RESEARCH ARTICLE

MARKERS OF IRON METABOLISM IN PEOPLE LIVING WITH HIV/AIDS: IMPLICATIONS IN DISEASE PATHOLOGY

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ABSTRACT

Conflicting reports on levels of markers of iron metabolism in HIV infections necessitated the need to investigate the risks of iron mediated severity in HIV- 1 infected Senegalese patients. Eighty untreated HIV-1 positive clients and 50 sero-negative controls were recruited and their serum total iron, transferrin saturation, total iron binding capacity, CD_4^+ T lymphocytes, vitamin c, zinc and selenium were estimated. The CD^{+4} T lymphocytes counts in our test subjects were significantly lower than those of the controls while the serum iron, total iron binding capacity and transferrin saturation were significantly higher in tests than controls ($P < 0.001$). Furthermore, there were significant differences between the test and control groups in the three antioxidants studied vas vitamin C, zinc and selenium ($p < 0.001$). The CD_4^+ T cell count had a positive significant correlation with the levels of antioxidants; vitamin C, zinc and selenium, but a negative correlation with serum iron. We conclude that derangement in iron metabolism, in addition to causing oxidative stress, may have contributed to the depletion of CD_4^+ T cell population in our subjects. Metabolic acidosis and serum iron level should be addressed in the management of HIV-1 infection and antioxidant support instituted.

INTRODUCTION

The human immunodeficiency virus 1 (HIV-1) infection is known to affect virtually all the organs of the body causing different metabolic derangements in addition to the depression of the immune system (Martinez and Gattell, 1999). Some of the metabolic derangements include oxidative stress due to persistent immune activation associated with uncontrolled HIV-1 replication that leads to excessive reactive oxygen species (ROS) generation (Pace and Leaf, 1997, Halliwell and Gutteridge, 1990), (Tang and Simt, 2000). Changes in metabolism as a result of immune response in HIV infections include biochemical parameters related to iron metabolism (Appelberg, 2001; Boela *et al.,* 1996). Increased oxidative stress can result in the release of bound iron from their apoproteins therefore further increasing the burden of plasma iron (Award, 2006). Free radicals generation has been shown to be the physiologic mediator of apoptosis associated with progression of HIV infection and correlates with the decrease in plasma zinc, selenium and vitamin E. (Favier *et al.,* 1994); (Buttke and Sandstrom 1994; Fang *et al.,* 2002; William, 2004).

In addition, excessive free radical production associated with HIV – infection was shown to participate in T – lymphocyte depletion by triggering apoptosis which may result in impaired antiviral defenses mediated by T helper cells (Roaderer *et al.,* 1991), and antioxidant depletion which lead to oxidative damage and cell death. Oxidative-stress also destroys T cells and causes the weakening of the immune system (Nakamura *et al.,* 2002). Iron is an essential trace element present in biological systems in either ferrous (Fe^{2+}) or Ferric (Fe^{3+}) state. Transferrin, a β1 glycoprotein synthesized in the liver, binds iron in the ferric form and transports it from the storage site for utilization through a receptor mediated pathway (Ponka *et al.,* 1998). The ability of transferrin to bind iron is called total iron binding capacity (TIBC) and the ratio of serum iron concentration to TIBC concentration determines the transferrin saturation. Body iron stores had been shown to increase in HIV infection due to their accumulation in tissues such as liver, heart, pancreas and cells like macrophages and could result in free radicals generation and accelerated catabolism of ascorbic acid (Larrea *et al.,* 1998; Delanghe *et al.,* 1998; Appelberg, 2001). Experimental evidences also revealed that serum iron may be increased or decreased depending on the stage of the disease. Although iron stores may decline in early asymptomatic HIV infection probably because of impaired absorption (Frii, 2001), they may increase

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with progression of the disease as iron accumulates in the macrophages and other cells (Boelaet *et al.,* 1996). Free radicals have also been implicated in the development of cancers and free iron can cause DNA double strand breaks and oncogene activation (Reizenstein, 1991); for example, breast cancer display five to fifteen times more transferrin receptors than normal breast tissue (Bullen *et al.,* 2005; Cazzola *et al.,* 1990; Weinberg, 1990; Muta *et al.,* 1990). The conflicting reports with regards to markers of iron metabolism in HIV infections underscore the necessity for this study (Larrea *et al.,* 1998; Delanghe *et al.,* 1998;) (Kreuzer *et al.,* 1997);(Arinola *et al.,* 2004); (Olaniyi and Arinola, 2001).

MATERIALS AND METHODS

Eighty newly diagnosed as HIV-1 positive patients comprising of 48 females (60%) and 32 males (40%) attending the sociale institute of health and hygiene of Dakar and Bio-Amarie laboratory of Kaolack who were recruited for this study after calculating the sample size, using the prevalence of 1% of the area. Fifty seronegative controls consisting of 30 females (60%) and 20 males (40%) were equally recruited for the study. Exclusion criterium for all subjects was ingestion of supplements including vitamin C and ferrous preparations over a preceding two month period. Two separate HIV screening kits were used for the detection of seropositivity using World Health Organization (WHO) criteria. A rapid ELISA was done on whole blood collected by finger prick using Determine ^{TM} HIV1/2 kit (Abbot Laboratories, Japan, and STAT-PAK assay kit (Chembio Diagnostic Systems, USA). The principle of the two test kits is based on antigen-antibody reactions with colour indicators. Samples were regarded as positive with a color change and negative without it.Ten milliliters of venous blood was collected from each participant into vacutainer tubes and 5mls was placed in each of EDTA and plain bottles. Serum was obtained from clotted blood in the plain bottle. After clot retraction and centrifugation at 25,000 rpm for five minutes using a bench centrifuge, the serum was separated and placed in another plain bottle and stored frozen at -70^0C for later analysis for iron, transferrin, total iron binding capacity (TIBC), zinc, selenium and vitamin C. The blood sample in the EDTA bottle was used immediately after collection for the determination of CD^+_4 T lymphocyte count. CD^+_4 T lymphocyte count was done using Partec Cyflow counter and Partec CD^+_4 T lymphocyte easy count kit. Twenty micro liters of whole blood was placed in a test tube, 20µl of CD4- MAB/PE (dye) was added and vortex mixed gently. The mixture was then incubated for 15 minutes at room temperature. To this, 80µl of no-lyse buffer was added and mixed. The mixture which was stained was aspirated into the Cyflow counter. The count result was displayed electronically.

Determination of Serum Iron

Serum Iron was determined by the method of Garcic (1979). The method is based on the formation of ternary complex: chromazurol B (CAB)- cetyltrimethyl ammonium bromide (CTMA)- iron complex which was read at 623nm. The concentration of iron in samples and controls were determined from the interpolation of the absorbance on the known concentration of standard, as follows:

$$\frac{\text{Absorbance of Test/Controls}}{\text{Absorbance of Standard}} \quad X \quad \frac{17.9\ \mu mol/L.}{1}$$

Estimation of Total Iron Binding Capacity (TIBC)

Serum TIBC was determined using the spectrophotometric method of Ramsay (1957). Serum was treated with excess Fe^{3+} ions to ensure complete Saturation of transferring. Unbound excess iron was adsorbed on aluminum oxide and precipitated. The Fe^{2+} ions in the supernatant was estimated by the previous method for serum iron. The TIBC was calculated as Serum iron x 3 µmol/L. One milliliter of ferric chloride was added to 0.5ml of serum in a tube and incubated at room temperature for 5 minutes, 1.0ml of aluminium oxide (Alox) was added, the mixture was covered and placed in a vortex mixer for 10 minutes. The tubes were removed and allowed to stand for 3 mins. The clear supernatant was used for Iron estimation as described earlier.

Transferrin Saturation

Transferrin Saturation was determined using the formula:

$$\frac{\text{Serum Iron (umol/L)}}{\text{TIBC}} \quad X \quad \frac{100}{1}\ \%$$

Estimation of Ascorbic Acid

Ascorbic Acid was estimated according to the spectrophotometric method of Henry et al., (1974). Ascorbic acid in serum is oxidized to its dehydroform by cupric sulfate. The dehydro- ascorbic acid was then reacted with 2, 4 dinitronphenylhydrazine in the presence of sulphuric acid to form a red colour whose intensity is proportional to the concentration of ascorbic acid and read at 520mm. Ascorbic acid concentrations were determined by interpolation of the absorbance of the standard and its concentration.

$$\frac{\text{Absorbance of Text/Controls}}{\text{Absorbance of Standard}} \quad X \quad \frac{2.0\ mg/dl.}{1}$$

Determination of Serum Zinc and Selenium levels

Serum zinc and selenium levels were determined using alpha-4 flame atomic absorption spectrophotometer (AAS) according to standard methods. Deproteinisation was done by placing 1.0 ml of serum in the test tube and adding 3ml of 2M HCl. The clear supernatant was aspirated into the flame atomic absorption spectrophotometer (AAS) after adjusting the wavelength at 213.9nm and 196.1nm for zinc and selenium estimation respectively. The concentrations were displayed electronically and the results were expressed in µmol/l.Data collected were analysed using descriptive and inferential statistics.

RESULTS

The mean CD_4^+ T cell, serum iron, TIBC, transferrin saturation for subjects and controls were 319 $\pm$ 22, 952 $\pm$ 57 cells/ul (p < 0.001); 35 $\pm$ 0.8, 11.8 $\pm$ 0.9 umol/l (p < 0.001); 58.5 $\pm$ 2.2, 45.2 $\pm$ 2.4 umol/l (p < 0.005) and 68.8 $\pm$ 3.3, 27.7 $\pm$ 2.2% (p <0.001) respectively (Table 1). The serum vitamin c, zinc and selenium of subjects and controls were 2.27 $\pm$ 0.57, 17.03 $\pm$ 2.27 (p < 0.001); 0.6 $\pm$ 0.05, 11.9 $\pm$ 0.26 umol/l (p < 0.001) and 0.1 $\pm$ 0.01, 1.2 $\pm$ 0.12 umol/l (p < 0.0001) respectively (Table 2). The CD_4^+ T cells count had a positive correlation with levels of vitamin C (r = 0.497, p < 0.001), zinc (r = 0.737, p < 0.001) and selenium (r = 0.639, p < 0.001)

Table 1: Mean concentrations of Markers of Iron Metabolism and CD$^+_4$ T cell count in Tests and controls

Parameters	Tests n=80	Controls n=50	P value
CD$^+_4$ T cells/ul	319.6 $\pm$ 22	951.5 $\pm$ 57	<0.0001
Serum Iron (umol/l)	35.3 $\pm$ 0.8	11.8 $\pm$ 0.9	<0.001
TIBC (umol/l)	58.5 $\pm$ 2.2	45.2 $\pm$ 2.4	<0.001
Transferrin Saturation (%)	68.0 $\pm$ 3.3	27.7 $\pm$ 2.2	<0.0001

Table 2: Mean Concentrations and p values of Serum Antioxidants in Tests and Controls

Antioxidants	Test n = 80 mean $\pm$ SEM	Control n = 50 mean $\pm$ SEM	P value
Vitamin C (umol/l)	2.27 $\pm$ 0.57	17.03 $\pm$ 2.27	<0.001
Zinc (umol/l)	0.6 $\pm$ 0.05	11.9 $\pm$ 0.26	<0.0001
Selenium (umol/l)	0.1 $\pm$ 0.02	1.2 $\pm$ 0.12	<0.0001

Table 3: Correlation between CD$_4^+$ T cells, serum iron and antioxidants in test subjects

Parameters	CD$_4^+$ T cell count	
	Correlation	P value
Vitamin C (umol/l)	r = 0.497	P <0.001
Zinc (umol/l)	r = 0.737	P <0.001
Selenium (umol/l)	r = 0.639	P < 0.001
Serum Iron (umol/l)	r = - 0.572	P < 0.001

but a negative correlation with serum iron (r = -0.572, p < 0.001) respectively (Table 3).

DISCUSSION

The significant differences in serum iron, transferrin saturation and total iron binding capacity (TIBC) obtained indicate derangement in iron metabolism. In addition, serum iron was found to have negative correlation with CD$_4^+$ T lymphocytes (Table 3). These findings corroborate with the results of some other workers (Delanghe *et al.,* 1998); (Fuchs *et al.,* 1993), who had previously observed that iron impaired the CD$_4^+$ T cell sub population *in vitro* (Fuchs *et al.,* 1993). Our data showed increased serum iron levels in HIV subjects which could have led to increased free radical generation and oxidative stress, and may play a significant role in the pathogenesis of HIV infection (Halliwell and Gutteridge, 1990; Hulgan *et al.,* 2003; Gordeuk *et al.,* 2001; Halliwell and Carrol, 1994; Van Asbeck *et al.,* 2001). Moreover, over 70% of the subjects in this study had mean transferring saturation levels above the reference value and this finding agrees perfectly with what was obtained in another study (Delanghe *et al.,* 1998). The various metabolic derangements predispose HIV patients to metabolic acidosis (Ajose *et al.,* 2008) and this promotes reduced binding of iron molecules to transferrin thereby resulting in an increased serum free iron (Award, 2006). Rich iron environment promotes the proliferation of bacteria like *Mycobacterium tuberculosis* with a significant contribution to the morbidity and mortality that accompanies HIV-1 infection. The generation of free radicals has been implicated in the depletion of the antioxidant and progressive loss of CD$_4^+$ T helper cells, which is indicated by the significantly lower levels of vitamin C obtained in our subjects. CD$_4^+$ T cell counts correlate positively with antioxidant levels (Table 3) in this study. This agrees with the findings of previous workers who showed that supplements with antioxidants such as zinc and selenium improve the immune status in HIV-1 patients (Shankar and Prassad, 1998; Durosinmi *et al.,* 2008; Fawzi, 2003; Fang *et al.,* 2002) and supports their usefulness as adjuvants to antiretroviral therapy.

Conclusion

Derangement in iron metabolism could contribute to the severity of HIV-1 infection and to the depletion of CD$_4^+$ T cell population in addition to the oxidative stress that might arise from depletion of antioxidant molecules. It is therefore recommended that iron supplementation should be discouraged in HIV infection while antioxidant supplementation could be recommended as adjuvant to antiretroviral therapy. Consideration should also be given to periodic evaluation of total antioxidant status (TAS), acid-base balance and serum iron in the treatment and management of HIV-1 infection. This would likely improve the prognosis and immune reconstitution of patients.

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