DETERMINATION OF HAEMATOCRIT LEVEL AND IRON PROFILE STUDY AMONG PERSONS LIVING WITH HIV IN UMUAHIA, ABIA STATE, NIGERIA

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ABSTRACT

The study was done to determine the haematocrit level and iron profile of the HIV positive patients in Umuahia.A total of 110 subjects were selected 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. Haematocrit was determined using microhaematocrit method.Statistical analysis was done using t-test and level of significance set at P<0.05.TIBC in HIV positive subjects (284.12±24.40µg/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%) were found to be statistically higher (p<0.05) when compared with HIV negative subjects (67.21±23.68µg/dl;29.12±10.50%) respectively.There was significant decrease (P<0.05) in haematocrit level of HIV positive patients (31.38±7.2%) compared to the control subjects (41.19±5.7%).The recorded increase in serum iron and transferrin saturation percent among HIV patients in this study were as a result of derangement in iron metabolism in addition to oxidative stress and the anaemia seen in the patients is as a result of decreased haemoglobin concentration.

KEYWORDS:HIV,AIDS,Haematocrit,Serum Iron,Total iron binding capacity,ferritin,Percentage transferrin saturation,Unsaturated Iron Binding capacity

No: of Tables :2 **No:of References:17**

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 haematocrit level and 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 3ml 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 and the remaining transferred to EDTA anticoagulated container for haematocrit estimation

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 560nm.

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 560nm 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

A2 Test-A1 Test xConc of Std =Total Iron(µg/dl)

A2 Std-A1 Std

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 ironx100=%Transferrin saturation

TIBC

Haemoglobin Estimation (Cynmeth Method)

Principle: The conversion of heamoglobin iron from the ferrous to the ferric state by the ferricynanide component of Drabkin's solution to form methaemoglobin combines with the potassium cynanide in the solution to produce the stable cynamethaemoglobin.The resulting coloured solution which is produced is then measured in a spectrophotometer at a wavelength of 540nm.

Procedure:1 in 201 dilution of blood was made by adding 20µl of blood to 4ml of diluent (Dbrabkin's solution) into a test tube.The test tubes were stoppered and inverted several times and were then poured into a cuvette and the absorbance were read in a spectrophotometer at 540nm against the reagent blank.The haemoglobin level was calculated.

Packed Cell Volume Determination

Method: microhaematocrit

Principle

Anticoagulated blood blood in a glass capillary of specified length, bore size and wall thickness is centrifuged in a microhaematocrit centrifuge at RCF 12000-15000xg for 3-5 minutes to obtain a constant packing of the red cells. The PCV value is read from the scale of a microhaematocrit reader or calculated by dividing the height of the red cell column by the height of the total column of blood (Cheesbrough, 2006).

Procedure

The plain capillary tubes were filled with EDTA venous blood samples, up to three-quarters of the tube.

The unfilled ends of the tubes were sealed with a plasticine sealant.

The tubes were balanced in a microhaematocrit centrifuge, taking note of the slot numbers.

They were centrifuged for 5 minutes at 12000 RCF for 5mins.

The PCV values were obtained by aligning the capillary tubes on the microhaematocrit reader, with the base of the red cell column on the zero line and the top of the plasma column on the 100 line.

Statistical Analysis: The analysis was done using t-test and the values were expressed as mean± SD with level of significance set at $P<0.05$

RESULT

Table1: Mean values of serum iron profile in the subjects

Parameters	HIV pos	HIV neg	P-level
Serum iron (µg/dl)	163.30±11.52	67.21±23.68	P<0.05
TIBC (µg/dl)	284±24.40	335.22±49.0	P<0.05
%Transferrin saturation(%)	51.10±13.02	29.12±10.50	P<0.05

Table2: Mean values of haematocrit level of the subjects

Parameter	HIV pos	HIV neg	P-level
Hb (g/dl)	3 1.38±7.2	41.19±5.7	P<0.05

DISCUSSION

Table 1 showed 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 the study done by McDermid *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).

Table 2 showed significant decrease ($P<0.05$) in the mean haematocrit level of HIV positive subjects when compared to those of HIV negative subjects.According to Rapaport (1987),erythropoiesis fails to increase in chronic disease because iron release from mononuclear phagocytes, which is the primary source of iron for making new red blood cells do not increase.Similarly,it was also reported that HIV status could result to decrease in erythropoiesis,which can have effect on other related parameters (example haemoglobin and haematocrit),independent of the effect on serum iron (Walsh *et al.,*2010).

CONCLUSION

The increase in serum iron and percentage transferrin saturation among HIV patients in this study were as a result of derangement i iron metabolism in addition to oxidative staress.The study equally showed that HIV infection decreases haematocrit level in the patients

infected.This will also affect erythropoiesis leading to anaemia of chronic disease.The haematocrit level of HIV patients should be monitored to improve their general wellbeing.

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