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RESEARCH PAPER

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Serum Iron Profile Study Among Persons Living With Hiv in Umuahia, Abia State,Nigeria

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ABSTRACT

The study was done to ascertain the iron profile level of the HIV positive patients in Umuahia. A total of 110 subjects were recruited for the study. The subjects were grouped into two: the HIV positive subjects (test group) and HIV negative subjects (control group). The blood samples were collected and analysed colorimetrically, while the percentage transferrin saturation was calculated. Statistical analysis was done using t-test and level of significance set at P<0.05. TIBC in HIV positive subjects (284.12 ± 24.40 µg /dl) were found to be significantly reduced (P<0.05) when compared with those of HIV negative subjects (335.22 ± 49.0 µg / dl). The mean serum iron and percentage transferrin saturation in HIV positive subjects (163.30 ± 11.52 µg /dl; 51.10 ± 13.02 % 0 were found to be statisticall higher (p<0.05) when compared with HIV negative subjects (67.21 ± 23.68 µg /dl; 29.12 ± 10.50%) respectively.

It could be inferred that the recorded increase i serum iron and transferrin saturation percent among HIV patients in this study were as a result of derangement i iron metabolism in addition to oxidative staress.
Keywords: HIV, AIDS, Serum Iron, Total iron binding capacity, ferritin, Percentage transferrin saturation and Unsaturated Iron Binding capacity.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a virus which causes Acquired Immunideficiency Syndrome(AIDS).This virus infects the cells that make up the human body and replicate themselves within those cells (Nielson et al., 2005). If untreated eventually,most HIV infected individuals develop AIDS (Buchbinder et al., 1994). AIDS is a set of symptoms and infections resulting from the damage to human immune system. The major consequence of this is such a progressive effective reduction of the immune system and leaves the individual prone to opportunistic infections.

Anaemia is one of the most common blood abnormalities seen in people with HIV infection. According to Levine (2003), the incidence of anaemia ranges from 10% in people who have no symptoms to 92% in patients with advanced AIDS. Semba (2001) stated that pathogenesis of anaemia during HIV infection is often multifactorial, and contributing factors include iron deficiency, the anaemia of chronic disease associated with HIV,malaria and opportunistic infections and it is responsive to iron supplementation. The anaemia though multifactorial, the anaemia of chronic disease appears to be the most frequent cause as opined by Coyle (1997).

Defects in iron metabolism are another common feature in HIV infection.Iron studies reveal a low serum iron level, a low total iron-binding capacity,low transferrin saturation,and or increased ferritin level. The presence of normal or increased iron stores usually indicates that a functional block of iron release may disturb iron utilization in HIV infection (Coyle, 1997; Means et al., 1999) but the study done by Semba et al. 2001 showed no relationship between iron level and HIV infection severity among HIV-positive women in sub-saharan Africa.

Anaemia are often overlooked and undertreated in patients with HIV infection. Meanwhile, clinical studies have shown that anaemai in these patients adversely affects functional ability and quality of life as opined by Levine et al. (2003). Moore (1999) stated that there is evidence that anaemia is related with decreased survival.Recovery from anaemia is associated with improved survival.

AIM

To determine serum iron profile level among persons living with hiv in Umuahia, Abia State, nigeria.

MATERIAL AND METHODS

Study Area: The study was done in Umuahia, Abia State, Nigeria. The subjects were recruited from HIV patients attending Health Services Department of Michael Okpara University of Agriculture, Umudike, Abia State and Daughters of Mary Mother of Mercy Hospital, Ahieke, Umuahia, Abia State and the control subjects were as well recruited from the same place.

Study Populationa and Enrolment: A total number of 110 subjects were recruited for the study.The subjects were divided into two groups,consisiting of 50 HIV positive subjects and 60 HIV negative subjects.

Selection criteria: The test subjects were selected after been established of having HIV infection using national algorithm and not reactive to any other viral infection and without any AIDS indicator conditions. The control group was selected after been established of not having HIV or reactive to any other viral infection. The subjects were known not to be on any iron supplementation for the previous one month prior to the study.

Exclusion Criteria: The subjects showing any underlying chronic illness, other HIV infection (for the test group) and reactive to other viral infections were excluded from the study.

Sample Collection: Using a 5ml sterile syringe, 5mls of venous blood was drawn from the subjects by a clean vein puncture from the anticubital vein and delivered into a plain container to retract serum.The sample in plain tubes were allowed to clot in the refrigerator overnight and serum was separated the next day. The serum was transferred into clean test tubes for estimation of serum iron profile in the subjects

Laboratory Methods and Procedures

The reagents were commercially purchased from a reputable supplier and the manufacturers' standard operating procedures were dully followed.Serum iron and TIBC were determined using TECO Diagnostic Kits (TECO Diagnostics, Anaheim, CA 92807, USA) and the subjects screened for HIV using Determine Rapid test strip and Uni-Gold test kits (Abbott Laboratories Diagnostics Division, USA).

Serum iron and TIBC Estimation: The serum ferritin was estimated by enzyme immunoassay method.Reagent kit was purchased from Biotec Laboratories Ltd.

Principle: The iron in the serum is dissociated from its Fe (iii)- transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the Fe (iii) to Fe (ii). The chromogenic agent, Ferene, forms a highly coloured Fe (ii)-complex that is measured photometrically at 560 nm.

The unsaturated iron binding capacity (UIBC) is determine by adding Fe (ii) to serum so that they bind to the unsaturated iron binding sites on transferrin. The excess Fe(ii0 ions are reacted with Ferrozine to form the colour complex,which is measured photometrically. The difference between the amount of Fe (ii) added and the amount of Fe (ii) measured represents the unsaturated iron binding capacity. The total iron binding capacity (TIBC) is determined by adding the serum iron value to the UIBC value.

Procedure

1. Serum iron: The tubes were labelled blank, standard,control and test accordingly.Into each tube,2.5ml iron buffer reagent was added 0.5ml sample was added to each tube for test and 0.05ml iron-freee water was added to blank.The spectrophotometer was zeroed with the blank and read at 560 nm wavelength. The absorbance of all tubes (A1 reading) were read and recorded. After recording A1 reading, 0.05ml of iron colour reagent was added to all tubes. The solution was mixed and placed in water bath at 37^0C for 10 minutes. The spectrophotometer was zeroed using the blank at 560nm wavelength. The absorbance of all tubes were read (A2 reading) and recorded.

Calculation

A=Absorbance

Std=Standard

$$\frac{\text{A2 Test-A1 Test}}{\text{A2 Std-A1 Std}} \times \text{Conc of Std} = \text{Total Iron}(\mu g/dl)$$

2.**UIBC (Unsaturated iron-binding capacity):** The tubes were labbed blank,standard,control and test accordingly. Into each tube, 2.0ml UIBC buffer reagent was added 0.5ml iron-free

water and 0.5ml of standard was added to the standard, while 0.5ml of sample and iron standard was added to the tube labelled test 1.0ml iron-free water was added to the blank. The spectrophotometer was zeroed with the blank and read at 560nm wavelength. The absorbance of all the tubes (A1 reading) were read and recorded 0.05ml of iron colour reagent was added to all tubes. The solution was mixed and placed in water bath at 37^0C for 10 minutes. The spectrophotometer was zeroed using the blank at 560nm wavelength. The absorbance of all the tubes were read (A2 reading) and recorded.

UIBC calculation

Conc of std- A2 Test-A1 Test x conc of std

A2 std-A1 std

= UIBC (µg/dl)

TIBC(Total iron binding capacity) = Iron value + UIBC=TIBC (µg/dl)

3. **Transferrin percentage saturation =**

Serum iron x 100 = % Transferrin saturation

TIBC

Statistical Analysis: The analysis was done using t-test with the statistical package for social science (SPSS) version 17 and the values were expressed as mean±SD with level of significance set at $P<0.05$.

Table. Mean values of serum iron profile in HIV positive subjects and HIV negative subjects.

Status	Serum iron(µg/dl)	TIBC(µg/dl)	%Transferrin saturation
HIV positive	163.30±11.52	284±24.40	51.10±13.02
HIV negative	67.21±23.68	335.22±49.0	29.12±10.50

DISCUSSION

There was a significant increase ($p<0.05$) in serum iron and percentage transferrin in HIV sero-positive subjects when compared to HIV negative subjects. This was in aggreement with th estudy done by Mc Dermid *et al.* (2007), serum iron and percentage transferrin saturation were reported to be significantly higher in HIV –positive subjects. Inversely,in a cross-sectional study of pregnant women in Malawi,it was revealed that iron status was not related to markers of HIV disease severity (Conor, 2007). In another study in sub-saharan Africa by Cono (2007), it was shown that iron deficiency coexists in populations with high prevalence of HIV. In similar retrospective studies by Doherty (2007) and Traore (2009) were evidences of relevant iron status in HIV infection, this accumulation on serum iron is not far from the release of bound iron from thier apoproteins occassioned by incraesed oxidative stress (Award, 2006).

Also,studies have shown that various metabolic derangement predispose HIV patients to metabolic acidosis (Ajose et al., 2008) which promotes reduced binding of iron molecules to transferrin with resultant increase in serum free iron,however there were contrasting studies (Semba et al., 2001). In similar studies by Salman et al. (2012) and Ajose *et al.* (2008), it was reported that high plasma iron and body iron stores have the potential of

promoting free radical generation and oxidative stress via the popularFenton/Baber-Weiss reaction. Transferrin as β1 glycoprotein synthesized in the liver, binds to iron in ferric form and transports it from the storage site for utilization through a receptor mediated pathway hence,whatever affects one, affects the other. The virus-host iron status interaction also plays an important role in the depletion. While some viruses selectively infect iron aquiring cells by binding to transferrin receptor during cell entry, others alter the expression of proteins involved in iron homeostasis protein and hepcidin.

It is worthy to note that the incraese or decrease in the iron status of HIV positive patients depend on the stage of the disease. Iron stores have been observed to decline in the early asymptomatic stage probably due ot impaired absorption (Frii, 2001), however, they may increase with progression of the disease as iron accumulates in the macrophages and other cells (Drakesmith and Prentice, 2008).

CONCLUSION

It could be inferred that the recorded increase i serum iron and transferrin saturation percent among HIV patients in this study were as a result of derangement i iron metabolism in addition to oxidative staress.

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