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Levels of some micronutrients in adult HIV-positive individuals receiving antiretroviral therapy in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria

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Abstract
Micronutrients are essential for immune function. In people living with Human Immune Virus (HIV), the virus affects their immune system, metabolism and nutritional requirements. In resource poor settings and among the food insecure, this challenge could be exacerbated. With increased access to Highly Active Antiretroviral Therapy (HAART) and improvements in immune well-being of these patients living with HIV, it is important to assess micronutrient status in this population, who are affected by food insecurity. Aim of this study was to assess some micronutrients and cytokines in adult HIV positive individuals. This cross-sectional study was conducted at HIV clinic Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State. Using simple random sampling technique, a total of 83 adults were recruited for the study. Forty-two HIV infected individuals on HAART and forty-one age and sex matched controls were recruited for the study. All subjects were between the age range of 18-49 years. CD_4^+ counts were assessed by Flow Cytometric method. The micronutrients copper, iron and zinc were assessed by AAS. Demographic data were obtained using questionnaires. Data were analyzed using SPSS version 26. Our findings show that micro-nutrients copper and iron were significantly lower in HIV infected subjects compared to control participants (P<0.001). Zinc was also significantly lower in the HIV positive group when compared to the control participants (P=0.038). HAART clearly has a positive impact on the immune well-being and stability of these infected individuals in this population. However, all micronutrients studied revealed disparity in the findings in both groups suggesting that despite HAART, micronutrients are depleted in HIV infection. In conclusion, HIV infection places a burden on the nutritional needs of people living with the disease. This is most likely exacerbated in resource poor settings with associated food insecurity. Monitoring micro-nutrients and possibly initiating micro-nutrient supplementation could be a useful adjunct therapy to HAART to ensure better health and well-being of this population living with HIV.

Keywords: Micronutrients, immune, HIV and AIDS, antiretroviral therapy and nutrition

Introduction

HIV, the virus that causes Acquired Immune Deficiency Syndrome (AIDS), has become one of the world's most serious health and development challenges since the first cases were reported in 1981. Approximately 84 million people have become infected with HIV since the start of the epidemic UNAIDS, (2022). Today, there are approximately 38 million people currently living with HIV, and tens of millions of people have died of AIDS-related causes since the beginning of the epidemic. (UNAIDS, 2022).

The virus is transmitted through certain body fluids and weakens the immune system by destroying cells that fight disease and infection, specifically CD_4^+ T cells. Left untreated, HIV reduces the number of CD_4^+ cells in the body, making it more difficult for the immune system to fight off infections and other diseases. HIV can lead to the development of AIDS, "acquired immunodeficiency syndrome." (UNAIDS, 2022).

Over the past two decades in particular, major global efforts have been mounted to address the epidemic, and significant progress has been made. The number of people newly infected with HIV, especially children, and the number of AIDS-related deaths have declined over the years, and the number of people with HIV receiving treatment increased to 28.7 million in 2021. (UNAIDS, 2022).

Although there have been significant declines in new infections since the mid-1990s, there were still about 1.5 million new infections in 2021, or about 4,000 new infections per day. Recent data show that progress has slowed and is unequal within and between countries. UNAIDS, (2022) Furthermore, the pace of decline varies by age group, sex, race, and region.

HIV remains a leading cause of death worldwide and the leading cause of death globally among women of reproductive age (UNAIDS, 2019).

Micronutrient deficiencies result from insufficient intake and absorption of vitamins and minerals to maintain good health and growth (Bouis *et al.,* 2017) [6]. Malnutrition and poor micronutrient levels especially in PLHIV are associated with an increased risk of progression to AIDS (Thimmapuram *et al.,* 2019) [19]. HIV/AIDS affects nutritional status by increasing energy requirements, reducing food intake, and adversely affecting nutrient absorption and metabolism (Alebel *et al.,* 2022) [3]. Malnutrition and food insecurity are associated with increased mortality and poor clinical outcomes among people living with HIV/AIDS (Benzekri *et al.,* 2015) [5]. HIV infection and malnutrition are part of a vicious cycle that contributes to immunodeficiency and negative health outcomes (Abba *et al.,* 2021) [1]. Malnutrition increases the risk of HIV pathogenesis, while HIV in turn activates malnutrition by reducing nutrient intake, absorption, and metabolism, which negatively affects the immune system. This process between malnutrition, the immune system, and HIV infection prompts dysfunctions within the immune system, makes the host to be more vulnerable to infection, and increases the severity of malnutrition (Abba *et al.,* 2021) [1]. Malnutrition may lead to reduced immunity and increased susceptibility to opportunistic infections, which can lead to further malnutrition. (Olson, 2021) [18].

For guaranteeing that micronutrient needs are met, the World Health Organization (WHO) advocates increasing access to a diversified diet, food fortification, and micronutrient supplementation, predominantly in areas where micronutrient deficiencies are endemic (Van *et al.*, 2019). The WHO and the United Nations Food and Agriculture Organization (FAO) have adopted four main strategies for improving dietary intake: food fortification, micronutrient supplementation, nutrition education, and disease control measures. The fortification of staple foods is key in improving dietary diversity and effectively decreasing micronutrient deficiencies (Mchiza *et al.*, 2015) [14].

Zinc deficiency may be as a result of malnutrition, one of the factors responsible for malnutrition in an HIV-positive subject is reduced appetite, opportunistic infections, fever, side effects of medications used to treat HIV, or depression. About 30%-50% of HIV-Positive subjects in developed countries and nearly 90% in developing countries complain of diarrhea and malabsorption (Duggal *et al.*, 2012) [7].

Finally, even minor deficiency of zinc might have an effect on immunity. In both the adaptive and innate immune systems, zinc is required for cell maintenance and development. Zinc deficiency impairs lymphocyte production, activation, and maturation, disrupts cytokine-mediated intercellular communication, and reduces innate host defence (Gammoh and Rink, 2017) [10].

Copper plays a major role in chemical reactions for energy production and prevention of oxidative stress in the cell, since enzymes (cytochrome c oxidase, copper-zinc superoxide dismutase) involved in electron transfer during the mitochondrial transport chain and in catalytic free radical reactions are copper-containing (Kardos *et al.*, 2018) [13].

Iron deficiency is the most common micronutrient deficiency worldwide, resulting in poor health outcomes, impaired development and increased susceptibility to infectious diseases and often co-existing with infection, including HIV (WHO, 2017).

Materials and Methods

Study location

This study was carried out at Nnamdi Azikiwe University Teaching Hospital located in Nnewi, Anambra State.

Study Design

This is a cross-sectional study designed to assess some micro-nutrient in adult HIV positive participants in Nnewi. A total of 83 adults were recruited for the study using purposive sampling technique which comprised 42 individuals as test group and 41 subjects as control. Information on socio-demographic, medical history and lifestyle was obtained using a questionnaire. All participants were between the ages of 18-49 years.

Subjects

Participants were selected for this study based on the following criteria; HIV individuals who are between the age range of 18-49 years and are on anti-retroviral therapy, inclusion, exclusion.

Inclusion Criteria

Confirmed adult HIV-positive participants who are on anti-retroviral therapy and age and sex-matched healthy control subjects. Age range: 18-49 years.

Exclusion

Transfused subjects and subjects with other co-morbidities were excluded from the study.

Ethical considerations

The ethical approval for this research was obtained from the Ethical committee Nnamdi Azikiwe University Teaching Hospital NAUTH, Nnewi and informed consent was also sought and obtained from the participants.

Sample Collection

Ten milliliters of whole blood was collected from the subjects using standard venipuncture technique. 5mls was dispensed into a plain container and centrifuged after clotting at 5000rpm for 5 minutes to obtain serum for assay of copper, zinc iron. Another 5mls was dispensed in an appropriate EDTA container for CD_4^+ T cells count. The labelled samples were frozen at -20^{0}C until analyses.

Principles and Methods

AAS

In the flame AAS, the principle is based on the dissociation of the element from its chemical bonds. This is then placed in an unexcited or ground state (neutral atom). Thus, the neutral atom is at a low energy level in which it is capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectrum. The amount of radiant energy absorbed at a characteristic wavelength in the flame is proportional to the concentration of the element present in the sample.

Serum sample in tube was digested using aqua ragia (1HNO3:3HCl) in a conical flask and allowed to cool. It was then filtered and made up to 50ml using deionized water.

The (Buck Scientific 205) Atomic Absorption Spectrophotometer was used for this analysis. Standards of the different elements were prepared and used to calibrate the machine just before analysis. Wave length and other parameters were set to optimize the performance of the machine as standard protocols.

The clear supernatant was aspirated into the flame atomic absorption spectrometer (AAS) after adjusting the wavelength at 213-nm.

After Analysis the concentration in ppm of the various element were subjected to statistical analysis.

Determination of CD_4^+ T cell count, principles and methods

Flow cytometer was used to determine CD_4^+ T-cells count. In the cyflow counter, the fluorescence monoclonal antibody (CD4mAbPE) binds to the CD_4-antigen on the monoclonal cells (T. lymphocytes and monocytes) and in buffer suspension. The complex is passed through the flow cuvette in a single steam of flow. The complex is executed by the solid state laser. High (Green laser at a wavelength of 522nm causing the complex to emit light which is captured by a photomultiplier tube and transmitted into digital read out as counts).

The basic principle of flow cytometer is the passage of cells in single file in front of a laser so they can be detected, counted and sorted. Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths.

Statistical Analysis

The Statistical Package for Social Science (SPSS Version 26, Chicago III-USA) was used for data analysis and the values expressed as mean and standard deviation.

The result of the test groups were compared within groups using ANOVA and between groups using student t-test at 95% level of confidence. Pearson's correlation: was used to assess for correlation and P value was set at ≤0.05 significance.

Results

Table 1: Demographic Characteristics of the Study Population

Variable	HIV Positive n = 42	HIV Negative n = 41	P-value
Age range (years) Mean Age (+ S.D.)	18-45 32 ± 6.3	18-49 34 ± 7.3	0.185
Sex			
Males	20 (47.6%)	20(48.8%)	0.909
Females	22 (52.4%)	21 (51.2%)	
Socio-economic status			
Low	30 (71.4%)	30 (73.2%)	0.946
Medium-high	12 (28.6%)	11 (26.8%)	

Table 1 shows the demographic characteristics of the study population. There are no significant differences in the mean age, sex and social economic status between the test group and the control group, P > 0.05.

Table 2: Levels of CD_4 count in low and medium-high income groups

Economic status	HIV positive Low (n=30)	HIV positive Medium-high (n=12)	p-value
CD_4^+ Range	400-550	400-600	
CD_4^+ mean ± S.D	464 ± 44.7	508 ± 61.5	0.200
Sex			
Males	14 (46.7%)	6 (50%)	0.883
Females	16 (53.3)	6 (50%)	

P-value for the mean difference is 0.200, this means that the mean value of CD_4^+in low income group did not differ significantly when compared to the medium-high income group.

The proportion of observations in different columns of the contingency table do not vary from row to row. The two characteristics that define the contingency table are not significantly related (P=0.883).

Table 3: Levels of Micronutrients in HIV sero-positive and HIV sero-negative control groups.

Micronutrients	Control	HIV positive	t-test	p-value
Zinc(mg/L)	0.63±0.22	0.50±0.33	2.107	0.038
Copper(mg/L)	0.85±0.58	0.18±0.37	6.143.	0.000
Iron(mg/L)	1.09±0.22	0.46±0.31	10.808	0.000

The mean levels of Zinc, Cu, Fe were significantly higher in control when compared to the HIV positive (p<0.05)

There were significant differences in the levels of the micronutrients assessed when compared in the HIV positive group and the control group as shown in Table 3.

For Zinc, the mean level was 0.50±0.33mg/l in the HIV positive group compared to 0.63±0.22mg/l (p=0.038) showing a significant difference.

The difference in the levels of copper and Iron were also markedly significantly different when compared in both groups with mean Copper levels in the HIV positive at 0.18±0.37mg/l compared to 0.85±0.58 mg/l (P=0.000). Similarly, the mean Iron levels in the HIV positive group was 0.46±0.31mg/l while in control group mean levels was 1.09±0.22 mg/l (p=0.000).

Discussion

This study revealed that HIV-positive subjects had significantly (P<0.05) lower mean ± SD values of Zinc when compared with control subjects (Table 3). This observation is in agreement with previous reports (Nsonwu-Anyanwu *et al.*, 2017 [17]; Enosakhare *et. al.*, 2018) [8]. This result is consistent with the study by Asemola *et al.*, (2018) which found that 100 HIV patient had zinc deficiency. However, this study is at variance with the report (Ndagije *et al.*, 2007) [16] who reported no significant difference in the zinc status of HIV-positive subjects and control.

The present study observed a significantly lower levels of copper among the HIV-Positive subjects when compared with negative control subjects (Table 3).

These findings are in agreement with previous report (Kassu *et. al.*, 2006 [12]; Akinola *et. al.*, 2012) [2] who observed significantly lower copper levels in HIV positive individual when compared with negative controls.

However, this study is variance with previous reports (Enosakhare *et. al.*, 2018) [8] who reported no significant difference between the HIV-positive subjects and Negative control subjects (p>0.05)

The present study demonstrated that HIV-infected participants had significantly lower iron levels when compared with the negative control participants (Table 3). This work is in variance with the works of Hsiang-chun *et al.*, (2015) [11] which stated that serum iron increase in HIV subjects can persists even in those on ART.

Conclusion

In conclusion, findings from the study have revealed that the studied trace elements copper, iron and zinc were decreased in the study population. It is therefore recommended that micronutrient supplementation may be beneficial among this population living with HIV.

Recommendations

It is thus recommended that these micro nutrients should be monitored in the treatment of HIV. Follow up study should be carried to assess long-term effect of HAART on these micro nutrients.

Nutrition Counselling and Education should be offered to encourage the development of appropriate nutrition habits and assist PLHIV in dealing with nutrition-related issues.

We therefore recommend that that HIV- infected individuals should be assessed intermittently for micronutrients deficiency to reduce the morbidity and mortality associated with HIV infection.

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