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Total Antioxidant Status is Related to the CD⁴⁺ Cell Count and the Clinical State of HIV/AIDS Patients in the Northeast, Nigeria

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In this case control study, we compared the Total Antioxidant Status (TAS) of two groups of HIV positive persons that consisted of 262 AIDS patients and 158 asymptomatic HIV-antibody positive subjects with a control group of 204 HIV-antibody negative subjects. The mean CD⁴⁺ cell counts per cubic millimeter were 187 for the AIDS patients, 495 for the asymptomatic HIV patients and 920 for the control group. TAS levels in mmol L⁻¹ were 0.34±0.08 for AIDS patients, 0.77±0.29 for asymptomatic patients and 1.4±0.13 for controls. The CD⁴⁺ counts and TAS showed no gender biases but they differed significantly between the groups; p<0.05. The TAS was progressively depleted in HIV infected persons as the disease progressed from asymptomatic state to AIDS.

Key words: Total antioxidant status, HIV, AIDS, CD⁴⁺ cells, Maiduguri

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INTRODUCTION

The sub-Saharan African countries have continued to bear the brunt of the Human Immunodeficiency Virus infection and the Acquired Immunodeficiency Syndrome (HIV/AIDS) pandemic. Those countries accounted for over 75% of the approximately 3 million AIDS deaths in 2003 worldwide (UNAIDS, 2004). The importance of micronutrient deficiency in determining the outcome of HIV infection is increasingly being recognized in the era of widespread antiretroviral drugs treatment (Gil *et al.*, 2005). When the balance between free radicals and antioxidant supply is tipped, the resulting oxidative stress can cause many disease conditions and in the persons infected with the Human Immunodeficiency Virus (HIV) infection, it is reported that there is increased viral replication and a variety of biochemical and physiologic changes, which often result in metabolic impairment and cell death (Kalra *et al.*, 1994). Antioxidants are compounds with chemical affinity for free radicals. They exist in abundance and bond with free radicals before free radicals can cause damage (Mates *et al.*, 2000). Compounds with antioxidant effects and replenishing mechanisms are in five classes, namely; enzymes peptides, phenolic compounds, nitrogen compounds and carotenoids. The potential impact of Reactive Oxygen Species (ROS) on plasma Total Antioxidant Status (TAS) in HIV infection is substantial according to previous reports (Varga and Matkovic, 1998; Lantos *et al.*, 1997). Consequently, excessive ROS production if not countered by antioxidant molecules can lead to oxidative stress which may play an important role in the progression of HIV infection (Kalra *et al.*, 1994). We are not aware of any study in Nigeria that has characterized any of the oxidative stress indicators in HIV/AIDS patients. The aim of this study is to determine the plasma total antioxidant status in patients infected with HIV at both ends of the spectrum HIV/AIDS disease namely the asymptomatic HIV infection and the advanced disease state of AIDS.

MATERIALS AND METHODS

Setting: Maiduguri is the capital of Borno State of Nigeria. The town is located on Latitude 11°50' and longitude 13°10'E and the state is bordered by both the Republics of Chad and Cameroon. The adjusted 2001 census showed that Maiduguri had over 590,000 residents. The population is made up of diverse congregating ethnic groups from Nigeria and other parts of sub-Saharan Africa, but the dominant groups are the Kanuri, Marghi, Bura, Shuwa and Fulani.

Subjects: Six hundred and twenty four individuals that included 204 control subjects, 158 asymptomatic HIV infected persons and 262 AIDS patients. The subjects were recruited prospectively and consecutively in a cross-sectional study carried out at the University of Maiduguri Teaching Hospital, Nigeria. The HIV-antibody negative apparently healthy controls were recruited from staff and blood donors. The HIV-antibody positive, asymptomatic non-AIDS included all who did not have symptoms or/and had generalized lymphadenopathy (Gill *et al.*, 2003; CDC, 1986) were recruited from prospective blood donors, counselees and occasional cases of self-request. The patients with Acquired Immunodeficiency Syndrome (AIDS) attended the medical clinics of the University of Maiduguri Teaching Hospital and were referred to the Immunology and/or Haematology Departments for investigations.

Methods: After counseling and obtaining informed consent in each case, blood was taken by venepuncture and stored in plain and heparinized containers. The initial screening test to determine HIV status of the study population was determined using Genescreen Elisa (Biorad, France) which employs the principle of Enzyme Linked Immunosorbent Assay. The HIV-antibody positive individuals had a further test that confirmed their status using Immunocomb II HIV-I and 2 ConFirm Kit (Organics, Israel). This Enzyme Immunoassay test was performed using blood samples. CD⁴⁺ T-lymphocyte cell count was determined using Dynabeads kits (Dyna, France) which employs the use of monoclonal antibodies coated beads. TAS levels were determined using a commercial kit (Randox, UK) based on colourimetric method (Koracevic *et al.*, 2001).

Statistical analysis: The results were summarized as means±SD and the means compared using non-parametric test (Mann Whitney and Kurskal Wallis). The level of significance in the differences between the means was inferred at $p < 0.05$.

RESULTS AND DISCUSSION

A total of 204 apparently healthy HIV-antibody negative controls, 158 asymptomatic non-AIDS HIV-antibody positive patients and 262 AIDS patients were studied (Table 1). The controls had a mean CD⁴⁺ count of $920.0 \pm 102 \text{ mm}^{-3}$ and TAS level of $1.4 \pm 0.13 \text{ mmol L}^{-1}$. The asymptomatic HIV-infected subjects had a mean CD⁴⁺ cell count of $495 \pm 94.0 \text{ mm}^{-3}$ and TAS level of $0.77 \pm 0.29 \text{ mmol L}^{-1}$ whereas the AIDS patients had CD⁴⁺ count of $187.0 \pm 51.0 \text{ mm}^{-3}$ and TAS level of $0.34 \pm 0.08 \text{ mmol L}^{-1}$ (Table 2, 3). The mean and standard deviations of the Total Lymphocyte Count (TLC) for the

Table 1: Age and sex characteristics of the population studied in Maiduguri, Nigeria

Age groups (Years)	AIDS group		Asymptomatic non-AIDS group		Controls	
	M	F	M	F	M	F
10-19	2	4	2	-	10	-
20-29	14	58	52	24	70	2
30-39	64	58	44	14	72	2
40-49	34	14	16	6	38	4
50+	14	-	2	-	2	2
Total	128	134	114	44	192	12
Grand total	262		158		204	
Mean age	34		31		32	

Table 2: CD⁴⁺ Counts for HIV patients and controls in Maiduguri, Nigeria

Groups	CD ⁴⁺ count (mm ⁻³)	
	Range	Mean
AIDS	80-217	187
Asymptomatic	399-600	495
Controls	896-1104	920

p<0.05 for all paired comparison, Kruskal Wallis, H = 313.310, df = 2, p = 0.000

Table 3: Levels of plasma total antioxidant status in AIDS patients, asymptomatic non-AIDS HIV patients and controls in Maiduguri, Nigeria

Groups	Value (mmol L ⁻¹)		
	N	Range	Mean±SD
Controls	204	1.21-1.70	1.40±0.13
Asymptomatic	158	0.43-1.51	0.77±0.29
AIDS	262	0.21-0.59	0.34±0.08

p<0.05 for all paired comparison, Kruskal-Wallis, H = 334.805, df = 2, p = 0.00 (all groups), Man-Whitney, U = 0.000, p = 0.000 (AIDS vs Controls), Man-Whitney, U = 2160.000, p = 0.000 (Asymptomatic vs Controls), Man-Whitney, U = 413.500, p = 0.000 (AIDS vs Asymptomatic)

groups were as follows; 2.83±1.4 for the controls, 2.07±0.40 for the asymptomatic HIV infected, 1.01±0.06 (10×9/L). The CD⁴⁺ cell counts as a fraction of the Total Lymphocyte counts for the groups were 32.5% for the controls, 23.9% for the asymptomatic HIV patients and 18.5% for the AIDS patients (Table 4). There were significant differences noted between the control group on the one hand and the asymptomatic HIV and AIDS group and between the AIDS and asymptomatic HIV infected on the other hand as tested by both the Mann-Whitney and Kruskal Wallis tests (p<0.05).

The data from this study provides evidence indicating that the progression from the asymptomatic HIV infection to the late stage of AIDS is associated with the progressive depletion of total antioxidant resources of affected individual. The depletion of the plasma total antioxidant status is more apparent in AIDS patients than in the asymptomatic persons with of HIV infection. The severe depletion of TAS in the AIDS stage of HIV infection apparently supports previous reports that indicate increased ROS production correlated with viral

Table 4: Means and standard deviations of White Blood Cell counts (WBC), Total Lymphocytes Counts (TLC) and CD⁴⁺ cell counts of the groups

Groups	Control	Asymptomatic HIV	AIDS
WBC	5.40±1.24	5.600±0.98	5.840±1.24 (10×9/L)
TLC	2.83±1.40	2.070±0.40	1.010±0.06 (10×9/L)
CD ⁴⁺ cells	0.92±0.10	0.495±0.09	0.187±0.05 (10×9/L)
CD ⁴⁺ cell/ TLC (%)	32.50	23.90	18.50

load in HIV infection (Elbim *et al.*, 1999). Like the present study, Gil *et al.* (2003) demonstrated a significantly lower TAS in HIV infected persons than a control group (CDC, 1992). In this study we showed that lower TAS was related to decreased CD⁴⁺ cell count and this too corroborated the findings of other reports (CDC, 1992). Viral Tat protein is known to increase the apoptotic index by increasing intracellular ROS. It thus appears that antioxidant molecules are depleted when they are consumed in the process of protecting cells against ROS induced oxidative damage in a magnitude that is related to advancement of the disease to AIDS. In an attempt to correlate the oxidative stress with the progression of HIV Gil *et al.* (2003) demonstrated that increasing the plasma levels of vitamins A, C and E was associated with significant reduction in CD³⁸⁺/CD⁸⁺ count (Gil *et al.*, 2005). Serum tissue fluids and host cells are reported to possess antioxidant mechanisms including ceruloplasmin, transferrin, catalase, superoxide dismutase and glutathione peroxidase among others (Varga and Matkovichs, 1998). However, this study suggests that the replenishing mechanisms for the total antioxidant molecules in advanced HIV infection are ineffective as the range of values for TAS in AIDS patients is very low.

One possible reason for the low level of TAS in the HIV-infection patients may be related to low intake of fruits vegetables and vitamins brought about by poverty scarcity ignorance and anorexia. A proactive approach will need to be instituted to forestall a situation of unhindered disease progression that maybe related to the increased oxidative stress. The importance of the abnormal levels of TAS in the pathophysiology of HIV infection in Nigeria has not been elaborated by any previous study. Therefore, the evidence provided by this study suggests that antioxidants may be beneficially applied to ameliorate the untoward effect of oxidative stress in HIV infection. The situation of decreasing antioxidant capacity in the plasma with the progression of HIV seen in present study seems to have a corollary in the erythrocyte cytoplasm. Repetto *et al.* (1996) showed significantly lower levels of red cell glutathione in patients with AIDS when compared to the HIV positive

but asymptomatic individuals. We reported earlier that glutathione peroxidase levels were low in AIDS patients from the same locality as those that participated in this present study (Ezimah *et al.*, 2005).

Persons living with HIV/AIDS (PLWHA) frequently have anorexia, nausea, vomiting and malabsorption. These conditions greatly contribute to the attendant weight loss and malnutrition. The thiol group of compounds that is situated in the albumin molecule and ascorbic acid provide the most important defence against oxidant injury (Doneke, 2000; Stenvinkel *et al.*, 1999). We hypothesize that the progressive HIV/AIDS disease (assessed by decline in the CD⁴⁺ cell count) and the consequent malnutrition, hypoalbuminaemia and low plasma total antioxidant level expose the PLWHA to enhanced oxidant injury. In addition, it is quite possible that the rapid deterioration and poor outcome of HIV infection in sub-Saharan Africa maybe linked to the pervading poverty, baseline under nutrition with both low dietary micronutrient and antioxidant intakes. The increased requirement of antioxidants by HIV infected persons only further aggravates the need for supplements.

This study supports some recent opinions that point to the benefits of increased intake of fruits providing micronutrient and antioxidants such as vitamins C (ascorbic acid) (Gil *et al.*, 2005). It is therefore not only safe but also justifiable to recommend the increased use of antioxidant vitamins such as vitamins C and E (alpha tocopherol) along with improved nutrition in the management of HIV/AIDS patients especially those who are residing in sub-Saharan Africa.

CONCLUSION

The total antioxidant status of persons infected with the human immunodeficiency virus was lower than that of a control group of apparently normal individuals. Moreover the total antioxidant status was depleted with the progression of the HIV/AIDS disease. The simple assay of TAS may complement other laboratory parameters in the staging and the monitoring of the progression of HIV infection in sub-Saharan Africa.

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