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Total antioxidant status and lipid peroxidation in HIV-1 infected patients in a rural area of south western Nigeria

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Summary

Total serum antioxidant status (TAS) and lipid peroxidation were evaluated in 62 HIV-1 seropositive patients before the commencement of antiretroviral drug therapy. Twenty-four (24) age-marched and apparently healthy HIV-1 seronegative subjects were recruited as control subjects. HIV-1 seropositive patients were classified according to the Centre for Disease Control and Prevention (CDC) Criteria. The mean serum malondialdehyde (MDA) concentrations of 5.58 ± 0.99nmol/ml and 4.24 ± 0.80nmol/ml were significantly elevated in HIV- 1 infected patients with CD4+ count of <200 lym/mm^3 and that of 200-499 lym/mm^3 respectively when compared with the control which is 3.37±0.56nmol/ml (P<0.001). Whereas when compared with control, the serum MDA concentration of 3.45 ± 0.48nmol/ml in HIV-1 patients with CD4+ count of >500 lym/mm^3 was not significant (*P*>0.05). In the intra groups comparison serum concentration of MDA were significantly elevated when compared with each other (*P*<0.001). The mean serum total antioxidant status (TAS) of 1.30 ± 0.11mmol/L, 1.12 ± 0.24 mmol/L and 0.95 ± 0.17mmol/L of HIV-1 seropositive patients with CD4+ count of >500 lym/mm^3, 200-499 lym/mm^3 and <200 lym/mm^3 respectively were significantly reduced compared with 1.69 ± 0.23mmol/L for the control group (*P*<0.001). Similarly in the intra groups comparison, serum concentration of TAS were significantly reduced when compared with each other (*P*<0.001). The weight of 54.81 ± 5.13 Kg for HIV-I seropositive patients with CD4+ count of <200 lym/mm^3 was significantly reduced when compared with 69.17± 4.38Kg for the control (*P*<0.05). Our results clearly show that severe oxidative stress occurs in the serum of seropositive patients in comparison with controls, and increases significantly with the progression of disease, i.e. AIDS > symptomatics > asymptomatics > controls. The inclusion of antioxidants in the therapeutic approach in managing HIV-1 seropositive patients will prevent the additional damage that free radicals could do to such patients.

Keywords: *Total antioxidant, lipid peroxidation, HIV-1/AIDS*

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Resume

Le status total du Sérum antioxydant (T,SA) et la peroxydation lipidique ont été évalués en 62 VHI-1 des patients séropositifs avant le début de la thérapie antirétrovirale des médicaments. 24 sujets séronégatifs d'ages -assortis et qui ne sont pas atteints du VIH-1 ont été recrutés sont des groupes témoins. Les patients ont été classes selon les critères du Centre de la Prévention et du Contrôle des maladies (CPC). Les concentrations moyenne du sérum malondialdéhyde (MDA) de 5,58+ - 0,99 nmol/ml et 4,24 + - 0,8, nmol/ml ont d'une façon significative élevée chez les patients infectée du VHI-1 avec CD4 + le calcul <200lym/mn3 et celui de 200-499 lym/mm3 respectivement, comparativement au contrôle qui est 3,37 + - 0,56nmol/ml (*P*<0,001). Pourtant, comparativement au contrôle, la concentration du sérum de 3,45+ - 0,48 nmol chez les patients de VHI-1 avec CD4 + calcul>500lym/mm3 n'était considérable (*P*>0,05). Dans la comparaison MDA était d'une façon significative élevée comparée entre chacun (*P*<0,001) Le status total du sérum antioxydant (STA) de 1,30+ - 0,11mn11/L , 01/L+ - 0,24 mmol/L et 0,95 + - 0,,17 mmol/L des patients séropositifs VHI-1 avec CD4 + calcul >500 lym/mm2, 200 -499lym/mm3 et 200lym/mm3 ont été réduits d'une manière significative, compare au 1,69 + - 0, 23mmol/L pour le groupe témoin (*P*<0,001). De la même façon, dans la comparaison intra groupes, la concentration du sérum du STA a été réduite d'une façon significative, comparativement entre chacun (P,0,001) Le poids de 54, 81 + - 5, 13kg pour les séropositifs VHI- avec CD4 + calcul <200lym/mm3 a été réduit d'une façon significative comparativement a 69,17+ -4, 38kg pour le contrôle (*P*<0,05). Nos résultats ont clairement montre que le stress oxydatif grave a lieu dans le sérum des patients séropositifs en comparaison aux contrôles, et des augmentations importantes avec une progression de la maladie. C'est a dire, le SIDA> symptomatiques>asymptomatiques>contrôles. L'inclusion des antioxydants dans l'approche thérapeutique dans la gestion VHI-1 des patients séropositifs préviendra le dommage additionnel que les radicaux pourraient faire aux patients pourraient faire aux patients.

Introduction

Reactive Oxygen Species (ROS), collectively described as free radicals (OFR) such as Superoxide (O_2^-), Hydroxyl (OH^-) and other non-radical oxygen derivatives such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOC1) play a significant role in the pathogenesis of many diseases such as atherosclerosis, diabetes mellitus, cancer, cardiovascular disease, liver disease, lung disease,

neurodegenerative disease, inflammatory and infectious diseases [1]. Free radicals are produced endogenously during cellular metabolism but their production may be greatly enhanced by exogenous factors like environmental pollutants, drugs, radiation and pathogens [2]. An increased free-radical generation has also been reported in several disease states [3]. During HIV infection, it has been suggested that an abundance of free radicals are produced in an environment in which the antioxidant compounds are progressively depleted [4]. A state of oxidative stress may contribute to several aspects of HIV disease pathogenesis, including viral replication, inflammatory response, decreased immune cell proliferation, loss of immune function, apoptosis, chronic weight loss and increased sensitivity to drug toxicities [4]. Oxidative stress has also been implicated in HIV-related muscle wasting and weight loss as well as dementia and neuropathy. The membranes on and within the immune cells contain a high percentage of polyunsaturated fatty acids (lipids) that are highly sensitive to oxidation [5]. The prime targets of ROS attack are the polyunsaturated fatty acids in the membrane lipids causing lipid peroxidation that may lead to disorganization of cell structure and function [6]. Decomposition of peroxidized lipid yields a wide variety of end products.

To circumvent the damage caused by ROS, multiple defence systems collectively called the antioxidants defence system is present in the human body [7, 8]. This system consists of substances that provide the much-needed stability to the free radical by allowing the pairing of electrons. Therefore protective efficiency of antioxidants in HIV-1/AIDS patients would depend on the balance between ROS and the availability of antioxidants themselves. The present study was therefore undertaken to evaluate the effect the Human Immunodeficiency Virus infection and the progression of AIDS on malondialdehyde (MDA), a marker of lipid peroxidation and total antioxidant status in HIV-1/AIDS patients and to determine if there is any correlation with the progression of the disease.

Materials and methods

The study was carried out after obtaining ethical approval from the authority of Daughter of Charity German Tuberculosis/Leprosy Center (NGO) at Iwaro-Oka, Ondo state, South-western Nigeria. A cross-sectional study was performed on sixty-two (62) HIV-1 infected subjects attending the center, the HIV-1 infected patients consist of 29 males and 31 females with the mean age of 31.7years (20 – 60) who were seropositive by HIV ELISA (Bio rad Greescreen 72375 HIV-1&11 Ag-Ab Batch no 1L0021) method [9,10]. The subjects were freshly diagnosed and had not commenced antiretroviral therapy. Twenty-four (24) control subjects were selected from healthy individuals matched for age and sex with mean age of 31.9years (19 – 59) who tested negative for HIV-1 and 11 and/or HCV infection.

Ten milliliter (ml) of venous blood was obtained without stasis from each subject after an overnight fast, 5mls into EDTA-anticoagulant bottle with minimum delay for estimation of absolute CD4 T lymphocyte using Dynabeads T4 quantification protocol (Dynal Biotech ASA Oslo, Norway) and HIV infected individual were classified according to the Centre for Disease Control and Prevention (CDC) Criteria [11] which states that; CD4+ T-cell counts >500 lymp/mm^3 asymptomatic, CD4+ T-cell counts 200-499 lymp/mm^3 symptomatic and CD4+ T-cell counts <200 lymp/mm^3 symptomatic with full blown AIDS.

The remaining 5mls of blood was put into plain bottle and the blood was allowed to clot and retract. Serum was obtained after centrifugation at 3500r.p.m for 10 minutes and immediately stored at -20°C until they were analyzed.Measurement of secondary products of lipid peroxidation such as malondialdehyde (MDA), often more correctly referred to as thio-barbituric reactive substances (TBARS), a simple and widely used assay [12], was employed to assess oxidative stress in this study. Malondialdehyde (MDA) was estimated according to the method of Satoh [13], which is based on the coupling of MDA with thio-barbituric acid. Measurement of total antioxidant status of serum was performed using a commercial kit from Randox Laboratories (Randox Laboratories Ltd, Diamond Road, Crumlin, Co. Antrim, Ireland) [14]. In this assay metmyoglobin reacts with H_2O_2 to form ferrylmyoglobin, a free-radical species. A chromogen 2,2-amino-dis-[3-ethylbenzthiazole sulfonate] is incubated with ferrylmyoglobin to produce a radical cation which has a blue-green colour and can be measured at 600 nm using Humalyzer 2000 analyzer. Antioxidants in the added serum cause suppression of this colour production proportional to their concentration. The assay was calibrated using 6-hydroxy-2,5,8-tetra-methylchroman-2-carboxylic acid (trolox). The results were expressed as mmo/L of trolox equivalent.

Statistics analysis: - Values were expressed as mean ± SD, the statistical significance of the mean differences between groups was assessed by Student's *t*-test and intra-group comparisons were made using one way analysis of variance (ANOVA). Method of correlation analysis (PEARSON) was also used to determine the degree of association between the variables of interest. A P value (<0.05) was regarded as significant.

Results

The MDA concentration (Table 1) was significantly elevated in the serum of HIV- 1 patients with CD4+ count of <200 lym/mm^3 and that of 200-500 lym/mm^3 (P<0.001) whereas there was no significant difference in HIV-1 patients with CD4+ count of >500 lym/mm^3 (P>0.05) when compared with the value in the control groups. Intra-group

Table 1. Biophysical data and serum concentration of MDA and total antioxidant status of HIH-1 positive and AIDS patients and control subjects

Parameters	Control (n= 24)	W_1 (n=20)	W_2 (n=24)	W_3 (n=18)	F-value	P
Age (years)	34±6.0	30±5.6	29±6.6	36±1.9	3.75	0.013
Weight (kg)	69.17±4.38	68.87±5.98	64.35±6.16	54.81±5.13	28.80	0.001
Height (m)	1.72±0.06	1.68±0.1	1.69±0.1	1.70±0.04	2.49	0.065
CD4 lymphcytes/mm3	972.25±167.12	601.46±101.39	391.91±127.42	189.87±67.21	147.48	0.0001
MDA (nmol/ml)	3.45±0.48	3.37±0.56	4.24±0.80	5.58±0.99	43.32	0.0001
Total antioxidant status (mmol/L trolox equivalent)	1.69±0.23	1.30±0.11	1.12±0.24	0.95±0.17	70.24	0.0001

W_1= Asymptomatic HIV infected subjects having a CD4 T lymphocyte counts > 500/mm³
W_2= Symptomatic HIV infected subjects having a CD4 T lymphocyte counts between 200 – 499 cells mm³
W_3= Symptomatic HIV infected subjects with full-blown AIDS having a CD4 T lymphocyte counts < 200 cells/mm³

Table 2. Showing the level of significance of Intra group comparison of CD4+ count, MDA and TAS in HIV- 1sero-positive with or without symptoms and HIV-1 sero-negative patients (controls).

Parameters	Con.Vs W_1	Con.Vs W_2	Con.Vs W_3	W_1Vs W_2	W_1Vs W_3	W_2Vs W_3
Age (years)	NS	NS	NS	NS	NS	NS
Weight (kg)	NS	NS	P<0.05	NS	P<0.05	P<0.05
Height (m)	NS	NS	NS	NS	NS	NS
CD4 lymphcytes/mm3	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001
MDA (nmol/ml)	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001
Total antioxidant status (mmol/L trolox equivalent)	P<0.0001	P<0.0001	P<0.0001	P<0.003	P<0.0001	P<0.0001

NS = Not significant.
Con = Control

comparisons showed that the serum concentration of MDA was significantly elevated in HIV- 1 patients with CD4+ count of <200 lym/mm³ when compared with HIV-1 patients with CD4+ count of 200-499 lym/mm³ (*P*<0.001). Similarly there is a significant increase in serum MDA concentration with HIV- 1 patients with CD4+ count of 200-499 lym/mm³ compared with HIV-1 seropositive patients with CD4+ counts of >500 lym/mm³ (*P*<0.001).

The intra-group comparisons showed that the mean serum concentrations of total antioxidant status (TAS) was significantly reduced in the HIV- 1 patients with CD4+ count of <200 lym/mm³ compared to that of CD4+ count of 200-499 lym/mm³ and HIV- 1 patients with CD4+ count of >500 lym/mm³ (*P*<0.001). Similarly there is a significant reduction in the mean serum concentration of TAS in HIV- 1 patients with CD4+ count of 200-499 lym/mm³ (*P*<0.001) when compared with HIV-1 patients with CD4+ count of >500 lym/mm³ (P<0.001). A positive correlation was observed (*P*<0.01, r= 0.077, n= 20) between lipid peroxidation and total antioxidant capacity in the serum of HIV-1 seropositive patients with CD4+ count of >500 lym/mm³, however a negative correlation was observed between lipid peroxidation and total antioxidant capacity in the serum of HIV-1 seropositive with CD4+ count of 200-499 lym/mm³ and that of <200 lym/mm³. (*P*<0.01, r= -0.121, n= 24); (*P*<0.01, r= -.0243, n= 18).

Discussion

During HIV infection, it has been suggested that an abundance of free radicals are produced in an environment in which the antioxidant compounds are progressively depleted [4]. Lipid peroxidation results from release of free radicals that can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes.

In this study, the concentration of serum malondialdehyde (MDA) which is the measure of lipid peroxidation was found to be increased in HIV-1 seropositive patients with CD4+ count <200 lym/mm³ and that of 200-499 lym/mm³ but there is no significant changes in those with CD4+ >500 lym/mm³ compared to the control, this finding is simi-

lar to the studies of Favier et al [15] and Jaruga et al [16] that there is increased lipid peroxidation in HIV- infected patients and lipid hydroperoxides induce apoptosis in T-cells. Tumour necrotic factor alpha (TNF-á) also plays an important role on the lipid peroxidation of blood plasma as it induces the production of ROS by polymorphonuclear leukocytes [17]. In addition to inducing lipid peroxidation, ROS activate the nuclear transcription factor NF-kB, which is obligatory for HIV replication in CD4+ lymphocytes [18]. Lipid peroxidation results from release of free radicals that can cause tissue damage by reacting with polyunsaturated fatty acid in cellular membranes. Free radicals can also cause oxidation of low-density lipoprotein (LDL) cholesterol [19] in the absence of protection provided by the antioxidants [20].

There was marked reduction in the serum total antioxidant of HIV-1 seropositive individual in this study, the degree of reduction was related to the degree in the reduction in CD4+ count which is the marker of the level of immunity in HIV-1 seropositive individual. Repetto et al [21] in their study reported that there was an oxidative stress in the blood of their HIV infected patients. During HIV infection, it has been suggested that an abundance of free radicals are produced in an environment in which the antioxidant compounds are progressively depleted [4]. There is negative correlation between MDA and total antioxidant status in serum samples of HIV patients ($P<0.001$, $r=-0.121$) and ($P<0.001$, $r=-.0243$) with CD4+ count of 200-500 lym/mm^3 and <200 lym/mm^3 respectively. In healthy conditions a balance exists between free-radical generation and antioxidant defence system that prevents occurrence of disease. However this study implies that HIV infection shifts the balance in favour of free radical generation that leads to oxidative tissue damage.

Since oxidative stress occurs to some degree after every tissue injury, the main question to be asked in evaluating its role in human disease is not 'can we demonstrate oxidative stress?' but rather 'does the oxidative stress that occurs make a significant contribution to disease activity?' Our results clearly show that severe oxidative stress occurs in the serum of HIV seropositive patients in comparison with healthy age and sex matched controls, and that this increases significantly with the degree of immune deficiency, i.e., AIDS > symptomatic > asymptomatic > controls.

Conclusion

In view of our present findings, a management strategy aimed at simultaneous control of lipid peroxidation and total antioxidant status in the serum of HIV-1 seropositive patients may be beneficial to avoid the damage that can be caused by free radicals. Since HIV infection and lipid peroxidation both directly relate to the severity of HIV infection, elimination of free radicals in the plasma before they can be taken up by the peripheral tissues might improve the prognosis for patients. All patients with HIV infection should be screened for nutritional problems and concerns at the time of their first contact with a health care professional, and routine monitoring should be performed on an ongoing basis.

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