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EFFECT OF ANTIRETROVIRAL DRUG THERAPY ON IRON STATUS, URIC ACID, CD4+T-CELLS AND WEIGHT OF HIV SEROPOSITIVE SUBJECTS

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ABSTRACT

Background: Human immunodeficiency virus (HIV) /Acquired immunodeficiency syndrome (AIDS) is one of the pandemic infections that ravage humans. HIV/AIDS diseases progress with decrease in weight, CD4+T cells count is lowered resulting to the production of uric acid which chelates the available iron leading to anemia. The use of antiretroviral agents further complicates the biochemical changes that take place.Methods: The study investigated the iron status, uric acid, CD4+T-cells and weight of HIV seropositive subjects and the effects of antiretroviral therapy on the parameters. A total number of 200 subjects between the ages of 20-65years were investigated out of which 20 subjects were HIV negative and they serve as the control. The remaining 180 subjects were grouped into six groups of 30 subjects per group depending on their antiretroviral drug combination. Serum iron and total iron binding capacity were estimated using commercial kit by TECO Laboratories while the uric acid level was estimated using kit by RANDOX Ltd, United Kingdom. CD4+Tcells were determined using flow cytometry. The weight was measured using high sensitive weighing balance.Results: This study revealed significant differences in the mean levels of total iron binding capacity (TIBC), iron, uric acid CD4+ T- cells and weight of HIV seropositive subjects when compared control whereas there was no significant difference when the mean levels of each of the drug combinations were compared with those not on antiretroviral therapies.Conclusion: Serum iron, total iron binding capacity uric acid, CD4+ T-cells count and weight should be assessed during HIV infection and during the use of antiretroviral therapy as these may predict HIV disease progression and treatment assessment.

Key Words: Human immunodeficiency virus, CD4+T-cells, antiretroviral therapy, iron, total iron binding capacity, uric acid, weight

INTRODUCTION

HIV is one of the pandemic infections that ravage human, it infects primarily the vital cells in the human immune system such as helper T-cells (specifically CD4+ T cells), macrophages, and dendrites, [1] thereby leading to low levels of CD4+ T-cells. HIV infects about 0.6% of the world'spopulation [2].UNAIDS and the WHO estimated that AIDS killed more than 25 million people between 1981, when it was first recognized, [3] and 2005. In 2009, AIDS claimed an estimated 1.8 million lives, down from a global peak of 2.1 million in 2004, approximately 260,000 children died of AIDS [4] in 2009. In Nigeria, an estimated 3.6 percent of the population is

living with HIV and AIDS.

Human immunodeficiency virus (HIV) infection is associated with oxidative stress and uric acid is one of the markers of oxidative stress [5]. In humans, uric acid is the final oxidation (breakdown) product of purine metabolism and is excreted in urine. In oxidative stress like HIV infection, there are biochemical changes in the concentrations of some analytes in the blood. Iron (for instance) is an integral part of many proteins and enzymes that maintain good health. In humans, iron (Fe) is an essential component of proteins involved in oxygen transport [6]. It is also essential for the regulation of cell growth and differentiation [7]. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity [8].On the other hand, excess amounts of iron can result in toxicity and even death. When the Iron level decreases, it causes anemia as total iron binding capacity and uric acid increases with response to HIV infection. An inverse relationship appears to exist on effort being made to boost immune system in HIV patient and the actual concentration of uric acid and iron. Uric acid forms a co-ordination complex with Fe^{3+} which does not support electron transport, thus it serves as an iron chelator and free radicals scavenger [9]. HIV/AIDS diseases progresses with decrease in weight, CD4+T cell count is lowered resulting to the production of uric acid which chelates the available iron leading to anemia [10]. The use of antiretroviral agents further complicates the biochemical changes that take place. The study investigated the iron status, uric acid CD4+ T- cells and weight of HIV seropositive subjects relative to control subjects; the effects of antiretroviral therapy on the parameters and to assess the relationship between iron status and CD4+T cells count of HIV seropositive subjects with or without antiretroviral therapy.

MATERIALS AND METHODS

Study area

This research work was carried out on HIV seropositive subjects attending HIV clinic at NnamdiAzikiwe University Teaching Hospital, Nnewi (NAUTH) and HIV seropositive individuals at NnamdiAzikiwe University Teaching Hospital, Nnewi (NAUTH). A total number of 200 subjects between the ages of 20-65 were investigated. The subjects were selected using a non-probability sampling technique from the HIV seropositive patient attending the HIV clinic at NnamdiAzikiwe University Teaching Hospital Nnewi (NAUTH). Ethical approval was obtained from the ethical committee of NnamdiAzikiwe University Teaching Hospital, Nnewi and also informed consent of the subjects participating in the research work was obtained. After obtaining the consent from the subjects, 5ml of venous blood was collected aseptically into dry plain containers. The serum was separated immediately after clot retraction and stored at -20^0 C for a period of 2 weeks until the time of assay.

Classification of the subjects:

The subjects were grouped based on antiretroviral therapy combination as follows:

HIV seropositive subjects on lamivudine, stavudine, nevirapine (LSN) therapy = 30

HIV seropositive on zidovudine, emitricitabine, tenofivir (ZET) therapy = 30

HIV seropositive subjects on lamivudine, zidovudine, emtricitabine (LZE) therapy = 30

HIV seropositive subjects on combivir, nevirapine (CN) therapy = 30

HIV seropositive subjects on nevirapine, tenofivir (NT) therapy = 30

HIV seropositive subjects not on antiretroviral therapy = 30

However, twenty (20) HIV seronegative subjects (apparently healthy individuals) served as controls.

Specimen analysis

Serum Iron / total iron were estimated using iron / total iron binding capacity kit Teco laboratories Ltd, United Kingdom according to the manufacturers' instruction. Uric acid was analyzed by enzymatic colorimetric method using Randox uric acid test kit manufactured by Randox laboratories Ltd, United Kingdom. CD4+Tcells were determined using flow cytometric method while the weight was measured using high sensitive weighing balance.

Statistical analysis

The statistical analysis was performed using the SPSS program (Statistical package for social science) version 16.0. The Mean and Standard Deviation (SD) were calculated for each parameter. Differences in the means for each parameter between the groups of the study were compared by student's t- test and Analysis of Variance. The changes were compared between HIV seropositive subjects on antiretroviral therapies (ON ART) and those not on antiretroviral therapies (NON ART).A probability <0.05 was considered to be significant.

RESULTS

There were significant differences in the mean levels of the parameters when compared among the groups whereas there was no significant difference when the mean levels of each of the drug combinations were compared with those not on antiretroviral therapies (see the tables below).

Table 1. CD4+ Tcells, iron, total iron binding capacity (TIBC), uric acid and weight of HIV seropositive subjects compared with control

	TIBC (µg/dl)	IRON (µg/dl)	URIC ACID(µmol/l)	CD4+T CELL(/mm^3)	WEIGHT
LSN N=30 Mean±SD	499.9±71.7	59.2±21.7	430.0±3.3	621.9±531.9	66.4±1.0
ZET N=30 Mean±SD	525.8±81.6	64.6±11.7	432.0±2.0	553.5±397.6	61.2±9.1
LZE N=30 Mean±SD	478.7±73.1	59.5±22.1	435.0±3.1	474.3±23.8	63.0±11.3
CN N=30 Mean±SD	487.6±120.3	66.0±25.0	430.5±3.4	549.1±265.1	65.1±12.5
NT N=30 Mean±SD	485.9±67.9	60.7±20.0	430.3±3.1	416.5±179.3	66.9±6.9
NON ART N=30 Mean±SD	484.2±95.5	72.6±11.7	425.0±2.3	630.0±40.3	68.2±8.2
CONTROL N=20 Mean±SD	577.8±22.3	86.2±13.7	305.7±1.2	653.9±115.6	80.2.2±1.0
F- value	4.0	3.8	10.6	3.4	5.5
P- value	0.00*	0.04*	0.01*	0.02*	0.01*

*significant at p<0.05

KEY: CN=Combivir and Nevirapine, **NT**=Nevirapine and tenofivir, **LSN**=Lamivudine, Stavudine and Nevirapine, **ZET**=Zidovudine, Emitricitabine and Tenofivir, **LZE**= Lamivudine, Zidovudine and Emitricitabine.

Table 2. CD4+ T cells, iron, total iron binding capacity (TIBC) and uric acid of HIV seropositive on combination of Lamivudine, Stavudine and Nevirapine (LSN) and those not on antiretroviral therapies (Non ART).

	On LSN Combinationn=30	Non ART n=30	T-value	p- value	I
TIBC	499.2±71.7	484.2±17.4	0.36	0.8413	NS
IRON	59.2±21.7	72.6±11.7	1.12	0.5825	NS
URIC ACID	430.0±3.3	425.0±0.4	1.32	0.1075	NS
CD4+	621.9±531.9	630±40.3	1.40	0.0700	NS
WEIGHT	66.4±1.0	68.2±8.2	.759	1.0549	NS

KEY: **I**= INFERENCE, **NS**=NOT SIGNIFICANT

Table 3. CD4+ T cells, iron, total iron binding capacity (TIBC) and uric acid of HIV seropositive on combination of Zidovudine, Emitricitabine and Tenofivir (ZET) and those not on antiretroviral therapies (Non ART).

	On ZET combinationn=30	Non ARTn=30	T-value	p-value	I
TIBC	525.8±81.6	484.4±95.5	1.77	0.0714	NS
IRON	64.6±11.0	72.6±11.7	1.35	2.000	NS
URIC ACID	432.0±2.0	425.0±2.3	4.86	0.5321	NS
CD4+T CELLS	553.5±397.6	630.0±40.3	.956	0.2310	NS
WEIGHT	61.1±9.1	68.2±8.2	-2.08	0.9598	NS

KEY: **I**= INFERENCE, **NS**=NOT SIGNIFICANT

Table 4. CD4+ T cells, iron, total iron binding capacity (TIBC) and uric acid of HIV seropositive on combination of Lamivudine, Zidovudine and Emitricitabine (LZE) and those not on antiretroviral therapies (Non ART).

	ON LZE combinationn=30	Non ART n=30	T-value	P -Value	I
TIBC	478.7±73.1	484.4±95.5	-250	0.1510	NS
IRON	59.5±22.1	72.7±11.7	-1.01	0.0777	NS
URIC ACID	435.4±3.1	425.0±2.3	.525	0.8140	NS
CD4+T CELLS	474.3±238.4	630.0±40.3	2.04	0.7190	NS
WEIGHT	63.0±11.3	68.2±8.2	-1.21	0.3200	NS

KEY: **I**= INFERENCE, **NS**=NOT SIGNIFICANT

Table 5. CD4+ T cells, iron, total iron binding capacity (TIBC) and uric acid of HIV seropositive on combination of Combivir and Nevirapine (CN), that not on antiretroviral therapy (Non ART).

	ON CN combinationn=30	Non ART n=30	T -value	p-value	I
TIBC	487.6±120.3	484.4±95.4	-250	0.3000	NS
IRON	66.0±25.0	72.6±11.7	-239	0.1240	NS
URIC ACID	430.5±3.3	425.0±2.3	1.97	0.0845	NS
CD4+T- CELLS	549.0±265.0	630.0±40.3	1.18	0.2150	NS
WEIGHT	63.0±12.5	68.2±8.2	-.441	0.4000	NS

KEY: **I**= INFERENCE, **NS**=NOT SIGNIFICANT

Table 6. CD4+, iron, total iron binding capacity (TIBC) and uric acid of HIV seropositive on combination of Nevirapine and Tenofivir (NT) and that not on antiretroviral therapy (Non ART).

	ON NT combination n=30	Non ART n=30	T-value	P- Value	I
TIBC	485.9±67.9	484.2±95.5	.081	0.1640	NS
IRON	60.7±20.1	72.6±11.7	-.826	0.5630	NS
URIC ACID	430.3±3.1	425.0±2.3	1.78	0.6455	NS
CD4+ T CELLS	474.0±265.0	630.0±40.3	-1.10	0.8400	NS
WEIGHT	66.9±6.9	68.2±8.2	-4.20	0.2153	NS

KEY: **I**= INFERENCE, **NS**=NOT SIGNIFICANT

DISCUSSION

Human immunodeficiency virus/Acquired immunodeficiency syndrome (AIDS) is one of the pandemic infections that ravage human, it infects primarily vital cells in the human immune system such as helper T-cells (specifically CD4+ T cells), macrophages, and dendrites,[1] thereby leading to low levels of CD4+ T-cells through three main mechanisms of: direct viral killing of infected cells, increased rates of apoptosis in infected cells and killing of infected CD4+ T-cells by CD8+T cells cytotoxic lymphocytes that recognize infected cells. When CD4+ T-cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections and tumours [11].

The study revealed that the control subjects had the highest mean CD4+ T- cell count; this is in line with a study [12] which reported that the control subjects had the highest CD4+ T-cells count compared to HIV seropositive subjects at various stages of HIV infection. The reason is that as HIV infects the human cells, it attacks the CD4+ T cells and become part of thecells and as they multiply, they make more copies of HIV which destroy them. When someone is infected with HIV for a long time, the CD4+ T-cells declines, this is a sign that that the immune system is weakening [13]. The lower the CD4+T-cells count, the more the progression of the diseases [13]. This study also revealed that there is more decreased iron level in HIV seropositive subjects on (LZE) and (ZET) combination when compared with Non ART subjects although not significant. This explains that iron level decreases in HIV seropositive subjects on antiretroviral therapy leading to iron deficiency anemia than HIV seropositive not on antiretroviral drug therapy. The decrease in iron level may be due to: changes in cytokine production with subsequent effects on hematopoiesis [14], decreased erythropoietin concentrations; [15] opportunistic infectious agents, such as *Mycobacterium avium* complex and parvovirus B-19, administration of chemotherapeutic agents such as zidovudine, ganciclovir and trimethoprim-sulfamethoxazole [16] and myelophthisis caused by cancers such as lymphosarcoma. Other mechanisms for HIV-associated anemia, although uncommon, include

vitamin B_{12} deficiency and the autoimmune destruction of red blood cells [17]. This study indicated that there was statistically significant decrease in iron with increase in total iron binding capacity which is in line with other works [16] that showed that there exists a significant decrease in serum iron (iron deficiency anemia) in relation to treatment of HIV infection. The incidence of anemia was strongly and consistently associated with the progression of HIV disease as measured by diagnosis of an AIDS-defining opportunistic illness and measurement of a CD4 count of <200 cells/μL. This association may likely be explained by the increasing viral burden as HIV disease progresses, which could cause anemia by increased cytokine-mediated myelosuppression. Alternatively, anemia may be a surrogate marker for some aspect of disease progression not captured by controlling the CD4+T cells count and clinical AIDS diagnosis [16]. There is also a significant difference between iron levels of control subjects and HIV seropositive subjects when compared. The control subjects had the highest mean value of iron which shows normal iron absorption and metabolism and blood concentration maintained at optimal level and this correlate with the research carried out where normal increase in serum iron in control subjects was reported [17].

The mean value of uric acid is highest in HIV seropositive on (LZE) and (ZET) antiretroviral therapy more than other antiretroviral drug combination therapy which means that the level of uric acid increase significantly in HIV disease with subjects on zidovudine combination therapy and this is in line with a work where a significant increase in uric acid of HIV seropositive subjects especially with patients on zidovudine combination therapy was reported [18]. The increase in uric acid with decrease in iron status was attributed to uric acid being a good antioxidant and that uric acid has ability to form stable co-ordination complexes with Fe ions. Formation of urate-Fe^{3+} complexes dramatically inhibits Fe^{3+}-catalysedascorbate oxidation, as well as lipid peroxidation in liposome [9]. Thus, in oxidative stress like HIV infection, the body in order to survive free oxygen radicals and oxygen singlet produced by lipid peroxidation, the body responds to the oxidative stress by increase in production of uric acid which in turn reduced iron level. However, a high level of uric acid is a major risk factor for the development of gout, a very painful condition called arthritis [18]. The study found out that traditional risk factors such as male sex, being overweight, kidney dysfunctions were associated with elevations in uric acid as well as the use of certain antiretroviral drugs like zidovudine. The uric acid level in the control subjects was reduced compared with HIV seropositive subjects; this indicates normal metabolism of uric acid and absence of oxidative stress as reported [18] that there exist a significant difference between serum uric acid in control subjects and HIV seropositive subjects.

The finding of reduced weight in the HIV seropositive subjects is also in line with the study that had been carried out [12,19] which reported that majority of people with HIV/AIDS weighed less than 90% of their ideal body or had lost more than 10% of their usual weight compared to the control subjects who are HIV seropositive subjects. There was a statistically significant difference ($p<0.05$) when their mean weight was compared. This study revealed that the control subjects had the highest mean weight compared to HIV seropositive subjects on antiretroviral therapy and those not on antiretroviral therapies.

CONCLUSION

This research study revealed a statistically significant difference in total iron binding capacity (TIBC), iron, uric acid CD4+ T- cells and weight of HIV seropositive subjects on therapy, those not on therapy and control subjects. The CD4+T-cells, iron and weight were significantly reduced in HIV seropositive subjects on antiretroviral therapy and highest in control subjects, TIBC was significantly higher among the HIV seropositive subjects on antiretroviral therapy and lowest in the control subjects. Uric acid was significantly higher in HIV seropositive subjects on antiretroviral therapy when compared with control subjects with normal serum uric acid. Therefore, serum iron, total iron binding capacity uric acid, CD4+ T-cells count and weight should be assessed during HIV infection and during the use of antiretroviral therapy as these may predict HIV disease progression and treatment assessment.

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