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Original Article

Zinc, copper, CD4 T-cell count and some hematological parameters of HIV-infected subjects in Southern Nigeria

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ABSTRACT

Background: Low concentration of trace elements has been associated with poor prognosis and mortality in HIV infection.
Methods: A cross sectional study was conducted among 100 HIV-infected subjects (70 were on ART treatment, while 30 were ART naïve). Fifty (50) apparently healthy controls were enrolled. Concentration of serum levels of zinc and copper was done using atomic absorption spectrometric method, while complete blood count was determined using automated blood analyzer. $CD4^+$ T-cell count was done using cyflow cytometer.
Aim and setting: The aim of this study was to investigate the level of some trace elements and some hematological parameters of HIV-seropositive subjects attending University of Calabar Teaching Hospital Clinic as well as prevalence of trace elements deficiency and anemic status and compare same with HIV-seronegative control.
Results: Mean serum zinc, $CD4^+$ T-cell count, Hb, PCV, RBC, MXD, were significantly ($p < 0.05$) reduced in the HIV-infected subjects, while copper/zinc ratio, MCV, MCH and platelet count were significantly ($p < 0.05$) raised in the HIV-infected subjects. The serum Cu level was comparable ($p > 0.05$) with the control. ART treatment had no effect on all the parameters assessed except $CD4^+$ T-cell count. Twenty five percent (25%), 3% and 56% of the HIV-infected subjects were zinc deficient, copper deficient and anemic, respectively. Gender was found as a predictor of zinc deficiency. Copper and zinc showed weak positive correlation with $CD4^+$ T-cell count.
Conclusion: ART treatment did not complement zinc status in HIV infection while improving $CD4^+$ T-cell count, hence the need to consider supplementation.

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1. Introduction

Trace elements are micronutrients that are essential for normal body metabolism.[1] They are required in minute amount by living organisms. They are essential for the host defense against infection[2] and act as activators in controlling biological functions.[3] Changes in the levels of micronutrients and its effects have been described in inflammatory responses, cancer cases, as well as parasitic and viral infections.[4–6] The human immune function has been reported to depend on nutritional status.[7,8] Serum trace elements such as copper and zinc have been reported to be useful in the diagnosis of viral hepatic disease.[9]

Zinc is an integral part of more than 200 enzymes (metalloenzymes) and play a crucial role in nucleic acid metabolisms, cell replication, tissue repairs, and growth. On one hand, its deficiency leads to severe alteration of the thymic function and subsequent loss of T-cell-mediated responses and increased susceptibility to infectious diseases.[10,11] On the other hand, copper functions as a scavenger of free radicals in biological membranes and structures via its presence in cytosolic erythrocyte superoxide dismutases.[12]

HIV/AIDS, despite the campaigns, has remained a public health concern in Sub-Saharan Africa, where an estimated 25.8 million adults and children are infected.[13] HIV patients have a large variety of physiological alterations at every level of the disease. These complications in synergy with related pathologies give rise to different nutritional problems.[14]

The present study sets off to investigate the usefulness of copper, zinc, copper–zinc relationship and some hematological parameters in managing HIV/AIDS patients.

2. Methods

2.1. Study design and subjects

In this cross-sectional study, a total of 150 subjects were enrolled. Seventy (70) of these were HIV-infected subjects on anti-retroviral therapy (ART), while 30 were HIV ART naïve subjects attending the HIV clinic, University of Calabar Teaching Hospital. Fifty (50) apparently healthy HIV seronegative individuals were recruited as controls. All subjects were residents in Calabar, Cross River State, Southern Nigeria. All subjects were between the age range of 18–65 years. Ethical approval was obtained from the University of Calabar Teaching Hospital Medical Ethics Committee. Informed consent was obtained from the subjects prior to the study.

2.2. Blood collection

Blood samples were collected from the patients after overnight fasting via the antecubital vein and separated into EDTA and plain containers for complete blood count/CD4$^+$T cell count and micronutrient assay respectively. The blood samples in plain container were centrifuged at $3500 \times g$ for 10 minutes to obtain serum. The complete blood count and CD4$^+$ T-cell count were analyzed immediately while the sera for copper and zinc analysis were frozen at -20°C and transported under cold chain to international Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria for analysis.

2.3. Complete blood count and CD4$^+$ T-cell assay

The complete blood count was performed using Sysmex KX-21N by Sysmex Corporation Kobe, Japan. The analysis was done following the manufacturers instruction. CD4$^+$ T-cell count was analyzed using Partex cyflow cytometer by Partec Cyflow, Germany. After booting the machine, 20 mL of CD4$^+$ T-cell count PEmAb was added to a Rohren tube followed by 20 μL of well-mixed EDTA blood sample. Both were mixed and incubated in the dark for 15 minutes at room temperature. This was followed by addition of 800 μL of the CD4$^+$ T-cell count buffer. The mixtures were mixed and read on the cyflow by plugging the sample tube to the sample port of the cyflow.

2.4. Determination of trace elements

The trace elements were assayed using atomic absorption spectrophotometric method using atomic absorption spectrophotometer by Buck Scientific (Model 205), USA. One thousand microliter of the serum were pipetted into the test tubes followed by equal volume of 10% trichloroacetic acid (TCA). Paraffin was used to cover the top of the tubes which after mixing were left to stand for 10 minutes. The tubes were than centrifuged. The supernatants were then transferred to another tube using Pasteur pipette where they were then aspirated into the spectrophotometer. Wave length of 324.8 nm and 213.9 nm were used for copper and zinc estimations, respectively.[15] All other settings were operated under conditions recommended by the manufacturers.

2.5. Statistical analysis

Data were analyzed using SPSS version 22 Package (SPCC Inc., Chicago, IL, USA). Categorical data were summarized into frequency and proportions while continuous data were expressed as means and standard deviations. One sample Kolmogorov–Smirnov test was used to assess the normality of the data. The complete blood count and trace element values were normally distributed in both the test and control subjects, hence parametric procedure. Comparison of the hematological parameters and serum trace elements between the test and control were performed using independent *t*-test while comparison among the various age ranges were performed using one-way ANOVA. Logistic regression was used to determine factors associated with copper and zinc deficiencies. *p*-values less than 0.05 ($p < 0.05$) were considered statistically significant.

3. Result

The mean age of the HIV-infected subjects was 35.97 ± 10.32 years, while that of the control was 29.84 ± 7.38 years. Thirty five percent (35%) of the HIV-infected subjects were males while the females constituted 65%. Of the 100 HIV-infected subjects analyzed for zinc, copper, and hematological