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CD4+ T-Cell Count, Serum Zinc, Copper and Selenium Levels in HIV Sero-Positive Subjects on ART and ART Naïve Subjects in Port Harcourt, Nigeria

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Abstract

Background: HIV infection results in depletion of immunocompetent cells such as CD4+ T-cells. Trace elements such as Copper, Zinc and selenium are known to be involved in immune function. In recent times, HIV-positive patients are treated with antiretroviral therapy (ART), with significant progress. This study was aimed at evaluating CD4+ T-cells levels, serum Copper, Zinc and Selenium levels in HIV seropositive subjects on ART and ART naive subjects (HIV positive subjects that have not started ART treatment) in Rivers State, Nigeria. **Methods:** 150 subjects aged 20 to 79 years were recruited after informed consent. 70 subjects were HIV-positive on ART, 30 subjects were HIV-positive ART naïve subjects, while 50 subjects were apparently healthy subjects. Ten (10) milliliters of blood was collected using a standard venipuncture technique from each subject for the analysis of CD4 T-cells using BD fluorescent activated cell sorter (FACSC count), serum Copper and Zinc were analyzed colorimetrically using semi auto-analyzer WP 21E, while selenium was analyzed using atomic absorption spectrophotometer ELICO, SL173. Data generated were analyzed using Graph-Pad Prism version 8.0.2 and $p < 0.05$ was considered significant. **Result:** This study revealed a significant reduction in mean zinc, selenium and CD4+ T-cell level respectively ($p = 0.0006$; 0.0001; 0.0001) in HIV-Positive subjects on ART and ART naive. There was also a significant increase in mean serum copper level in the HIV-positive subject as compared to control subjects ($p = 0.0001$). ART treatment improved the CD4+ T cell count and serum levels of selenium and zinc; however, ART did not correct the imbalance. Furthermore, female subjects on ART have a significantly higher CD4+ T-cell count than the males (p

< 0.001). **Conclusion:** Selenium and Zinc deficiency are associated with HIV disease despite the role of ART hence micronutrient supplementation is advised for HIV-positive subjects on ART.

Keywords

Anti Retroviral Therapy (ART), Human Immunodeficiency Virus (HIV), Trace Elements, CD4 T-Cells

1. Introduction

The human immunodeficiency virus (HIV) infection can impair nutritional status of the patient [1], by causing a reduced intake and absorption of micronutrients, it can also cause increased utilization of nutrients leading to nutrient deficient status. A good number of these micronutrients are involved in buffering the antioxidant status, especially zinc, copper and selenium. These micronutrients are also involved in immunomodulatory functions. Therefore suboptimal levels of these antioxidant nutrients during HIV infection can contribute to immune dysregulation and increased HIV replication and progression [2]. Malnourishment could lead to immune dysfunction and higher susceptibility to various opportunistic infectious diseases which most times coexist with HIV infection [2] [3]. Selenium (Se), Copper (Cu), and zinc (Zn) are the key trace elements that serve as antioxidants, and have a role in HIV-1 disease progression [2] [4]. Trace elements especially zinc (Zn) and selenium (Se) are known to be important for maintaining a healthy immune system, hence Zinc deficiency is associated with a decline in T cells generation especially $CD4^+$ and $CD8^+$ T cells resulting in a concomitant depression of humoral and cell-mediated immunity and progression to AIDS [5] [6] [7]. Selenium deficiency has also been implicated in several medical complications including impaired immune response [7]. This means that Immune function is highly dependent on nutritional status, because the large mass and a high rate of cellular turnover of the immune system make it a major user of nutrients. With the opportunistic infection, as an added burden in HIV/AIDS patients, the nutrient requirement may increase manyfold. The introduction of ART in the management of HIV patients has changed the scenario of HIV infection and pathogenesis, however, information is lacking on the nutritional status as regards the serum levels of essential trace elements such as selenium, zinc and copper in HIV patients on ART, and ART naïve HIV subjects. This study is therefore aimed at evaluating the effect of ART treatment on $CD4^+$ T-cell levels and some nutritional minerals (selenium, zinc and copper) in HIV-positive subjects living in Port Harcourt Nigeria.

2. Material and Methods

Methods: This study was conducted in Port Harcourt, Rivers State Nigeria. Patients were recruited by random sampling among HIV positive subjects attend-

ing Rivers State University teaching hospital, between March and December 2021. The Cochran formula was used to determine the sample size with 3.8% HIV prevalence in Rivers State.

A total of 150 subjects within the age of 20 and 80 years were recruited in this study; of which 70 subjects were HIV-positive on antiretroviral therapy (ART), 30 subjects were HIV-positive that are ART naïve, while 50 subjects were apparently healthy subjects who served as controls. These subjects willingly gave their consent to participate in the study. Ten milliliters of blood were collected aseptically from all HIV-infected and healthy subjects under trace elements free condition. $CD4^+$ T-cell count was done same day with whole blood. The remaining blood samples were centrifuged at 3000 g for five minutes. The collected serum was stored at −70˚C until analysis. All glassware and bottles used for separation of serum and further analysis were previously soaked in 10% nitric acid and rinsed thoroughly with deionized water. Serum Copper and zinc were measured colorimetrically while serum Selenium levels were measured using atomic absorption spectrophotometer.

2.1. $CD4^+$ 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 suggested by the manufacturer and CD4 T cells count was analyzed using a Becton Dickinson fluorescent activated cell sorter count (FACSC count) automation.

2.2. Selenium

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

2.3. Zinc

Serum Zinc was determined colorimetrically using Human Zinc Monoliquid kit purchased from Fortress Diagnostics, United Kingdom, with Product code: BXC0462, following manufacturers instruction. 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. 50 µL of the sample was added only to the sample tube, 50 µL of the QC was added to the QC tube, and then 50 µL of the standard was added to the standard tube. 1000 µL 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 560 nm.

2.4. Copper

Serum copper was determined colorimetrically using human copper kit pur-

chased from Fortress Diagnostics, United Kingdom with product key BXC0341A, following manufacturer's instructions. Briefly; all reagents were allowed to attain room temperature and 50 µL of distilled water, standard and samples were added to the respective tubes for blank, standard and tests. 1ml of R1 was pipette into all the tubes and mixed, then incubated for 5 minutes using a water bath at 20˚C - 25˚C. The concentration of each sample was determined using a semi auto-analyzer WP 21E (China) at 570 nm within 5 minutes after adding the working reagent.

2.5. Statistical Analysis

The generated data were analyzed using Graph-pad prism version 8.0.2. The results were expressed as mean and standard deviation of the study groups. Analysis of variance was used to determine the statistical difference between the HIV positive subjects group and the control group. P-value of <0.05 were considered to be statistically significant. Results are presented in tables as mean ± standard deviation (M ± SD).

3. Results

In Table 1, the results show that mean ± SD of CD4 T-cell count in control subjects (1399 ± 390.4 cells/ml) is significantly higher than the mean ± SD of CD4 T-cell count of HIV-positive subjects (546.9 ± 277.7 cells/ml) on ART which is also higher than the mean ± SD of $CD4^+$ T-cell count in ART naïve HIV positive subjects (297.5 ± 244.6 cells/ml) ($p < 0.0001$).

On the contrary, the mean ± SD of serum Copper levels of the control subjects (198.3 ± 40.23 µg/dl) is significantly lower than the HIV-positive subjects on ART (258.5 ± 65.68 µg/dl), which also lower than ART naive HIV-positive subjects (285.5 ± 85.70 µg/dl) ($p < 0.0001$). The mean ± SD of plasma Zinc levels in the control subjects (8.19 ± 0.47 µmol/l) is significantly higher than the levels in HIV positive subjects on ART (7.89 ± 0.69 µmol/l) ($p < 0.0006$). However, the levels are not significantly different between the subjects on ART and the ART naïve subjects (7.79 ± 0.70 µmol/l). The result for mean ± SD of serum Selenium in the control subjects (0.47 ± 0.40 µmol/l) is significantly higher than mean ±

Table 1. Mean ± SD of $CD4^+$ T-cell count, serum Cu^{2+}, Zn^{2+} and Se of Control, ART Naïve HIV subjects and HIV subjects on ART.

Parameters	Control	Naïve	On ART	F value	P value	Remark
CD4 (cells/ml)	1399 ± 390.4^a	297.5 ± 244.6^b	546.9 ± 277.7^c	136.0	<0.0001	S
Cu^{2+} (µg/dl)	198.3 ± 40.23^a	285.5 ± 85.70^{bc}	258.5 ± 65.68^{bc}	17.29	<0.0001	S
Zn^{2+} (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

Post-Hoc Turkey's Analysis: Values in the same row with different superscript (a, b) differ significantly from one another when control was compared with Naïve and those on ART, at $p < 0.05$. CD = cluster differentiation Cells, Cu^{2+} = Copper II ion, Zn^{2+} = Zinc II ion, Se = Selenium.

SD of the HIV subjects on ART (0.058 ± 0.07 µmol/l) which is also higher than the levels in ART naïve HIV-positive subjects (0.006 ± 0.004 µmol/l).

In Table 2, the results were analyzed with respect to gender. The female control subjects have a significantly higher $CD4^+$ T-cell count (1420 ± 383.7 cell/ml) than the male counterparts (1306 ± 405.6 cell/ml). Similarly, the female HIV subjects on ART have a higher CD4 T-cell count (577.5 ± 272.0 cell/ml) than the males (451.2 ± 282.4 cell/ml) and also the female ART naïve (301.8 ± 269.7 cell/ml) and male ART naïve (244.3 ± 237.2 cell/ml). The serum Copper level was not significantly different in both sexes (male 197.2 ± 39.83 µg/dl and female 195.8 ± 40.47 µg/dl) for the control and the HIV-positive subjects on ART. However the female HIV-positive ART naïve subjects (296.3 ± 111.7 µg/dl) has higher serum Copper levels than the male (273.1 ± 64.87 µg/dl). The serum Zinc levels are not significantly different in all the groups.

The female control subjects has serum Selenium levels (0.48 ± 0.38 µmol/l) significantly lower than the male control subjects (0.59 ± 0.49 µmol/l), conversely, the female HIV-positive subjects on ART (0.07 ± 0.06 µmol/l) has a higher serum selenium levels when compared to the males (0.03 ± 0.02 µmol/l) but female ART naïve subjects (0.007 ± 0.005 µmol/l) has lower Selenium levels than male ART naïve (0.01 ± 0.005 µmol/l).

4. Discussion

HIV infection and severity have been estimated using the decline in immunomodulatory and immunocompetent cells such as $CD4^+$ T cells, which is found to correlate with an increase in viral load. In this study, we report a significantly lower $CD4^+$ T cell count in subjects who are HIV positive when compared with the control subjects who are HIV-negative ($p < 0.05$) (Table 1). We also report a further decline of $CD4^+$ T cells in HIV-positive subjects who are ART naïve when compared with HIV-positive subjects on ART. This finding implies that ART improves the $CD4^+$ T cell count of subjects receiving ART, even though the levels are still suboptimal as compared to the control subjects. This report is consistent with other reports [8] [9] [10]. The reduction in $CD4^+$ T-cell in HIV

Table 2. Comparative analysis of Mean ± SD of $CD4^+$ T-cell count, serum Cu^{2+}, Zn^{2+} and Se of male and female Control, ART Naïve HIV subjects and HIV Subjects on ART.

Parameters	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 ± 237.2^b	451.2 ± 282.4^c	1420 ± 383.7^d	301.8 ± 269.7^e	577.5 ± 272.0^f	52.79	<0.0001	S
Cu^{2+} (µg/dl)	197.2 ± 39.83^a	273.1 ± 64.87^{bc}	262.7 ± 77.27^{bc}	195.8 ± 40.47^a	296.3 ± 111.7^d	256.7 ± 60.63^c	6.846	<0.0001	S
Zn^{2+} (µmol/l)	8.17 ± 0.52^a	8.33 ± 0.79^a	7.69 ± 0.39^a	8.16 ± 0.43^a	8.13 ± 0.49^a	7.83 ± 0.87^a	2.776	0.0503	NS
Se (µmol/l)	0.59 ± 0.49^a	0.01 ± 0.005^b	0.03 ± 0.02^c	0.48 ± 0.38^a	0.007 ± 0.005^d	0.07 ± 0.06^e	21.36	<0.0001	S

Post-Hoc Turkey's Analysis: Values in the same row with different superscript (a, b, c) differ significantly from one another when control was compared with Naïve and those on ART. Subjects on ART: CD = cluster differentiation Cells, Cu^{2+} = Copper II ion, Zn^{2+} = Zinc II ion, Se = Selenium.

subjects is because the hallmark of HIV infection and subsequent AIDS pathogenesis is a progressive depletion of $CD4^+$ T-cell populations in close association with progressive impairment of cellular immunity and increasing susceptibility to opportunistic infection [11]. ART has been reported to interfere with viral replication, reducing the viral load drastically and improving the $CD4^+$ T-cell count. This is because ART treatment is geared towards interfering with the HIV virus pathogenesis and infection thereby boosting the immune system as shown by the increased $CD4^+$ T-cell count in the HIV-positive subjects on ART when compared with the ART naive HIV-positive subjects. This report is also in agreement with the work of [10].

This study demonstrated in **Table 1** a significantly higher mean ± SD in serum Copper levels in HIV-positive subjects when compared with the control HIV-negative subjects ($p < 0.05$). This observation is in agreement with [12] [13] [14] [15]. This increase in serum Copper may reflect a non-specific increase in serum concentration of copper binding protein ceruloplasmin [16] High levels of copper in HIV-positive subjects may suggest its possible role as a useful marker of HIV activity and progression to AIDS [17]. However, this study is at variance with [10] [18], who reported no significant difference between the HIV-positive subjects and the control HIV-negative subjects ($p > 0.05$). This work also reports a higher mean ± SD of serum Copper levels in HIV-positive subjects who are ART naïve when compared to HIV-positive subjects on ART, an indication that ART might be helpful in reducing serum Copper.

Furthermore, this study revealed that HIV-positive subjects had significantly ($p < 0.05$) lower mean ± SD values of serum zinc when compared with the control subjects (**Table 1**). This observation is in agreement with previous reports of [8] [10] [19]. However, this study is at variance with the report of [20] who reported no significant difference in the zinc status of HIV-positive children and control. The reason for a decline in zinc status of HIV positive subjects may be attributed to three mechanisms: 1) high demand for zinc in HIV positive subjects may be because HIV nucleocapsid and integrase proteins that are essential for assembly of infectious virions contain zinc fingers which may require zinc for normal structure and functioning [21] [22] [23]; 2) Zinc deficiency may also be as a result of malnutrition. One of the factors responsible for malnutrition in an HIV-positive subject is reduced appetite which could be due to difficulty in ingesting food as a result of infections such as oral thrush or Osophagitis caused by Candida: a common opportunistic infection in HIV-positive subjects, fever, side effects of medicines or depression; and 3) Poor absorption of nutrients may be due to accompanying diarrhea which may be because of bacterial infections such as *Salmonella* or *Mycobacterium avium* or viral infections such as *Cytomegalovirus* (CMV) or parasitic infection such as *Giardia*, *Cryptosporidium parvum* and *Enterocytozoon bieneusi*; due to nausea/vomiting as a side effect of medications used to treat HIV. About 30% - 50% of HIV-positive subjects in developed countries and nearly 90% in developing

countries complain of diarrhea and malabsorption [24]. It was further observed that plasma Zinc levels in HIV-positive subjects were similar in ART subjects and ART naïve subjects. This implies that ART did not change the status of plasma zinc levels in the HIV-positive subjects. Zinc deficiency has been associated with impaired immune function and an increased susceptibility to infection [25] and zinc supplementation has been reported to have a beneficial effect on HIV subjects [26].

HIV-positive subjects also had significantly lower mean ± SD serum Selenium levels ($p < 0.05$) when compared to the control subjects (**Table 1**). This report is in agreement [22] [27] [28]. It is also in agreement with the reports of [19] [29]. This finding may be attributed to fact that HIV virus may be capable of incorporating host selenium into viral selenoproteins that have glutathione peroxidase activity [28] both as an integral component of glutathione peroxidase and thioredoxin reductase. Another reason could be that Selenium plays an important role in decreasing oxidative stress in HIV-infected cells possibly suppressing the rate of HIV replication, by this mechanism, the Selenium may be depleted [22]. Therefore, decreasing plasma selenium concentration in HIV-positive subjects are sensitive markers of disease progression and severity, low levels of plasma selenium have been associated with a significantly increased risk of death from HIV infection [30]. However, this study is at variance with the reports of [31] who reported higher mean ± SD of selenium in HIV-positive subjects when compared with the control. The reason for this disparity is unknown. It was also observed that HIV-positive subjects on ART had a higher mean ± SD plasma Selenium levels when compared with ART naïve subjects. The implication of this finding is that ART improves serum Selenium levels; this is because a reduction in viral load will imply an increase in $CD4^+$ T-cells and a reduction in nutrient requirements of the host.

In **Table 2**, the parameters were analyzed with respect to sex having sorted the patients into male and female. The mean ± SD levels for $CD4^+$ T-cells were significantly lower in the males when compared to the females in all categories. The control subjects in the females have a significantly higher $CD4^+$ T-cell count when compared to the males ($p < 0.05$). Similarly, the HIV-positive female subjects also had higher levels of CD4 T-cells than the HIV-positive males. This same picture was obtained across board for those on ART and ART naïve subjects. This study is in agreement with [32] [33]. The increased mean ± SD of $CD4^+$ T-cell in the female than that of the male may be as a result of the sex hormone, 17-Beta estradiol's (E_2) effect in female according to [34]. E_2 is the main estrogen found in the blood of women and exerts its actions through binding to the estrogen receptors present in the reproductive tract tissues and in immune cells in peripheral blood including $CD4^+$ T-cells and macrophages, the two HIV target cells [35]. Binding of E_2 to its receptors results in modulation of the expression of multiple genes. Earlier studies have illustrated the broad spectrum of E_2 on immune cells and the innate and adaptive immune responses [36]. According to a report by [37] treatment of $CD4^+$ T-cells with E_2 from women

significantly reduced HIV infection.

The mean ± SD levels of serum Copper did not show any significant difference in both sexes ($p > 0.05$) (Table 2). Gender was not a predictor of serum copper hence no significance ($p > 0.05$) in the mean ± SD levels of copper between male and female subjects. This same scenario was recorded across board for HIV-positive subjects on ART and ART naïve subjects. This study is consistent with the reports of [10] however it is at variance [38] [39] who reported a significant difference ($P < 0.05$) in the level of copper, with females having a higher copper level than male. Similarly, the serum mean ± SD of zinc levels when analyzed with respect to gender did not show any significant difference.

In this study, there was no significant ($p > 0.05$) difference in serum Selenium levels with respect to gender in the control subjects, however HIV-positive subjects on ART has a higher plasma selenium compared to ART naïve. This study is in agreement with [18] and a possible explanation for the similarity in the mean ± SD selenium levels in the controls may be because a wide variety of foods are rich in selenium which is consumed by both sexes. However, this study is at variance with the study of [40] that showed that men had slightly higher mean ± SD selenium than women although no explanation for this disparity was given.

5. Conclusion

Conclusively, trace elements such as copper, zinc and selenium play a critical role in the proper functioning of the immune system. They are also important constituents of the antioxidant mechanism. Selenium and zinc deficiency are significantly associated with HIV disease severity and progression hence micronutrient supplementation will be beneficial for HIV-positive subjects despite being on ART. It is evident that ART improves the health status of the HIV-positive subjects, but it does not ameliorate the trace element deficiency. Thus Zinc/selenium supplementation can be used to give a boost to ART regimen.

Author Contributions

Conceptualization: Donatus Onukwufor Onwuli; Methodology: Ugochukwu Chioma and Donatus Onukwufor Onwuli; Software: Anyalebechi Eberechukwu Okwuchi and Helen Waribo-Anthony; Validation: Donatus Onukwufor Onwuli; Formal analysis: Ugochukwu Chioma; Investigation: Donatus Onukwufor Onwuli; Resources: Anyalebechi Eberechukwu Okwuchi; Ugochukwu Chioma and Helen Waribo-Anthony; Data curation: Ugochukwu Chioma and Helen Waribo-Anthony; Writing: Ugochukwu Chioma and Donatus Onukwufor Onwuli; Original Draft: Ugochukwu Chioma; Writing Review and Editing: Ugochukwu Chioma, Donatus Onukwufor Onwuli, Anyalebechi Eberechukwu Okwuchi and Helen Waribo-Anthony; Visualization: Donatus Onukwufor Onwuli and Anyalebechi Eberechukwu Okwuchi.; Supervision: Donatus Onukwufor Onwuli and Helen Waribo-Anthony; Project administration: Donatus Onukwufor Onwuli; Funding acquisition: Ugochukwu Chioma.

Ethical Statement

This research was reviewed and approved by the institutional review board of Rivers State University Teaching Hospital in Port Harcourt, Nigeria (registration number: RSUTH/REC/2020033). Informed consent was obtained from all participants.

Support

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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