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Original Research Paper

To determine the correlation of zinc, copper, ceruloplasmin, and metallothionein in HIV-infected subjects: A Case Control Analysis

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ABSTRACT:

Background: The aim of this study is to test the role of zinc, copper and their respective transporters in HIV-infected individuals and to compare and correlate the association of zinc, copper, ceruloplasmin and metallothionein between each variable respectively in healthy individuals and HIV-infected individuals. Methodology: The human ethics committee approved the study protocol. Since there is no computerized data, collecting it manually is cumbersome, so the sample size is 100 HIV-infected people. The control group has 100 healthy people. Serum zinc, copper, ceruloplasmin, and metallothionein were estimate. Results: We have shown the increased mean values of copper, ceruloplasmin, and metallothionein levels in the HIV-infected subjects than the non-HIV healthy controls. In addition, we observed significant statistical difference in the levels of serum copper, ceruloplasmin, and metallothionein when compared between the two groups of the study. On the other hand, we observed lower levels of serum zinc levels in HIV-infected subjects along with statistical difference in the mean levels of serum zinc level when compared between the two groups of the study. Conclusion: The results of this research indicate that an imbalance in the absorption of zinc and copper in HIV patients is the root cause of the changes that were observed in the study parameters. This imbalance is due to the lower levels of zinc transporters that are found in HIV patients in comparison to healthy controls.

Keywords: HIV; Ceruloplasmin; Metallothionein; Zinc; Copper; CD4

INTRODUCTION:

Since the beginning of the epidemic, 79.3 million [55.9–110 million] people have been infected with the Human Immunodeficiency Virus (HIV) and 36.3 million [27.2–47.8 million] people have died of HIV [1,2]. Globally, 37.7 million [30.2–45.1 million] people were living with HIV at the end of 2018 [1,2]. With nearly 27% of its population HIV-positive, Eswatini has the highest HIV prevalence of any country in the world [1,2]. On the more fortunate end of the spectrum, Samoa is reported as having the smallest infected population (reporting only 12 cases), with Saudi Arabia and Afghanistan being reported to have the lowest prevalence of the disease, among reported nations, at approximately 0.01% of their populations, respectively [2]. HIV can disrupt trace element homeostasis, causing immunosuppression [3-5]. Trace element status may affect insulin resistance and secondary complications. Circulating monocytes show an apoptotic resistance phenotype during HIV viremia, along with altered ceruloplasmin and metallothionein expressions [6-9]. Ceruloplasmin affects copper metabolism and immune function [3]. Metallothioneins aid in zinc metabolism and immune function [10,11]. During acute viremia following interruption of suppressive ART, ceruloplasmin and metallothionein expression is altered [12,13]. According to studies [14-17] a mineral-rich diet and mineral supplementation could help with HIV-related zinc and copper levels. Copper and zinc are inversely related. In HIV infection, elevated ceruloplasmin levels increase copper absorption and decrease zinc levels. In recent years, researchers have become more interested in zinc, copper, and their

transporters. Trace metals are essential for humans, and their normal concentrations affect metabolic processes. Trace metals deficiency is linked to diabetes, anemia, depression, aging, low sexual potency, heart disease, HIV, and cancer. Zinc and copper bioavailability in HIV patients is interesting. Literature shows that during immunocompromised states, HIV-affected people lose zinc and copper. Frequent urination, insulin resistance, absorption, and inadequate intake can cause this loss. The aim of this study is to test the role of zinc, copper and their respective transporters in HIV-infected individuals and to compare and correlate the association of zinc, copper, ceruloplasmin and metallothionein between each variable respectively in healthy individuals and HIV-infected individuals.

METHODOLOGY:

The study protocol was approved by the institutional Human ethics committee. Since there is no computerized data available; it is a cumbersome task to collect the data manually, therefore, the sample size has been confined to 100 HIV-infected individuals. 100 healthy control individuals were included in the control group. The HIV subjects in this study were positive for HIV in serum (positivity was determined by ELISA using two different antigens) and were not under antiretroviral treatment. The HIV RNA load was not able to execute due to a lack of facilities at our institution. HIV-positive individuals were screened in accordance with the guidelines established by the National AIDS Control Organization and recruited from an antiretroviral facility or ICTC located within the college. Exclusion criteria were type 1 or type 2 diabetes mellitus, HIV infection, pathological complications, and HAART treatment. The control group consisted of 100 individuals were otherwise healthy and free of HIV infection. Healthy controls (non-HIV group) must be on a normal diet, non-diabetic, not taking supplements, free of other health complications, non-smokers, and non-drinkers. HIV subjects must not be on HAART. After informed written consent from all study group subjects, 5ml venous blood was drawn into plane vials (red top). Blood will be centrifuged at 3000 rpm for 20 minutes to separate serum, which will be frozen until assayed. Serum zinc and copper estimated by Zasoski et al (1977). The atoms which when passed through the flame, get excited and absorb the specific wavelength confined to them and the concentration is determined. Glassware was soaked in nitric acid for 24 hours, rinsed for five times in milli-Q water, dried and used. Copper and zinc standards: From the copper and zinc stock solutions (1000ppm), the calibration curve concentrations (50, 100, 150, 200, 250 µg/dL) were freshly prepared by serial dilution. The absorbencies of the samples were estimated by atomic absorption spectrophotometer. The absorbencies of the samples were compared with known reference standards. Serum Ceruloplasmin was estimated by using the Elisa Assay method purchased from Eagle Biosciences laboratories. Serum metallothionein were estimated with ELISA kit obtained from LSBiotech Research laboratories.

Statistical Analysis:

Statisticians used the latest version of IBM SPSS. When comparing two groups' variable means, use the unpaired t-test. The authors used one-way analysis of variance to compare group medians (ANOVA). Pearson correlation determined the relationship between two variables. 0.05 is significant.

RESULTS:

Zinc, copper, ceruloplasmin, and metallothionein details of the present study population (Fig. 1):

We have shown the increased mean values of copper, ceruloplasmin, and metallothionein levels in the HIV-infected subjects than the non-HIV healthy controls. In addition, we observed significant statistical difference in the levels of serum copper, ceruloplasmin, and metallothionein when compared between the two groups of the study. On the other hand, we observed lower levels of serum zinc levels in HIV-infected subjects along with statistical difference in the mean levels of serum zinc level when compared between the two groups of the study.

Significant Correlation between the parameters of the present study (Figs. 2 & 3):

The present study observed a stable incline, with a positive regression of $y = 0.1852x + 58.71$ when compared between serum ceruloplasmin and serum copper levels in HIV-infected subjects. On the other hand, the present study observed a stable decline, with a negative regression of $y = -0.2508x + 72.08$ when compared between serum ceruloplasmin and serum zinc levels in non-HIV healthy control subjects.

Study parameters in different stages of HIV-infection:

The study observed no statistical significance when compared between the sub-group subjects with regards to the variables of the present study.

DISCUSSION:

In the current study, researchers found that subjects who did not have HIV had lower levels of zinc than individuals who had HIV. On the other hand, our research showed that HIV-positive patients had a higher plasma concentration of copper and metallothionein than HIV-negative patients did. This increase was consistent with the findings of other subjects that showed it in HIV-positive patients, as shown by the concentration of ceruloplasmin. Studies [17-19] found that there were lower levels of zinc when compared to control subjects. This finding is consistent with the findings of the current study. Silva et al., 2019 [20] found that there were higher levels of metallothionein in HIV patients compared to subjects who did not have HIV. The atrophy of the thymus, impaired cell-mediated cutaneous sensitivity, and lymphopenia, along with altered production of tumor necrosis factor, all of which have been implicated in the pathophysiology of cachexia and wasting in acquiring immune deficiency syndrome are all results of zinc in in-vitro experiments with human T-cell lines. Schreck et al., 1992 [21], demonstrated that the addition of antioxidant compounds prevented activation of nuclear factor kB and inhibited HIV replication. Zinc exploitation by the HIV-1 virus for gene expression, multimerization, and interaction portray HIV-1 as a zinc reliant virus, which at least in part explained the dwindling plasma zinc levels frequently observed in HIV-1 infected patients [22]. According to John et al., 2010 [23], we state that a lack of zinc in HIV-1 infection may compromise the production of Zn-dependent molecules, which in turn may affect the immunogenic response toward the damage in the early phase of the disease. In HIV infection and inflammatory states, serum copper is higher compared to normal individuals [24.25]. This mounting has been attributed toward the elicited hepatic synthesis and release of ceruloplasmin [26]. We hypothesize that copper needs an ATP transporter for ingress into the cell. In HIV infection ATPs that are produced in the body will also be involved in the central dogma of cell for the assembly of new viral proteins, hence ATP adequacy decreases, and it may hamper the intake of copper into the cell via ceruloplasmin leading to heightened copper in blood with concomitant hampered absorption of zinc [27,28]. In addition, the present study also observed a positive correlation between copper levels and ceruloplasmin in HIV-infected subjects and on the contrary negative correlation zinc with ceruloplasmin in non-HIV healthy control subjects. No study has similar correlation like the present study observed and this is the first study to acknowledge this novelty. We infer that as the concentration of copper levels increased in HIV-infected subjects because of copper levels induced the synthesis of metallothionein and ceruloplasmin levels. This compensatory increase nullifies the altered concentrations of zinc and copper levels in the study subjects.

CONCLUSION:

The results of this research indicate that an imbalance in the absorption of zinc and copper in HIV patients is the root cause of the changes that were observed in the study parameters. This imbalance is due to the lower levels of zinc transporters that are found in HIV patients in comparison to healthy controls. In addition, this research discovered a connection between HIV and the presence of ceruloplasmin and metallothionein in the body. Using biomarkers from this study, such as ceruloplasmin and metallothionein, HIV patients may be able to identify and diagnose secondary complications.

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Figure 1: Mean values of different variables HIV-infected subjects and control subjects of the study

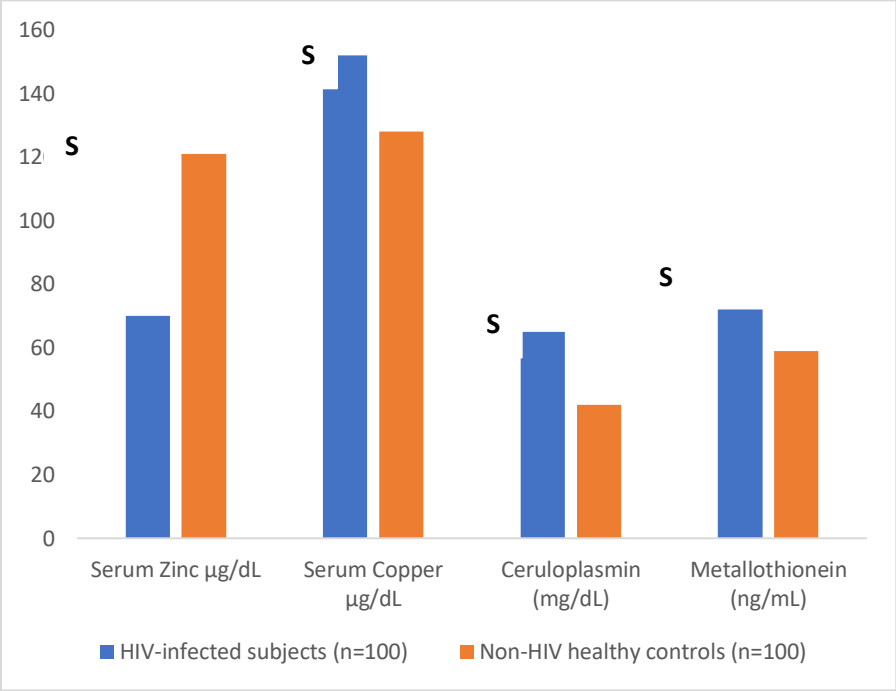

Figure 2: Scatter diagram showing relationship between copper and ceruloplasmin levels in HIV-infected subjects

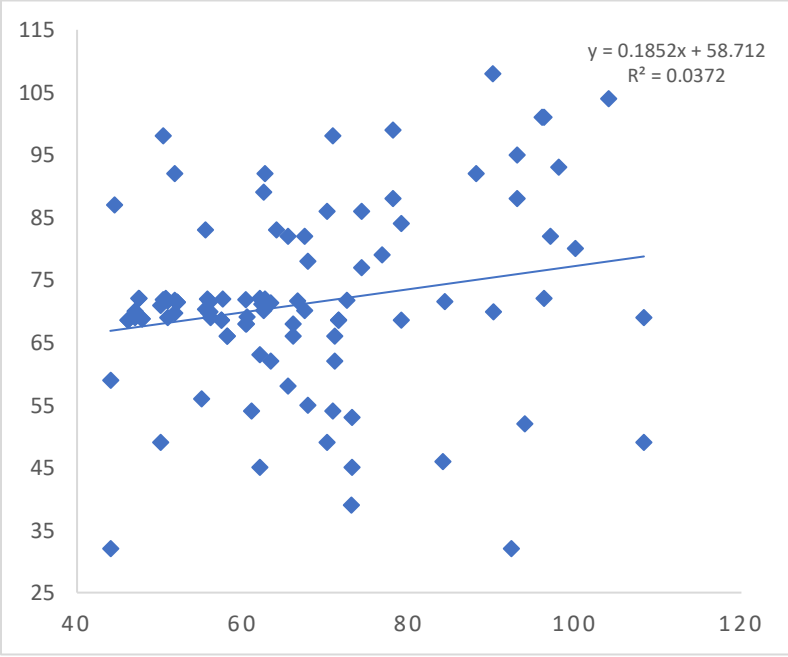

Figure 3: Scatter diagram showing relationship between zinc and ceruloplasmin in Non-HIV healthy control subjects