences, Imo State University, Owerri
[4]Department of Optometry, Faculty of Health Sciences, Imo State University, Owerri, Nigeria
[5]Department of Animal and Environmental Biology, Imo State University, Owerri, Nigeria
Corresponding author

KEYWORDS

HIV seropositive, Antiretroviral therapy,

A B S T R A C T

Changes in some biochemical parameters of HIV seropositive patients on antiretroviral therapy in Dalhatu Araf Specialist Hospital. Lafia. Nasarawa State was carried out using some biochemical parameters like total protein, albumin, urea. Creatinine, ALT and AST. Eighty subjects were recruited for this study with 50 subjects being HIV seropositive on ART while 30 subjects were HIV seronegative subjects between ages 18 - 60 years and were both male and female. Their serum total protein, albumin, urea. Creatinine, ALT and AST were measured and the results showed a significant difference of ($P<0.05$) in total protein, albumin and urea (84.5±1.40 59.8±2.5. 46.75±2.38 33.8±3.07, 6.1±7.35 4.0±9.9) of the HIV seropositive subjects on ART over the control subjects. These values do no suggest any damage to the kidney and the liver.

Introduction

Human immunodeficiency virus infection / acquired immunodeficiency syndrome (HIV/AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV) (Sepkowitz, 2001). During the initial infection a person may experience a brief period of influenza-like illness. This is typically followed by a prolonged period without symptoms. As the illness progresses it interferes more and more with the immune system, making people much more likely to get infections, including opportunistic infections, and tumors that do not usually affect people with working immune systems.

HIV is transmitted primarily via unprotected sexual intercourse (including anal and even oral sex), contaminated blood transfusions and hypodermic needles and from mother to

child during pregnancy, delivery, or breastfeeding. Some bodily fluids, such as saliva and tears^ do not transmit HIV (CDC, 2003). Prevention of HIV infection, primarily through safe sex and needle-exchange programs, is a key strategy to control the spread of the disease. There is no cure or vaccine; however, antiretroviral treatment can slow the course of the disease and may lead to a near-normal life expectancy. While antiretroviral treatment reduces the risk of death and complications from the disease, these medications are expensive and may be associated with side effects.

Genetic research indicates that H1V originated in west-central Africa during the early twentieth century (Sharp and Hahn, 2011). AIDS was first recognized by the Centers for Disease Control and Prevention (CDC) in 1981 and its cause-HIV infection-was identified in the early part of the decade (Gallo, 2006). Since its discovery, AIDS has caused nearly 30 million deaths (as of 2009) *(UNAIDS,* 2010). As of 2010, approximately 34 million people have contracted HIV globally *(UNAIDS,* 2011). AIDS is considered a pandemic—a disease outbreak which is present over a large area and is actively spreading (Kallings, 2008).

HIV/AIDS has had a great impact on society, both as an illness and as a source of discrimination. The disease also has significant economic impacts. There are many misconceptions about HIV/AIDS such as the belief that it can be transmitted by casual non-sexual contact. The disease has also become subject to many controversies involving religion.

The pathophysiology of AIDS is complex (Guss, 1994). Ultimately, HIV causes AIDS by depleting $CD4^+$ T helper lymphocytes. This weakens the immune system and allows opportunistic infections. T lymphocytes are essential to the immune response and without them; the body cannot fight infections or kill cancerous cells. The mechanism of $CD4^+$T cell depletion differs in the acute and chronic phases (Hel *et al.,* 2006).

During the acute phase, HIV-induced cell lysis and killing of infected cells by cytotoxic T cells accounts for $CD4^+$ T cell depletion, although apoptosis may also be a factor. During the chronic phase, the consequences of generalized immune activation coupled with the gradual loss of the ability of the immune system to generate new T cells appear to account for the slow decline in $CD4^+$ T cell numbers.

Although the symptoms of immune deficiency characteristic of AIDS do not appear for years after a person is infected, the bulk of $CD4^+$ T cell loss occurs during the first weeks of infection, especially in the intestinal mucosa, which harbors the majority of the lymphocytes found in the body. The reason for the preferential loss of mucosal $CD4^+$ T cells is that a majority of mucosal $CD4^+$ T cells express the CCR5 coreceptor, whereas a small fraction of $CD4^+$ T cells in the bloodstream do so (Brenchley *et al.,* 2004).

HIV seeks out and destroys CCR5 expressing $CD4^+$ cells during acute infection. A vigorous immune response eventually controls the infection and initiates the clinically latent phase. However, $CD4^+$ T cells in mucosal tissues remain depleted throughout the infection, although enough remain to initially ward off life-threatening infections.

Continuous HIV replication results in a state of generalized immune activation persisting throughout the chronic phase. Immune activation, which is reflected by the increased activation state of immune cells

and release of pro-inflammatory cytokines, results from the activity of several HIV gene products and the immune response to ongoing HIV replication. Another cause is the breakdown of the immune surveillance system of the mucosal barrier caused by the depletion of mucosal $CD4^+$ T cells during the acute phase of disease (Brenchley *et al.*, 2004).

This results in the systemic exposure of the immune system to microbial components of the gut's normal flora, which in a healthy person is kept in check by the mucosal immune system. The activation and proliferation of T cells that results from immune activation provides fresh targets for HIV infection. However, direct killing by HTV alone cannot account for the observed depletion of $CD4^+$ T cells since only 0.01-0.10% of $CD4^+$ T cells in the blood are infected.

A major cause of $CD4^+$ T cell loss appears to result from their heightened susceptibility to apoptosis when the immune system remains activated. Although new T cells are continuously produced by the thymus to replace the ones lost, the regenerative capacity of the thymus is slowly destroyed by direct infection of its thymocytes by HIV. Eventually, the minimal number of $CD4^+$ T cells necessary to maintain a sufficient immune response is lost, leading to AIDS (Pantaleo *et al.,* 1997).

Aims and objectives

The objective of this work is to access the changes in some biochemical parameters of HIV seropositive patients on antiretroviral therapy in Dalhatu Araf Specialist Hospital, Lafia. Nassarawa State. This is premised on the following aims:

1. To determine the overall levels of biochemical parameters- total protein, albumin, urea, Creatinine, AST and ALT.
2. To determine changes of the biochemical parameters mentioned above in respect of age.
3. To determine the changes that occur in these biochemical parameter as it relates to sex.

Materials and Methods

Study area

This study was carried out in Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State. Nasarawa state is located within the North central Nigeria with a population of 1.8 million people (NPC,2009). The state boarders with Plateau and Taraba states in the east, Benue state in the south, Abuja (FCT) in the west and Kaduna state in the north. The major ethnic groups in the state are Eggon,Afo, Mada, Gbagyi, Migil, Rindre, Hausa and many other tribes. Lafia, the state capital has a population of about 400,000 people. It has four tertiary institutions of learning which are Federal University Lafia, College of Agriculture Lafia, Nasarawa State polytechnic Lafia and School of Nursing Lafia. Most of the government agencies and parastatals are situated in the city and has a good representation of the tribes in the state - a good background for this study. The inhabitants are predominantly Christians and Muslims with few pagans.

Study population

Fifty blood samples were collected from patients who are confirmed to be HIV positive (using the National Aligorithm) and are on Antiretroviral therapy (ART) of all sexes from the age of 18 and above in Dalhatu Araf Specialist Hospital, Lafia. Similarly, thirty control blood samples were collected within Lafia metropolis from apparently healthy subjects who tested

negative for HIV using simple antibody screening test.

Selection criteria inclusion

1. Patients on (ART) from age 18 years and above were selected for this study.
2. Patients that have been on ART for at least one year were selected.
3. Patients whose consent was obtained were selected for the study.

Exclusion

1. Those who refused consent
2. Those that are less than one year on ART.
3. Those that have other diseases like hepatitis virus and renal disorder.

Specimen collection

Blood samples were collected from the test subjects by venopuncture. The skin surface was swabbed with methylated spirit and about 5ml venous blood was collected through the median cubital vein using sterile needle and swab. The blood collected into plain bottles was allowed to stand for about 30 minutes to clot and further centrifuged approximately at 3000 rpm for three minutes. The serum was separated from the clot using Pasteur pipette into plain sterile bottle and stored at - 20°C until used.

Laboratory procedure

All reagents were commercially purchased and the manufacturers' standard operational procedures (SOPs) were strictly followed.

Urea (modified urease-berthlot method)

Spectrum reagent kit with catalogue number REF: 321 was used.

Procedure:

Test tubes for test, standard and blank were arranged and 1ml of urea working reagent was placed on each tube. 0.01ml of serum and standard were placed on the corresponding tubes. They were mixed and incubated at 37°C for 5 minutes. 1ml of colour reagent was added to all tubes and mixed. Then it was incubated for another 5 minutes at 37°C and 1ml distilled water was added to all tubes.

The absorbance of sample and standard were taken against the reagent blank at 600nm.

Creatinine (Jaffe reaction)

Spectrum reagent kit with catalogue number REF: 234 was used. Fixed rate colorimetric assay.

Procedure:

Tubes were arranged in test and standard. Exactly 1.0 ml working reagent was mixed with O.1ml serum and standard. After 30 seconds, the absorbance Al of the test and standard were read. And exactly 2 minutes later absorbance A2 of test and standard were read all at 492nm.
Normal range = 62-133 umol/L

Total protein (Biuret reagent)

Spectrum Total protein Biuret reagent kit with catalogue number REF: 310 was used.

Procedure

Test tubes were arranged for test, standard and blank. 1ml of protein reagent was placed into each tube and 0.02ml of serum sample and standard were placed into the corresponding tubes. They were mixed and incubated for 10 minutes at room temperature. The absorbance of the sample and standard was taken against reagent blank within 30 minutes.

Normal range = 40 - 80g/dl

Alanine aminotransferase (ALT) (Colorimetric)

Agappe reagent with catalogue number 51214003 was used. Kinetic assay. **(Modified Reitmen Frankel)**

Procedure:

Test tubes for tests were arranged and 1ml of agape ALT working reagent was placed on the tubes. O.lml serum sample was added to the tubes. These were well mixed and incubated at 37°C for 1 minute. The change in absorbance was taken per minute for three minutes at 340nm.

Aspartate aminotransferase (AST) (Colorimetric)

Agappe AST reagent with catalogue number 51213003 was used. Kinetic assay.
(Modified Reitmen Frankel)

Procedure:

Test tubes for tests were arranged and 1ml of agape AST working reagent was placed on the tubes. 0.1ml serum sample was added to the tubes. These were well mixed and incubated at 37°C for 1 minute. The change in absorbance was taken per minute for three minutes at 340nm.

Albumin - BCG

Spectrum Albumin reagent with catalogue number REF: 211 was used.

Procedure:

Test tubes were arranged for test, standard and blank. 1ml of albumin reagent was placed into each tube and 0.01ml of serum sample and standard were placed into the corresponding tubes. They were mixed and incubated for approximately 5 minutes at room temperature. The absorbance of the sample and standard was taken against reagent blank within 60 minutes.

Normal range = 20 - 42mg/dl

Statistical analysis

The results were expressed as mean and standard deviations. The level of significance was calculated by student T-test. Values with $P<0.05$ were considered statistically significant.

Results and Discussion

Table 1 shows the serum mean and standard deviation of total protein, albumin, urea, Creatinine, ALT and AST of both the HIV positive individuals on ART and the HIV negative controls. The analysis showed there is statistical difference ($P<0.05$) in Total protein of the test group (84.5±1.40) when compared controls (59.8±2.5); albumin of the test group (46.75±2.38) when compared with controls (33.8±3.07) and urea of the test group (6.1±7.35) when compared with controls (4.0±9.95) and no significant difference in Creatinine, ALT and AST ($P>0.05$) in test and control groups respectively.

Table 2 shows the serum levels and standard deviation of total protein, albumin, urea, creatinine, AST and ALT in relation to age. There is statistical difference ($P<0.05$) in ages 18 - 30 of total protein (70.1 ±2.21) for test subjects over control subjects (59.1±3.74), albumin (45.1±2.21) for test subjects over control subjects (34.2±3.22) and urea (5.8±7.08) for test subjects over controls (4.6±7.84). There is also significant different in ages 31-45 for test protein (70.5±2.30) over control subjects l.8±3.65), albumin for test subjects (43.8±2.24) over control subjects (33.8±3.55) id urea for test subjects (6.0±6.92) over control subjects

(4.6±7.82). There is significant different in ages 46 above for test protein (69.9±2.52) over control subjects (60.3±3.57), albumin for test subjects (44.9±2.19) over control subjects (33.8±3.11) and urea for test subjects (6.2±6.85) over control subjects (4.6±7.84).There is also no statistical difference (P>0.05) in the values of Creatinine, ALT and AST in all the age distribution for both test subjects and controls.

Table 3 shows the serum levels and standard deviation of total protein, albumin, urea, Creatinine, AST and ALT in relation to sex. There is statistical difference (P<0.05) in males of total protein (68.3.05) for test subjects over control subjects (55.1±4.25), albumin (46.4±2.42) for test subjects over control subjects (33.1±3.09) and urea (5.0±7.34) for test subjects over controls (4.7±7.82). There is also statistical difference (P<0.05) in females of total protein (73.8±.2.83) for test subjects over control subjects (65.5±3.02), albumin (46.7±2.01) for test subjects over control subjects (36.0±3.11) and urea (7.1 ±4.92) for test subjects over controls (4.6±7.34). There is also no statistical difference (P>0.05) in the values of Creatinine, ALT and AST for both test subjects and control subjects of male and female groups.

Renal disease has been recognised as a common and intimately associated complication of human immunodeficiency virus (HIV) infection. It is now known that there are several syndromes and diseases associated with HIV infection (Cohen and Kimmel, 2007).

In this study conducted on HIV infected subjects on Antiretroviral Therapy (ART) and apparently healthy (HIV negative) controls at Dalhatu Araf Specialist Hospital Lafia, the Total Protein and albumin of the infected subjects on ART are higher than the HIV negative subjects (P<0.05). This might be due to dietary intake of protein as encouraged by the physicians. There is a slight increase in serum urea of the HIV infected individuals on ART than those of HIV negative subjects (P<0.05). This may also result from dietary intake. There is no significant difference (P>0.05) in serum Creatinine between the HIV positive subjects and the negative controls.

The liver enzymes (AST & ALT) levels of HIV positive subjects on ART were normal compared to the negative controls. This may probably mean that ART on HIV positive subjects does not damage the liver. Some studies suggested that liver injury in HIV may be associated with co-infection with hepatitis C virus (Jecotot *et* al.,2000; Balasbbramian *et al.*, 2005 and Gross *et al.*, 1998).

When age groups were compared in Table 2, there is a significant difference (P<0.05)between the total protein, albumin and urea of the HIV positive subjects on ART over HIV negative controls. This may be due to the above earlier mentioned reason for high protein and albumin. Urea also has a slight significant difference (P<0.05) between the age groups of both the HIV positive subjects on ART over apparently healthy subjects.Likewise the liver enzymes (AST&ALT) did not show a level of Significance (P>0.05) between the HIV positive subjects on ART and the negative controls of all age groups.

The study of sex distribution showed a significant difference (P<0.05) in total protein, albumin and urea of HIV positive subjects on ART over the HIV negative control subjects. There is no significant difference in Creatinine as well liver enzymes (AST & ALT).

Table.1 Overall Levels of Total Protein, Albumin, Urea, Creatinine, AST and ALT of HIV Seropositive Subjects on ART with Controls

	HIV+VE(n=50)	HIV-VE(n-30)	LEVEL OF SIGNIFICANCE
PARAMETERS	MEAN±SD	MEAN±SD	
Protein(g/dl)	84.8±1.4	59.8±2.5	P<0.05
Urea(Mmol/L)	5.0±7.35	4.0±9.95	P<0.05
Albumin(g/dl)	46.75±3.07	33.8±2.38	P<0.05
Creatinine(µmol/L)	77.20±3.96	77.8±3.89	P>0.05
ALT(U/L)	10.1±1.47	9.5±1.54	P>0.05
AST(U/L)	12.8±1.82	13.2±1.66	P>0.05

Table.2 Age related levels total protein, albumin, urea, Creatinine, AST and ALT of HIV positive subjects on ART and controls

AGE(Yeras)	TOTAL PROTEIN(g/dl)	ALBUMIN (g/dl)	UREA (Mmol/L)	CREATININE (Mmol/L)	ALT(U/L)	AST(U/L)
18-30(n=27)	70.1 ±2.21	45.1±2.21	5.8±7.08	67.6±4.55	14.5±1.65	13.0±1.75
Control(n=13)	59.1±3.74	34.1±3.22	5.8±7.08	91.2±2.77	13.8±1.61	15.0±1.22
P-Value	t=-9.90 p<0.05	T=1.26 p<0.05	t=0.48 p<0.05	T=20.3 p>0.05	t=0.04 p>0.05	T=0.42 p>0.05
31-45(n=19)	70.5±2.30	43.8±2.24	6.0±6.92	67.1±4.70	11.9±2.09	12.2±1.91
Control(n=11)	59.8±3.65	33.8±3.55	6.0±6.92	93.4±2.53	14.2±1.63	13.5±1.63
P-Value	t=9.44 p<0.05	t=-9.47 p<0.05	t=0.57 p>0.05	t=18.0 p>0.05	t=-3.38 p>0.05	t=-0.20 p>0.05
45 Above(n=6)	69.9±2.52	49.9±2.19	6.2±6.85	66.2±4.93	14.6±1.64	14.9±1.19
Control(n=6)	60.3±3.57	33.8±3.42	6.2±6.85	98.7±2.07	15.1±1.46	11.3±2.25
P-Value	t=-8.29 p<0.05	t=-6.69 p<0.05	t=0.66 p<0.05	t=15.6 p>0.05	t=0.05>0.05	t=2.49 p>0.05

Table.3 Sex related levels total protein, albumin, urea, Creatinine, AST and ALT of HIV seropositive subjects on ART and controls

SEX	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)	UREA (Mmol/L)	CREATININE (Mmol/L)	ALT (U/L)	AST (U/L)
Males(n=18	68.2±3.05	46.4±3.09	4.7±7.82	75.4±4.03	16.6±1.17	12.2±1.90
Controls(n=19)	55.2±4.25	33.1±2.42	5.0±7.34	83.7±3.15	13.2±1.75	11.7±2.08
P-Value	t=10.7 p<0.05	t=-1.44 p<0.05	t=-0.12 p<0.05	t=6.9 p>0.05	t=-0.64 p>0.05	t=-0.08 p>0.05
Females(n=32)	73.8±2.83	46.7±3.11	4.6±7.84	60.8±5.34	12.0±2.01	12.4±1.87
Controls(n=11)	65.5±3.02	36.0±1.73	7.1±4.92	75.4±4.83	15.3±1.33	13.7±1.62
P-Value	t=-7.98 p<0.05	T=-1.21 p<0.05	t=1.05 p<0.05	t=8.3 p>0.05	t=0.61 p>0.05	t=-0.22 p>0.05

Conclusion

From the results of the study, one may deduce that antiretroviral therapy may not pose any danger to the kidney and the liver. Any damage to the liver may be as result; co-infection with hepatitis C virus; and the kidney may be due to several renal syndromes and diseases as suggested by some other studies. The significant high values sometime seen in total protein, albumin and urea of HIV positive individuals n ART may be dietary.

References

Balasubramian A; Koziel M; Goopman J.E; Ganju R.K;(2005). Molecular mechanism of hepatic injury in coinfection hepatitis C virus and HIV. *Clin Infect Dis,* 41 (supl 1): S32-S37.

Brenchley, J.M., Schacker, T.W., Ruff, L.E., Price, D.A., Taylor ,J.H., Beilman, G.J., Nguyen, P.L., Khoruts, A., Larson, M., Haase, A.T. and Douek, D.C. (2004). "$CD4^+$ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract". *J. Exp. Med.* 200 (6): 749-59.

Centers for Disease Control and Prevention. (2003). "HIV and its Transmission".

Cohen, S.D. and Kimmel, P.L. (2007). HIV associated renal diseases in Africa -A desperate need for additional study. *Nephrol. Dial. Transpl.,* 22(8):2116-2119.

Gallo,R.C.(2006). "A reflection on HIV/AIDS research after 25 years". *Retrovirology* 3: 72.

Gross,A.,Jockel J.,Wei,M.C;and Korsmeyer, S.J. (1998). Enforced demerization of bax results in its translocation, mitochondrial dysfunction and apoptosis. *EMBO J,* 17:3878-85.

Guss, D.A., (1994). "The acquired immune deficiency syndrome: an overview for the emergency physician, Part 1". *J EmergMed* 12 (3): 375-84.

Hel, Z., McGhee ,J.R. and Mestecky J., (2006). "HIV infection: first battle decides the war". *Trends ImmunoL* 27 (6): 274-81.

Jacotot, E; Ravagnan, L; Loeffler, M; Ferri ,K.F; Viera ,H.L; Zamzami ,N; Constantini, P; Braid, J.P; Douglas, E; Susin, S.A; Cointe, D; Xie ,Z.H; Reed ,J.C; Roques, B.P.and Kroemer, G. (2000). The HIV 1 viral protein R induces apoptosis via direct effect on the mitochondrial permeability transition pore. *J Exp Med,191:33-44*

Kallings ,L.O., (2008). "The first postmodern pandemic: 25 years of HIV/AIDS". J[*Intern Med* **263** (3): 2 1 8s3.

NPC(2009).

Pantaleo ,G., Demarest, J.F., Schacker, T., Vaccarezza, M., Cohen,O.J., Daucher, M., Graziosi, C., Schnittman, S.S., Quinn, T.C., Shaw, G.M., Perrin, L., Tambussi, G., Lazzarm, A., Sekaly ,R.P., Soudeyns, H., Corey, L. and Fauci, A.S. (1997). "The qualitative nature of the primary immune response to HIV infection is a prognosticator of disease progression independent of the initial level of plasma viremia". *Proc Natl AcadSci USA.* 94 (1): 254-258.

Sepkowitz, K.A. (2001). "AIDS-the first 20 years". *N. Engl. J. Med.* 344 (23): 1764-72.

Sharp, P.M. andHahn, B.H.(2011). "Origins of HIV and the AIDS Pandemic". *Cold Spring Harbor perspectives in medicine* 1 (1).

UNAIDS (2011) Joint United Nations Programme on HIV/AIDS. Global HIV Response, Epidemic update and health sector progress toward universal access. Pg 1-10;60-80;150-160.

UNAIDS. (2010). "Global Report Fact Sheet".