ORIGINAL ARTICLE

Antiretroviral concentration, redox indexes and progression markers in HIV-infected patient's blood samples follow-up during six month

Rosario G. Hernandez[1], Ivon G. Blanco[2], Alicia T. Reyes[1], Daniel P. Correa[1], Dayme H. Requejo[1], Yusimit B. Alfonso[1], Lizette G. del Valle[1], Olga S. L. Fernandez[2]

[1]"Pedro Kouri" Tropical Medicine Institute; [2]Institute of Pharmacy and Food, Havana University; Havana, ***Cuba***

Received March 5, 2014
Accepted March 21, 2014

Published Online April 28, 2014

DOI 10.5455/oams.210314.or.060

Corresponding Author
Lizette Gil del Valle
Clinical Pharmacology Department,
"Pedro Kouri" Tropical Medicine Institute (IPK),
P.O. Box 601, Marianao 13,
La Habana, Cuba.
lgil@ipk.sld.cu

Key Words
Antiretroviral;
Human immunodeficiency virus;
Redox indexes

Abstract

Objective: Human immunodeficiency virus (HIV) infection is accompanied by metabolic and immunological dysfunctions. Oxidative stress has been associated to HIV infection, and also its relation to highly active antiretroviral therapy (HAART) is suggested. This study assessed the effect of HAART combination (zidovudine/lamivudine/nevirapine) on redox indexes (RI) and progression markers of disease while monitoring also drugs concentration in blood.

Methods: Eighty HIV subjects (40 taking HAART and 40 non-HAART) and 40 supposedly healthy voluntaries (SHV) were recruited. Blood antiretroviral drug concentrations were assessed by high performance liquid chromatography for each patient. Also peroxidation potential (PP), glutathione, malondialdehyde, hydroperoxides, superoxide dismutase (SOD), catalase (CAT), advanced oxidation protein products, viral load (VL) and CD4 T lymphocyte subsets were measured at baseline and at 6 months.

Results: Drug concentration follow-up verified compliance of therapeutic range in 76% and 79% of patients studied at baseline and at 6 months without statistical difference. The comparison between SHV and HIV patients groups showed significant differences in almost all RI (except in SOD activity). Significantly modified values were found in almost all RI (except in CAT activity and PP) at 6 months compared to the baseline. Non-significant differences were found between HIV-infected patients with respect to CD4. 78% of patients receiving treatment showed a VL reduction.

Conclusion: The confirmation of therapeutic range and its benefits in HIV-infected patients combined with additional oxidative stress impact promoted an integral view approach to follow-up the infection.

INTRODUCTION

Human immunodeficiency virus (HIV) infection has worldwide proportions with long-term significance [1, 2]. The HIV infection is characterized by numerical and functional impair in CD4 cells, which result in progressive immunodeficiency. The complex immune dysfunction in HIV-positive individuals predisposes them to both pathogenic and opportunistic infections [3, 4].

The most significant advance in the medical management of HIV infection has been the treatment of patients with antiretroviral (ART) drugs. In the latest years, a relevant decline of the morbidity and mortality of HIV infection has been observed due to the use of combined therapy named high active antiretroviral therapy (HAART) [5-8]. This treatment can suppress HIV replication to undetectable levels (<50 copies/ml) and improve the immune function in patients, specially CD4 T lymphocyte subsets, having as a consequence a decrease of infectious complications and a global clinical improvement [2, 6].

To ensure an optimal net benefit, the HAART effects need to be follow-up with individually dose adjustments accordingly. Therapeutic drug monitoring (TDM) should be evaluated for rapidly achieving and adequately maintaining serum levels in between effective range. TDM is generally assessed by high performance liquid chromatography (HPLC) and allows maximizing the probability of a successful outcome and minimizing the probability of toxicity and resistance. Adherence can also be tested or verified using repeated TDM. In order to relate any or some effect to the chronic use of ART, it is necessary to verify its bioavailability in therapeutic range [9-13].

But HAART does not completely solve the immune and metabolic alterations during HIV evolution [7, 14-16]. Instead of hepatic toxicity from ART reported early in the epidemic, recent reports continue to point out the mitochondria as toxic target and oxidative stress as consequences of the therapy [17-22]. Since HAART does not completely eliminate HIV, it is likely that the final outcome of treatment will depend not only on the

efficacy of treatments in reducing viral load (VL), but also on the immune system ability to recover and control residual virus [23]. Previous studies have suggested a role of oxidative stress in the stimulation of HIV replication, the development of immunodeficiency and also in the consequences of treatment [24-28].

Oxidative stress can be defined as an imbalance between the oxidant and antioxidant system, with predominance of the former: a variety of enzymatic and non-enzymatic antioxidants present in human serum become insufficient to avoid cellular reactive oxygen species (ROS) interaction. ROS are linked to various physiological processes too. In order to maintain a state of homeostasis; living organisms are striving to keep those highly reactive molecules under tight control with the help of an intricate system of antioxidants. ROS are deeply involved in both arms of the immunological defense system, the innate and the acquired responses. Its generation represents one of the first lines of defense mounted against the invading pathogens and constitutes essential protective mechanisms that the living organisms use for their survival. Some physiological roles in immune defense, antibacterial action, vascular tone and signal transduction are argued. ROS over-production in viral infections is generally related to pro-oxidant effect of inflammatory cytokines and/or polymorphonuclear leukocyte activation exerting also intracellular signal transduction cascades within cells and thereby up-regulating the cellular response; in regulatory mode, also contribute to decrease the cell activation threshold [23, 29-34].

Several researchers have demonstrated that humans infected with HIV are under chronic oxidative stress characterized by perturbations of the antioxidant defense system. Those include changes in glutathione (GSH), thioredoxin, superoxide dismutase (SOD), catalase (CAT), ascorbic acid, glutathione peroxidase (GPx), tocopherol and selenium levels. In addition, elevated levels of hydroperoxides (HPO) and malondialdehyde (MDA) were found in both pediatric and adult patients [3, 25, 35-37].

In HIV-infected patients treated with HAART, the role of oxidative stress in disease progression has become intricate. Virus control with HAART may not, as one might expect, reduce oxidative stress levels; on the contrary, it may increase oxidative stress [14, 15, 17, 22, 28, 38].

Combinations of anti-HIV drugs containing nucleoside reverse transcriptase inhibitors (NRTI) are used during HIV infection evolution as clinical guidelines recommended. Additional adverse effects and/or regimen adherence difficulties have serious consequences such as loss of serum HIV suppression, development of drug-resistant HIV strains and increased probability of developing opportunistic illness [6, 12, 15, 39]. NRTI are associated with lactic acidosis, hyperlipidemia, glucose intolerance, diabetes mellitus, atherosclerosis, fat redistribution and wasting syndrome; all of these could be related to its toxicity by increasing oxidative stress [4, 21, 40]. The phophorylated NRTI mitochondrial toxicity may amplify some of the pathophysiologic and phenotypical events in infection [7, 41, 42].

The study and enhancement of surrogate markers of HIV disease progression continues to be an important area of research particularly with the advent of therapies that claim to halt or slow the process of immunological decline [4, 43-45]. Additional markers and combinatorial analysis that add value to CD4 T lymphocyte subset would therefore be useful, *i.e.* in the decision of when to start/stop or change therapy [22, 41, 46-48].

Considering the above backgrounds, this study follows ART concentration and redox indexes in relation to VL and CD4 T lymphocyte subsets in blood samples of HIV patients with HAART in order to explore simultaneous variables processing and contributing to the existing results.

MATERIALS AND METHODS

Study design and ethical consideration

An observational cohort study was designed enrolling HIV-infected (asymptomatic and AIDS patients) and uninfected subject. All the patients were attended at the out-patients clinic at the Institute "Pedro Kouri" Hospital. They all gave written informed consent to take part in the study after verbal and written explanation of the methods and risks involved were given. The work was developed by a multidisciplinary group, including clinical experts in HIV/AIDS management. Procedures and protocols were previously reviewed and approved by the Institute "Pedro Kouri" Ethical Committee for Research on Human Subjects. The study is in accordance with the principle of the Declaration of Helsinki and its modification in 2004 and 2008 and also national and international regulations concerning the Ethical Principles for Medical Research Involving Human Subjects, Clinical Practices and Drugs Quality [49, 50].

Subjects and methods

Subjects were eligible if they had no active opportunistic infection. Exclusion criteria were: smoke habits, initiation of antioxidant vitamin therapy prior to study, hiperlipidemia, diabetes, kidney/liver dysfunction, intractable diarrhea (at least six liquid stools daily), vomiting or evidence of gastrointestinal bleeding. All patients had not family history of coronary heart disease. They were recruited

 DOI 10.5455/oams.210314.or.060

sequentially. Doses were in accordance also of World Health Organization (WHO) guidelines and were as follows: azidothymidine (AZT, zidovudine), 300 mg twice daily; lamivudine (3TC), 150 mg twice daily; nevirapine (NVP), 200 mg twice daily. This ART combination was the most frequently prescribed with 49.8% of total treated AIDS patients in Cuba. The compliance with the HAART intake was verified each month recovering the pills not taken in its flask before receives the new one.

Eighty subjects were HIV+; 40 of them had no started HAART and 40 were approved to take HAART for 6 months. Subjects were advised to continue their normal activity and to report any unusual symptom. Physicians reported HIV-associated opportunistic and other infections in the clinical charts during 6 months once the study began.

Data collection

One hundred twenty subjects ranging from 30-50 years of age were included. All subjects were assessed at their initial clinic visit and 6 months later. AIDS cases were eligible for starting antiretroviral treatment during 2011-2012 according to the health national policy and were assessed 14 days after treatments start. Patients underwent an initial screening, which included the evaluation of their medical, diet, and supplemental intake history, anthropometrics data (weight, height, *etc*), and review of clinical lab results (complete blood analysis, glucose, creatinine, urea, liver enzymes). This screening was done at the 6 months of study too.

Laboratory analyses

Blood and serum samples from the 120 subjects were processed. The blood was sampled at least 12 h after fasting. The serum samples were stored at –25ºC until the analyses were carried out.

HIV positiveness was diagnosed by a reactive enzyme-linked immunosorbent assay (ELISA) for HIV (Vironostika Uni-Form I/II Plus O; Organon), which was confirmed by positive Western Blot analysis (DAVIH Blot VIH-I).

Flow cytometry analysis

A study of $CD3^+/CD4^+$T-lymphocytes subsets, in total blood with lysing solution (Becton, Dickinson & Co; BD) was carried out. For each T lymphocyte subsets Tritest™ (BD) CD3/CD4 were used. These analyses were performed on a fluorescence-activated cell sorting (FACS) flow cytometry (BD) [51].

Viral load

VL was determined with polymerase chain reaction and nucleic acid sequence-based amplification (PCR-NASBA; Biomerieux) ultrasensitive assay with the lower limit of quantification of 50 IU.

Antretroviral Quantification

Apparatus and HPLC-conditions

The HPLC system which consists of a stainless steel gradient pump, a diode-array detector, a degasser and a multimode autosampler (all from Konik-Tech; Barcelona, Spain) was employed. The ultraviolet detector was used to monitor the drugs at wavelengths of 271 nm (3TC), 267 nm (AZT) and 265 nm (NVP). The mobile phase was different for each drug and was vacuum degassed before use. Acetonitrile and water (9:91, v/v) at a flow-rate of 0.7 ml/min was used for 3TC. Methanol and water (20:80, v/v) at a flow rate of 1.2 ml/min for AZT [52], and buffer phosphate (pH 5.5) and acetonitrile (80:20, v/v) with 0.2% triethylamin at a flow rate of 1.2 ml/min for NVP [53]. A C18 reversed-phase column, 250 x 4 mm (inside dimension) with 5 µm particle size (Merck, Darmstadt, Germany) packing was used.

Samples preparation and quantification

HIV subject's samples were routinely heated at 57ºC for 40 min to inactivate the virus prior to handling [54, 55]. The solid-phase extraction cartridges (C18 LiChrolut®, 1 ml, 100 mg) were purchased from Merck. It was used for the extraction of the 3TC and AZT concentration conditioning with 2 ml of methanol and 2 ml of bidistilled water before 500 µl of patient's plasma samples were loaded into the column and were allowed to pass through the bed with minimal suction followed by 500 µl of bidistilled water and collected in 500 µl of methanol. The eluent was evaporated to dryness under a nitrogen stream at 40ºC, the residue was reconstituted in the mobile phase (200 µl), and an aliquot (50 µl) was injected onto the HPLC system.

The NVP sample pre-treatment consisted of protein precipitation (500 µl plasma sample) with 250 µl of tricloroacetic acid at 20%, mixed on a vortex mixer for 1 min and then centrifuged at 10,000 rpm for 5 min and an aliquot (50 µl) of the supernatant was injected onto the HPLC system.

An automatic injector system facilitated the samples injections. Plasma drug concentrations were quantified by isocratic HPLC [52, 53, 55].

Biochemical measurements

Glutathione: the method described by Tietze [56], was used for the quantification of reduced GSH in serum. GSH (Sigma; St. Louis, MO, USA) was used to generate standard curves.

Malondialdehyde: MDA concentrations were analyzed by stable chromophore production after 40 min of incubation at 45°C. It was spectrophotometrically measured at a wavelength of 586 nm. Freshly prepared solutions of malondialdehyde bis(dimethyl acetal) (Sigma) assayed under identical conditions

were used as reference standards. Concentrations of MDA in serum samples were calculated using the corresponding standard curve and values were expressed as nmol/g-hemoglobin (Hb) [57].

Peroxidation potential (PP): for the determination of the susceptibility to lipid peroxidation, serum samples were incubated with a solution of cupric sulphate (final concentration of 2 mM) at 37ºC for 24 h. The PP was calculated by subtracting the MDA concentration at zero time from the one obtained at 24 h [58, 59].

Total hydroperoxide: HPO was measured by the assay based on the oxidation of ferrous ions to ferric ions by hydroperoxides under acidic conditions. The ferric ions binds with the indicator dye xylenol orange (3,3'-bis(N,N-di(carboxymethyl)-aminomethyl)-o-cresolsulfone-phatein, sodium salt) to form a stable colored complex, which can be measured at 560 nm [60].

Superoxide dismutase: evaluation of SOD activity was determined by the method which employs xanthine and xanthine oxidase to generate superoxide radicals, which in turn reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltretazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is then measured by the inhibition of this reaction [61].

Catalase: CAT activity was measured using a molar extinction coefficient of 43.6 M/cm, the rate of the first 30 s was used to calculate the activity. CAT activity was expressed as U/mgHb [62].

Advanced oxidation protein products: serum AOPP was measured according to the method of Witko-Sarsat *et al* [63]. The values were expressed in chloramines-T equivalents and corrected by serum albumin concentrations.

Unless otherwise stated, all chemicals were obtained from Sigma Chemical Company.

Data analysis and statistics

Data are expressed by mean ± standard deviation (SD). The normality of variables was evaluated by the Kolmogorov-Smirnov test. The variance homogeneity was evaluated by the Levene test. Comparison of patients' variables related to baseline and 6 months with HAART respect healthy people group and asymptomatic HIV group were assessed using analysis of variance (ANOVA). Statistical significance was defined as $P < 0.05$.

Second, simultaneous change of markers combination during treatment was analyzed by three ways in each patient. For that the difference between parameters at baseline and 6 months for the treatment group was computed. Success (positive) was considered every time modification of any indicators (VL $\leq$ 250 IU; MDA, HPO, PAOP $\leq$ 1 U; PP $\geq$ 1 U) show beneficial effects. First variable include positive change in VL. The second combinatorial variable evaluates positive change in antioxidant system and VL (simultaneous beneficial change in VL and PP). The third combinatorial variable evaluates beneficial change in antioxidant system, VL and damage of biomolecules (simultaneous positive change in PP, VL, HPO, AOPP and MDA). For each patient global success was considered every time as a simultaneous change was positive, but if at least one of the parameters result in negative modification it was considered as a global failure. Frequency of global success was reported for each group. An exploratory factorial analysis was performed combining redox and progression indexes [64, 65]. The SPSS software version 20.0 was used for all statistical analyses.

RESULTS

The baseline characteristics of the 120 subjects are to see in Table 1. There were no statistical significant differences ($P > 0.05$) between the groups at baseline with respect to demography, gender and number of participants.

Subjects continue their normal activity and physicians follow-up reported on clinical charts showed the concurrence of characteristic symptoms for those using antiretroviral drugs (nauseas in 10, sleeping in 6, stomach ache in 8, and headache in 13 patients out of 40, *etc*) during the first two months for almost all patients. Fourteen treated patients were given metamizole 300 mg (non-steroidal anti-inflammatory drug) during consecutively four days in a frequent of 3 times a day in different dates during the first two months. Dolor symptoms were ameliorated. No other drug was used in patients to treat any morbidity during the 6 months of the study.

The mean value of each ART concentration from two extractions and their therapeutic range are shown Table 2. The comparison between concentrations of each determination showed significant differences ($P < 0.05$) for NVP and AZT. All ART mean concentrations were in the therapeutic range in AIDS patients.

The patients' percentages that could reach the therapeutic range for each ART in both determinations are shown in the Fig.1. In the first extraction 74%, and in the second extraction 77% of patients could reach the therapeutic range for NVP's concentration. In both extractions 100% of patients could reach the therapeutic range for AZT's concentration. In the first extraction 77% of patients and 79% of patients in the second extraction could reach the therapeutic range for 3TC's concentration.

 DOI 10.5455/oams.210314.or.060

Table 1. Age, gender, ethnicity and others characteristics of participants (IPK, January 2011 – March 2012)

		Seronegative HIV (SHV)	Asymptomatic HIV patients	AIDS patients
Case number (n)		40	40	40
Age (years)		40.3 ± 5.07	36.25 ± 7.26	41.41 ± 11.38
Gender	**Male**	26	33	31
	Female	14	7	9
Ethnicity	**White**	21	30	27
	Black	9	2	3
	Mixed race	10	8	10
High active antiretroviral therapy (HAART)		-	No	AZT/3TC/NVP
Antioxidant therapy or nutritional supplementation		No	No	No

SHV; supposedly healthy volunteers SD: standard deviation; no differences were detected for comparisons between any of the groups (P > 0.05)

Table 2. Therapeutic range for each drug and antiretroviral quantification according extraction in AIDS patients

Drug	Therapeutic range			Concentration 1 (µg/ml)	Concentration 2 (µg/ml)
	C_{max} (µg/ml)	C_{min} (µg/ml)	Variability		
Zidovudine	1.8	0.02	high	0.61 ± 0.06	0.16 ± 0.02*****
Lamivudine	1.4-1.8	0.1-1	high	0.25 ± 0.01	0.27 ± 0.03
Nevirapine	20	3-5	45%	3.42 ± 0.26	5.5 ± 0.53*****

Drug concentrations were assessed by HPLC in serum; C_{max}, maximum concentration; C_{min}, minimum concentration; concentration 1, extraction 1 after 14 days with HAART; concentration 2, extraction 2 after 6 month with HAART; *****P < 0.05 for differences between extractions.

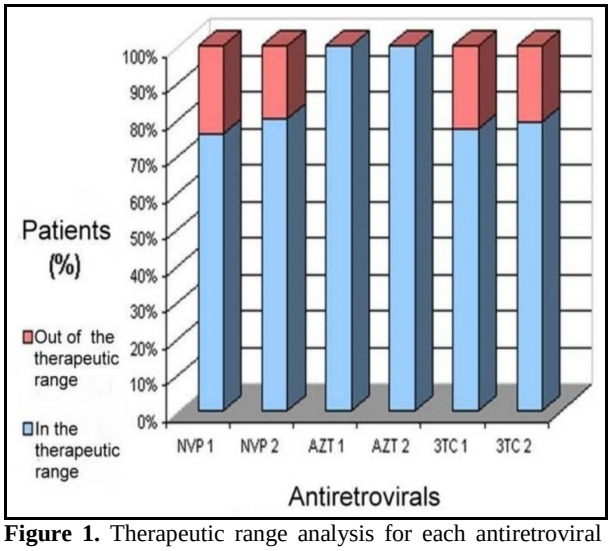

Figure 1. Therapeutic range analysis for each antiretroviral drug according to extraction (NVP1: nevirapine concentration for extraction 1, after 14 days with HAART; NVP2: nevirapine concentration for extraction 2, after 6 month with HAART; AZT1: zidovudine concentration for extraction 1, after 14 days with HAART; AZT2: zidovudine concentration for extraction 2, after 6 month with HAART; 3TC1: lamivudine concentration for extraction 1, after 14 days with HAART; 3TC2: lamivudine concentration for extraction 2, after 6 month with HAART). Note that drug concentrations were assessed by HPLC in serum. No significant differences were detected in comparison between extractions for the same drug (P < 0.05).

The mean value of all redox indexes and HIV progression markers evaluated for supposedly healthy voluntary (SHV) control and HIV groups are shown in Table 3. MDA, HPO and AOPP serum concentrations were significantly higher (P < 0.05) in HIV and AIDS groups with respect to control group. Serum GSH levels in HIV and AIDS individuals compared to HIV-control value were significantly lower (P < 0.05). The activities of the erythrocyte antioxidant enzyme SOD and CAT were significantly higher in HIV and AIDS groups compared to HIV-control (P < 0.05). PP is a global index which reflects serum susceptibility to lipid peroxidation. HIV and AIDS patients had significantly higher PP (P < 0.05), suggesting reduced lipid-serum antioxidant capacity with respect to control value.

MDA, HPO and AOPP serum levels were significantly higher (P < 0.05) in AIDS patients (after 14 days with HAART) compared with HIV asymptomatic patients. For GSH, CAT, SOD and PP no significant differences were found (P > 0.05). The comparison between HIV asymptomatic patients and AIDS patients (after 6 month with HAART) groups showed significant differences (P < 0.05) in almost all redox indexes (except in activity of SOD).

The comparison between the two extractions of AIDS patients group (after 14 days and after 6 month with HAART) showed significant differences (P < 0.05) in almost all redox indexes (except in PP and activity of SOD).

Table 3. Redox indexes and HIV progression markers data of different groups

Indexes	Seronegative HIV (SHV)	Asymptomatic HIV patients	AIDS patients	
			Extraction 1	Extraction 2
MDA (nmol/gHb)	2.33 ± 0.11	5.43 ± 0.35[a]	6.92 ± 0.7[ab]	8.79 ± 0.76[abc]
HPO (μM)	117.01 ± 3.47	135.67 ± 11.36[a]	163.19 ± 7.43[ab]	198.7 ± 16.11[abc]
AOPP (μM cloramine-T)	12.93 ± 0.66	25.98 ± 2,53[a]	33.8 ± 3.2[ab]	50.13 ± 5.51[abc]
GSH (μM/gHb)	1247.58 ± 21.51	420.04 ± 17.18[a]	378.65 ± 26.48[a]	319.55 ± 26.54[abc]
CAT (U/mgHb/min)	135.1 ± 2.76	277.05 ± 16.89 [a]	295 ± 20.1 [a]	366.22 ± 20.32[abc]
SOD (U/mgHb/min)	2.53 ± 0.19	3.35 ± 0.04[a]	3.48 ± 0.3[a]	3.22 ± 0.24[a]
PP (μM)	6.8 ± 0.32	15.15 ± 1.02[a]	13 ± 1.23[a]	13.2 ± 1.01[a]
T-CD4 total count (cell/mm^3)	1300,47 ± 56.35	454.36 ± 24.44[a]	439.5 ± 44.08[a]	483.33 ± 39.76[a]
VL (IU)	-	29786.31 ± 2202.75	6518.5 ± 277.89[b]	333.5 ± 16.26[bc]

Different letter represents significant differences ($P < 0.05$): compared to; [a]control, [b]asymptomatic HIV group, and [c]extraction 1.
Extraction 1, after 14 days with HAART; extraction 2, after 6 month with HAART.

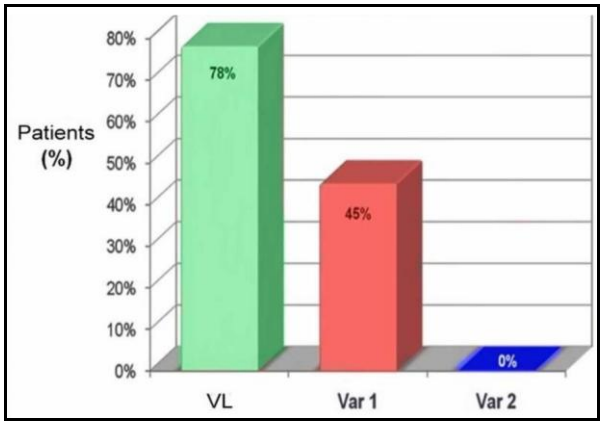

Figure 2. Global analysis considering beneficial and simultaneous changes in viral load and combinatory variables (Var 1 and Var 2) after 6 months with HAART (AZT/3TC/NVP). [VL: viral load; Var 1: differences between extractions in VL ≤ 250 IU and PP ≥ 1 U; Var 2: differences between extractions in VL ≤ 250 IU, MDA, HPO and AOPP ≤ 1 U, and PP ≥ 1 U]

CD4 number was significantly lower ($P < 0.05$) in HIV and AIDS groups with respect to control group. Non-significant differences were recorded between HIV-infected patients groups (with and without HAART) compared to CD4. VL was significantly lower ($P < 0.05$) in AIDS patients with HAART in comparison with HIV asymptomatic patients. VL was significantly lower ($P < 0.05$) in the second extraction of AIDS patients (after 6 month with HAART) group than the first one (after 14 days with HAART).

The comparison between extractions showed that 78% of patients with HAART had a beneficial change in the VL (VL ≤ 250 IU). But if PP is added to this analysis then only 45% of the patients with HAART had a beneficial change in both (VL ≤ 250 IU and PP ≥ 1 U). If MDA, HPO and AOPP are also added to analysis, then none of the patients (0%) with HAART had beneficial change in indexes and the VL simultaneously (VL ≤ 250 IU, MDA, HPO and PAOP ≤ 1 U, and PP ≥ 1 U) (Fig.2).

The factorial analysis result is shown in Table 4. Variances accumulate values were from 80-98% and acceptable samples adequacies (Kaiser coefficient of variance > 0.7) were determined for all analyzed groups. For SHV group, one main component was found with interdependence between MDA, PP, AOPP and CAT activity related inversely to GSH and CD4. When data of asymptomatic HIV patients were processed combined with the healthy group one main component was also found but with interdependence between MDA, PP, AOPP, CAT activity and CD4 related inversely to GSH and VL. In the case of HIV with HAART (extraction 1) three main components were found:

-first, showed interdependence between PP and GSH inversely related to CAT and VL;

-second, MDA and CD4 were inversely related to VL;
-and third, interdependence between AOPP and MDA were observed.

In the case of HIV with HAART (extraction 2) two main components were found:

-first, showed interdependence between SOD activity and GSH inversely related to CD4;

-second, PP, AOPP and CD4 were inversely related to MDA and CAT activity.

DISCUSSION

Voluntary screening for HIV infection is implemented as an integrated rights-based strategy by the Cuban national public health system. Most of the individuals are diagnosed as HIV+ in asymptomatic condition. National therapeutic guideline recommends and supports free HAART for AIDS condition (CD4 less

 DOI 10.5455/oams.210314.or.060

Table 4. Factorial analysis of redox indexes, viral load and T-CD4$^+$ lymphocyte absolute count

Variables	Seronegative HIV (SHV)	SHV and asymptomatic HIV patients	AIDS patients				
			Extraction 1			Extraction 2	
Kaiser coefficient	0.908	0.876	0.795			0.702	
Variance values accumulates for main components	89%	98%	80%			93%	
	Charges for main component						
	Comp. 1	**Comp. 1**	**Comp. 1**	**Comp. 2**	**Comp. 3**	**Comp. 1**	**Comp. 2**
MDA	0.897	0.87	0.21	0.515	0.561	0.22	-0.439
PP	0.796	0.822	0.777	0.165	-0.015	0.084	0.427
GSH	-0.918	-0.981	0.726	-0.033	0.207	-0.591	0.186
CAT	0.819	0.803	-0.64	0.22	0.171	0.157	-0.815
AOPP	0.587	0.577	-0.113	-0.115	0.885	-0.195	0.492
SOD	0.445	0.433	-0.06	-0.053	-0.007	-0.533	0.063
T-CD4$^+$ count	-0.961	0.508	-0.303	0.792	-0.011	0.996	0.064
VL	-	-0.876	-0.513	-0.602	0.219	-0.08	0.115

Extraction 1: values for AIDS group after 14 days with HAART; extraction 2: values for AIDS group after 6 months with HAART.

than 250 cell/mm^3 or VL over 55,000 IU). Universal ART coverage to all patients with AIDS clinical criteria has been done using Cuban-produced generic. Also ART not produced in Cuba are acquired with the support from the Global Fund to fight aids, tuberculosis and malaria. This last one was given up to patients who needs other combinations not included in the present study [66].

There are several studies of disturbed redox metabolism in HIV-infected patients [24, 25, 28, 37]. In the present study, we have shown that, in HIV infection and during HAART, the decrease in VL and the stabilization in CD4 are accompanied by both an abnormal antioxidant-redox status and an increase in the levels of damaged redox indexes.

In previous studies evidences of HAART effectiveness of pharmaceutical leaders is reported around 70-80% [6, 12, 53, 55, 67]. In the present study, Cuban generic drugs were given and the general effectiveness was in the 78%. High variability has been found for NVP and AZT corroborating results previously reported [67]. AZT is considered as pro-drug so its metabolic process could impact in the plasma concentration variability. The compliance of therapeutic range has been detected in 74-77% for NVP and 100% for AZT. To corroborate that the obtained therapeutic effect was related with the effective dose in blood, TDM was developed as recommended. Drug concentration follow-up verifies compliance of therapeutic range.

Concomitant drugs used in the present study have no impact in the subsequent indexes assessed taking into consideration the frecuency, doses employed and the fact that second extraction was done after 6 months with HAART. Simultaneously to the therapeutic effect associated toxicities have been suggested and reported. Oxidative stress has been one of these theories related to mitochondrial toxicity. HIV infection increases the oxidative stress process in relation to chronic activation of inflammation related to chronic virus exposure. This status is further influence by the use of HAART. This was observed by the significantly higher and modified MDA (lipid peroxidation), HPO, AOPP concentrations and CAT activity (Table 3), suggesting an increase in oxidant stimuli [31, 68]. Also lower and modified PP and GSH concentrations were detected suggesting antioxidant consumption.

It is therefore speculated that, the observed effect may be a consequence of the association of infection evolution and therapeutic regimen. Several pre-HAART studies found that both asymptomatic HIV-infected individuals and AIDS patients had higher levels of oxidative stress, as indicated by increased plasma metabolites of lipid peroxidation and/or reduced antioxidant levels, compared with healthy controls [68]. Another study reported that HAART may increase oxidative stress levels above and beyond levels caused by the virus itself [34]. HAART may induce; ***(i)*** an increase in oxidant generation, ***(ii)*** a decrease in anti-oxidant protection, or ***(iii)*** a failure to repair oxidative damage. Oxidative stress-mediated cell damage occurs, in part by ROS, an unstable biomolecule configuration which quickly reacts with other molecules to achieve the stable configuration. In HIV infection, ROS reactions could result also from non-enzymatic protein oxidation and the subsequent oxidative degradation of proteins.

Abnormally high levels of ROS as well as the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles

and enzymes as well as increased lipid peroxidation [69-71]. ROS and their metabolites are avoided and processed from the cell by enzymatic systems including SOD, CAT and GPx, or the nonenzymatic system including alpha-tocopherol, ascorbic acid, GSH and uric acid. GPx plays an important role as defense mechanism in mammals, against oxidative damage by catalyzing the reduction of a variety of HPO, using GSH as the reducing substrate. GSH redox cycle also acts as a direct endogenous scavenger of hydroxyl radicals, involved in detoxification and metabolism of a number of substances in the liver [42, 47, 72]. HAART may reduce GSH synthesis, enhance GSH utilization, or limit intracellular reduction of its oxidized form (GSSG) [73]. GSH reduction modify related functions such as reducing capacity, protein biosynthesis, immune function, accumulations of lipid peroxidation products and detoxification capacity leading to the accumulation of hepatotoxic metabolites and to liver damage [28, 48, 73]. HAART could also have a role in oxidative stress resulting from the destruction of tissues and liver cells and the activation of neutrophils and macrophages.

The major damage to cells results from the oxidative stress alteration of macromolecules in membrane lipids, essential proteins and DNA [42, 72]. The differences in oxidative stress as well as in the plasma concentrations of antioxidants between pre-HAART and HAART patients may be explained by HAART influences. Although HAART results in suppression of viral replication and dramatic improvement in clinical and immunological status [15, 16], weight loss and wasting may still be observed in some HAART patients. Certain aspects of HAART toxicity may be associated with oxidative stress, thereby increasing the body's demand for certain antioxidants [22, 40, 43].

These previous studies have been carried out using statistical comparisons considering the oxidative stress indexes as independent variables. In the present analysis, seven oxidative stress indexes and two progression markers were computed to evidence simultaneous modification regarding to the clinical condition and the HAART period, as an integral view. Indeed, individual effect was computed in a global way in each group to evidence the simultaneous variation of combined variables in patients. The factorial multivariate analysis showed direct and inverse exploratory relationship between some variables evaluated in diverse clinical condition. The physiological (SHV group) relation was modified in the case of asymptomatic condition showing evidences that viral factor can influence on the variable relationship between evaluated indexes. In the case of clinical AIDS conditions, evidences showed modification of the variable relationship as consequences of the treatment factor.

Meanwhile, HAART did not induce normalization of these parameters. These findings lend further support to the idea that enhanced oxidative stress contributes to the pathogenesis of HIV infection, and the findings also suggest that antioxidant intervention could influence and counteract these oxidative disturbances, even in the "HAART era" [32, 74, 75]. However, it remains to be proven that such intervention or supplementation effects result effective in HIV-infected patients receiving HAART.

There is growing evidence that ROS, oxidized molecules, redox regulators, related active mediators, cellular organelles functions and surrounding environments are all tied together in intricate networks affecting the whole body energetic, metabolism and state of health and disease. Long-term clinical implications of oxidative stress, and how it is related to HAART associated complications as fat atrophy, insulin resistance, lipid abnormalities and others are becoming encouraged. The oxidative stress evaluations will, therefore, become potential utility factors to follow antiviral combinations effects, as well as the usefulness of antioxidant and alternative therapies.

The ability to combine diagnostic indexes in order to integral view approach represents a valuable tool for both understanding the pathogenic actions of the virus and for the clinical monitoring of HIV-infected patients. The optimal usage of this tool in the clinical setting, however, still remains to be defined [4, 76-78].

REFERENCES

1. WHO. Global summary of AIDS epidemic 2012. Available via http://www.who.int/hiv/data/epi_core_Dec2013_en.png?ua=1 (Accessed 27 April 2014).
2. UNAIDS report on the global AIDS epidemic 2013. WHO Library, UNAIDS/JC2502/1/E, November 2013.
3. Le Douce V, Herbein G, Rohr O, Schwartz C. Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. Retrovirology 2010; 7:32.
4. Capeau J. Premature Aging and Premature Age-Related Comorbidities in HIV-Infected Patients: Facts and Hypotheses. Clin Infect Dis 2011; 53:1127-9.
5. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents. Centres for Disease Control and Prevention, July 2013.
6. De Clercq E. The history of antiretrovirals: key discoveries over the past 25 years. Rev Med Virol 2009; 19:287-99.
7. Lozano F, Domingo P. Antiretroviral therapy for HIV infection. Enferm Infecc Microbiol Clin 2011; 29:455-65.
8. Barreiro P, Simarro N, Rodriguez-Novoa S. Antirretrovirales. Medicine 2006; 9:3845-51.
9. Bean P, Patnaik M, Graziano FM, Aziz DC. Therapeutic drug monitoring of antiretroviral agents. Am Clin Lab 2000; 19:20-2.

 DOI 10.5455/oams.210314.or.060

10. Burger D, Aarnoutse RE, Hugen PW. Pros and cons of therapeutic drug monitoring of antiretroviral agents. Curr Opin Infect Dis 2002; 15:17-22.

11. Marchei E, Valvo L, Pacifici R, Pellegrini M, Tossini G, Zuccaro P. Simultaneous determination of zidovudine and nevirapine in human plasma by RP-LC. J Pharm Biomed Anal 2002; 29:1081-8.

12. Khoo S, Lloyd J, Dalton M, Bonington A, Hart E, Gibbons S, Flegg P, Sweeney J, Wilkins EG, Back DJ. Pharmacologic optimization of protease inhibitors and nonnucleoside transcriptase inhibitors (POPIN) – a randomized controlled trial of therapeutic drug monitoring and adherence support. J Acquir Immune Defic Syndr 2006; 41:461-7.

13. la Porte C, Back DJ, Blaschke T, Boucher CA, Fletcher CV, Flexner C, Gerber J, Kashuba AD, Schapiro JM, Burger DM. Updated guideline to perform therapeutic drug monitoring for antiviral agents. Rev Antivir Ther 2006; 2006:4-14.

14. Hulgan T, Morrow J, D'Aquila R, Raffanti S, Morgan M, Rebeiro P, Haas DW. Oxidant stress is increased during treatment of human immunodeficiency virus infection. Clin Infect Dis 2003; 37:1711-7.

15. Hulgan T, Hughes M, Sun X, Smeaton LM, Terry E, Robbins GK, Shafer RW, Clifford DB, McComsey GA, Canter JA, Morrow JD, Haas DW. Oxidant stress and peripheral neuropathy during antiretroviral therapy: an AIDS clinical trials group study. J Acquir Immune Defic Syndr 2006; 42:450-4.

16. Gutierrez F, Padilla S, Masia M, Iribarren JA, Moreno S, Viciana P, Munoz L, Gomez Sirvent JL, Vidal F, Lopez-Aldeguer J, Blanco JR, Leal M, Rodriguez-Arenas MA, Perez Hoyos S; CoRIS-MD. Clinical outcome of HIV-infected patients with sustained virologic response to antiretroviral therapy: long-term follow-up of a multicenter cohort. PLoS One 2006; 1:e89.

17. Lagathu C, Eustace B, Prot M, Frantz D, Gu Y, Bastard JP, Maachi M, Azoulay S, Briggs M, Caron M, Capeau J. Some HIV antiretrovirals increase oxidative stress and alter chemokine, cytokine or adiponectin production in human adipocytes and macrophages. Antivir Ther 2007; 12:489-500.

18. Buccigrossi V, Laudiero G, Nicastro E, Miele E, Esposito F, Guarino A. The HIV-1 transactivator factor (Tat) induces enterocyte apoptosis through a redox-mediated mechanism. PLoS One 2011; 6:e29436.

19. Day BJ, Lewis, W. Oxidative stress in NRTI-induced toxicity. Cardiovasc Toxicol 2004; 4:207-16.

20. Caron M, Auclairt, M. Vissian, A. Vigouroux, C. Capeau, J. Contribution of mitochondrial dysfunction and oxidative stress to cellular premature senescence induced by antiretroviral thymidine analogues. Antivir Ther 2008; 13:27-38.

21. Feng JY, Johnson AA. Johnson KA, Anderson KS. Insights into the molecular mechanism of mitochondrial toxicity by AIDS drugs. J Biol Chem 2011; 276:23832-7.

22. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol 2012; 2012:645460.

23. Gostner J, Becker K, Fuchs D, Sucher R. Redox regulation of the immune response. Redox Rep 2013; 18:88-94.

24. Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J 1991; 10:2247-58.

25. Favier A, Sappey C, Leclerc P, Faure P, Micoud M. Antioxidant status and lipid peroxidation in patients infected with HIV. Chem Biol Interact 1994; 91:165-80.

26. Dobmeyer T, Findhammer S, Dobmeyer JM, Klein SA, Raffel B, Hoelzer D, Helm EB, Kabelitz D, Rossol R. *Ex vivo* induction of apoptosis in lymphocytes is mediated by oxidative stress: role of lymphocyte loss in HIV infection. Free Radic Biol Med 1997; 22:775-85.

27. Elbim C, Pillet S, Prevost MH, Preira A, Girard PM, Rogine N, Hakim J, Israel N, Gougerot-Pocidalo MA. The role of phagocytes in HIV-related oxidative stress. J Clin Virol 2001; 20:99-109.

28. Aukrust P, Muller F, Svardal AM, Ueland T, Berge RK, Froland SS. Disturbed glutathione metabolism and decreased antioxidant levels in human immunodeficiency virus-infected patients during highly active antiretroviral therapy-potential immunomodulatory effects of antioxidants. J Infect Dis 2003; 188:232-8.

29. Gendron K, Ferbeyre G, Heveker N, Brakier-Gingras L. The activity of the HIV-1 IRES is stimulated by oxidative stress and controlled by a negative regulatory element. Nucleic Acids Res 2011; 39:902-12.

30. Larbi A, Kempf J, Pawelec G. Oxidative stress modulation and T cell activation. Exp Gerontol 2007; 42:852-8.

31. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39:44-84.

32. Aquaro F, Scopelliti, F, Pollicita, M, Perno CF. Oxidative stress and HIV infection: target pathways for novel therapies? Future HIV Therapy 2008; 2:327-38.

33. Wanchu A, Rana SV, Pallikkuth S, Sachdeva RK. Short communication: oxidative stress in HIV-infected individuals: a cross-sectional study. AIDS Res Hum Retroviruses 2009; 25:1307-11.

34. Coaccioli S, Crapa G, Fantera M, Del Giorno R, Lavagna A, Standoli ML, Frongillo R, Biondi R, Puxeddu A. Oxidant/antioxidant status in patients with chronic HIV infection. Clin Ter 2010; 161:55-8.

35. Gil L, Gonzalez I, Diaz M, Bermudez AY, Hernandez RD, Abad LY. Evaluation of paediatric patients HIV/AIDS. Rev OFIL 2012; 22:172-83.

36. Gil L, Martinez G, Gonzalez I, Alvarez A, Molina R, Tarinas A, Leon OS, Perez J. Contribution to characterization of oxidative stress in HIV/AIDS patients. Pharmacol Res 2003; 47:217-24.

37. Stephensen C, Marquis GS, Douglas SD, Wilson CM. Immune activation and oxidative damage in HIV-positive and HIV-negative adolescents. J Acquir Immune Defic Syndr 2005; 38:180-90.

38. Ngondi JL, Oben J, Forkah DM, Etame LH, Mbanya D. The effect of different combination therapies on oxidative stress markers in HIV infected patients in Cameroon. AIDS Res Ther 2006; 3:19-26.

39. Elbim C, Pillet S, Prevoste MH, Preira A, Girard PM, Rogine N, Matusani H, Hakim J, Israel N, Gougerot-Pocidalo MA. Redox and activation status of monocytes from human immunodeficiency virus-infected patients: relationship with viral load. J Virol 1999; 73:4561-6.

40. Vassimon HS, Deminice R, Machado AA, Monteiro JP, Jordao AA. The association of lipodystrophy and oxidative stress biomarkers in HIV-infected men. Curr HIV Res 2010; 8:364-9.

41. Awodele O1, Olayemi SO, Nwite JA, Adeyemo TA. Investigation of the levels of oxidative stress parameters in HIV and HIV-TB co-infected patients. J Infect Dev Ctries 2012; 6:79-85.

42. Block G, Dietrich M, Norkus EP, Morrow JD, Hudes M, Caan B, Packer L. Factors associated with oxidative stress in human populations. Am J Epidemiol 2002; 156:274-85.

43. Birben E, Murat U, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012; 5:9-19.

44. Ma Q. Transcriptional responses to oxidative stress: pathological and toxicological implications. Pharmacol Ther 2010; 125:376-93.

45. Suresh DR, Annam V, Pratibha K, Maruti BV. Total antioxidant capacity a novel early bio-chemical marker of oxidative stress in HIV infected individuals. J Biomed Sci 2009; 16:61-72.

46. Alfadda AA, Sallam RM. Reactive oxygen species in health and disease. J Biomed Biotechnol 2012; 2012:936486.

47. Lushchak VI. Glutathione homeostasis and functions: potential targets for medical interventions. J Amino Acids 2012; 2012:736837.

48. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol 2002; 30:620–50.

49. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Seoul, 2000.

50. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline - Guideline for. Good Clinical Practice E6(R1), June 1996. Available via http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf (Accessed 27 April 2014).

51. Giorgi J. Characterization of T lymphocytes subset alterations by flow cytometry in HIV disease. Ann NY Acad Sci 1993; 677:126-37.

52. Tarinas A, Tapanes RD, Ferrer G, Perez J. Validation of high-performance liquid chromatography methods for determination of zidovudine, stavudine, lamivudine and indinavir in human plasma. Farm Hosp 2007; 31:243-7.

53. Tarinas A, Tapanes RD, Gonzalez D, Ferrer G, Abreu D, Perez J. Bioequivalence study of two nevirapine tablet formulations in human immunodeficiency virus-infected patients. Farm Hosp 2007; 31:165-8.

54. Burger D, Rosing H, van Gijn R, Meenhorst PL, van Tellingen O, Beijnen JH. Determination of stavudine, a new antiretroviral agent in human plasma by reversed-phase high-perfromance liquid chromatography with ultraviolet detection. J Chromatogr 1992; 584:239-47.

55. Tarinas A, Tapanes RD, Gil L, Gonzalez D, Castro O, Saul A, Martinez A, Orta M, Ferrer G, Perez J. Bioequivalence study: generic and trade formulations of stavudine, lamivudine, zidovudine and indinavir in Cuban HIV-infected subjects. Rev Cub Farm 2006; 40:2-9.

56. Tietze F. Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Anal Biochem 1974; 27:502–22.

57. Erdelmeier I, Gerard D, Yadan JC, Chaudiere J. Reactions of N methyl-2-phenyl-indole with malondialdehyde and 4-hydroxialkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation. Chem Res Toxicol 1998; 11:1184-94.

58. Bartosz G. Total antioxidant capacity. Adv Clin Chem 2003; 37:219-92.

59. Ozdemirler G, Mehmetcik G, Oztezcan S, Toker G, Sivas A, Uysal M. Peroxidation potential and antioxidant activity of serum in patients with diabetes mellitus and myocard infarction. Horm Metab Res 1995; 271:194-6.

60. Jiang Z, Woollard AC, Wolff SP. Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids 1991; 26:853-6.

61. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay form superoxide dismutase. Eur J Biochem 1974; 47:469-74.

62. Clairborne A. Catalase activity. In: Green-Wald R (ed) Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, FL, pp 283-284, 1986.

63. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, Nguye-Knoa, Nguyen AT, Zingraff J, Jungers P, Deschamp-Latscha D. Advanced oxidation protein product as novel mediators of inflammation and monocytes activation in chronic renal failure. J Immunol 1998; 161:2524 -32.

64. Gorsuch RL. Common factor analysis vs. components analysis: some well and little known facts. Multivar Behav Res 1990; 25:33-9.

65. Garcia E, Gil J, Rodriguez G. Factorial Analysis - Statistical Book, La Muralla, Madrid, 2000.

66. The Global Fund to Fight AIDS, Tuberculosis and Malaria. Available via http://www.theglobalfund.org/en/ (Accessed 27 April 2014).

67. Bramuglia F, Curras V, Hocht C , Rubio MC. Therapeutic drug monitoring in the treatment of HIV infection. Actual SIDA 2008; 16:128-35.

68. Mandas A, Lorio E L, Congiu MG, Balestrieri C, Mereu A, Cau D, Curreli N. Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy. J Biomed Biotechnol 2009; 2009:749575.

69. Devasagayam T, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India 2004; 52:794-804.

70. Halliwell B. The wanderings of a free radical. Free Radic Biol Med 2009; 46:531-42.

71. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 2004; 142:231-55.

72. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signalling, and cell injury. Free Radic Biol Med 2000; 28:1456-62.

73. Sen CK. Cellular thiols and redox-regulated signal transduction. Curr Top Cell Regul 2000; 36:1-30.

74. Deresz LF, Sprinz E, Kramer AS, Cunha G, de Oliveira AR, Sporleder H, de Freitas DR, Lazzarotto AR, Dall'Ago P. Regulation of oxidative stress in response to acute aerobic and resistance exercise in HIV-infected subjects: a case-control study. AIDS Care 2010; 22:1410-7.

75. Milazzo L, Menzaghi B, Caramma I, Nasi M, Sangaletti O, Cesari M, Poma BZ, Cossarizza A, Antinori S, Galli MO. Effect of antioxidants on mitochondrial function in HIV-1-related lipoatrophy: a pilot study. AIDS Res Hum Retroviruses 2010; 26:1207-14.

76. Roberts R, Smith RA, Safe S, Szabo C, Tjalkens RB, Robertson FM. Toxicological and pathophysiological roles of reactive oxygen and nitrogen species. Toxicology 2010; 276:85-94.

77. Arguelles S, Garcia S, Maldonado M, Machado A, Ayala A. Do the serum oxidative stress biomarkers provide a reasonable index of the general oxidative stress status? Biochim Biophys Acta 2004; 1674:251-9.

78. Kregel K, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 2007; 292:R18-36.

 DOI 10.5455/oams.210314.or.060