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Research Article

Serum Hepcidin levels and Iron indices in HIV Infected Patients in a Nigerian Community

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ABSTRACT

Human immunodeficiency virus (HIV) infection is a pandemic disease that could affect and alter many cellular and systemic components of the host. Anemia has been shown to be a common complication of HIV infection. The study assessed the impact of HIV infection on serum levels of hepcidin and iron indices since hepcidin is an iron regulatory protein. A total of ninety subjects were investigated which consisted of fifty subjects living with HIV and forty apparently healthy subjects as control. Twenty-nine of the HIV subjects were on antiretroviral therapy (ART); emtricitabine, zidovudine, tenofovir and lamivudine in different combinations. Hepcidin was estimated using enzyme-linked immunosorbent assay (ELISA) while serum iron, total iron-binding capacity (TIBC) and unsaturated iron-binding capacity (UIBC) were estimated using spectrophotometric method. The result showed a significant increase ($p<0.05$) in unsaturated iron-binding capacity (UIBC) of HIV subjects compared with control. Although there was no significant negative impact of HIV infection on hepcidin and the iron indices, the study concluded that the parameters should be measured to monitoring the progress of management in order to prevent anemia which may occur during HIV infection.

Keywords: *HIV, hepcidin, iron, ART*

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INTRODUCTION

Human immuno deficiency virus (HIV) infection is a pandemic disease that could affect and alter many cellular and systemic components of the host. HIV virus targets and alters the immune system thereby increasing the risk to develop other infections and diseases (Adam, 2018). It was reported that more than 35 million people worldwide were affected with HIV infection and Sub-Saharan Africa was the most affected in a report released in 2014 (Bekolo, 2014). Over the decades, the virus had spread through Africa to other parts of the world (Alex *et al.*, 2019). Furthermore, the number continued to increase and it was estimated that more than 37 million people globally were living with HIV as at 2020 [The Joint United Nations Programme on HIV/AIDS (*UNAIDS*), 2021a]. However, only a little more than 27 million people living with HIV were accessing antiretroviral therapy (ART) globally (UNAIDS, 2021b) and majority of the people living with HIV reside in developing countries. Although scientists are still working to get a cure for HIV infection; the increasing access to effective HIV prevention, diagnosis, treatment and management has made HIV infection become one of the manageable health conditions enabling those affected to live healthy lives (WHO, 2021).

Iron deficiency is a common cause of anemia in the developing world. Anemia has been shown to be common in HIV-infected and Acquired Immune Deficiency Syndrome (AIDS) patients until the introduction of antiretroviral therapy (ART) (Hsiang-Chun, 2015). While anemia has been shown to be a common complication of HIV infection in the work of Wisaksana *et al.* (2011), another finding associated HIV infection with positive iron balance among subjects (Patrick *et al.*, 2016). Hepcidin, a 25-amino acid peptide exclusively synthesized by the liver, was initially identified as part of a search for novel antimicrobial peptides (Park, 2001). Human hepcidin is a mediator of innate immunity and iron-regulatory hormone and its synthesis is greatly stimulated by inflammation or iron overload (Tomas, 2019). The blood level of Hepcidin could be regulated by several factors, such as the blood concentration of iron, anemia, hypoxia and inflammation (Donovan, 2005; Cunha *et al.*, 2015). Hepcidin exerts its functions by blocking iron flows into plasma, duodenal absorption, as well as release from macrophages and mobilization of stored iron from hepatocytes (Ganz, 2011). Thus, it prevents the export of iron from enterocytes, macrophages, and hepatocytes thereby resulting into the

reduction of available systemic iron. Hepcidin levels could be suppressed by factors such as erythropoiesis which is stimulated during acute blood loss (Pasricha *et al*., 2016) and chronic endurance exercise in healthy, non-anemic, iron-sufficient men (Moretti *et al*., 2018). Therefore, the study set to determine the impact of HIV infection on the serum levels of hepcidin and iron indices.

MATERIALS AND METHODS

Subjects: Ninety subjects between the ages of 30-60 years were investigated which consisted of fifty subjects living with HIV and forty apparently healthy individuals served as control. Ethical approval was sought for and obtained from Federal Teaching Hospital, Ido-Ekiti (FETHI), Ekiti State before the collection of blood samples. Samples were collected from the subjects who voluntarily gave their consent. Men and women who have HIV with or without antiretroviral therapy (ART) and apparently healthy subjects were investigated. Sample analysis was carried out at the laboratory section of the Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti (ABUAD), Ekiti State.

The HIV subjects were divided into three groups namely: HIV subjects on therapy, HIV subjects not on therapy and control subjects. Subjects with diabetes mellitus, kidney disease, and other conditions were excluded from this study. The HIV subjects on ART took emtricitabine (200 mg orally once daily); tenofovir (300 mg orally once daily); zidovudine (300 mg orally twice a day) and lamivudine (300 mg orally once daily)

Methods of determination of parameters

Estimation of hepcidin enzyme-linked immunosorbent assay (ELISA): The hepcidin in patient's sample competes with the added hepcidin-biotin conjugate for binding to the coated antibody in the microwell. After incubation the unbound conjugate is washed off. Incubation with a streptavidin-peroxidase enzyme complex and a second wash step follows. The addition of substrate solution results in a colour development which is stopped after a short incubation. The intensity of colour developed is inversely proportional to the concentration of Hepcidin in the patient sample (Kroot, 2011).

The standards (50µl) were added to the microwells designated for standards at different concentrations. 40µl of sample diluents and 10 µl of the sample were added to sample wells. 50 µl of horseradish peroxidase was then added to each well except the blank and the microplate was well covered with an adhesive lid and incubated for 30 minutes. The solution was discarded and washed 4 times with the wash buffer (300µl) using ELISA washer. After the last wash, removal of any remaining wash buffer was done by blotting. 50µl of chromogen A and B solutions were added respectively to each well. It was gently mixed and covered properly with an adhesive lid and incubated for 20 minutes at 370C. 50µl of stop solution was added to each well. The absorbance was read at 450nm wavelength within 30 minutes. The concentration of human hepcidin for each sample was displayed on the digital monitor.

Estimation of serum iron: Transferrin-bound iron is released and reduced from ferric to ferrous ions at an acidic pH. The ferrous ions react with ferrozine to form a violet coloured complex which is measured spectrophotometrically at 560 nm and is proportional to serum iron concentration (Henry, 1984). Iron buffer reagent (2.5ml) was added to all tubes (blank, standard, control, samples). 500µl of standard/control/sample was added to designated tubes and mixed properly. The absorbance of the mixture was read on a spectrophotometer at 560 nm wavelength and recorded as A1. Then 50µl of iron colour reagent was added to all tubes and placed in the water bath at 370C for 10 minutes. Samples were read again at 560 nm and the absorbance was recorded as A2.

The concentration of iron =

$$\frac{\text{Absorbance A2 Test or control - Absorbance A1}}{\text{Absorbance A2-A1 Standard}} \text{ Test or control x Conc of Std}$$

Estimation of unsaturated iron binding capacity (UIBC): Ferrous iron is added to serum at alkaline pH which then binds with transferrin at the unsaturated iron binding sites. The unbound ferrous ions are measured using ferrozine reaction. The difference between the added ferrous ions and the unbound ions is the unsaturated iron-binding capacity.

All tubes (blank, standard, control, samples) were labeled and 2.0ml of unsaturated iron-binding capacity buffer was added. Then 0.5ml of respective samples were added into each tube and mixed properly. Absorbance was read at 560 nm and recorded as A1. 0.05ml of iron colour reagent was then added to all tubes. All tubes were placed in a heating water bath at 370C for 10 minutes. Absorbance was read at 560 nm and recorded as A2. The values obtained were used to calculate the unsaturated iron-binding capacity as in serum iron.

Total iron binding capacity (TIBC) : This was obtained by adding the values of serum iron concentration and unsaturated iron binding capacity.

Statistical analysis

The data obtained were analyzed statistically using a software package for social sciences (SPSS) version 23.0 (SPSS Inc. Chicago, Illinois, USA). All parameters were expressed as mean ± standard deviation (SD) and values were statistically significant at $p<0.05$

RESULTS

The mean ± SD of Iron (µmol/L), UIBC (µmol/L), TIBC (µmol/L) and Hepcidin (ng/ml) for HIV subjects on ART, HIV subject not on ART and control are shown on table 1. UIBC showed significant increase at $p<0.05$ when HIV subjects on ART and HIV subjects not on ART were compared with control group. There was no significant difference ($p<0.05$) in other parameters when HIV subjects on ART and HIV subjects not on ART were compared with control group.

Figure 1 shows the level of Iron, UIBC, TIBC and Hepcidin in HIV seropositive subjects based on gender. The chart shows that there was an elevated level of iron and hepcidin in males compared with females. Also the chart shows that there were elevated levels of UIBC and TIBC in females compared with males.

Figure 2 shows the level of iron, UIBC, TIBC and hepcidin in HIV seropositive subjects on antiretroviral drugs. The chart shows that there was an elevated level of iron in HIV subjects on emtricitabine compared with those on emtricitabine + zidovudine, tenofovir and zidovudine + lamivudine. The chart also shows an elevated level of hepcidin in HIV subjects on emtricitabine + zidovudine compared with those on emtricitabine, tenofovir and zidovudine + lamivudine.

Table 1:
Serum levels of hepcidin and iron indices in HIV seropositive subjects and control

Variables	HIV subject on ART (N=29)	HIV subject not on ART (N=21)	Control subjects (N=40)	P values
Iron (µmol/L)	24.01 ± 5.39	21.85 ± 4.17	20.82 ± 6.71	0.082
UIBC (µmol/L)	15.68 ± 5.67	13.15 ± 4.98	19.40 ± 7.69	0.002*
TIBC (µmol/L)	39.67 ± 10.05	35.02 ± 8.26	40.20 ± 11.77	0.169
Hepcidin (ng/ml)	18.43 ± 14.78	24.17 ± 7.88	20.73 ± 6.33	0.148

*---Significant at P<0.05

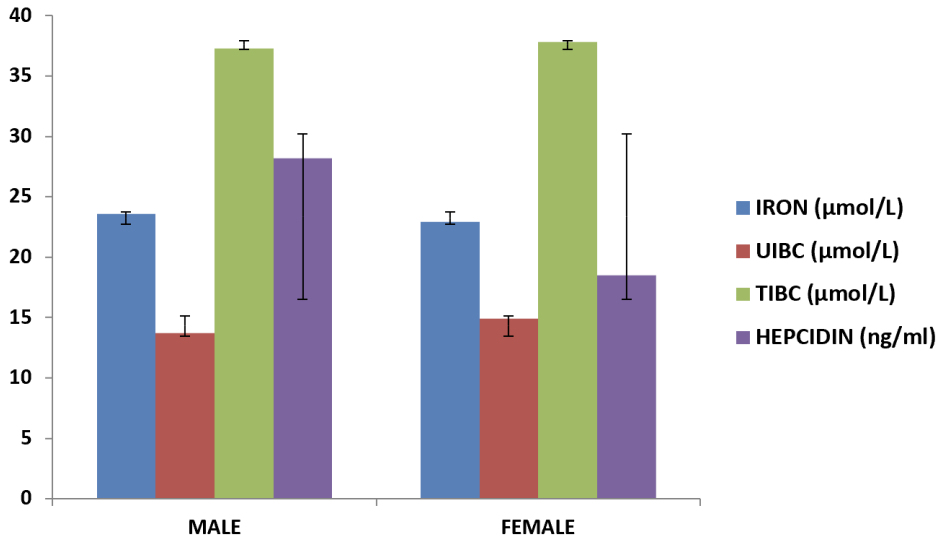

Figure 1
Hepcidin and Iron status in HIV seropositive subjects based on gender

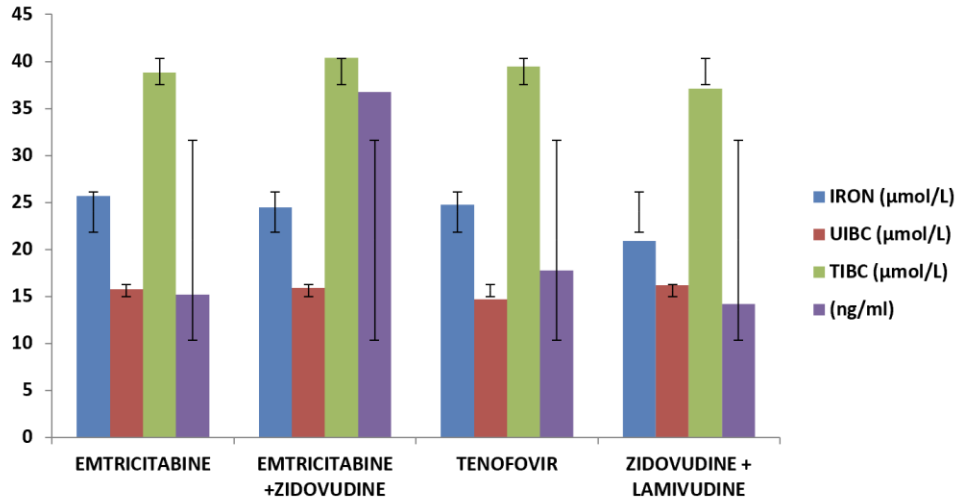

Figure 2 :
Hepcidin and Iron status in HIV seropositive subjects based on ART

DISCUSSION

This research assessed the impact of HIV on the levels of hepcidin and iron indices in HIV subjects. Hepcidin showed no significant difference when HIV subjects were compared with control group. The finding is partly in support of the works of Andrew *et al*. (2014) which stated that hepcidin is raised in both HIV subjects with or without ART because there was increase in hepcidin levels in subjects not on ART although not significant. This could be due to other inflammatory and infectious diseases which were not investigated in this study. This finding is not in line with the works of Cunha *et al*. (2015) which stated that hepcidin was significantly lower in subjects with HIV subjects who were not on ART. Hepcidin levels could be suppressed by factors such as erythropoiesis which is stimulated during acute blood loss (Pasricha et al., 2016). There was no significant increase in serum iron level when HIV subjects on ART and those not on ART were compared with control subjects. This slight increase in hepcidin in HIV subjects on ART may downregulate serum iron in the subjects in order to hinder the replication of the viruses. This work is in line with the works of Hsiang-Chun *et al*. (2015) which stated that serum iron increase in HIV subjects and persist even in those on ART. This increase may be due to the different combinations of the antiretroviral drugs as well as the multivitamins intake which are normally prescribed for HIV subjects. There was a significant decrease in UIBC when HIV subjects on ART and those not on ART were compared with control groups. This is still in support of Hsiang-Chun *et al. (*2015) which reported that UIBC level reduces in subjects with HIV on ART and those not on ART. There was no significant decrease in TIBC level when HIV subjects on ART and those not on ART were compared with control subjects. This finding is partly in line with the works of Patrick *et al*. (2015) which stated that TIBC was significantly lower in patient with HIV compared with control subjects. Although, this study did not record significant decrease in TIBC, the levels were lower in the HIV subjects compared with control subjects. This may be due to the fact that HIV infection causes redistribution of iron in HIV subjects. In this study, there was no significant difference in TIBC levels in both male and female HIV subjects while hepcidin level is higher in male than in female HIV subjects. However, UIBC was higher in female subjects than in male HIV subjects. The study also observed that the level of iron was higher in male than in female HIV subjects. This is in line with the works of Le (2016) which shows that iron level is higher in males than female HIV subjects. The slight increase in hepcidin recorded in male HIV subjects than in female HIV subjects may have occurred from moderate increase in iron stores in the males.

TIBC and hepcidin levels were higher in HIV patients taking emtricitabine + zidovudine than in HIV subjects taking tenofovir, emtricitabine, zidovudine+lamivudine. The level of iron was higher in HIV patient taking emtricitabine than in HIV subjects taking tenofovir, emtricitabine+zidovudine and zidovudine+lamivudine while the level of UIBC was higher in HIV patients taking zidovudine+lamivudine than in HIV subjects taking tenofovir, emtricitabine+zidovudine and emtricitabine. This is in line with the report of Angel *et al*. (2017) which suggested higher efficacy occurs with Tenofovir / Emtricitabine at 96 weeks versus Zidovudine / Lamivudineat 48 weeks. The different HAART regimens or combinations might have restored the hepcidin levels as well as iron homeostasis in the HIV subjects on ART.

In conclusion, there was no significant difference in Hepcidin, Iron and TIBC in the HIV subjects investigated. However, there was significant increase in levels of UIBC in HIV subjects on ART and HIV subjects not on ART compared to apparently healthy subjects. The findings from this research suggest that the slight increase in hepcidin may downregulate serum iron in HIV subjects in order to hinder the replication of the viruses. There was no significant increase in iron and hepcidin in males compared with females when iron indices and hepcidin in HIV seropositive subjects were compared based on gender while there were elevated levels of UIBC and TIBC in females compared with males. Although there was no significant negative impact of HIV infection on hepcidin and the iron indices, the study concluded that the parameters should be measured to monitoring the progress of management in order to prevent anemia which may occur during HIV infection

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