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ORIGINAL ARTICLE

Redox indexes and progression markers in HIV-infected patients treated with three different HAART combinations

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Key Words
Antiretroviral therapy; HIV; Oxidative stress; Redox indexes

Abstract

Objective: Highly active antiretroviral therapy (HAART) is the choice to treat cases with an established HIV-infection and to prevent establishment of infection after exposure. Oxidative stress has been associated to HIV infection and, depending of therapy combination, effectiveness and adherence, to HAART. This study assessed the effect of three different HAART combinations, *i.e.* zidovudine/lamivudine/nevirapine **(I)**, zidovudine/lamivudine/indinavir **(II)** and stavudine/lamivudine/nevirapine **(III)**, on redox indexes and progression markers of disease on blood.

Methods: We recruited 200 individuals of which 160 were HIV+ (40 never treated and 120 were taking different HAART combinations for at least six months), and the other 40 were supposedly healthy voluntaries (SHV). Peroxidation potential, glutathione, malondialdehyde, hydroperoxides, superoxide dismutase (SOD), catalase, advanced oxidation protein products (AOPP), viral load and CD4 lymphocytes T subsets were measured at baseline for all individuals and after 6 months.

Results: The comparison between SHV and HIV treated or non-treated patients groups showed significant differences in almost all redox indexes with different molecular significance. Statistically modified values were found in almost all redox indexes at 6 months compared to the baseline except in SOD activity and peroxidation potential for combination I and III combinations and AOPP and SOD activity for combination II. Insignificant differences were found between HIV+ patients groups with respect to CD4 T lymphocyte subsets. The 78% of patients receiving treatment showed a viral load reduction. The multivariate statistical model clearly separated treated groups according progression indexes and redox profile.

Conclusion: Therapeutic effectiveness confirmation and its benefits in HIV-infected patients combined with additional oxidative stress impact promoted integral view approach to follow up infection.

INTRODUCTION

The full understanding of the interplay between immune activation, HIV replication and therapies are encouraged worldwide. The complex immune dysfunction in HIV-positive individuals even in antiretroviral era predisposes them to both pathogenic and opportunistic infections and continue to impact in world schedule of health [1, 2].

The knowledge on HIV virology has expanded tremendously with continues increase of antiviral therapy arsenal. Since HIV-infected patients are using antiretrovirals (ART), clinically relevant changes in body metabolism and pathologies with different patterns have been described [3]. In the latest years, an important decline of the morbidity and mortality of HIV infection in first world has been observed due to the use of combined therapy named highly active antiretroviral therapy (HAART) [4, 5]. This treatment can suppress HIV replication to undetectable levels (< 50 copies per ml) and improve patient's immune functions, especially the CD4 T lymphocyte subsets (CD4), resulting in a decrease of infectious complications and a global clinical improvement [6-9]. However, HAART does not eliminate HIV, nor either solves the immune and metabolic alterations during infection evolution on the contrary, increases the infection metabolic toxicity. The main pathogenic mechanism through which nucleoside analogues and protease inhibitors contribute to the metabolic changes and organ toxicities is by mitochondrial dysfunction [3, 10]. The ability to quantify viral load and to perform sequence analyses represent valuable tools both for understanding the pathogenic actions of the virus and for the clinical monitoring of HIV-infected patients [5, 11].

Previous studies have suggested a role of oxidative stress in the stimulation of HIV replication, the development of immunodeficiency through apoptosis and in the consequences of treatment [12-22]. 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 [23-26]. 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 [27-29]. 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 [24-30]. Some physiological roles of ROS in immune defense, antibacterial action, vascular tone and signal transduction are argued. ROS overproduction in viral infections is generally related to pro-oxidant effect of inflammatory cytokines and/or polymorphonuclear leukocyte activation using also intracellular signal transduction cascades within cell and thereby up-regulating the cellular response. In regulatory mode, ROS also contribute to decrease the cell activation threshold [28, 31-34].

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 malondyaldehyde (MDA) were found in both pediatric and adult patients [18, 20, 35-43].

In HIV-infected patients treated with HAART, the role of oxidative stress in disease progression is complex. Virus control with HAART would not reduce oxidative stress levels as one may expect; on the contrary it would increase [12-14, 16, 21, 44-46].

As per clinical guidelines, combinations of anti-HIV drugs containing nucleoside reverse-transcriptase inhibitors (NRTI) are widely used during HIV infection follow up [5]. Additional adverse effects and treatment adherence difficulties have serious consequences such as failure of serum HIV suppression, development of drug-resistant HIV strains and increased probability of developing opportunistic illness [44, 47, 48]. NRTI are associated with lactic acidosis, hyperlipidemia, glucose intolerance, diabetes mellitus, atherosclerosis, fat redistribution and wasting syndrome. All of these morbidities could increase oxidative stress related to NRTI toxicity [6, 13, 27, 47]. The phophorylated-NRTI mitochondrial toxicity may amplify some of the pathophysiologic and phenotypical events in infection [17, 45].

Study of new 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 deterioration [24, 49]. It would be useful to identify additional markers that, along with the count of CD4+ T-lymphocytes, support the decisions taking; for example as to when to start, stop or change therapy [5, 29, 50].

Considering backgrounds, this study follows redox indexes in relation to viral load and CD4 T lymphocyte subsets in blood samples of HIV patients. Patients were treated with three different combination of HAART in order to explore simultaneous variables processing and contribution to the existing results: ***(I)*** zidovudine (AZT), lamivudine (3TC) and nevirapine (NVP); ***(II)*** AZT, 3TC and indinavir (IDV); ***(III)*** stavudine (d4T), 3TC and NVP.

METHODS

Study design and ethical consideration

A longitudinal study was designed with different groups composed of individuals with HIV (asymptomatic), AIDS patients treated with HAART and a control group with supposedly healthy voluntaries (SHV). All the individuals were attended at the out-patients clinic in the "Pedro Kourí" Institute Hospital. They all gave written informed consent to take part in the study after verbal and written explanation of 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 "Pedro Kourí" Institute Ethical Committee for Research on Human Subjects. The study is accordance with the Declaration of Helsinki principles and its modifications in 2004 and 2008, and also with national and international regulations concerning the ethical principles for medical research involving human subjects, clinical practices and drugs quality [51, 52].

Subjects and methods

One-hundred sixty subjects were HIV+ of which 40 had no started HAART (asymptomatic) and 120 that are indicated to take HAART. All subjects were followed up for 6 months. Subjects were advised to continue their normal activity and to report any unusual symptom.

Two-hundred subjects (including 40 SHV) ranging from 30-50 years of age were recruited sequentially. Exclusion criteria were smoke habits, initiation of antioxidant vitamin therapy prior to study, hyperlipidemia, diabetes, kidney/liver dysfunction, intractable diarrhea (at least six liquid stools daily), vomiting or evidence of gastrointestinal bleeding. None

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of the patients had family history of coronary heart disease. Physicians were encouraged to report HIV-associated opportunistic and other infections in the clinical charts during 6 months once the study began. AIDS cases were eligible for starting HAART during 2011-2012 according to the health national policy. Doses were in accordance also of WHO Guidelines and were as follow: AZT, 300 mg twice daily; 3TC, 150 mg twice daily; NVP, 200 mg twice daily; IDV, 800 mg thrice daily; d4T, 40 mg twice daily [4]. This three HAART combinations were the most frequently prescribed with 80.2% of total AIDS treated patients in Cuba. The compliance with the HAART intake was verified each month recovering the pills which weren't taken in its flask.

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

The blood was sampled at least 12 h after fasting. The serum samples were stored at –20ºC until the analyses were carried out.

HIV positiveness was diagnosed by a reactive enzyme-linked immunosorbent assay (ELISA) for HIV (Vironostika HIV Uni-Form II plus O kit, Organon Technika, Netherlands) which was confirmed by positive Western blot analysis (DAVIH-BLOT, DAVIH Laboratories, Cuba).

Flow cytometry analysis

CD3+/CD4+ T lymphocyte subsets quantification in total blood with lysing solution (Becton Dickinson, BD) was carried out. For each T lymphocyte subsets T memory CD3 and CD4 were used. These analyses were performed on a FACScan flow cytometry (BD) [53].

Viral load (VL)

VL was determined with the Biomerieux polymerase chain reaction (PCR-NASBA) ultrasensitive assay with the lower limit of quantification of 50 IU.

Biochemical measurements

Glutathione concentration

The method described by Tietze [54] was used for the quantification of reduced GSH in serum. GSH was used to generate standard curves.

Malondialdehyde concentration

MDA concentrations were analyzed by stable chromophore production after 40 min of incubation at 45°C. It is measured at a wavelength of 586 nm by Pharmacia spectrophotometer. Freshly prepared solutions of malondialdehyde bis(dimethyl acetal) assayed under identical conditions were used as reference standards. Serum MDA concentrations were calculated using the corresponding standard curve and values were expressed as nmol/gHb [55].

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 0 time from the one obtained at 24 h [56, 57].

Total hydroperoxides

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(carboxy methyl)-aminomethyl)-o-cresolsulfone-phatein, sodium salt) to form a stable colored complex, which can be measured at 560 nm [58].

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-phenyl tretazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is then measured by the inhibition of this reaction [59].

Catalase

CAT activity was measured using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}, the rate of the first 30 seconds was used to calculate the activity. CAT activity was expressed as U/mgHb [60].

Advanced oxidation protein products (AOPP)

Serum AOPP was measured according to the method of Witko-Sarsat *et al* [61]. The values were expressed in chloramines-T equivalents and corrected by serum albumin concentrations.

Unless otherwise stated, all chemicals were obtained from Sigma (St. Louis, MO, USA).

Data analysis and statistics

Results were 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 ANOVA test, followed by a post hoc Newman-Keuls methods. Statistical significance was defined as $P < 0.05$.

An exploratory factorial analysis was performed combining redox and progression indexes [62, 63]. Also a canonical discriminate analysis was performed combining redox and progression indexes with others markers evaluated.

RESULTS

The baseline characteristics of the 200 subjects are shown 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. Principal symptoms during the first two months for patients were nauseas in 31, sleeping in 16, stomach ache, headache in 42 patients out of 120, *etc*. Fourteen treated patients used the non-steroidal anti-inflammatory drug methamizole 300 mg consecutively during four days in a frequency of 3 times a day during the first two months; pain symptoms were ameliorated. No other drug was used in patients to treat any morbidity during 6 months of the study.

Effectiveness analyses of the three HAART combinations used are shown in Table 2. The CD4 number was significantly lower ($P < 0.05$) in HIV and AIDS groups compared to SHV group. Insignificant differences were found between HIV-infected patients groups (with and without HAART) with respect to CD4. VL was significantly lower ($P < 0.05$) in AIDS patients with HAART compared to asymptomatic HIV patients.

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

		SHV	Asymptomatic HIV patients	AIDS patients		
N		40	40	40	40	40
Age, years (median ± SD)		40.3 ± 5.07	36.25 ± 7.26	41.41 ± 11.38	42.41 ± 7.34	41.77± 7.31
Gender	**Male**	26	33	31	33	34
	Female	14	7	9	7	6
Ethnicity	**White**	21	30	27	33	30
	Black	9	2	3	3	5
	Mixed race	10	8	10	4	5
HAART		No	No	AZT/3TC/NVP	AZT/3TC/IDV	d4T/3TC/NVP
Antioxidant therapy or nutritional supplementation		No	No	No	No	No

SHV, supposedly healthy voluntaries; HAART, highly active antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; IDV, indinavir; d4T, stavudine. IPK, 2011-2012: Period of consults in Institute of Tropical Medicine "Pedro Kouri" was from January 2011 to March 2012. Note: no significant differences were detected in comparison between variables for the different groups ($P > 0.05$)

Table 2. HIV progression markers in different study groups

	Time of extraction (months)	CD4 (cell/ml; mean ± SD)	Patients with stabil./increa. CD4	Viral load (VL, IU; mean ± SD)	Patients with non-detectable VL	Patients with non-detectable VL and stabil./increa. CD4
SHV	-	1300.47 ± 56.35	-	-	-	-
Asymptomatic HIV patients	-	454.36 ± 24.44[a]	-	29786.31 ± 2202.75	-	-
AZT/3TC/NVP	0	439.5 ± 44.08[a]	65.5%	6518.5 ± 277.89[b]	80%	76%
	6	483.33 ± 39.76[a]		333.5 ± 16.26[bc]		
AZT/3TC/IDV	0	280.53 ± 166.12[a]	80%	27919.1 ± 686.25[b]	98%	78%
	6	402.6 ± 227.53[a]		4076.18 ± 145.84[bc]		
d4T/3TC/NVP	0	311.16 ± 206.68[a]	82%	31832.77 ± 544.07[b]	98%	80%
	6	478.23 ± 251.98[a]		3665.81 ± 739.66[bc]		

SHV, supposedly healthy voluntaries; HAART, highly active antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; IDV, indinavir; d4T, stavudine. Different letters represent significant differences ($P < 0.05$): [a]compared to SHV group; [b]compared to asymptomatic HIV patients; [c]differences between extractions.

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The VL was significantly lower ($P < 0.05$) in the second extraction of AIDS patients (after 6 month with HAART) group compared to the first one (after 14 days with HAART). The HAART effect were mainly stabilization on CD4 reaching 65.5, 80 and 82% and VL reduction with 80, 98 and 98% for combinations I, II and III, respectively ($P < 0.05$). The global analysis showed that the 78% of patients with HAART had a beneficial change with VL reduction (250 UI at least).

The mean value of all redox indexes evaluated for SHV and HIV groups are shown in Table 3. The comparison between SHV and asymptomatic HIV patients groups (after 6 months respect baseline for each group) showed not significant differences ($P > 0.05$) in all redox indexes (data not shown).

Serum MDA, HPO and AOPP concentrations were significantly higher ($P < 0.05$) in HIV and AIDS groups compared to SHV group. Also significantly modified MDA and HPO 6 months extraction values ($P < 0.05$) were detected for combinations I, I and III with respect to the baseline. The AOPP 6 months extraction values were significantly modified ($P < 0.05$) for combinations I and III.

Serum GSH levels in HIV and AIDS individuals compared to SHV group were significantly lower ($P < 0.05$). Activities of the erythrocyte antioxidant enzymes SOD and CAT were significantly higher in HIV and AIDS groups compared to SHV group ($P < 0.05$). Also, significantly modified 6 months extraction values of CAT were detected for combinations I, II and III ($P < 0.05$).

The PP index is an assay for serum antioxidant capacity showing serum susceptibility to lipid peroxidation. HIV and AIDS patients had significantly higher PP ($P < 0.05$), suggesting reduced lipid-serum antioxidant capacity compared to SHV group.

Serum MDA, HPO and AOPP levels were significantly higher ($P < 0.05$) in AIDS patients (after 14 days with HAART) compared to asymptomatic HIV patients for combinations I and III. MDA concentration showed significantly higher values ($P < 0.05$) than asymptomatic HIV patients only for combination II. For GSH and SOD no significant differences were found ($P > 0.05$). PP reflected significant differences ($P < 0.05$) in combinations II and III. Significantly higher CAT values were found in combination III compared to asymptomatic HIV patients ($P < 0.05$).

The comparison between asymptomatic HIV patients and AIDS patients after 6 months with HAART showed significant differences ($P < 0.05$) in all redox indexes except SOD activity. Also exceptions ($P > 0.05$) were found in AOPP for combination II and in PP for combination I. Comparison between the two extractions of AIDS patients group after 14 days and after 6 months with HAART showed significant differences ($P < 0.05$) in all redox indexes except PP and SOD. Also exception ($P > 0.05$) was found for AOPP in combination II group.

Table 3. Redox indexes of different study groups (mean ± SD)

	SHV	Asymptomatic HIV patients	AZT/3TC/NVP		AZT/3TC/IDV		d4T/3TC/NVP	
			E1	E2	E1	E2	E1	E2
MDA (nmol/gHb)	2.33 ± 0.11	5.43 ± 0.35^{a}	6.92 ± 0.7^{ab}	8.79 ± 0.76^{abc}	6.92 ± 0.59^{ab}	8.8 ± 0.49^{abc}	7.07 ± 0.54^{ab}	10.52 ± 1.74^{abc}
HPO (μM)	117.01 ± 3.47	135.67 ± 11.36^{a}	163.19 ± 7.43^{ab}	198.7 ± 16.11^{abc}	139.4 ± 5.89 ^{a}	152.63 ± 5.27^{abc}	160.11 ± 6.4^{ab}	202.4 ± 17.32^{abc}
AOPP (μM cloramine-T)	12.93 ± 0.66	25.98 ± 2.53^{a}	33.8 ± 3.2^{ab}	50.13 ± 5.51^{abc}	27.43 ± 0.79^{a}	27.36 ± 1.5^{a}	16.82 ± 1.09^{ab}	37.61 ± 1.71^{abc}
GSH (μM/gHb)	1247.6 ± 21.51	420.04 ± 17.18^{a}	378.65 ± 26.48^{a}	319.55 ± 26.54^{abc}	426.6 ± 26.6^{a}	334.11 ± 33.07^{abc}	457.32 ± 12.1^{a}	350.16 ± 33.95^{abc}
CAT (U/mgHb)	135.1 ± 2.76	277.05 ± 16.89^{a}	295 ± 20.1^{a}	366.22 ± 20.32^{abc}	261.43 ± 13.89^{a}	439.2 ± 14.7^{abc}	342.86 ± 22.39^{ab}	448.7 ± 23.02^{abc}
SOD (U/mgHb)	2.53 ± 0.19	3.35 ± 0.04^{a}	3.48 ± 0.3^{a}	3.22 ± 0.24^{a}	3.12 ± 0.35^{a}	3.2 ± 0.31^{a}	3.22 ± 0.35^{a}	3.21 ± 0.33^{a}
PP (μM)	6.8 ± 0.32	15.15 ± 1.02^{a}	13 ± 1.23^{a}	13.2 ± 1.01^{a}	10.47 ± 1.41^{ab}	12.1 ± 1.43^{abc}	11.44 ± 1.87^{ab}	12.59 ± 1.41^{ab}

SHV, supposedly healthy voluntaries; HAART, highly active antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; IDV, indinavir; d4T, stavudine; E1 (extraction 1), after 14 days with HAART; E2 (extraction 2): after 6 month with HAART; PP, peroxidation potential; CAT, catalase; SOD, superoxide dismutase; HPO, hydroperoxide; MDA, malondialdehyde; GSH, glutathione; AOPP, advanced oxidation protein product. Different letters represents significant differences (p<0,05): [a]compared to SHV group; [b]compared to asymptomatic HIV patients; [c]differences between extractions.

The factorial analysis was done resulting in 80-98% of acceptable samples adequacies (Kaiser coefficient > 0.7) that were determined for SHV, HIV and AIDS pre-HAART analyzed groups. In the case of treated group including the three combinations non-acceptable adequacy were determined (Kaiser coefficient < 0.7). For SHV 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 AIDS condition (baseline analyses prior of three HAART combinations consumption, extraction 1), one main component were found with interdependence between PP, CD4 and GSH inversely related to CAT, MDA and VL (data not shown).

Discriminate analysis using redox indexes and HIV progression markers revealed that 98% of the variation between groups was accounted by two first discriminate functions ($P < 0.001$) (Fig.1). Biochemical redox indexes and HIV progression markers were related in these two functions with discriminate loadings of 0.983 and 0.811, respectively. Also 88.5% of original grouped cases were correctly classified by reanalysis using the two functions.

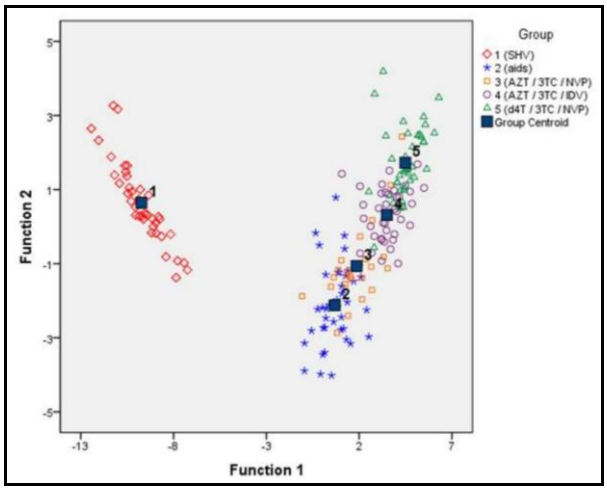

Figure 1. Canonical discriminate analysis representation related to redox indexes in different studied groups. AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; IDV, indinavir; d4T, stavudine. **(1)** SHV: seronegative HIV-presumable healthy subjects; **(2)** AIDS: subjects with acquired immunodeficiency syndrome; **(3)** group of individuals treated with AZT/3TC/NVP; **(4)** group of individuals treated with AZT/3TC/IDV; **(5)** group of individuals treated with d4T/3TC/NVP. **Function 1:** 1.624 MDA + 1.521 PP + 0.122 GSH + 0.039 CAT + 0.055 AOPP +0.295 HPO + 1.273 SOD + 0.31 CD4 – 125.259. **Function 2:** 4.74 MDA + 3.024 PP + 0.046 GSH + 0.077 CAT + 0.027 AOPP + 0.393 HPO + 1.881 SOD + 0.003 CD4 – 89.905.

DISCUSSION

There are several studies of disturbed redox metabolism in HIV-infected patients with and without treatment [12-14, 18, 21, 35-43]. In the present study, we have shown that, in HIV infection and during HAART, VL decrease and CD4 stabilization are accompanied by both abnormal antioxidant-redox status and increase in the levels of redox indexes with different behavior according to HAART combinations.

Voluntary screening of HIV infection is implemented as an integrated rights-based strategy by the Cuban national public health system. Most of the individuals in the present study are diagnosed as HIV+ in asymptomatic condition. National therapeutic guideline recommends and supports free HAART for AIDS condition (CD4 less than 250 cell/mm^3 or VL over 55000 IU). Universal ART coverage to all patients with AIDS clinical criteria has been done using Cuban-produced generics. Also ART not produced in Cuba are acquired with the support from 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 [38].

In previous studies evidences of HAART effectiveness of pharmaceutical leaders is reported around 70-80% [1, 7, 8, 11]. Cuban generic drugs were used during the research and the general effectiveness was 78% accordingly to previous reports. Concomitant drug used in the present study have no impact in the subsequent indexes assessed, taking into consideration the frequency, doses employed and the fact that second extraction was done after 6 months with HAART.

HIV infection increases the oxidative stress process in relation to chronic activation of inflammation related to chronic virus exposition [4, 19, 20, 29, 40]. This status is further influenced by the use of HAART. Oxidative stress has been one of the theories related to mitochondrial toxicity and ART toxicities. This was observed by the significantly higher and modified values of MDA, HPO, AOPP and CAT activities (Table 2), suggesting an increase in oxidant stimuli. Also lower and modified antioxidant capacity inversely to PP values and GSH concentrations were detected in HAART combinations.

Associated toxicities simultaneously to the therapeutic effect have been previously reported [16, 34, 47]. 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 [38-42, 49]. Other studies reported that HAART may increase oxidative stress levels above and beyond levels caused by the virus itself inducing ***(i)*** increase in oxidant generation,

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(ii) decrease in antioxidant protection or ***(iii)*** failure to repair oxidative damage [44, 47, 48]. Oxidative stress-mediated cellular damage occurs, in part by ROS, unstable bimolecular configurations which quickly react with other molecules to achieve stable configuration [65, 66]. In HIV infection ROS reactions could result also from non-enzymatic protein oxidation and the subsequent oxidative degradation of proteins [32, 67].

ROS and its 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 [24-27]. Abnormally high ROS levels as well as simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes as well as increased lipid peroxidation [20, 23, 30].

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 [31, 33, 34]. HAART may reduce GSH synthesis, enhance GSH utilization, or limit intracellular reduction of its oxidized form (glutathione disulfide, GSSG) [21, 24]. 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 [23, 34, 40]. HAART could also have a role in oxidative stress resulting from tissues and liver cells destruction, neutrophils and macrophages activation [20, 30, 39, 45, 67].

Major cellular damage is a result from macromolecules oxidative stress alteration in membrane lipids, essential proteins and DNA [25, 34, 67]. The differences in oxidative stress indexes as well as in the plasma concentrations of antioxidants between pre- and post-therapy status may be explained by HAART influences. Certain aspects of HAART toxicity may be associated with oxidative stress consequences, although HAART results in suppression of viral replication and both clinical and immunological status improvement [3, 7], thereby increasing the body's requirements for certain antioxidants [23, 25, 68].

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

Discriminate analyses meanwhile showed that HAART did not induce normalization of these parameters, resulting in different consequences accordingly with drug combination [62, 63]. To our knowledge, this is the first time reporting multivariate and integral analysis considering redox and progression indexes in the HIV field. These findings lend further support to idea that enhanced oxidative stress contributes to the pathogenesis of HIV infection even with ART, and findings also suggest that antioxidant intervention could influence and counteract these oxidative disturbances, even in the HAART era [23, 49, 68]. However, it remains to be proven that such intervention or supplementation effects result effective in HIV infected patients receiving HAART. In our study, differences in some redox indexes related to progression markers and also to HAART, evaluated by multivariate and discriminant analysis, proved the association of underlying oxidative mechanism.

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 [24, 29]. 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. Oxidative stress evaluation will, therefore, become a potential utility aspect to follow antiviral combinations' effects, as well as to evaluate 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 virus pathogenic actions and for HIV-infected patients clinical monitoring. Optimal usage of this tool in clinical setting, however, still remains to be defined.

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COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

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