Changes in antioxidant profile among HIV-infected individuals on generic highly active antiretroviral therapy in southern India

Muthu Sundaram a, Suneeta Saghaya a, Bhaskar Priya a, Kartik K. Venkatesh b, Pachamuthu Balakrishnan a, Esaki Muthu Shankara a, Kailapuri G. Murugavela a, Suniti Solomon a, Nagalingeswaran Kumarasamy a,*

a Infectious Diseases Laboratory, YRG Centre for AIDS Research and Education (YRG CARE), VHS Hospital Campus, Rajiv Gandhi Salai, Taramani, Chennai 600 113, India
b Brown University Medical School, Providence, Rhode Island, USA

Received 10 October 2007; received in revised form 26 March 2008; accepted 14 April 2008
Corresponding Editor: William Cameron, Ottawa, Canada

KEYWORDS
Antioxidants; Oxidative stress; HIV; Generic HAART; India

Summary
Objective: The role of oxidative stress in disease progression has been shown to be more complicated in HIV-infected individuals receiving highly active antiretroviral therapy (HAART) compared to those who remain treatment-naïve. This study examined the changes in the antioxidant profile of HIV-infected subjects who remained HAART-naïve due to a high CD4 cell count and HIV-negative controls, over a 12-month follow-up period at YRG CARE, a tertiary HIV referral centre in southern India.

Methods: We prospectively studied 35 HIV-infected participants (18 on d4T+3TC+EFV ( stavudine + lamivudine + efavirenz), eight on AZT+3TC+EFV ( zidovudine + lamivudine + efavirenz), and nine who were antiretroviral therapy-naïve) and 20 HIV-negative controls. Antioxidant profile (total antioxidant status, glutathione reductase, glutathione peroxidase, uric acid, ceruloplasmin, zinc, and albumin), CD4 cell count, plasma viral load, dietary intake, and history of smoking and alcohol use were determined at baseline and at twelve months.

Results: At 12 months, participants on HAART showed a significant increase in glutathione peroxidase (baseline: 1765 vs. 12 months: 2850 U/l; p < 0.001) and albumin (3.6 vs. 4.4 g/dl; p < 0.001), and a significant decrease in glutathione reductase (52.6 vs. 50.5 U/l; p = 0.054) and uric acid (5.4 vs. 4.8 mg/dl; p = 0.027) compared to baseline. Also HAART-naïve participants had a significant increase in albumin (baseline: 3.7 vs. 12 months: 4.3 g/dl; p = 0.023) and a significant decrease in zinc levels (baseline: 79.0 vs. 12 months: 74.5 µg/dl; p = 0.052) from baseline to 12 months. HIV-negative subjects had a significant increase in glutathione reductase at 12 months from baseline (baseline: 37 vs. 12 months: 39 U/l; p = 0.002). No significant difference in total

* Corresponding author. Tel.: +91 44 22542929; fax: +91 44 22542939.
E-mail address: kumarasamy@yrgcare.org (N. Kumarasamy).

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doi:10.1016/j.ijid.2008.04.004
Introduction

HIV disease results in considerable morbidity and mortality due to opportunistic infections (OIs) that can be effectively managed with the prompt initiation of highly active antiretroviral therapy (HAART) in HIV-infected individuals. However, recent studies have documented the possibility of increasing toxicities and metabolic changes with the long-term use of HAART. Cells contain several enzymatic and non-enzymatic antioxidants to prevent or limit reactive oxygen species (ROS)-associated damage. The defense system involves many compounds with vastly different properties: enzymes (superoxide dismutase, catalase, glutathione peroxidase), macromolecules (albumin, ceruloplasmin), and small molecules (vitamin C, vitamin E, b-carotene, reduced glutathione, and uric acid).

ROS may enhance viral replication by activating nuclear transcription factors such as nuclear factor-kB (NF-kB), which might ultimately lead to an increased rate of viral gene expression. In the pre-HAARTera, several studies found that both asymptomatic and symptomatic HIV-infected individuals are at increased risk of oxidative stress, including raised plasma metabolites of lipid peroxidation (LPO) and reduced antioxidant levels, compared with non-HIV-infected individuals. Certain micronutrients may play a key role in maintaining normal immune functions that may protect immune effector cells from oxidative damage.

As the natural history and disease progression of HIV can vary across ethnic and racial groups, it is necessary to identify the exact role of oxidative stress and changes in the antioxidant profile among the HIV-infected on HAART in southern India. The present study was undertaken to examine the changes in antioxidant profile among HIV-infected participants on generic HAART, participants who remained HAART-naive, and HIV-negative controls.

Patients and methods

Setting and subjects

This prospective study was conducted at YR Gaitonde Centre for AIDS Research and Education (YRG CARE), a non-profit HIV referral centre in southern India, providing medical and psychosocial care to over 10,000 individuals with HIV infection. A total of 35 HIV-infected subjects (26 on HAART, nine HAART-naive) and 20 HIV-negative controls were included in the study. All the participants were involved in other clinic-based research studies. Study participants from prior studies were selected due to limitations in funding. All the laboratory investigations were done at baseline and at 12 months. Dietary data were collected using a 24-h recall, and the nutritive value was calculated using indigenous software ‘DIGEST’.

HIV-infected participants met the following inclusion criteria: >18 years of age, HIV-positive, antiretroviral therapy (ART)-naive prior to initiating HAART, and without any active OIs. HIV-infected participants who remained naive did not require HAART due to their high CD4 count. HIV-positive participants were either teetotalers or had quit smoking and drinking on knowing their HIV status. All HIV-infected participants were on vitamin and mineral supplementation.

HIV-negative control participants had no serological evidence of HIV and/or HCV-infection, were not smokers or drinkers, had no abnormal laboratory findings (complete blood count, AST and ALT levels within normal range), and had normal blood pressure (120/80 mmHg), a body mass index (BMI) in the range 18.6–25.2 kg/m², normal chest X-ray, and normal electrocardiogram.

The YRG CARE institutional review board approved the study protocol. Written informed consent was obtained from all the individuals enrolled in the study.

Specimens

To analyze serum albumin, uric acid, total antioxidant status (TAS), ceruloplasmin, and glutathione reductase (GR), 5 ml of peripheral blood was drawn after an overnight fast. Additionally, 3.5 ml of blood was collected into EDTA-containing tubes (BD VacutainerR⃝, BD, Franklin Lakes, NJ, USA) to determine glutathione peroxidase (GPx). Two aliquots of serum and plasma were stored at –80°C, protected from light, and thawed only once.

Immunological and virological markers

Plasma viral load (PVL) was determined by the Cobas Ampli-cor HIV-1 Monitor Assay version 1.5 (Roche Molecular Systems, Branchburg, NJ, USA); results below the limit of detection were assigned a value of <400 copies/ml. Absolute CD4 T-lymphocyte counts were determined using the Guava Personal Cell Analyzer (Guava Technologies, Inc., USA).

Estimation of antioxidant levels

TAS, GR, GPx, and ceruloplasmin levels were measured using commercial kits procured from Randox Laboratories, UK, and Aptec Diagnostics, Belgium.

The TAS test measures the total antioxidant effect; ABTS (2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonate)) reacts with all of the defense systems in the circulation, namely superoxide dismutase (SOD), GPx and metal-binding protein (e.g., ceruloplasmin), uric acid, albumin, and DNA repair enzymes. For TAS quantitation, Randox kit Cat. No. NX2332 (Randox Laboratories Ltd, Crumlin, UK) was used.
Evaluation of GPx activity was determined using Randox kit Cat. No. R5014 (Randox Laboratories Ltd). GPx reduces H$_2$O$_2$ to H$_2$O by oxidizing glutathione (GSH). Re-reduction of the oxidized form of glutathione (GSSG) is then catalyzed by glutathione reductase. The decrease in absorbance at 340 nm was measured.$^{10}$

The activity of GR was determined by using Randox diagnostic reagents (GR 2368; Randox Laboratories Ltd).

The level of ceruloplasmin was determined by immunoturbidometric method using an Olympus AU 400 autoanalyzer (Olympus Optical, Japan). It has been shown that ceruloplasmin, a copper-dependent acute phase protein, has antioxidant functions that can prove beneficial in several pathological conditions.

The level of uric acid was determined using Raichem diagnostic reagents (Hemagen Diagnostics Inc., San Diego, CA, USA) by uricase method.

It has been shown that albumin levels <3.0 g/dl are associated with decreased survival in HIV-infected individuals. Serum albumin was estimated using Olympus diagnostic reagents (Olympus Diagnostica, GmbH, Ireland) by bromocresol green (BCG) method.

Low levels of plasma zinc predict a three-fold increase in HIV-related mortality. Zinc acts as an antioxidant or helps such functions, which not only regulate the immune responses of the host, but may also alter the genome of the viruses. Zinc was estimated using Chema diagnostic reagents (Chema Diagnostica, Italy) by photometric method.

**Statistical analysis**

Descriptive statistics, Chi-square statistics, and the Mann—Whitney U-test were used. Statistical analyses were performed with Statistical Package for Social Sciences software (SPSS, version 13.0, Chicago, IL, USA). A $p$ value of less than 0.05 was considered statistically significant.

**Results**

Among the 35 HIV-infected subjects investigated, 26 were treated with HAART and nine were HAART-naïve; there were 20 HIV-negative controls. The mean age in the HAART-experienced, HAART-naïve, and the control groups was 32 ± 7.1, 29 ± 6.3, and 29 ± 6.0 years, respectively. Over two-thirds (65%) of HAART-experienced participants, 44% of HAART-naïve participants, and 50% of controls were male. The baseline characteristics of the study population are shown in Table 1.

At baseline, the median BMI was 16.9, 19.9, and 21.2 kg/m$^2$ in the HAART-experienced, HAART-naïve, and the control groups, respectively. HAART-experienced participants had a median CD4 count of 167 cells/$\mu$l (interquartile range (IQR) 116–219) and a higher viral load of 286 500 copies/ml (IQR 117 500–696 710) compared to HAART-naïve participants who had a median CD4 of 418 cells/$\mu$l (IQR 293–624) and viral load of 22 400 copies/ml (400–193 000). Also at baseline, HAART-experienced participants had a higher mean calorie intake of 2879 kcal and a protein intake of 94.1 g than the HAART-naïve participants whose calorie intake was 2320 kcal and protein intake 75.1 g. Among the HAART-experienced group, 69% of participants received d4T+3TC+EFV ( stavudine + lamivudine + efavirenz) and 31% received AZT+3TC+EFV ( zidovudine + lamivudine + efavirenz).

The antioxidant profiles of all three groups are shown in Table 2. At baseline, albumin (3.6 vs. 3.7 vs. 4.8 g/dl) and zinc (71.0 vs. 79.0 vs. 92.5 mg/dl) were significantly lower and GR (52.6 vs. 46.0 vs. 37.0 U/l), uric acid (5.4 vs. 4.8 vs. 4.0 mg/dl) were significantly lower in the HAART-naïve group compared to the HAART-experienced and control groups.
4.1 mg/dl), and ceruloplasmin (31 vs. 25 vs. 26 mg/dl) were significantly higher among the HIV-infected participants who were at a more advanced stage of HIV disease and about to initiate HAART as compared to the HIV-infected participants who would remain ART-naive and the HIV-negative controls.

Participants in the three groups did not experience significant changes in TAS from baseline to 12 months. From baseline to 12 months, GPx significantly increased (1765 U/l to 2850 U/l) in the HAART-experienced group ($p < 0.001$). Additionally from baseline to 12 months, HAART-experienced participants underwent a significant decrease in their uric acid levels (5.4 mg/dl to 4.8 mg/dl; $p = 0.027$).

From baseline to 12 months, HAART-experienced participants experienced a significant increase in their median albumin levels (3.6 g/dl to 4.4 g/dl; $p < 0.001$). Similarly HAART-naive participants experienced a significant increase in their median albumin levels (3.7 g/dl to 4.3 g/dl; $p = 0.002$) and significant decrease in their median zinc levels from baseline to 12 months (baseline: 79.0 vs. 12 months: 74.5 mg/dl; $p = 0.052$). At 12 months, GR significantly increased in the control group (37 U/l to 39 U/l; $p = 0.002$) and significantly decreased in the HAART-experienced group from baseline (52.6 U/l to 50.5 U/l; $p = 0.054$). HAART-experienced participants and HIV-negative controls did not experience significant changes in their median zinc and ceruloplasmin levels from baseline to 12 months. There was also no significant difference in the dietary intake of the HIV-infected subjects from baseline to 12 months.

**Discussion**

This study documents alterations in the levels of GPx, albumin, GR, and uric acid between baseline and 12 months among HAART-experienced participants. Studies from the developed world have documented biochemical changes that could be a result of greater utilization of antioxidant micronutrients subsequent to increased oxidative stress rather than inadequate dietary intake or malabsorption, inadequate nutrient release from the liver, acute infections, and/or inadequate availability of carrier molecules that may influence circulating antioxidant concentrations.

This study suggests that enhanced oxidative stress and disturbed glutathione metabolism occur after initiating HAART. These effects appear to be related to persistent tumor necrosis factor-α (TNF-α) activation in HIV-infected patients. HIV-infected individuals have been shown to have decreased antioxidant concentrations, disruption in glutathione metabolism, and enhanced spontaneous generation of ROS. Furthermore, a significant decrease in GR levels was documented in treated patients at 12 months. To be cognizant, lower GR levels and oxidative stress in general are known to upregulate inflammatory cytokine activities in patients receiving HAART. HIV-infected individuals can have significantly higher GPx activity than HIV-uninfected individuals. We found that HIV-infected individuals receiving HAART experienced increased levels of GPx over time. GPx plays an important role in the metabolism of ROS as a defense mechanism against oxidative damage by catalyzing the reduction of a variety of hydroperoxides via glutathione as a defense mechanism against oxidative damage by catalyzing the reduction of a variety of hydroperoxides via glutathione peroxidase.
the reducing substrate. In addition to its role as a substrate in the GSH redox cycle, glutathione also acts as a direct endogenous scavenger of hydroxyl radicals, and is involved in the detoxification and metabolism of a number of chemicals and drugs in the liver. The changes observed in the current study therefore reveal that antioxidant supplementation, including glutathione, might have an effect on oxidative stress in HIV-infected individuals. Antioxidant enzyme levels are sensitive to oxidative stress; alterations were observed in the present study, which prove the cell damage and weakened antioxidant defense in HIV-infected individuals.

The current study found that HIV infection can increase the oxidative stress process, which is then further increased by HAART usage; this finding is in line with other studies. In HIV infection, ROS species may enhance viral replication by activating nuclear transcription factors, which ultimately could lead to viral gene expression. The deficiency of total antioxidant status might markedly increase oxidative stress, possibly adversely affecting the immune response and predisposing to drug toxicity.

The current study found significant changes in antioxidants such as GR, GPx, uric acid, and albumin among HAART-experienced participants over a period of time. Supplementation with glutathione or antioxidants may improve immunological and virological indices in HIV-infected individuals. These agents may positively act on the individuals’ immunological status and decrease HIV replication. Further, a greater understanding of the formation of ROS and the subsequent oxidative stress is important for effective clinical care of HIV-infected individuals. Clinical guidelines that indicate cellular damage due to oxidative stress need to be established and monitored during HAART treatment. Current drug therapies and other therapeutic measures such as administration of N-acetylcysteine supplements, may improve the quality of patient care, in addition to diet counseling.

This study demonstrates that changes in antioxidants are observed among HAART-experienced participants in the southern India setting. Further long-term prospective studies on a large population must be designed to substantiate and evaluate the relationship between antioxidants and disease progression in persons receiving HAART among HIV-infected individuals, especially in resource-limited settings.

This study had certain limitations viz. limited funding, small sample size, and inadequacy of follow-up (for 12 months). Furthermore, there were certain constraints with regard to including more parameters related to oxidative stress, largely due to lack of funds. The analytical stability of the kits and pre-analytical variables were also a challenge. In addition, the study was probably underpowered to detect some of the parameter changes over a period of time.

Acknowledgements

The authors gratefully acknowledge the statistical assistance of Ms Anitha J. Cecelia, and the clinical and laboratory staff of YRG CARE. This paper was presented at the 8th International Congress on AIDS in Asia and the Pacific (ICAAP), Colombo, Sri Lanka, August 2007 (Abstract No. 1602).

Conflict of interest: The authors declare no conflict of interest for the present study.

References

