RESEARCH ARTICLE

Age and gender-dependent response in levels of CD4+ T-cell levels, viral load, and some trace elements in HIV sero-positive subjects on ART and ART naïve subjects in Rivers State, Nigeria

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ABSTRACT

Background: Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) infection results in depletion of immune cells and micronutrients. Recently, HIV patients are treated with Antiretroviral Therapy (ART) with remarkable progress. This study was aimed at evaluating Cluster of Differentiation ($CD4^+$) T-cells levels, viral load, serum Copper, Zinc and Selenium levels in HIV sero-positive subjects on ART and ART naïve subjects in Southern Nigeria with respect to age and gender.

Methods: 150 subjects aged from 20 years to 79 years were recruited after informed consent 70 subjects were HIV-positive on ART, 30 subjects were HIV-positive ART negative, while 50 were apparently healthy subjects. Ten (10) milliliters of blood was collected from each subject for the analysis of CD4 T-cells using fluorescent activated cell sorter, serum Copper and Zinc were analyzed colorimetrically using semi auto-analyzer WP 21E, while selenium was analyzed using atomic absorption spectrophotometer.

Results: $CD4^+$ T-cells was significantly lower in HIV subjects, and administration of ART improved the count significantly (546.9 ± 277.7 cells/ml) when compared with HIV ART naïve (297.5±244.6 cells/ml) [p<0.001]. The viral load 2.93 ± 1.39 was significantly higher in the ART naïve when compared to the HIV positive on ART 0.33 ± 0.19 (p<0.0001.). Serum selenium levels in control subjects (0.47 ± 0.40 µmol/l) was significantly higher than in HIV positive subjects on ART (0.058 ± 0.07 µmol/l) which is also significantly higher than ART naïve subjects (0.006 ± 0.004 µmol/l).[p<0.001]. Serum copper was significantly higher (285.5 ± 85.70 µg/dl) in ART naïve subjects than the HIV positive subjects on ART (258.5 ± 65.68 µg/dl) and the control subjects (198.3 ±40.23 µg/dl) [p<0.001]. Female subjects on ART (577.5 ± 27.0cells/ml) has a significantly higher CD4 T-cell count than male subjects on ART (451.2 ± 28.4 cells/ml) (p,0.001) and age interval of ART medication did not significantly differ.

Conclusion: There is significant reduction in $CD4^+$ T-cell count, with elevated viral load and alteration in serum trace elements levels in HIV infection, while ART treatment improves the condition.

Key Words: *CD4 T-cells; ART; Viral load; HIV; Micronutrients*

INTRODUCTION

Human Immunodeficiency Virus (HIV) infection has become a global health problem and HIV positive individuals are susceptible to malnutrition [1] due to several factors, such as poor nutritional intake (gastrointestinal complications such as nausea and vomiting, oral and esophageal sores), loss of nutrient (diarrhea and/or malabsorption), alteration in the metabolic process (changes in fatty acid metabolism and increased protein turnover), and interaction between drug and nutrient [2]. HIV infects mainly the cells of the immune system particularly $CD4^+$T-helper cells, macrophages, and dendritic cells. The progressive depletion of the CD4+T-cells plays a critical role in the pathogenesis of HIV [3]. Trace elements (zinc (Zn), iron (Fe), copper (Cu), selenium (Se), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn), and magnesium (Mg)) are dietary mineral that are present in very minute quantities (less than 0.01%) of the mass of the organism [4] and are involved in the regulation of vital functions of the body at all stages of development [5]. Zinc and Selenium are essential trace elements required for maintaining a healthy immune system. The deficiency of Zinc can result in decreased T-cells generation, suppression of humoral and cell-mediated immunity [6,7], progression to AIDS, and mortality in HIV-infected adults [8-9]. Deficiency of Selenium is associated with the increased mortality rate in HIV-positive individuals [10-15]. Hurwitz et al., in a randomized trial, reported improvement in CD4 T-cell count following Selenium supplementation in HIV-positive adults receiving Antiretroviral Therapy (ART) [16]. According to the National HIV/AIDS plan report in 2019, Rivers State is among the first three states with the highest incidence of HIV cases in Nigeria [17], with Port Harcourt having the largest number of cases in the state. There are limited data on the levels of plasma Zinc, Selenium, viral load, and $CD4^+$ cells in HIV-seropositive subjects in this locality. This study is therefore aimed at evaluating the plasma levels of $CD4^+$ T-cells, viral load, Zinc, and Selenium levels in HIV-Sero-positive individuals on ART and ART naïve subjects resident in Port Harcourt with respect to age and gender.

MATERIALS AND METHODS

Study Design

Case-control and cross-sectional study designs were adopted on HIV-seropositive subjects on antiretroviral therapy (ART) and those that were not on antiretroviral therapy (ART naïve). This study was carried out in the Department of Medical Laboratory Science and Rivers State University Teaching Hospital, and Subjects were drawn from outpatients attending the hospital.

Methodology

Ethics

Ethical clearance was obtained from Rivers State Ministry of Health, and Rivers State university teaching hospital (RSUTH) ethical clearance committees, with approval file numbers: MH/PRS/391/VOL.2/636 and RSUTH/REC/2020033 respectively.

A total of one hundred and fifty (150) subjects, aged (20-79) were recruited for the study. Fifty (50) apparently healthy individuals were used as the control group, while one hundred (100) HIV-seropositive subjects made up of 70 on ART and 30 ART naïve subjects were used as the test group. Both the control and Test group subjects were recruited after informed consent was obtained.

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CD4+ T-cell count

Immunophenotyping of lymphocytes for CD4+ T-cell count was carried out by automated FACS count (Becton Dickinson, Singapore (BD). The Lymphocytes were stained according to the protocol recommended by the manufacturer and CD4 T-cells count was analyzed using a Becton Dickinson Fluorescent Activated Cell Sorter Count (FACSC count) automation.

Viral load

HIV viral load was analyzed using Real-Time Polymerase Chain Reaction (RT-PCR) COBAS TaqMan 48 Analyzer, according to manufacturer's instructions, which involves specimen preparation to isolate HIV-1 RNA, reverse transcription of the target RNA to generate complementary DNA (cDNA), and simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide probe specific to the target, and HIV viral load quantification, processes that are automatically conducted.

Selenium

Serum Selenium levels were measured by graphite furnace atomic absorption spectrometry (Atomic absorption Spectrophotometer ELICO, India, Model No. SL173), following the manufacturer's instructions.

Zinc

Serum Zinc was determined colorimetrically using Human Zinc Monoliquid kit purchased from Fortress Diagnostics, United Kingdom, with Product code: BXC0462, following manufacturers' instructions. Briefly; all reagents were brought to room temperature before use. Glass tubes were arranged and labeled as blank, standard, Quality Control (QC), and samples respectively. 50µL of the sample was added only to the sample tube, 50µL of the QC was added to the QC tube, and then 50µL of the standard was added to the standard tube. 1000µL of R1 zinc reagent was added to the blank, standard, QC, and sample tubes. It was then mixed and incubated for 5 minutes at 37°C. The concentration of each sample was determined within 5 minutes using a semi auto-analyzer WP 21E (China) at 560nm.

Statistical Analysis

The generated data were analyzed using Graph-pad prism version 8.0.2 to obtain the mean and standard deviation of the study groups. Analysis of variance was used to determine the statistical significance between the HIV-positive subjects group and the control group. Sample T-test was used where only two variables were compared and a zp-value of <0.05 was considered to be statistically significant. Results are presented in tables as mean ± standard deviation (M ± SD).

RESULTS

Table (1-6)

TABLE 1
ANOVA Result of Control, ART Naïve, and HIV Subjects on ART

Parameters	Control (Overall)	Naïve (Overall)	On ART (Overall)	F value	P-value	Remark
CD4 (cells/ml)	1399 ± 390.4^{a}	297.5 ± 244.6^{bc}	546.9 ± 277.7^{bd}	136	<0.0001	S
Viral Load (cp/ml)x105	0.00 ± 0.00^{a}	2.93 ± 1.39^{bc}	0.33 ± 0.19^{bd}	19.83	<0.0001	S
Cu2+ (µg/dl)	198.3 ± 40.23^{a}	285.5 ± 85.70^{bc}	258.5 ± 65.68^{bc}	17.29	<0.0001	S
Zn2+ (µmol/l)	8.19 ± 0.47^{a}	7.89 ± 0.69^{bc}	7.79 ± 0.70^{bc}	7.748	0.0006	S
Se (µmol/l)	0.47 ± 0.40^{a}	0.006 ± 0.004^{bc}	0.058 ± 0.07^{bc}	38.37	<0.0001	S

Key: Values in the same row with different superscripts differ significantly from one another when control was compared with Naïve and those on ART. However, values with the same superscript do not differ significantly when Naïve subjects were compared against subjects on ART at p<0.05

TABLE 2
ANOVA Result of Female Control, Female ART Naïve, and Female HIV Subjects on ART

Parameters	Control (Female)	Naïve (Female)	ART (Female)	F value	P value	Remark
CD4 (cell/ml)	1306 ± 383.7^{a}	301.8 ± 269.7^{bc}	577.5 ± 272.0^{bd}	48.21	<0.0001	S
Viral Load (cp/ml)*105	0.00 ± 0.00^{a}	2.48 ± 2.38^{bc}	0.31 ± 0.28^{bd}	11.54	<0.0001	S
Cu^{2+} (µg/dl)	195.8 ± 40.47^{a}	296.3 ± 111.7bc	256.7 ± 60.63^{bc}	7.943	0.0007	S
Zn^{2+} (µmol/l)	8.160 ± 0.43	8.13 ± 0.49	7.83 ± 0.87	1.565	0.2156	NS
Se (µmol/l)	0.49 ± 0.48^{a}	0.007 ± 0.005^{bc}	0.07 ± 0.05^{bd}	23.95	<0.0001	S

Key: values in the same row with different superscripts differ significantly from one another when control was compared with Naïve and those on ART. However, values with the same superscript do not differ significantly when Naïve subjects were compared against subjects on ART at p<0.05.

TABLE 3
ANOVA result of male control, male art naïve, and male HIV subjects on art

Parameters	Control (Male)	Naïve (Male)	ART (Male)	F value	P-value	Remark
CD4 (cell/ml)	1420 ± 405.6^{a}	244.3 ± 237.2^{bc}	451.2 ± 282.4^{bc}	78.27	<0.0001	S
Viral Load (cp/ml)*105	0.00 ± 0.00^{a}	4.99 ± 4.423^{bc}	0.42 ± 0.26^{bd}	68.23	<0.0001	S
Cu^{2+} (µg/dl)	197.2 ± 39.83^{a}	273.1 ± 64.87^{bc}	262.7 ± 77.27^{bc}	9.161	0.0004	S
Zn^{2+} (µmol/l)	8.17 ± 0.52^{a}	8.33 ± 0.79^{ac}	7.69 ± 0.39^{bd}	6.508	0.0028	S
Se (µmol/l)	0.59 ± 0.49^{a}	0.01 ± 0.005^{bc}	0.02 ± 0.02306^{bc}	24.8	<0.0001	S

Key: values in the same row with different superscripts differ significantly from one another when control was compared with Naïve and those on ART. However, values with the same superscript do not differ significantly when Naïve subjects were compared against subjects on ART at p<0.05.

TABLE 4
Comparative Analysis (ANOVA) of Male and Female Subjects on ART and ART Naïve

	Control (male)	Naïve (male)	ART (male)	Control (female)	Naïve (female)	ART (Female)	F value	P value	Remark
CD4 (cell/ml)	1306 ± 405.6^{a}	244.3 ± 23.2^{bc}	451.2 ± 28.4^{bce}	1420 ± 38.7^{bdfg}	301.8 ± 26.7^{bcfhi}	577.5 ± 27.0^{bdehj}	52.79	<0.0001	S
Viral Load (cp/ml)x105	0.00 ± 0.00^{a}	4.99 ± 2.42^{bc}	0.42 ± 0.26^{bde}	0.00 ± 0.00^{adfg}	0.0008 ± 0.0003^{bdfh}	0.14 ± 0.11^{bdeh}	10.32	<0.0001	S
Cu^{2+} (µg/dl)	197.2 ± 39.83^{a}	273.1 ± 64.87^{bc}	262.7 ± 77.27^{bce}	195.8 ± 40.47^{adfg}	296.3 ± 111.7^{bcehi}	256.7 ± 60.63^{bcehi}	6.846	<0.0001	S

Zn^{2+} (µmol/l)	8.17 ± 0.52	8.33 ± 0.79	7.69 ± 0.39	8.16 ± 0.43	8.13 ± 0.49	7.83 ± 0.87	2.776	0.0503	NS
Se (µmol/l)	0.59 ± 0.49[a]	0.01 ± 0.005[bc]	0.03 ± 0.02[bce]	0.48 ± 0.38[adfg]	0.007 ± 0.005[bcehi]	0.07 ± 0.06[bcehi]	21.36	<0.0001	S

Key: values in the same row with different superscripts differ significantly from one another when control was compared with Naïve and those on ART. . However, values with the same superscript do not differ significantly when Naïve subjects were compared against subjects on ART at p<0.05.

TABLE 5
Comparative Analysis (ANOVA) of Female Subjects on ART Based on Age Interval (years)

Parameters	20-29 yrs	30-39 yrs	40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs	F value	P value	Remark
CD4 (cells/ml)	621.1 ± 297.4	611.0 ± 315.8	508.3 ± 241.6	749.0 ± 164.4	540.7 ± 282.0	412.5 ± 156.3	0.756	0.586	NS
Zn^{2+} (µmol/l)	7.57 ± 0.38	8.00 ± 0.99	8.05 ± 1.23	7.60 ± 0.22	7.60 ± 0.32	7.55 ± 0.49	0.616	0.688	NS
Se (µmol/l)	0.1 0 ± 0.05	0.04 ± 0.02	0.08 ± 0.01	0.06 ± 0.05	0.07 ± 0.01	0.03 ± 0.002	0.709	0.62	NS
Viral Load (cp/ml)*105	0.30 ± 0.26	0.08 ± 0.07	0.48 ± 0.11	0.05 ± 0.03	0.66 ± 0.15	0.10 ± 0.09	0.636	0.673	NS

Key: All parameters and values in the same row without superscript do not differ significantly when the various age groups were compared against one another at p<0.05.

TABLE 6

Comparative Analysis (ANOVA) of Male Subjects on ART Based on Age Interval (years)

Parameters	20-29 yrs	30-39 yrs	40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs	F value	P value	Remark
CD4 (cells/ml)	729.5 ± 28.99	271.0 ± 336.0	574.3 ± 227.0	409.7 ± 337.9	523.0± 192.3	270.5 ± 105.4	1.007	0.4496	NS
Zn^{2+} (µmol / l)	7.65 ± 0.21	7.26 ± 0.12	8.03 ± 0.40	7.54 ± 0.28	8.05 ± 0.49	7.80 ± 0.42	2.7 29	0.0 634	N S
S e (µmol / l)	0.03 ± 0.001	0.05 ± 0.04	0.02 ± 0.01	0.02 ± 0.003	0.02 ± 0.005	0.02 ± 0.002	0.8 96	0.50 96	N S
Viral Load (cp/ml) * 105	0.07 ± 0.006	0.48 ± 0.4	0.05 ± 0.03	0.74 ± 0.41	0.07 ± 0.05	0.68 ± 0.41	0.96	0.4743	NS

Key: All parameters and values in the same row without superscript do not differ significantly when the various age groups were compared against one another at p<0.05.

DISCUSSION

The key feature of untreated HIV/AIDS infection is the progressive depletion of $CD4^+$ cell lymphocytes and resultant severe immunosuppression. Initiation of ART gives better clinical improvement and leads to an increase in CD4+ cell count, dramatically decreases the incidence of opportunistic infections, and leads to immune restoration (partial or complete), that is, reversal of HIV-associated immunological alterations [18, 19]. This leads to a better quality of life for the patients [18-20]. The present study reveals that there is a higher level of CD4 T-cells in patients on ART compared to the level in ART naïve patients. This corresponds with the findings of Otieno et al., [21]. The improvement in CD4 T-cell count in patients on ART was age-related as subjects within the age range of 20-29 years had the highest number of CD4 T-cell count compared to the older subjects. This study also revealed gender-based differences in CD4 T-lymphocyte count. CD4 T-cells were higher in females than in males on ART and ART naïve subjects. This finding could be explained by the fact that women are likely tested for HIV earlier than men and had earlier access to treatment and clinical care compared to men, because of the increasing availability of antenatal testing as part of ongoing expansion in voluntary counseling and testing [22-23]. Early access to treatment and clinical care in female improve their health condition compared to their male counterparts at enrollment into care. Patients on ART showed greater improvement in CD4 lymphocyte count compared to ART naïve patients of both sexes, which confirmed the earlier report by Kumarasamy et al., [24] that both men and women showed consistent improvement in the HIV patients on initiating ART.

Micronutrients play a critical role in the proper functioning of the immune system. Thus in HIV where there is profound immune suppression, there occurs a deficiency of many micronutrients such as Zinc and Selenium. In HIV-positive cases, there is zinc deficiency, an essential element for the functioning of CD4 T-cell counts as seen in Tables 1-3, where zinc levels in control subjects (healthy individuals) were significantly higher when compared to HIV-infected subjects (ART naïve and subjects on ART). Zinc level was not affected by sex and age as there was no significant difference across the various age ranges and sex in the ART naïve and subjects on ART (Tables 4-6). Decreased Zinc levels have been reported in HIV infection at different stages [25]. Hence, low levels of Zinc may contribute to susceptibility to HIV or clinical features of HIV; also, HIV infection does suppress Zinc levels [26].

The deficiency of Selenium is associated with the early progression of HIV disease and mortality [27]. Deficiency of Selenium has been reported in HIV/ AIDS patients. This study reports decreased levels of Selenium in HIV seropositive subjects, this was independent of age and sex. This agrees with the reports of other researchers [28-32].

CONCLUSION

Low levels of CD4 lymphocytes, Zinc, and Selenium in HIV-infected subjects have been established. This scenario can enhance disease progression, complications, and mortality. Plasma Zn and Se deficiency are common in Port Harcourt HIV-infected patients and early evaluation of the nutritional status of these HIV positive subjects as well as provision of appropriate nutritional support and mineral supplementation along with antiretroviral therapy are recommended.

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