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Comparative Evaluation of the Levels of $CD4^+$ and $CD8^+$ T-Cells, Viral Load, and Some Immunomodulatory Trace Elements in ART and ART-naïve HIV Patients in Port-Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the levels of CD4+ and CD8+ T-cells, viral load, and some immunomodulatory trace elements in ART and ART-naïve HIV patients in Port-Harcourt, Nigeria.
Methodology: A total of 150 subjects (males and females) between the ages of 20 and 79 were recruited for the study, out of which 50 subjects were apparently healthy (those who tested negative for HIV), and were used as the control group, while the remaining 100 subjects were those who tested positive for HIV, and were used as the test group; out of this 100 subjects, 70 subjects were on anti-retroviral therapy (ART), while the remaining 30 subjects were not on anti-retroviral therapy (ART naïve). About 13 mls of venous blood was collected from the antecubital fossa of each subject. 3mls was dispensed into an EDTA-anticoagulant bottle, and used for the estimation of $CD4^+$ and $CD8^+$ counts using a BD fluorescent activated cell sorter count (FACSC count)

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automation. Also, 5mls of the venous blood was dispensed into another EDTA-anticoagulated bottle; it was spun to obtain the plasma which was used to analyze the viral load using real time polymerase chain reaction (RT-PCR) COBAS TaqMan 48 Analyzer. Then, another 5 mls of the venous blood was dispensed into lithium heparin bottle; it was spun to obtain the plasma, which was used for the analysis of copper, iron, zinc and magnesium by colorimetric method using semi auto-analyzer WP 21E, and selenium using atomic absorption spectrophotometer with graphite furnace technique SN-SG 710690.

Results: The results showed that there was no significant difference ($p>0.05$) in the mean levels of $CD8^+$ T-cell, iron and magnesium between the HIV-positive subjects (ART HIV-positive and ART-naïve HIV-positive) and the control. However, the mean levels of $CD4^+$ T-cell and plasma copper were significantly lower ($p<0.05$) in the HIV-positive subjects compared to the control; also, the mean levels of the $CD4^+$ T-cell in the ART-naïve subjects were significantly lower compared to the ART subjects. The viral load in ART-naïve subjects were significantly higher compared to the ART subjects and control. However, the mean levels of zinc and selenium were significantly lower in the HIV-positive subjects compared to the control.

Conclusion: Based on these results, it may be stated that some immunomodulatory trace elements such as zinc and selenium were deficient in HIV-positive subjects, and as such, addition of zinc and/or selenium supplements in the treatment regimen for HIV-positive subjects may be helpful in boosting their immunity and effective management.

Keywords: HIV-positive subjects; ART-naïve; HIV subjects on ART; immunomodulatory trace elements.

1. INTRODUCTION

The human immunodeficiency virus (HIV) is one of the leading infectious causes of death in the world [1]. Two types of HIV have been identified and characterized as HIV-1 and HIV-2; HIV-1 being the more virulent and infective one, happens to be the cause of the majority of HIV infections globally [2]. The human immunodeficiency viruses (HIV) are two species of lentivirus (a sub group of retrovirus) that causes HIV infection and over time AIDS [3]. The virus infects vital cells in the human immune system such as T-helper cells (specifically CD_4^+ T-cells), macrophages and dendritic cells [4].

HIV/AIDS remains a leading contributor to the burden of disease and a significant public health threat for the country with about an estimated 1.9 million persons living with HIV [5], of which Rivers state has a prevalence rate of 3.8% of the total number of people living with HIV in Nigeria making it the third highest in the country [5]. The distribution of HIV burden across age bands indicates that 12% of persons living with HIV are between the ages of 0-14 years while 75% are between 15-49 years and 13% `are 50 years and above with females having a significantly higher burden compared to men, [5].

The symptoms of HIV vary depending on the stage of infection. In the first few weeks after initial infection, people may be asymptomatic, or an influenza-like illness including fever, headache, rash or sore throat may be observed [1]. As the infection progressively weakens the immune system, they can develop other signs and symptoms such as swollen lymph nodes, weight loss, fever, diarrhea and cough, tuberculosis, cryptococcal meningitis, severe bacterial infections and cancers such as lymphomas and kaposi's sarcoma [1]. Nigeria is one of the countries in the world with the highest number of people living with HIV [6]. Thus, HIV/AIDS remains a leading contributor to the burden of disease and a significant public health threat for the country with about an estimated 1.9 million persons living with HIV [5].

Trace elements such as selenium, magnesium, iron, copper and zinc have been reported to regulate and improve immune functions [7], thereby playing a significant role in cell homeostasis [8]. They also increase survival, reduce oxidative stress, and improve birth outcomes [9]. It is unknown if the HIV-positive patients on ART have a higher level of $CD4^+$, $CD8^+$, and some of these immunomodulatory trace elements. It is also unknown if the levels of trace elements has correlation with viral load suppression. Therefore, this study was aimed at evaluating the levels of $CD4^+$ and $CD8^+$ T-cells, viral load, and some immunomodulatory trace elements in ART and ART-naïve HIV patients in Port-Harcourt, Nigeria. However, there is a dearth of information on the levels of

Immunomodulatory trace elements and viral load in HIV-infected subjects in Rivers State as Information gathered in this study will be useful in tailing regimen to obtain better immunosuppression.

2. MATERIALS AND METHODS

2.1 Experimental Design

This is a case-control and cross-sectional study carried out in Port-Harcourt, Rivers State, Nigeria. A total of 150 subjects (males and females) between the ages of 20 and 79 were recruited from the Rivers State University Teaching Hospital, and used for the study; 50 subjects were apparently healthy (those who tested negative for HIV), and were used as the control group, while the remaining 100 subjects were those who tested positive for HIV, and were used as the test group; out of this 100 subjects, 70 subjects were on anti-retroviral therapy (ART), while the remaining 30 subjects were not on anti-retroviral therapy (ART naïve). The study was carried out from April 2019 to April, 2021. Relevant information from each subject was obtained using a well-structured questionnaire.

Individuals who tested positive for HIV were included in the study as the test subject, while the control subjects are apparently healthy individuals who tested negative for HIV screening, and not on any medication for the past two weeks. Pregnant women, children less than 20 years of age, and individuals who declined consent were excluded from this study.

2.2 Blood Sample Collection

About 13 mls of venous blood was collected from the antecubital fossa of each subject using a vacutainer. 3 mls were transfered into a sample bottle containing 0.5 ml of 1.2 mg/ml dipotassium ethylene diamine tetra-acetic acid (K_2EDTA); it was mixed by several gentle inversions, and used for the estimation of $CD4^+$ and $CD8^+$ counts. Again, 5mls of the venous blood was transferred into another bottle containing 0.5ml of 1.2 mg/ml K_2EDTA; this was spun to obtain the plasma, which was used for the estimation of viral load within 24 hours after collection. Finally, 5 mls of the venous blood was transferred into lithium heparin sample bottle; it was spun to obtain the plasma, which was used for the analysis of zinc, magnesium, selenium, iron and copper.

2.3 Sample Analysis

The $CD4^+$ and $CD8^+$ counts were analyzed using a BD fluorescent activated cell sorter count (FACSC count) automation, while viral load was analyzed using real time polymerase chain reaction (RT-PCR) COBAS TaqMan 48 Analyzer. Also, copper, iron, zinc and magnesium were analyzed by colorimetric method using semi auto-analyzer WP 21E, while selenium was analyzed using atomic absorption spectrophotometer with graphite furnace technique SN-SG 710690.

2.4 Statistical Analysis

The generated data were analyzed using Graph-pad prism version 8.0.2.263, and the results were expressed as mean ± standard deviation. The results from the different parameters were compared using the one-way ANOVA, and significant differences among groups were further checked using Tukey comparison tool. Results were considered statistically significant at 95% confidence interval ($p<0.05$).

3. RESULTS

3.1 Comparison between the Mean Levels of CD^{4+} T-Cell, CD^{8+} T-Cell and Viral Load of Control, ART-Naïve HIV Subjects and HIV Subjects on ART

Details of this are shown in Table 1 below. It shows that there was no significant difference in the mean levels of CD^{8+} T-cell (897.2±379.2, 857.5±361.0 and 867.2± 366.3, $p>0.05$) between the control, HIV subjects on ART and ART-Naïve HIV-positive subjects respectively. However, there was a significant reduction in the $CD4^+$ of the HIV ART naïve and ART subjects when compared to control. The $CD4^+$ count was also significantly higher in HIV-positive subjects on ART compared to ART naïve. Similarly, the viral load was significantly higher in the ART naïve compared to those on ART ($p<0.05$).

3.2 Comparison between the Mean Levels of Iron, Magnesium, Copper, Zinc and Selenium of Control, ART-Naïve HIV Subjects and HIV Subjects on ART

Details of this are shown in Table 2 below. It shows that there was no significant difference in the mean levels of iron (6.59±2.32 µmol, 6.54±1.81 µmol, 6.76±2.74 µmol, $P>0.05$) and

magnesium (2.13±0.19 mg/dl, 2.12±0.16, 2.12±0.18, p>0.05) between the control, ART-positive and ART-naive. However, there was a significantly higher mean levels of copper in the ART-positive and ART-naïve subjects compared to the control, and a significantly lower mean levels of zinc and selenium in the ART-positive and ART-naïve subjects compared to the control.

4. DISCUSSION

Results from this study showed a statistically significant higher CD^{4+} T-cell in the control group when compared to those in the test subjects. This finding is consistent with other reports such as Nsonwu-Anyanwu et al. [10], Ekwempu et al. [11] and Enosakhare et al. [12]. The reduction in CD^{4+} T-cell in the HIV-positive subjects may be attributed to the hallmark of HIV infection and subsequently AIDS pathogenesis, which is a progressive depletion of CD^{4+} T-cell populations in close association with progressive impairment of cellular immunity and increasing susceptibility to opportunistic infection [13]. There was also a significantly higher CD^{4+} T-cell in the HIV subjects on ART compared to the ART-naïve subjects, which may be attributed to the fact that ART treatment centers on boosting the immune system as shown by the increased $CD4^{+}$ T-cell count [12].

There was a significant increase in the viral load in the HIV-positive subjects compared to the control; this reported agrees with the fact that, the viral infection would lead to its replication in the biological system of the individual. Also, a significant increase was noted in the HIV-naïve patients compared to those on ART. This may be attributed to the fact that the anti-retroviral therapy may have inhibited the replication of the human immunodeficiency virus to a reasonable level.

There was a significantly lower level of plasma zinc in HIV-positive subjects compared to control subjects. This observation is in agreement with previous reports and the reports of Anyabolu et al. [14], Nsonwu-Anyanwu et al. [10] and Enosakhare et al. [12]. This finding may be attributed to high demand in zinc, because HIV nucleocapsid and integrase proteins that are essential for assembly of infectious virions contain zinc fingers that require zinc for normal structure and functioning [15,16]. Zinc deficiency is associated with impaired immune function and an increased susceptibility to infection [17].

Similarly, there was a significantly lower level of plasma selenium in HIV-positive subjects compared to the control subjects. This study is in agreement with the reports of Fawzi, [18], Kiremidjian et al., [19] and Zhang et al., [20]. This finding may be attributed to the fact that HIV may be capable of incorporating host selenium into viral selenoproteins that have glutathione peroxidase activity [20]. As an integral component of glutathione peroxidase and thioredoxin reductase, selenium plays an important role in decreasing oxidative stress in HIV-infected cells possibly by suppressing the rate of HIV replication [19]. Therefore, decreasing plasma selenium concentration in HIV-positive subjects are sensitive markers of disease progression and severity, as low levels of plasma selenium has been associated with a significantly increased risk of death from HIV infection (Stefano et al*.,* 2010). However, this study is at variance with the reports of Nwegbu et al. [21] and Khalili et al. [22] who reported significantly increased selenium in HIV-positive subjects when compared with the control. This disparity may be due to the fact that Nwegbu and Khalili may have used HIV-positive subjects that were in their early course (low severity) of the infection for their study.

Table 1. Mean levels of CD^{4+} T-cell, CD^{8+} T-cell and viral load of control, ART-naïve HIV subjects and HIV Subjects on ART compared

Parameters	CD_4 (cells/ml)	CD_8 (cells/ml)	Viral Load (cp/ml) x 10^5
Control	1399 ± 390.4^{bc}	897.2±379.2	0.00 ± 0.00^{b}
ART-Naïve	297.5 ± 244.6^{ac}	857.5±361.0	2.93 ± 1.39^{ac}
On ART	546.9 ± 277.7^{ab}	867.2±366.3	0.33 ± 0.19^{b}
F-value	136.0	0.118	19.83
P-value	<0.0001	0.8885	<0.0001
Remark	S	NS	S

Key: a=significantly different from Control; b= significantly different from ART- Naïve; c= significantly different from On ART

Table 2. Mean levels of iron (Fe^{2+}), magnesium (Mg^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}) and selenium (Se) of control, ART-naïve HIV subjects and HIV Subjects on ART compared

Parameters	Fe^{2+} (µmol/l)	Mg^{2+} (mg/dl)	Cu^{2+} (µg/dl)	Zn^{2+} (µmol/l)	Se (µmol/l)
Control	6.59±2.32	2.13±0.19	$198.3±40.23^{bc}$	$8.19±0.47^{bc}$	$0.47±0.40^{bc}$
ART-Naïve	6.76±2.74	2.12±0.18	$285.5±85.70^{a}$	$7.89±0.69^{a}$	$0.006±0.004^{a}$
On ART	6.54±1.81	2.12±0.16	$258.5±65.68^{ab}$	$7.79±0.70^{a}$	$0.058±0.07^{ab}$
F-value	0.120	0.118	17.29	7.748	38.37
P-value	0.8865	0.8881	<0.0001	0.0006	<0.0001
Remark	NS	NS	S	S	S

Key: a=significantly different from Control; b= significantly different from ART- Naïve; c= significantly different from On ART

Furthermore, there was a statistically significant increase in plasma copper in HIV-positive subjects compared to the control subjects. This observation is in agreement with Bogden et al. [23], Ahmad et al. [24], Ciftci et al. [25], and Koyanagi et al. [26]. This may reflect a non-specific increase in serum concentration of copper binding protein ceruloplasmin [27]. High levels of copper in HIV-positive subjects suggest its possible role as a useful marker of HIV activity and progression to AIDS [28]. However, this study is at variance with that of Enosakhare et al. [12] and Amare et al. [29] which reported no significant difference in plasma copper between the HIV-positive subjects with the control subjects.

There was no statistically significance difference in plasma magnesium and iron, and CD^{8+} T-cell in the HIV-positive subjects compared to the control subjects. This may be that the patients were already on magnesium supplements alongside maintaining a healthy diet in foods rich in magnesium. However, this study is at variance with the reports of Okwara et al., (2012), which stated a reduced plasma magnesium levels in the HIV-positive subjects compared to the control subjects; their report may be attributed to the fact that in the study, the HIV-positive subjects were grouped according to the stages of the infection. Also, the non-significant difference noted in plasma iron level agrees with the report of Drain et al. [30], and this may be attributed to the fact that HIV infection alone resulted in an increased cellular iron irrespective of ART treatment [31].

5. CONCLUSION

The deficiency of some immunomodulatory trace elements such as zinc and selenium associated with HIV-positive subjects was noted from this study. Although ART has remarkably improved the survival of HIV-positive subjects by improving the CD^{4+} T-cell count, zinc/selenium status of the HIV-positive subjects on ART treatment was comparable with ART naïve HIV-positive subjects. This showed that the ART treatment did not complement these micronutrients hence the need for supplementation therapy of these micronutrients since they have the potential to boost the immune system in more than one way. These micronutrients could yet have a role in slowing the disease progression by reducing the incidence of opportunistic infections and HIV mortality. Thus Zinc/selenium supplementation alone or in combination with other micronutrients can be used to give a boost to ART, they can also be a part of nutritional program in HIV-positive subjects and this may have a particularly useful application in HIV patients living in countries with poor economic resource.

CONSENT

Informed consent was gotten from persons recruited in this study.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the Rivers State Ministry of Health and the Rivers State University Teaching Hospital (RSUTH) with a file no of MH/PRS/391/VOL.2/636 and RSUTH/REC/2020033.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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