

**HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN PATIENTS TAKING ANTI -
RETROVIRAL DRUGS IN UMUAHIA METROPOLIS**O. D. Omodamiro^{1*}, C. I. Nwakaego² and Dr. O. Ajah¹¹Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.²Department of Optometry, Federal University of Technology Owerri, Imo State, Nigeria.***Corresponding Author: O. D. Omodamiro**

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ABSTRACT

Background: Haematological and biochemical abnormalities are among the most common clinical pathological manifestations of HIV patients on HAART (Highly Antiretroviral therapy). Not only that the illness itself is fatal but the potential antiretroviral drugs meant at least to prolong the life of hopeful victims still predisposes them to other possible adverse effects that are detrimental. **Experimental design:** Ten (10) confirmed HIV positive HAART patients who have taken the drugs for 4 months, 7 months, a year, 2 years, 4 years, 5 years and 7 years and a HIV negative patient were screened for haematological and biochemical changes. Haematological changes were assessed using Coulter Ac-T differential analyzer and biochemical parameters assayed spectrophotometrically. **Results:** Kidney assessment shows that the urea values for most of the HIV patients are significantly ($p < 0.05$) greater than the control while only two are relatively normal. But in creatinine all are relatively normal. The electrolyte values indicate that Na^+ , Cl^- and HCO_3^- are significantly ($p < 0.005$) lower than the control value while K^+ are relatively normal compared to the control. The ratio of high urea to low creatinine and low electrolyte indicate mild kidney toxicity. For liver function test, the ALP values are significantly ($p < 0.05$) higher than the control value. Most of the ALP and AST values also increased compared to the control value. But the bilirubins are normal. This indicates a mild liver disturbance as drug administration is prolonged. For haematological value, there is a low PCV value and variable value of CD4 level, but mostly high for some i.e. significantly ($p < 0.05$) increased to that of the control. The Haemoglobin is normal for the first 1-7 years of drug administration. **Conclusion:** Administration of Highly Anti-retroviral Drugs showed progressive immune system repairs, but the adverse effect is severe as it tends to hepatotoxicity, renal kidney injury and anemia if administration of drug is prolonged.

KEYWORDS: biochemical, haematological, pathological, HIV, HAART and spectrophotometrically.**INTRODUCTION**

Retroviridae is a family of enveloped viruses that replicate in a host cell through the process of reverse transcription. A retrovirus is a single stranded positive sense RNA virus with a DNA intermediate and as an obligate parasite targets a host cell. Once inside the host cell cytoplasm, the virus uses its own reverse transcriptase enzyme to produce DNA from its RNA genome, the reverse set of the usual pattern, thus retro (backwards). This new DNA is then incorporated into the host cell genome by an integrase enzyme, at which point the retroviral DNA as part of its own genome, translating and transcribing the viral genes along with the cell's own genes, producing the proteins required to assemble new copies of the virus. It is difficult to detect the virus until it has infected the host. At that point the infection will persist indefinitely.^[1] These are infectious RNA containing viruses which are transmitted from human to

human. Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics for bacteria, specific antivirals are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen, instead they inhibit their development. Most of the antiviral drugs now available are designed to help deal with HIV, herpes viruses, the hepatitis B and C viruses, and influenza A and B viruses. The management of HIV/AIDS normally includes the use of multiple antiretroviral drugs in an attempt to control HIV infection. There are several classes of antiviral agents that act on different stages of the HIV life-cycle. The use of multiple drugs acting on different viral targets is known as highly active antiretroviral therapy (HAART). HAART decreases the patient's total burden of HIV, maintains function of the immune system, and prevents opportunistic infections that often lead to death. Some of the retroviral drugs include:

Zidovudine (ZDV) (INN), also known as azidothymidine (AZT), is an anti-retroviral medication used to prevent and treat HIV/AIDS. It is of the nucleoside analog reverse-transcriptase inhibitor (NRTI) class. AZT inhibits the enzyme (reverse transcriptase) that HIV uses to synthesize DNA, thus preventing viral DNA from forming. AZT was the first U.S. government-approved treatment for HIV, marketed under the brand name Retrovir. AZT was the first breakthrough in AIDS therapy, significantly reducing the replication of the virus and leading to clinical and immunologic improvements.^[2] It can also be used to prevent HIV transmission, such as from mother to child during the period of birth or after a needle stick injury. Used by itself in HIV-infected patients, AZT slows HIV replication in patients, but does not stop it entirely.^[3] HIV may become AZT-resistant over time, and therefore AZT is now usually used in conjunction with other anti-HIV drugs in the combination therapy called highly active antiretroviral therapy (HAART). AZT is included in Combivir and Trizivir, and is included in the World Health Organization's Model List of Essential Medicines, which suggests the minimum medicinal needs for a basic health care system.^[4] Abacavir is an antiretroviral medication used to prevent and treat HIV/AIDS. It is of the nucleoside analog reverse transcriptase inhibitor (NRTI) type. Viral strains that are resistant to zidovudine (AZT) lamivudine (3TC) are generally, but not always, sensitive to abacavir. It is well tolerated: the main side effect is hypersensitivity, which can be severe, and in rare cases, fatal. Genetic testing can indicate whether an individual is likely to be hypersensitive; over 90% of people can safely take abacavir. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.^[5] Indinavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Protease inhibitors block the part of HIV called protease. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Indinavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two others.^[6]

Nelfinavir mesylate (Viracept, formally AG1343) is a potent and orally bioavailable human immunodeficiency virus HIV-1 protease inhibitor ($K_i=2nM$) and is widely prescribed in combination with HIV reverse transcriptase inhibitors for the treatment of HIV infection. Nevirapine (NVP), also marketed under the trade name Viramune (Boehringer Ingelheim), is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used to treat HIV-1 infection and AIDS. As with other antiretroviral drugs, HIV rapidly develops

resistance if nevirapine is used alone, so recommended therapy consists of combinations of three or more anti-retrovirals. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.^[5]

The aim of this present study is to compare between HIV patients that have taken antiretroviral drugs for months to years, in order to determine the toxicological effects of these drugs they are taking using an HIV negative individual as a control.

METHODOLOGY

Experimental design

A total of 11 samples were collected from 10 HIV positive patients both males and females from HIV department of Federal Medical Centre (FMC), Umuahia, Abia state and one HIV negative patient. Five (5) of the samples are from those that started taking the antiretroviral drugs recently from (1-2) years and those that have taken it for a long period of time, from (3-8) years. The sample of HIV negative patient served as control.

Blood sample collection

Blood samples were taken in accordance with the Nigerian National Ethics and Operational Guidelines for Research on Human subjects (NNEOGRHS). Five (5) ml of those blood samples were collected from the individuals into a polystyrene sample bottle, after clotting, the blood samples were centrifuged at 10,000rpm for 5 min, then the serum were used in the determination of the biochemical parameters.

Biochemical parameter assessment

Determination of biochemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin (BIL), plasma urea (mmol/l), plasma creatinine (mmol/l) and electrolyte balance levels in the blood serum were measured by enzymatic method using Randox Diagnostic Kits Spectrophotometrically^{[7][8]} while the fresh blood/plasma samples were used for the determination of the haematological parameters such as CD4 count, Haemoglobin and Packed cell volume using standard methods and procedures.

Statistical analysis

Values for the biochemical parameters were expressed as Mean \pm Standard Deviation (S.D). The data obtained were statistically analyzed using one way analysis of variance (ANOVA) with Duncan's multiple comparison post Hoc tests (LSD) to compare the level of significance between control and experimental groups. All statistical analysis was evaluated using SPSS version 17.0 software. The values $p < 0.05$ were considered significant.

RESULTS

DRUGS: Zidovudine, Abacavir, Indinavir, Nelfinavir, Efavirenz, Lamivudine, Tenofovir.

Table 1: Kidney Function Test Parameters.

PATIENTS	D. D.	UREA (mg/dl)	CREATININE (mg/dl)	Na ⁺ (mg/dl)	K ⁺ (mg/dl)	Cl ⁻ (mg/dl)	HCO ₃ ⁻ (mg/dl)
HIV P 14 yrs.		23.35±0.49*	1.35±0.35	137.60±0.85*	4.10±0.42	100.35±0.49*	25.40±0.57
HIV P 25 yrs.		14.36±0.49	1.15±0.64	143.60±0.85	4.75±0.49	105.40±0.57*	26.60±0.85
HIV P 35 yrs.		22.60±0.85*	1.45±0.49	145.40±0.57	4.90±0.85	107.35±0.49	24.40±0.57
HIV P 45 yrs.		57.40±0.57*	1.90±0.85	144.35±0.49	4.40±0.57	104.60±0.85*	23.35±0.49
HIV P 57 yrs.		33.53±0.49*	1.40±0.57	142.60±0.85	4.40±0.57	106.30±0.42*	20.60±0.85*
HIV P 61 yr.		19.35±0.49*	1.20±0.57	141.60±0.85	4.10±0.42	102.35±0.49*	20.40±0.57*
HIV P 74 mnths.		18.30±0.42*	1.20±0.87	140.35±0.49	4.00±0.57	102±60±0.85*	27.35±0.49
HIV P 8 1 yr.		12.35±0.49	0.90±0.57	139.60±0.85*	5.15±0.49	100.30±0.49*	27.60±0.85
HIV P 9 2 yrs.		18.60±0.85*	1.50±0.49	138.30±0.42*	4.60±0.85	103.35±0.49*	25.30±0.42
HIV P 107 mnths.		20.30±0.42*	1.30±0.71	139.049±0.49*	3.90±0.57	99.60±0.85*	23.35±0.49
Control---		14.35±0.49	0.80±0.14	143.60±0.85	3.75±0.21	110.60±0.85	25.25±0.49

D.D = Duration of Drug taken. * within each column are significantly (p < 0.05) different from the control.

In the Urea value of the HIV patients, HIV P 1, HIV P 3, HIV P 4, HIV P 5, HIV P 6, HIV P 7, HIV P 9, HIV P 10 are significantly (p < 0.05) greater than the Control. While HIV P 2 and HIV P 8 had no significant difference when compared to the Control. In the creatinine value of the patients, there was no significant difference compared the control. In the sodium ion value of the HIV patients, HIV P 1, HIV P 8, HIV P 9 and HIV P 10 are significantly (p < 0.05) lower than the Control. While HIV P 2, HIV P 3, HIV P 4, HIV P 5, HIV P 6 and HIV P 7 have no significant difference compared to the Control.

In the potassium ion value of the patients, all the HIV patients showed no significant (p > 0.05) difference. In the chloride ion value of HIV patients, all the samples were significantly (p < 0.05) lower than the Control. In the bicarbonate ion value of the HIV patients, HIV P 5 and HIV P 6 are significantly (p < 0.05) lower than the Control. HIV P 1, HIV P 2, HIV P 3, HIV P 4, HIV P 7, HIV P 8, HIV 9, HIV P 10 have no significant difference when compared to the control.

Table 2: liver function test Parameters.

PATIENTS	D.D	TB (mg/dl)	CB (mg/dl)	ALP (μ/l)	ALT (μ/l)	AST (μ/l)
HIV P 1	4yrs.	0.75±0.49	0.11±0.05	24.40±0.57*	5.60±0.85*	6.35±0.49*
HIV P 2	5yrs.	0.75±0.49	0.55±0.64	72.60±0.85*	10.35±0.49*	18.40±0.57
HIV P 3	5yrs.	1.00±0.85	0.09±0.05	30.40±0.57*	20.60±0.85*	12.35±0.49*
HIV P 4	5yrs.	0.80±0.57	0.68±0.85	49.35±0.49*	9.40±0.57*	12.60±0.85*
HIV P 5	7yrs.	2.15±0.49	0.98±0.05	35.60±0.85*	15.35±0.49	8.30±0.42*
HIV P 6	1yr.	0.90±0.85	0.95±0.35	25.40±0.57*	15.60±0.85	12.35±0.49*
HIV P 7	4mnths.	0.70±0.57	0.11±0.05	50.35±0.49*	16.40±0.57	14.60±0.85*
HIV P 8	1yr.	0.65±0.49	0.67±0.85	19.60±0.85*	10.35±0.49*	16.30±0.42*
HIV P 9	2yrs.	0.90±0.85	0.09±0.06	23.30±0.42*	11.60±0.85	17.35±0.49
HIV P 10	7mnths.	0.80±0.57	0.67±0.85	32.35±0.49*	27.30±0.42*	26.60±0.85*
CONTROL	---	1.30±0.42	0.70±0.57	16.35±0.49	14.30±0.42	19.60±0.85

D.D = Duration of drug taken, T.B = Total Bilirubin, C.B = Conjugated Bilirubin, * within each column are significantly (p < 0.05) different from control.

In the total bilirubin value of the HIV patients, HIV P 1, HIV P 2, HIV P 3, HIV P 4, HIV P 5, HIV P 6, HIV P 7, HIV P 8, HIV P 9 and HIV P 10 had no significant difference compared to the control. In the conjugated bilirubin value of HIV patients, HIV P 1 to HIV P 10 had no significant difference when compared to the Control.

In the Alkaline phosphatase value of the HIV patients, HIV P 1 to HIV P 10 are significantly (p < 0.05) higher than the Control. In the Alanine transaminase value of the HIV patients, HIV P 1, HIV P 2, HIV P 4, and HIV P

8 are significantly (p < 0.05) lower than the Control. While HIV P 3 and HIV P 10 are significantly (p < 0.05) higher than the Control. But HIV P 5, HIV P 6, HIV P 7 and HIV P 9 had no significant difference compared to the Control. In the aspartate transaminase value of the HIV patients, HIV P 1, HIV P 3, HIV P 4, HIV P 5, HIV P 6, HIV P 7 and HIV P 8 are significantly (p < 0.05) lower. While HIV P 10 was significantly (p < 0.05) higher than the control. But HIV P 2 and HIV P 9 had no significant difference compared to the Control.

Table 3: Haematological test parameters.

PATIENTS	D.D	PCV (%)	HB (mg/dl)	CD4
HIV P 1	4yrs.	34.35±0.49	11.55±0.49	488.60±0.85*
HIV P 2	5yrs.	32.35±0.49*	10.95±0.64	1008.60 ±0.85*
HIV P 3	5yrs.	33.60±0.85*	11.35±0.49	169.40±0.57*
HIV P 4	5yrs.	30.40±0.57*	10.60±0.85	301.35±0.49*
HIV P 5	7yrs.	35.35±0.49	11.90±0.57	553.60±0.85*
HIV P 6	1yr.	30.35±0.49*	10.30±0.42	158.60±0.85*
HIV P 7	4months	36.30±0.42	12.60±0.85	550.35±0.49
HIV P 8	1yr.	36.35±0.49	12.40±0.57	483.60 ±0.85*
HIV P 9	2yrs.	32.60±0.85*	11.05±0.49	58.30±0.42*
HIV P 10	7months	30.30±0.42*	10.30±0.71	512.35±0.49*
Control	---	36.35±0.49	12.45±0.64	550.60±0.85*

D.D = Duration of drug taken, * within each column are significantly ($p < 0.05$) different from the control.

In the PCV value of the HIV Patients, HIV P 2, HIV P 3, HIV P 4, HIV P 6, HIV P 9, and HIV P 10 are significantly ($p < 0.05$) lower than the Control. While there is no significant difference between the control and others. In the Hb value of the patients, all the HIV patients value from HIV P1 to HIV P 10 had no significant difference compared to the control. In the CD4 value of the patients, HIV P 1, HIV P 3, HIV P 4, HIV P 6, HIV P 8, HIV P 9 and HIV P 10 are significantly ($P < 0.05$) lower than the Control. While HIV P 2 and HIV P 5 are significantly ($p < 0.05$) greater than the Control. But HIV P 7 had no significant difference to the Control.

DISCUSSION

In this present study, the effect of some haematological and biochemical parameters of abnormalities in HIV infected patients administered with Highly active antiretroviral therapy (HAART) drugs which include Zidovudine, Lamiduvine, Abacavir, Emtricitabine, Tenofovir, Combivir, Nevirapine, Nelfinavir, Efavienz, Indinavir etc used in treatment of HIV/AIDS in southern part of Nigeria.

These anti-retroviral drugs are given in combination to yield substantial benefit. This is achieved by combining two Nucleoside reverse transcriptase inhibitor with a protease inhibitor. These drugs are given to the patients in stages, at a space of two months interval. In table 3.1, the result of the urea test showed that 80% of the HIV positive patients have values that are significantly ($p < 0.05$) greater compared to that of the control with duration of drug administered. Though among those that have taken the drugs for 5 years each, one of them only have a relatively normal value compared to the control and this might be as a result of drug metabolism variation in individual. The creatinine values of the patients are all relatively normal. While the electrolyte test showed that 40% of the value of the sodium ion are significantly ($p < 0.05$) greater than the control with time. The potassium ions value are relatively normal, but the chloride ion value of 80% patients showed an increase compared to the control value with duration of drug given. Bicarbonate ion value showed that 2% of the

patients have values that are significantly ($p < 0.05$) greater compared to the control. This study showed that the integrity of the kidney is tampered with as drug administration continued in the 1st 7 years of drug administration, and may likely to exacerbate with time.^[9]

In table 3.2, the total bilirubin and conjugated bilirubin values of all the patients have no significant difference compared to the control. All the patients have ALP value that are significantly ($p < 0.05$) greater compared to the control value. Though this can account for liver injury or bone disorder, further test on ALT showed that 80% of the patient's showed significant ($p < 0.05$) decrease in value compared to that of control value. While the remaining 20 percent showed a sharp increase compared to that of control with time. 80% of the patients also have significantly ($p < 0.05$) lower value compared to the control value, 1% relatively normal and 1% significantly ($p < 0.05$) greater compared to the control with time. This showed that the liver has a mild liver disturbance and do not actually affect the integrity of the liver since the total and conjugated bilirubin are normal in the first 7 years of drug administration.

In table 3.3, the haematological test showed that 60% of the value are significantly ($p < 0.05$) lower compare to the normal while 40% are relatively normal compared to the control with time. But the haemoglobin values for all the patients are relatively normal. This showed that they are not likely to be anaemic in the first 7 years of drug administration.

The CD4 count value showed that 70% are significantly ($p < 0.05$) lower compare to the control, 2% have values that are significantly ($p < 0.05$) greater than the control value and 1% that is relatively number. This showed that the drug administered are progressive except in HIV P 2, HIV P 3, HIV 6 who has taken the drug for 5 years, 2 years, and 1 year respectively which can be as a result of not starting medication on time.^[10]

CONCLUSION

This study showed that there is a positive response effect of the HAART for reducing the HIV positive patients viral load, that is why their CD4 count is higher than the control (HIV negative patients), though few showed a drastic reduction, which may be because of not taking drugs in time. It is obvious from this study that the kidney toxicity increases as the drug administration is prolonged and will tend to exacerbate with time. While the liver showed a mild liver disturbance which may likely to increase with time. The haematological effect are still normal and do not generally have a significant change.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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