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Profile of Micronutrients, Electrolytes, Haematology and CD4 Count in HIV Infected Subjects on Highly Active Antiretroviral Therapy

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ABSTRACT

Human Immunodeficiency Virus (HIV) infection compromises the immune status, diminishes micronutrients availability and causes electrolyte imbalance in individuals. Highly Active Antiretroviral therapy (HAART) is currently used in the management of HIV patients. This work was designed to determine the profile of micronutrients, electrolytes, haematology and CD4 Count in HIV Infected Subjects on HAART. One hundred and sixty-three (thirty-six males and one hundred and twenty-seven females) subjects of whom one hundred and eleven (twenty-five males and eighty-six females) were HIV positive (grouped as Pre-HAART subjects). Fifty-two (eleven males and forty-one females) HIV- negative controls were used in this study. Blood samples (10 mL) were collected by veni-puncture from Pre-HAART subjects and the control group. 5 mL was added to clean sterile bottle to yield serum for micronutrient and electrolyte determination while 5 mL were added to EDTA bottles for haematological and CD4 count. The HIV subjects were then placed on HAART for six months. Blood samples were then collected. Our findings showed a significant ($p \leq 0.05$) increase in CD4 count and the haematological parameters of HAART subjects when compared to pre-HAART HIV subjects. The same results were obtained for the serum levels of vitamins A and C, zinc, copper, selenium and potassium ion. However, the serum levels of vitamin E, sodium and chloride ions did not show any significant different ($p \geq 0.05$) between the pre-HAART and HAART subjects. Mineral supplements rich in vitamin E, sodium and chloride ions may be required in combination with HAART in HIV management.

Keywords: HIV, HAART, Micronutrients, Haematology.

Introduction

The Human Immunodeficiency Virus (HIV) has been of immense concern over the years. It belongs to a larger group, the Retroviruses, based on the mode of replication, and to a smaller group, the Lentiviruses, based on duration for onset of symptoms.[1] To help combat the infection, the use of three or more combinations of antiretroviral agents was introduced. This was termed Highly Active Antiretroviral Therapy (HAART). This has dramatically altered the treatment and life expectancy of HIV infected individuals.[2,3] The use of antiretroviral therapies (ART) is recommended globally for the management of HIV/AIDS since a permanent cure is still elusive. Different types of ART or combination therapies are currently in use and the prescription and use of a particular therapy depends on tolerability, the cost, and the therapeutic objectives.[4] In recent times, WHO has recommended first-line therapy with two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitors (NNRTI).[5] A combination of nevirapine, stavudine, and Lamivudine or lamivudine with zidovudine is frequently prescribed.[5] The efficacy of ART is routinely assessed by measuring the blood plasma viral load and CD4+T-cell count.

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Essentially ART usage is geared towards achieving suppression of plasma viral load in a sustainable fashion. While it cannot be assumed that effective ART would eliminate transmission with individual exposure, evidence from several cohort, observational and mathematical modeling studies suggests that effective ART may be a promising way to reducing HIV transmission within the population.[6] Micronutrient status of the HIV patients has been shown to be negatively affected. Top on the list are those micronutrients that are involved in immune functions and antioxidant system.[7, 8-10] Antioxidant vitamins such as vitamins C and E often present a negative correlation between normal and individuals suffering from pathogenic infections. The levels of these parameters in subjects undergoing HAART therapy will serve as an indicator to determine its effectiveness. There is largely insufficient data as regards determining whether HAART ameliorates micronutrient deficiency or to recommend or refute the benefit of providing micronutrient supplements to HIV-positive persons receiving HAART.[11] The aim of this study was to assess the levels of antioxidant micronutrients: zinc, selenium, vitamin C and vitamin E in HIV positive patients on HAART. Furthermore, hematological abnormalities are common complications of human immunodeficiency virus infection. These abnormalities increase as the disease progresses.[12-14] Hematological parameters and immune status are important monitoring tools for assessing treatment and prognosis in HIV patients. These parameters were measured in this study.

Materials and Methods

Study Area

The study area was Emuoha General Hospital Emuoha, it is the headquarters of Emuoha Local Government Area of River State, Nigeria. The LGA has an area of 706 km² and Emuoha is situated on latitude 5° 1' 54" North and longitude 6° 24' 1" East. The 2006 national census put the population of the study area at 266,008 people. Projected to 2015 at 2.8

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percent national growth rate, the 2015 population of the study area is 333,042 people.[15]

Study design
This is a comparative study of subjects with HIV (pre-HAART and HAART) without TB co-infection. A reference group of healthy HIV negative subjects was also recruited. Blood samples were collected for serum analysis of micronutrient levels of Selenium, Copper and Zinc, electrolytes (sodium, potassium and chloride ions), white blood cells, haemoglobin, platelets and CD4+ cell count.

Selection of subjects
A total of one hundred and sixty-three (163) participants were involved in the study, of which 111 were HIV positive (designated as pre-HAART and HAART) and 52 HIV-negative controls. The controls were recruited such that there was no significant difference in age when compared to HIV positive individuals.

Ethical considerations
The proposal for this study was approved by Emuoha General Hospital Emuoha, Ethical Committee. Informed consent was obtained from the subjects before they were enrolled in the study.

Inclusion criteria
Only HIV positive and HIV negative individuals who consented were recruited for this study.

Exclusion criteria
HIV positive subjects and HIV negative subjects with tuberculosis, pregnancy, hepatitis, hypertension, kidney problem diabetes and any other ailment that has significant effects on the parameters of interest were excluded from the study.

Specimen collection
Veinous blood (10 mL) were collected by veni-puncture without stasis from HIV patients (Pre-HAART) and HIV negative control. Similarly, 10 mL of blood was taken from the HAART group after six months on HAART therapy. In each case, 5 mL was added to a clean sterile bottle to yield serum for micronutrients (copper, zinc and selenium) and electrolyte (sodium, potassium and chloride) determination while 5 mL was added to EDTA for the haematological and CD4-T cell determination. The blood dispensed into the plain bottles were allowed to stand for about an hour to clot and for retraction and then centrifuged at 3,500 rpm for 10 minutes at room temperature. The serum was separated and stored at -20°C until time of analysis.

HIV testing
HIV testing for all patients was performed using Determine Alere rapid tests Kits. Positive results with this test were confirmed with Statpack (HIV 1/2 Stat-Pak, Chembio Diagnostic Systems, New York, USA); if this test showed a negative result, further testing was done with Unigold (Uni-Gold TM HIV, Trinity Biotech, Wicklow, Ireland). Patients who tested positive for HIV were referred to an ART clinic in the same facility for enrolment in HIV care and consideration of ART initiation.

CD4+ T cell count
CD4+ T cells were analyzed using a BD FACS Count flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) with two monoclonal antibodies (aCD4 and aCD8; Becton Dickinson Immunocytometry Systems).

Haematological Parameters
Full blood cell counts were done using Sysmex Kx-21 (Sysmex Corporation; Kobe Japan).

Estimation of Serum Electrolyte Levels
Electrolyte levels were analyzed using an automated Chemistry Analyzer (Model 3000) manufactured by Buck Scientific Corporation, Connecticut, USA.

Micronutrient Analysis
Serum concentrations micronutrient (Se, Zn and Cu) were determined using atomic absorption spectrophotometry (Buck Scientific, 210, Atomic Absorption Spectrophotometer, Connecticut, USA) as previously described.[16]

Data analysis
Data obtained were analyzed using SPSS version 20 statistical software package. Results generated were expressed as mean ± SD and P-value < 0.05 were considered significant. The significance difference among the groups was assessed by repeated-measures analysis of variance (ANOVA).

Results and Discussion

Socio-demographic characteristics of the study participant
Table 1 shows the socio-demographic profile of both HIV-positive (subjects) and HIV-negative (controls) participants. 163 participants were recruited for the study, 111 (68.1%) were HIV positive and 52 (31.9%) were HIV-negative. These subjects were further characterized as reported in the table.

Serum levels of some micronutrients of HIV positive (Pre-HAART and HAART) and controls
Our results show a statistically significant ($p < 0.05$) depletion of the micronutrients in Pre-HAART HIV subjects when compared with HAART HIV subjects and the controls.

Serum electrolytes of HIV positive (Pre-HAART and HAART) and controls
Our findings show that the HAART HIV and control subjects had significant higher presence of potassium ions.

CD4 Counts and Some Haematological Parameters of HIV Positive (Pre-HAART and HAART) and controls
Our findings indicate that the HAART HIV subjects had very significant ($p \leq 0.05$) increase in CD4 counts and other haematological parameters considered in this study when compared with the Pre-HAART HIV subjects.

The reduction in vitamins A and E observed in HIV positive subjects in this present study is in agreement with the works of Lawal *et al*.[16] who also reported lower levels of these vitamins. Lower levels of these vitamins in HIV positive patients compared with controls is not unconnected with increased absorption and utilization of free radicals.[16] However, the use of HAART ameliorated the depletion of these vitamins and hence its availability for other physiological functions. The activity of HAART may have led to the reduced generation of free radicals or other metabolic processes that could lead to depletion of the vitamins.
The decrease in vitamin C in the present study is in consensus with the works of Stephensen *et al*.[17] and Anyabolu *et al*.[18]. Similarly, it was reported that antioxidant role of vitamin C in combating the prooxidant activity of HIV infection is believed to be largely responsible for vitamin C deficiency in HIV infection. [6]
Our results show that patients with HIV showed significant ($p \leq 0.05$) increase in the mean serum copper level when compared with the control subjects. This finding was consistent with previous studies by Lawal *et al*.[16] and Nwegbu *et al*.[19] who reported that there was a significant increase in the mean plasma copper concentration when compared with control group. Copper as a compound required for immune complex formation, blood and coagulation factors formation is a major micronutrient required by the body.[20] HIV infection has been reported to be associated with increased proinflammatory cytokines[21] which was reported to lower the plasma levels of zinc but raises the level of Copper.[22] The results of the present study showed that there is significant ($p \leq 0.05$) decrease in the mean plasma zinc levels in patients when compared with mean plasma zinc level of control group. This finding agreed with the studies of Khalili *et al*.,[8] Lawal *et al*.[16] and Mohamed *et al*.[23] who reported that there was a significant ($p \leq 0.05$) decrease between zinc levels in HIV positive patients when compared with zinc level of the control group.
From this present study, the serum level of selenium is markedly reduced in HIV positive subjects when compared with control subjects. The reduction was statistically significant ($p < 0.05$). This result was in agreement with studies by Bobat *et al*.[24] and Ogunro *et al*.[25] The reduction in selenium level may be caused by several factors such as oxidative state induced by the virus, malabsorption,[26] altered metabolism, gut infection,

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Table 1: Socio-demographic characteristics of the study participant

Variable	Categories	HIV-Positive (%)	Controls (%)	Total (%)
Age	11-20	20 (18.0)	12 (23.0)	32 (19.6)
	21-30	36 (32.4)	17 (32.7)	53 (32.5)
	31-40	31 (27.9)	12 (23.1)	43 (26.4)
	41-60	24 (21.6)	11 (21.2)	35 (21.5)
Sex	Male	25(22.5)	11(21.2)	36 (22.1)
	Female	86(77.5)	41(78.8)	127 (77.9)
Marital status	Married	41 (37.0)	23 (44.2)	64 (39.3)
	Unmarried	70 (63.1)	29 (55.8)	99 (60.7)
Occupation	Civil Servant	2 (1.8)	2 (3.8)	4 (2.5)
	Farming	23 (20.7)	8 (15.4)	31 (19.0)
	Trading	47 (42.3)	26 (50.0)	73 (44.8)
	Artisan	6 (5.4)	12 (23.1)	18 (11.0)
	Unemployed	34 (30.6)	3 (5.8)	37 (22.1)
Education	Illiterate	28 (25.2)	7 (13.5)	35 (21.5)
	Primary	48 (43.2)	22 (42.3)	70 (43.0)
	High School	34 (30.6)	21 (40.40	55 (33.7)
	Tertiary Educated	1 (0.9)	2 (3.8)	3 (1.8)

Table 2: Serum levels of some micronutrients of hiv positive (pre-HAART and HAART) and controls in the study.

Parameters	Control N=52	Pre-HAART N=111	HAART N=111
Vitamin A (µg/dL)	9.70 ± 0.93^a	6.32 ± 1.11^b	8.02 ± 0.70^c
Vitamin C (mg/dL)	1.73 ± 0.16^a	0.75 ± 0.20^b	1.21 ± 0.42^c
Vitamin E (mg/dL)	0.67 ± 0.09^a	0.35 ± 0.04^b	0.41 ± 0.02^b
Zinc (µg/dL)	98.48 ± 5.93^a	76.20 ± 9.16^b	89.20 ± 4.73^c
Copper (µg/dL)	26.43 ± 3.34^a	35.12 ± 2.82^b	31.66 ± 2.33^c
Selenium (µg/dL)	55.17 ± 6.02^a	23.30 ± 5.91^b	44.42 ± 5.23^c

Mean ± SD values with different superscript are statistically different at $p \leq 0.05$

Table 3: Serum electrolytes of HIV positive (pre-HAART and HAART) and controls.

Parameters	Contro N=52	Pre-HAART N=111	HAART N=111
Potassium (mmol/L)	4.07 ± 0.57^a	3.29 ± 0.33^b	3.92 ± 0.43^c
Sodium (mmol/L)	137.46 ± 3.35^a	132.61 ± 2.46^b	134.50 ± 3.27^b
Chloride (mmol/L)	100.42 ± 6.28^a	98.92 ± 5.65^a	99.59 ± 6.53^a

Mean ± SD values with different superscript are statistically different at $p \leq 0.05$

Table 4: Cd4 Counts and Some Haematological Parameters of HIV Positive (Pre-HAART and HAART) and controls

Parameters	Control N=52	Pre-HAART N=111	HAART N=111
CD4(cells/mm^3)	934.48 ± 324.42^a	288.52 ± 186.20^b	475.44 ± 147.16^c
WBC ($x10^3/\mu L$)	4.18 ± 0.17^a	7.84 ± 1.14^b	6.07 ± 0.46^c
PLT ($x10^3/\mu L$)	329.80 ± 55.61^a	141.65 ± 17.47^b	216.76 ± 8.62^c
HB (g/dL)	13.84 ± 0.26^a	11.11 ± 0.64^b	12.59 ± 0.34^c
PCV (%)	45.78 ± 2.43^a	36.66 ± 0.87^b	39.16 ± 0.87^c

Mean ± SD values with different superscript are statistically different at $p \leq 0.05$

altered gut barrier function, and the hypermetabolic state produced by chronic HIV infection.[27] It has also been suggested that a possible cause of selenium depletion among HIV positive subjects is the utilization of selenium by HIV-1 virus to produce its own selenoenzymes.[28]

Results obtained from this study also showed a statistically significant decrease ($p < 0.05$) in the mean values of K^+ and Na^+ when compared with the normal subjects. This is in agreement with the findings of Ansgar,[29] Ross and Klothman[30] and Eshiet *et al*.[31] who observed that HIV infection may present fluid electrolytes and acid-base abnormalities, acute renal failure, glomerulopathy directly related to underlining HIV infection at different stages.

The observed lower levels of plasma K^+ in this study may be attributed to the dilution of the extracellular space, movement of K^+ into cells or loss from the body or kidney usually associated with HIV infections as earlier reported by.[32]

The mean value of plasma chloride was low although the value was not statistically significant ($p > 0.05$) in this study. Heavy sweating, vomiting,

adrenal and kidney diseases, pyelonephritis, diarrhea and dehydration leads to reduced levels of chloride as reported by Kleyman *et al*.[33] These symptoms were not severe and in some cases totally undiagnosed in the HIV subjects that were used.

Haematological abnormalities frequently encountered in HIV-infected individuals are anaemia, granulocyte disorders, thrombocytopenia, lymphomas, coagulopathies and vascular malignancies like Kaposi sarcoma. Although in the majority of cases, haematologic abnormalities are detected in middle or advanced stages of HIV infection, some of these like anaemia and thrombocytopenia have been reported to occur in early stages of HIV infection.[34]

In this study, we found that before the initiation of HAART, anemia was the commonest hematological derangement in HIV positive HAART naïve. Haemoglobin concentration (Hb) and packed cell volume (PCV) were higher in the control subjects when compared with HIV positives on HAART and pre-HAART HIV positive subjects in each case. This agrees with the works of Wuba *et al*.[35] and Munyazesa *et al*.[36] Anaemia has been shown to be a risk factor for early death in patients with AIDS, and the causes of HIV-related anaemia are multifactorial.[37] This might also be due to generalized pancytopenia usually caused by chronic condition like HIV infection and some changes in cytokine production, decreased erythropoietin concentrations, opportunistic infectious agents.[38]

Our study shows that HAART naïve patients have significantly higher value ($p < 0.05$) of WBC when compared to HAART patients and controls subjects. This is in agreement with works of [39,40] who in their studies observed an increase in WBC in HIV subjects. White Blood Cell (WBC) count is important to monitor because the elevation of WBC may indicate infection, lack of response to treatment or an abnormality. The total WBC count shows a significant reduction in HAART patients although the reduction, progressive reduction observed in total WBC may indicate suppressive activity of the antiretroviral drug on the virus. This may be due to suppression of bone marrow and direct infection of T cells.

We also observed a significant decrease ($p < 0.05$) in platelet count in HIV HAART naïve patients when compared to HIV HAART patients and controls. This is in agreement with the works of Munyazesa *et al*.[36] and Vannappagari *et al*.[41] who reported higher prevalence of thrombocytopenia in HIV-infected individuals. The marked reduction in platelet counts may directly be attributed to the ability of HIV to directly suppress platelet production by infecting megakaryocytes and indirectly suppress production by causing alterations in the production of cytokines (cell-produced chemicals) and growth factors needed for proper platelet production and function.[42] It was reported that HIV infection induces macrophages to produce more of a chemical called macrophage-derived chemokine (MDC) which appears to alter the function of platelets. HIV may also cause platelets to be targeted by anti-platelet antibodies, a process that results in what is termed immune destruction.[41] The result is immune thrombocytopenic purpura (ITP). With ITP, it appears that the platelets have become coated with proteins from HIV and are thus, recognized as foreign invaders by the immune system when they pass through the spleen. The immune system then targets them for destruction. There can also be non-immune destruction with thrombotic thrombocytopenic purpura (TTP). It is thought that TTP may result from the blood vessel-damaging effect of HIV proteins.[43] Degree of thrombocytopenia in this study was higher in HAART naïve patients than those on HAART. It was reported that highly active antiretroviral therapy results in a sustained increase in the platelet count in HIV-infected patients with thrombocytopenia.

The present study has shown that prevalence of anemia is higher in treatment naïve patients compared to patients on treatment. This is consistent with the previous study done by Owiredu *et al*.[44] and Mildvan *et al*.[45] The findings of this study affirm that hematological disorders are corrected by combination antiretroviral therapy which also decreases the viral load. Thus, HIV patients who were on HAART had a greater number of blood cells within six months of beginning treatment and hematological disorders were corrected.[46] The present study shows that patients on HAART had statistically significant increase in WBC compared to their HAART-naïve counterparts. This study shows that CD4 cell counts of patients on HAART were significantly higher compared to HAART-naïve, this study supports the fact that HAART resulted in the significant increase in CD4 T cell counts in the majority of patients. This possibly was due to suppression of plasma HIV RNA.[42]

Conclusion

This study has shown that the use of HAART therapy reduced haematological and biochemical abnormalities and increase CD4+ count significantly, an addition to the growing knowledge in this line of study. Most importantly, despite the huge benefits of the use of HAART, it is suggestive that supplementation with vitamins especially vitamin E and electrolyte such as sodium and potassium will be necessary.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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