Characterization of oxidative stress in different clinical conditions, using redox indexes of diagnostic value

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ABSTRACT

The redox status imbalance could be related to diverse clinical entities. Recognized analytic methodologies quantifying both: oxidative biomolecular damage and antioxidant activity were used for the characterization of redox balance in human samples (urine, lymphocytes, plasma and serum) in relation with progression markers of diverse clinical conditions. Case and control studies of Human immunodeficiency virus (HIV), Dengue, Diabetes mellitus (DM) type 1, Human-T lymphotropic virus type-1 patients and apparently healthy individuals (18-84 years) were carried out. The evaluation was also applied in intervention designs. The results evidenced oxidative alterations and antioxidant capacity decreased significantly (p < 0.05) in patients compared to healthy individuals related in age and gender. Studies of nutritional intervention and antioxidant supplementation with Vimang® in 40 and 81 HIV Cuban patients respectively showed significant beneficial changes (p < 0.05) in 56% and 43.9% of cases respectively. An observational study in 56 HIV Cuban patients was carried out involving two combinations of antiretroviral therapy. The study showed evidences of oxidative modifications significant (p < 0.05) in 87% of the cases, finding differences among combinations. An observational study involving 40 Cuban DM type 1 patients with change of neutral protamine Hagedorn insulin from pig to human recombinant, showed a significant beneficial change (p < 0.05) in 81% of the cases after the change. The integral characterization could be useful for follow up and individuals’ management but also contribute to the knowledge of the molecular mechanisms underlying in these illnesses.

Keywords: oxidative stress, HIV, HTLV-1, diabetes type 1, dengue, integral diagnosis

RESUMEN

Caracterización del estrés oxidativo en diferentes condiciones clínicas mediante índices redox de valor diagnóstico. El desbalance del estado redox pudiera estar relacionado con diversas condiciones clínicas. Para la caracterización del estado redox en muestras humanas (plasma, suero, eritrocitos, linfocitos y orina) se emplearon metodologías analíticas para cuantificar el daño oxidativo a biomoléculas y la actividad antioxidante. Se hicieron controles de pacientes con virus de inmunodeficiencia humana (VIH), con diabetes mellitus (DM) tipo 1, con virus linfotrópico de células T humanas tipo 1, y de individuos aparentemente sanos (entre 18 y 84 años de edad). La caracterización también se aplicó a diseños de intervención. Se evidenciaron alteraciones oxidativas y significativa disminución de la capacidad antioxidante (p < 0.05) en los enfermos con respecto al grupo presumiblemente sano, relacionados en edad y género. Los estudios de intervención nutricional y suplementarios con Vimang® en 40 y 81 pacientes con VIH, mostraron beneficios significativos (p < 0.05) en el 56% y 43.9% de los casos, respectivamente. En el estudio observacional en 56 pacientes con VIH con dos combinaciones de terapia, se incrementó el daño oxidativo y disminuyó la capacidad antioxidante significativamente (p < 0.05) en el 87% de ellos, con diferencias según las combinaciones. Se efectuó un estudio observacional en pacientes con DM tipo 1 con cambio de insulina protamina neutral de Hagedorn de cerdo a insulina recombinante humana, que evidenció beneficios significativos (p < 0.05) en el 81%. Ello contribuyó no solo a la atención integral de los pacientes, sino también al conocimiento de los mecanismos moleculares involucrados en estas enfermedades.

Palabras clave: estrés oxidativo, VIH, VLTH-1, diabetes tipo 1, dengue, diagnóstico integral

Introduction

The study and refinement of surrogate markers of disease progression continues to be an important area of research particularly with the advent of therapies that claim to halt or slow the immunological influence [1].

The immunological process is tightly complex considering the plethoric tissues, cell lines and molecular mechanism involved for such pathology. The generation of reactive oxygen species (ROS) is widely recognized as an effector’s system of it, exerting physiological roles in cellular response at low/moderate ROS concentrations such as: defence against pathogens agents, signalling pathways, induction of mitogenic response and contributing to the homeostasis. ROS are generated endogenously by mitochondria and other specialized enzymes [1, 2]. Also it could be generated by exogenous agents as microbes, environmental carcinogens, xenobiotic and toxic food ingredients. The interaction of ROS and the antioxidant system establishes

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or maintain a redox balance. Increased ROS production could overwhelm the antioxidant capacity of the cell producing oxidative stress (OS) and damage to cell structures as: nucleic acids, lipids, proteins and carbohydrates. Oxidative damage to macromolecules is detectable under normal physiological conditions in healthy individuals suggesting that the efficiency of antioxidant and repair mechanisms cannot avoid completely ROS reactions with macromolecules. Therefore, cells also under normal physiological conditions manifest a certain level of oxidative damage. The sustained cumulative oxidative damage related to OS status has been associated to aging and more than a hundred different diseases [2, 3].

Aging is the process of change accumulation by age and it could be defined as a complex chronological and multifactor event [4]. The OS hypothesis of aging postulated by Harman in 1956 [5] recognize that accrual of macromolecular damage accumulation is due to the redox imbalance. OS within mitochondria can lead to a vicious cycle in which ROS production progressively increase with age leading, in turn, to progressive increase of damage and suggested that biomolecule oxidation processes could be the central cause factor promoting aging process. Age-related diseases are often considered to be distinct pathologies, rather than inevitable part of aging and a consequence of redox deregulation. Indeed a chronic inflammatory process is crucial associated with degenerative conditions during the process. The functional consequence of an age related modifications in such biochemical’s markers of OS has been little studied and remains therefore largely unknown [6].

Human immunodeficiency virus (HIV), Human-T lymphotropic virus type 1 (HTLV-1) and Dengue infection are worldwide problems; all of them occurring in relation to immune system function [7]. OS, induced by the increased production of ROS, may play a critical role in the stimulation of virus replication and the degenerative evolution of individuals. Excessive production of ROS may be related to both an increased activation of polymorphonuclear leukocytes in infections or influenced by the prooxidant effect of tumor necrosis factor-alpha produced by activated macrophages during the course infection, and to activate caspases and mitochondria. Several investigators have proposed that apoptosis, initiated by OS, is a direct cause in the pathophysiology in these infected patients [1, 7].

In the latest years, a relevant morbidity and mortality decline of HIV infection has been observed due to the use of potent combined therapy named High Active Antiretroviral Therapies (HAART). It has led to a decrease of viral load, and a quantitative and qualitative improvement of the immune functions in patients, specially T-CD4+ lymphocyte count and viral load, having as a consequence a decrease of infectious complications and a global clinical improvement [8].

But HAART did not completely solve immune and metabolic alterations during HIV evolution instead hepatic toxicity was reported early in the epidemic, and recent reports continue to point mitochondria as toxic targets. Since ART therapies do not completely eliminate HIV, it is likely that the final outcomes of treatment will depend not only on the efficacy of treatments in reducing viral load, but also on the immune system’s ability to recover and control residual virus. Different nutritional and antioxidant intervention used to counteract OS in HIV/AIDS patients were developed with limited advantages.

Diabetes mellitus (DM) type 1 is an autoimmune disease involving T cell-mediated dysfunction of pancreatic β-cells. The dysfunction results in a lack of insulin generation and prolonged exposure of cells and tissues to hyperglycaemia. Four major mechanisms are recognized in recent reports that reflect a single hyperglycaemia-induced process of superoxide overproduction by the mitochondrial electron-transport chain [1, 2]. This overproduction contributes to the OS in DM patients and it may be related to endothelial dysfunction and could be related to complications.

Insulin used for therapeutic purposes was obtained first from beef and pork pancreas, which have been superseded by recombinant human insulin more recently. The first recombinant human insulin was approved by the Food and Drug Administration (FDA) in 1982. Human insulin was introduced for routine treatment taking into account animal insulin known to produce insulin antibodies that can slow the action of the insulin and cause problems such as insulin allergies.

A large amount of data about OS implications in some tissues damage, certain diseases, biological variables and life habits has been shown but reports on changes of OS markers in blood and blood plasma of healthy populations during aging are rare, also referred to Dengue and HTLV-1 [9]. Otherwise an integral characterization which includes injury markers as well as protection indicators of the redox status has been applied poorly in the literature. The aim of the present work was to study the status of an extensive array of redox indexes as proposal of an integral and dynamic characterization of redox status. The indexes evaluate the concentration of oxidized biomolecules and antioxidant activity in relation to progression disease markers. For this purpose, we compared the blood levels of these markers in HIV/AIDS, dengue, HTLV-1 and DM type 1 patients to those of healthy aged-matched control. Also the integral redox diagnostic is applied in certain interventions in HIV and diabetic patients.

### Materials and methods

#### Subjects and blood collection

**Healthy, HTLV-1, HIV/AIDS, Dengue and DM groups**

The integral diagnosis was applied in convenience samples of subjects that gave written informed consent to take part in the study after verbal and written explanation of the methods and risks involved were given. Procedures were previously reviewed and approved by the Tropical Medicine Institute Pedro Kouri Committee for Research on Human Subjects, also by the Center for Pharmacoepidemiology Development (DM studies). The studies were in accordance with the principles of the Declaration of Helsinki concerning to the Ethical Principles for Medical Research Involving Human Subjects. All these studies are observational or case-control studies.
Patients underwent an initial screening, which included medical history, toxic habits (smoking, alcohol) alimentary habits and history of supplemental vitamin intake, anthropometrics data (weight, height), and a biochemical check up (complete blood count, glucose, creatinine, urea, liver enzymes). Blood was sampled at least 12 h after fasting. Samples were stored in freezer at –70 °C until analysis.

**Healthy group**

The purpose of this study was to examine redox status in plasma and erythrocytes of 194 healthy individuals (women and men ranging from 18 to 84 years of ages varying ethnicity) and determine correlation between these parameters and aging.

**DM type 1 patients**

The Diabetic patients were selected from Database of Diabetic consult of the Elpidio Berovides Care Center (San Agustin Community of Havana City, Cuba) where the change of neutral protamine Hagedorn (NPH) insulin pork for NPH human insulin would be generalized. Forty diabetic patients were enrolled. All patients had been treated from time of diagnosis and during the study with 2 daily insulin injections.

Administrations of insulin were done at the Care Center. Nurses followed accomplishment of injections and incidence of adverse reactions by interviewing patients daily. For adverse reactions control, the model 33–36–1 from Public Health Ministry of Cuba was used, based on the Karch and Lasagna methodology [10]. All patients completed the study.

**DM type 1 patients with insulin change**

The study period of 2 months was considered taking into account previous results of glucose control during transfers from animal to human insulin and possible effects on oxidative indexes were considered.

Blood and serum samples from 40 patients (48 median age, range 38–64) before and 2 months after change of insulin treatment were used. The groups were from both sexes and varying ethnicity. Healthy sex- and age matched subjects values of biochemical indexes previously determined and reported were used as references values.

**Dengue patients**

Integral diagnosis were applied in 22 serologically confirmed Dengue patients that would permit examination of the role of stress target which cause damage to biomolecules related with classification of Dengue. Blood samples were taken at days 3, 5 and 7 after the onset of fever. For this purpose, we compared the blood levels of these markers in Dengue patients classified as: dengue fever, dengue fever with hemorrhagic manifestations, and dengue hemorrhagic fever to those of healthy aged-matched control.

**HTLV-1 patients**

Comparison of redox balance indicators assessed in 9 asymptomatic HTLV-1 seropositive (HTLV-1+) and 10 apparently healthy individuals were done. These parameters were related to changes in the levels of CD3, CD4, CD8 and CD25 T cell subsets in patients.

**HIV patients**

Seropositive individuals were identified with a reactive enzyme-linked immuno sorbent assay for HIV (ELISA-Uniform VIH I/II plus O test, Organon Technica, Netherlands), which was confirmed by positive Western Blot analysis (DAVIH BLOT VIH-I, DAVIH Lab, Cuba).

**HIV group with nutritional intervention**

Stable HIV-positive subjects from the Santiago de Las Vegas Sanatorium (SSV-Havana) ranging in age from 20–47 years were approached and 18 females and 22 males recruited for study. Subjects were eligible if they had no active opportunistic infection. The aim of the present study was to examine the impact of improved nutrition on HIV progression during 3 months. To achieve our aim, we took advantage of two opportunities. First, we recruited institutionalized HIV positive subjects as their diet is known and controlled. Second, we examined these subjects at the beginning and end of a seasonal period when fresh fruit and vegetables become common dietary items. During this 3-month period we applied the integral diagnosis.

Patients were in relatively good health and functioning well, with non-clinical evidences that nutrient metabolism was compromised by wasting or severe diarrhea. Thirty six patients completed the study, the remaining 4 being unable to keep the 3-month follow-up appointment.

**HIV group with antioxidant supplementation**

This study is a randomized double-blind placebo-controlled trial. Stable HIV-positive subjects from SSV-Havana were approached for the study between September 2000 and April 2001. Patients could be on any combination of antiviral therapy providing that any therapy was started 1 month prior to study entry and remained stable for the duration of the study. Subjects were eligible if they had no active opportunistic infection. Eighty two subjects were enrolled. Patients involved (82) were placed on a controlled diet 2 weeks prior to randomization and continued during the study period. They also received dietary counseling to maximize and standardize their intake of food rich in vitamins.

Patients were randomly assigned (using a random number table) to receive daily eight tablets or Vimang® (Mangifera indica extract, 300 mg) or eight tablets of placebo. Therefore subjects, observers and laboratory personnel were unaware of the nature of supplements given. The placebo contained corn-starch. Supplements and placebo were both coded, had the same taste, appeared identical and were prepared specifically for this study. The code was not broken until all patients completed the therapy and all the analysis were performed.

Compliance with the supplements or placebo intake was verified by counting leftover medication. Subjects were advised to continue their normal activity and to report any unusual symptoms. Patients were required to record their food intake for 7 days at the beginning and the end of the study, including all food and beverages consumed their portion size and method of preparation. Patients were assessed at baseline and at 6 months. At each study visit, anthropometry, 10. Karch FE, Lasagna L. Toward the operational identification of adverse drug reactions. Clin Pharmacol Ther. 1977; 21(3):247-54.
review of food records and laboratory test were also performed. Physicians reported HIV-associated opportunistic and other infections in the chart during 6 months after randomization.

**HIV group with ART**

Subjects were eligible if they had no active opportunistic infection. Eighty two subjects ranging in age from 30-50 years were enrolled. Patients were ambulatory patients recruited on the Institute Pedro Kourí Hospital. Data were processes by the SIDATRAT software (IPK, version 2008). Fifty six were taking 2 different HAART combinations during 6 months; therapy I: zidovudine (AZT) + lamivudine (3TC) + indinavir (IDV); and therapy II: stavudine (d4T) + lamivudine (3TC) + nevirapine (NVP). Twenty eight patients were HAART-free. Subjects were advised to continue their normal activity and to report any unusual symptoms. Patients were assessed at baseline and at 6 months. At each study visit, anthropometry and laboratory test were also performed.

Physicians reported HIV-associated opportunistic and other infections in the chart during 6 months after beginning the study.

**Flow cytometry analysis**

A study of T lymphocytes subsets CD3+/CD4+, CD3+/CD38+, CD4+/CD95+ in total blood with lysing solution Becton Dickinson (BD) was carried out. For each T lymphocyte subset, TriTEST™ CD3 CD45 CD4; CD3 CD45 CD38; CD3 CD45 CD95 were used. Analyses were performed on a FACSscan flow cytometer (Becton Dickinson Immunocytometry System) by MULTICELL program.

**Assessment of dietary intake**

Food and beverage diaries were converted to standard portion sizes at 0 and 3 months for seven consecutive days. Nutrient intake was calculated using the Cuban CERES food composition database, which contains the nutrient contents of the commonly consumed food and beverages in Cuba [11].

**Viral load**

Viral load was determined with the Biomerieux’s nucleic acid sequence-based amplification polymerase chain reaction (NASBA®) ultrasensitive assay, at the lowest limit of quantification of 50 U.

**Biochemical and hematologic measurements**

Blood samples were obtained after at least 12-h fasting. Samples were collected into heparin-treated tubes. Centrifugation was performed at 1000 x g. Samples were collected into heparin-treated tubes. Centrifugation was performed at 1000 x g.

**Measurement of glycemic control**

Glycosylated hemoglobin (HbA1c) was evaluated by using a commercial available kit (Human GmbH D65205, Germany; Cat No: 10657). This kit used a fast ion-exchange resin separation and spectrophotometer. The normal range was 4.7-6.3% of total Hb.

Fructosamine was assessed by using a commercial available kit (CENTIS Diagnostic, Cuba). This kit used a colorimetric assay and spectrophotometer. The normal range was 265-315 μmol/L.

**Glutathione concentration**

Plasma reduced glutathione (GSH) was analyzed with the method described by Sedlak and Lindsay. GSH (Sigma, St. Louis, MO, USA) was used to generate standard curves [12].

**Malondialdehyde concentration**

Malondialdehyde (MDA) concentrations were analyzed by the LPO-586 kit obtained from Calbiochem (La Jolla, CA, USA). In this assay, stable chromophore production after 40 min of incubation at 45 °C is measured at a wavelength of 586 nm by Pharmacia Spectrophotometer. Freshly prepared solutions of MDA bis [dimethyl acetal] (Sigma, St Louis, MO, USA) assayed under identical conditions were used as reference standards. Concentrations of MDA in plasma samples were calculated using the corresponding standard curve and values were expressed as nmol/g Hb [13].

**Peroxidation potential**

For the determination of the susceptibility to lipid peroxidation, plasma samples were incubated with a solution of cupric sulfate (final concentration of 2 mM) at 37 °C for 24 h. The peroxidation potential (PP) was calculated by subtracting the MDA concentration at time 0 from the one obtained at 24 h [14].

**Total hydroperoxide**

The total hydroperoxide (HPO) was measured based on the oxidation of ferrous ions to ferric ions by hydroperoxides under acidic conditions. Ferric ions bind with the indicator dye xylenol orange (3,3'-bis(N,N-di(carboxymethyl)-aminoethyl)-o-cresolsulfonephthalein, sodium salt) to form a stable coloured complex which can be measured at 560 nm.

**SOD**

The enzymatic activity of SOD was evaluated by using Randox Ltd. (Diamond Road, Crumlin, UK) Kit Cat. No.SD125. In brief, the method employs xanthine and xanthine oxidase to generate O2⁴⁻, which reacts with 2-(4-iophenyl)-5-(4-nitrophenyl)-3-(4-trithiazolium chloride (INT) to form a red formazan dye. SOD activity was then measured by the inhibition degree of this reaction and was expressed as U/mg Hb.

**CAT**

The enzymatic activity of CAT was measured according to the method of Aebi. Using a molar extinction
coefficient of 43.6 M⁻¹ cm⁻¹, the rate of the first 30 s was used to calculate the activity. Catalase activity was expressed as U/mg Hb [15].

**Advanced oxidation protein products**

Plasma advanced oxidation protein products (AOPP) was measured according to the methods of Witko-Sarsat. The values were expressed in Chloramine T equivalents and corrected by serum albumin concentrations [16].

**DNA fragmentation**

Quantitation of DNA fragmentation was determined by colorimetric diphenylamine assay as described by [17]. Leukocytes were obtained from whole blood samples by osmotic shock and lysed in lysis buffer (0.2% v/v Triton X-100, 10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Lysates were centrifuged at 13 000 x g for 10 min. The supernatant, containing small DNA fragments, was separated from the pellet of intact DNA. The pellet was resuspended in 1 mL of lysis buffer and perchloric acid was added to a DNA. The pellet was resuspended in 1 mL of lysis buffer and perchloric acid was added to a final concentration of 0.5 M, to both the supernatant and the pellet, followed by 2 volumes of diphenylamine solution (0.088 M diphenylamine, 98% v/v glacial acetic acid, 1.5% v/v sulfuric acid concentration and 0.5% v/v of 1.6% acetaldehyde solution). Samples were store at 4 °C for 48 h and were quantified(188,466),(457,501) by spectrophotometry at 575 nm. DNA fragmentation was expressed as percentage of total DNA appearing in the supernatant fractions (DNAF%).

**Therapeutic or intervention effects**

The intervention effect was analyzed calculating the simultaneous change in two combinations of variables for each patient in every study. The first variable included simultaneous change in some evolution markers. The second included, additionally to the first variable, simultaneous significant changes in redox markers. The significant change in every marker between the initial and the last evaluation was computed. The intervention effect was considered if every time simultaneous change, according to indicators, show beneficial effects in patients. The opposite was considered as a failure. However, if at least one of the parameter failed, it was considered as a global failure.

**Statistics**

Initially the outliers preliminary test for detection of error values was applied as a first step in the statistical analysis. Data were expressed as means ± standard deviation (SD). After this, the homogeneity of variance test (Bartlett-Box) was used. Analysis of variance and comparison of means were performed to detect statistical differences between the control group and patients in each study.

The biochemical indexes values before and after the study in the different groups were compared using dependent or independent t test, accordingly. The numbers of patients with success or failure evaluated by the combinational variables was reported in contingency tables. Comparison in each variables were done using χ² test. The minimal level of significance was identified at p < 0.05. The SPSS software package (IBM; version 13.0, 2007) was used for all statistical analyses.

**Results**

**Healthy group**

The results associated to healthy group indicate that the balance of oxidant and antioxidant systems in plasma shifts in favour of accelerating oxidation during aging (Table 1). That is demonstrated by erythrocytic glutathione disulfide and HPO increased together with slight decrease of erythrocytic GSH with age. Besides, biomolecules injury such as lipid peroxidation (MDA), protein damage (AOPP) and DNAF% were observed.

**HTLV-1 group**

Significant differences were found (p < 0.05) between HTLV-1+ and control group regarding to stress indicators, except the concentration of GSH (Table 2). The CD4+ T lymphocytes count inside the normal intervals whereas the CD8+ T lymphocytes count was slightly increased. Relative values of CD25 also showed significant differences between HTLV-1 and healthy subjects (data not shown). These results reveal both the presence of a pro-oxidant state with the activation of the cytotoxic cellular response and the activation of cellular gene expression.

**Dengue group**

A reduction of glutathione peroxidase and an increase of SOD activity were found in Dengue patients. In this condition, the detoxification capacity evaluated as PP was reduced and it suggests hydroxyl radicals’ formation which may promote lipid damage in accordance with high levels of lipid peroxidation. In line with this, HPO showed a reduction compared to the control group. This is a stable intermediate that could generate hydroxyl, peroxyl or other ROS by interacting with organic metal. Generally in OS conditions, this index showed higher values of HPO because of its accumulation. In this case, the lower values could indicate an enhanced peroxidation due to metal concentration increase in the media, which may produce lipid by-product as MDA. The hydrophilic antioxidant GSH showed significantly modifications in these individuals. Significantly change was observed in hydrophilic PP too. The observed increase of OS processes in these patients resulting from cytotoxic lipid product would modify proteins and membranes by adding reactions contributing to the physiopathologic results from a descriptive analysis, although there were no significant differences noted associated to different days of clinical condition and classification of fever.

**DM group**

Related to the DM group, there were no differences in the male/female ratio or in the proportion of those with hypertension or current smoking between diabetec patients and study controls (p > 0.05). Compliance of human insulin injection was excellent on the basis of the applied data (94%). Adverse reactions reports during the 2 months of insulin change were computed and were generally considered as hypoglycaemic manifestations. It represented a 0.7% of incidence in the 17% of patients.

The mean value of all biochemical markers evaluated before and 2 months after change of insulin and oxidation in different clinical conditions.

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references values are showed in table 3. The statistical analysis showed a significant modification (p < 0.05) in all indexes except in activity of antioxidant enzymes SOD and CAT from erythrocyte cytoplasm origin (p > 0.05). Mean statistical comparisons between references values and biochemical markers evaluated at 2 months showed persistent significant difference (p < 0.05). The values of HbA1c before (8.74 ± 0.3) and 2 months after (6.51 ± 0.25) showed persistent significant differences when compared to study controls too. Plasma levels of Glucose control indexes were improved positively (satisfied 110-90% of the individual requirements) in 60% of patients (24), insufficient (satisfied 90-70% of the individual requirements) in 35% (14) and critical (satisfied < 70% of the individual requirements) in 3% (2). Protein intake was adequate (satisfied 110-90% of the individual requirements) in 35% (14), while 42% (17) and 21% (9) of the patients had insufficient (satisfied 90-70% of the individual requirements) and critical (satisfied < 70% of the individual requirements) low protein intakes, respectively. Lipid intake was greater that recommended in 95% (38) but carbohydrate intake was insufficient and critical in 71% (28) of HIV positive patients (data not shown). Micronutrient intake did not differ significantly between baseline and at 3 months (data not shown). Likewise, there was no significant difference in body mass index (BMI) in kg/m² at day 0 compared with the end of the study (data not shown).

Significant improvements in vitamins A, C, E intakes were noted during the study period (p < 0.05 in all parameters, even its level was still maintained critical (satisfied < 70% of the individual requirements)). This occurred in part due to the increased fruits and vegetable intake related to experimental period.

HIV nutritional intervention

At baseline, mean energy intake was adequate (satisfied 110-90% of the individual requirements) in 60% of patients (24), insufficient (satisfied 90-70% of the individual requirements) in 35% (14) and critical (satisfied < 70% of the individual requirements) in 3% (2). Protein intake was adequate (satisfied 110-90% of the individual requirements) in 35% (14), while 42% (17) and 21% (9) of the patients had insufficient (satisfied 90-70% of the individual requirements) and critical (satisfied < 70% of the individual requirements) low protein intakes, respectively. Lipid intake was greater that recommended in 95% (38) but carbohydrate intake was insufficient and critical in 71% (28) of HIV positive patients (data not shown). Micronutrient intake did not differ significantly between baseline and at 3 months (data not shown). Likewise, there was no significant difference in body mass index (BMI) in kg/m² at day 0 compared with the end of the study (data not shown).

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Table 1. The effect of age on different redox indexes in healthy subjects (mean ± SD)

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<tbody>
<tr>
<td>1 (n = 35)</td>
<td>18-29</td>
<td>1.42 ± 0.27</td>
<td>2.7 ± 0.3</td>
<td>67.5 ± 16.6</td>
<td>12.11 ± 2.4</td>
<td>45.0 ± 4.5</td>
<td>7.7 ± 2.2</td>
<td>3.3 ± 1.3</td>
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<tr>
<td>2 (n = 28)</td>
<td>30-39</td>
<td>1.45 ± 0.17</td>
<td>2.7 ± 0.3</td>
<td>70.3 ± 13.9</td>
<td>15.9 ± 2.8</td>
<td>51.2 ± 26.9</td>
<td>9.41 ± 2.9</td>
<td>3.7 ± 1.7</td>
</tr>
<tr>
<td>3 (n = 31)</td>
<td>40-49</td>
<td>1.51 ± 0.23</td>
<td>2.6 ± 0.4</td>
<td>72.0 ± 20.1</td>
<td>16.2 ± 3.1</td>
<td>51.1 ± 24.1</td>
<td>9.38 ± 3.14</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>4 (n = 35)</td>
<td>50-59</td>
<td>1.62 ± 0.30</td>
<td>2.5 ± 0.4</td>
<td>83.0 ± 20.6</td>
<td>17.14 ± 2.6</td>
<td>56.4 ± 17.9</td>
<td>9.06 ± 2.8</td>
<td>4.6 ± 1.9</td>
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<tr>
<td>5 (n = 48)</td>
<td>60-69</td>
<td>1.89 ± 0.55</td>
<td>2.4 ± 0.4</td>
<td>96.3 ± 22.2</td>
<td>19.57 ± 3.4</td>
<td>57.2 ± 15.1</td>
<td>11.43 ± 3.1</td>
<td>4.7 ± 1.5</td>
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<tr>
<td>6 (n = 17)</td>
<td>70-81</td>
<td>1.69 ± 0.27</td>
<td>2.4 ± 0.7</td>
<td>105.3 ± 32.2</td>
<td>18.9 ± 2.7</td>
<td>68.2 ± 11.1</td>
<td>13.0 ± 4.2</td>
<td>5.8 ± 1.5</td>
</tr>
</tbody>
</table>

Table 2. Redox indexes of HTLV-I, HIV, Dengue and Diabetes mellitus type 1 patients (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA [nmol/g Hb]</th>
<th>GSH [nmol/g Hb]</th>
<th>HPO [μM]</th>
<th>SOD [U/mg Hb]</th>
<th>CAT [U/mg Hb]</th>
<th>AOPP [μmol Cloramin T]</th>
<th>PP [nmol/g Hb]</th>
<th>DNAF%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>1.65 ± 0.57</td>
<td>989.45 ± 75.3</td>
<td>70.3 ± 13.9</td>
<td>1.41 ± 0.72</td>
<td>2.35 ± 0.4</td>
<td>15.9 ± 2.8</td>
<td>9.41 ± 2.95</td>
<td>3.9 ± 1.7</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>4.52 ± 0.21</td>
<td>202.08 ± 28.65</td>
<td>-</td>
<td>2.83 ± 0.84</td>
<td>-</td>
<td>46.29 ± 7.31</td>
<td>13.24 ± 1.19</td>
<td>-</td>
</tr>
<tr>
<td>HIV</td>
<td>3.98 ± 1.42</td>
<td>393 ± 146</td>
<td>242.4 ± 57.5</td>
<td>-</td>
<td>-</td>
<td>11.09 ± 3.17</td>
<td>7.19 ± 1.16</td>
<td>-</td>
</tr>
<tr>
<td>Dengue</td>
<td>3.22 ± 0.53</td>
<td>687.5 ± 241.53</td>
<td>119.6 ± 34.61</td>
<td>2.26 ± 0.71</td>
<td>-</td>
<td>-</td>
<td>15.09 ± 2.60</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes mellitus type 1</td>
<td>5.52 ± 0.73</td>
<td>518.72 ± 107.51</td>
<td>172 ± 26.29</td>
<td>4.1 ± 0.9</td>
<td>3.8 ± 0.5</td>
<td>31.85 ± 2.4</td>
<td>24.50 ± 3.81</td>
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a,b,c Represent significant differences in comparison with an apparently healthy group.

MDA: malondialdehyde.
GSH: reduced glutathione.
HPO: hydroperoxide.
SOD: superoxide dismutase.
CAT: catalase.
AOPP: advanced oxidation of proteins products.
PP: peroxidation potential.
DNAF%: Percentage of DNA fragmentation.
During this season fruits and vegetables with high quantities of micronutrients, including polyphenols, are available. All OS markers showed significant differences between healthy group baseline data and the seropositive group at both times, 0 and 3 months of the study (p < 0.05; table 3).

In HIV patients plasmatic PP was significantly (p < 0.05) increased at 3 months. Lipid peroxidation measured by MDA and HPO was not however significantly different after 3 months of dietary change. SOD and glutathione peroxidase activity were significantly different (p < 0.05) between baseline data and the seropositive group at both times, 0 and 3 months.

GSH was significantly higher (p < 0.05) at 3 months when compared at baseline while DNA damage measured as DNAF% was not changed.

Lymphocyte counts of T CD4+ and CD95+ were not significantly different between at baseline and at 3 months. A significant statistical decrease (p < 0.01) was detected in CD38/CD8+ compared to values at baseline.

PP improved positively in 68% (27) of patients as a consequence of the increased intake of fruits and vegetables from diet (Table 3). When glutathione peroxidase and GSH were also factored into this analysis, a positive change in 56% (22) of patients was noted. Sample sizes were not large enough to detect differences in micronutrient intake between stage A2B2 and A3B3 subjects.

**HIV antioxidant supplementation**

At baseline, there were no differences detected between placebo and Vimang® supplemented groups. Sixty eight subjects completed the study. Among the 14 who did not complete the study, six were randomized on the supplemented group (all was unable to keep the 6-month follow-up appointment) and eight were randomized to the placebo group (four had epigastria discomfort and four were unable to keep their 6-month follow-up appointment). All 14 patients have baseline and follow-up measurements (month 3 or 4 of the study) performed for all parameters and were thus included in the statistical analysis as an intention-to-treat basis.

The supplemented group (n = 36) had an increase in plasma GSH (p < 0.05) and a reduction in PP (p < 0.01), MDA (p < 0.01), HPO (p < 0.01), DNAF% (p < 0.01) and SOD (p < 0.01) when compared with placebo group (n = 32). Change simultaneous analysis results in 81% of patients with improved positively the antioxidant capacity (Table 3). When MDA, HPO and DNAF% were also factored into this analysis 43.9% of patients improved positively antioxidant capacity with simultaneous reduction of oxidative damage on biomolecules (lipids and DNA) in HIV supplemented group (Table 3). No significant differences were detected in CD4 and CD8/CD38 between placebo and supplemented groups. There was also a trend towards a reduction in CD95 receptor (mean ± SD changes over 6 month −6.12 ± 2.30 vs. −13.47 ± 3.31 , p = 0.08; data not shown). Nutritional index of macro and micronutrients intake during the period not showed differences between groups and no significant differences were detected during clinical behavior. No adverse reaction was detected in supplemented group (data not shown).

**HIV with two ART combinations**

Eighty four HIV+ individual, from whom 56 were treated with ARTs, were enrolled to evaluate two different antiviral combinations, 28 subjects for each
one. The other 28 HIV+ individuals were not treated with antivirals. Eighty four healthy subjects were processed as control. There were no significant differences between the groups at baselines with respect to demography and number of patients.

All HIV+ patients enrolled were classified as A3B3 and during the 6 months of study period were kept stable.

Treatment compliance was excellent on the basis of patients’ reports (94%). The statistical analysis of mean value of all biochemical redox and HIV progression markers evaluated in control and HIV group before and 6 months of HAART treatment showed significant difference between control and HIV group before and 6 months after HAART treatment and significantly modification respect HIV group treated (p < 0.05) in all indexes except in activity of CAT (p > 0.05).

Plasma GSH levels were significantly lower at 6 months compared to both the initiation of study and control value (p < 0.05). Lipid peroxidation evaluated as MDA and HPO plasma concentration were significantly higher (p < 0.05) at the end of the study. Plasma levels of AOPP were significantly (p < 0.05) higher in HIV patients at the end of the study. The activity of the erythrocyte SOD antioxidant enzyme was significantly higher at the end of the study (p < 0.05). HIV patients had PP significantly lower at the beginning, suggesting reduced lipid-serum antioxidant capacity. It was modified significantly (p < 0.05) at the end of the study, and DNAF% was also significant higher (p < 0.05).

The immunologic and virologic influence of HAART in HIV population has been several reported previously and as in this study HIV-RNA copies per millilitre of plasma showed a significant decrease (p < 0.05). Concomitant increase in both CD4+ and CD8+ T cell count in peripheral blood were reported too. In this study CD4+ T cell counts increase significantly (p < 0.05) but CD8+ T cell not (data not shown). Non-significant changes were found with respect to CD95+ and CD38+ T cells compared to the beginning of the experiment either (p > 0.05). But trends toward a reduction were observed in both (data not shown).

CAT values for the both treated groups did not show significant difference for determination at initial and at 6 months (p > 0.05). Measures of the others OS indexes showed a significant difference (p < 0.05) between values determined for the two HAART combinations at 6 months and respect HIV+ no treated at both determinations. For therapy II, all values were significantly higher compared to the therapy I group and HIV+ non-treated, except for GSH values for therapy II which were lower than therapy I values.

No significant differences were found between groups treated respect to CD4+, CD8+, CD38+, CD95+ T cell subset, viral load and BMI at 6 months (data not shown).

Multivariate linear regression coefficients for different variables on each HAART combinations were evaluated. For therapy I, this analysis confirmed that BMI and CD4+ T cell count did not have significant impact on MDA, GSH, HPO, AOPP, DNAF%, SOD and CAT but CD4+ T cell subset significantly influenced (p < 0.05) the antioxidant capacity evaluated as PP. Besides, viral load significantly influenced (p < 0.05) on MDA, HPO and GSH but did not on CAT, SOD, AOPP, PP and DNAF%.

For combinatory analysis of treatment, simultaneous beneficial changes were computed. In the first combination, VL and CD4+ T cell counts were considered. For therapy I, 22 patients (80%) had beneficial positive change in both factors considered as a success, as for 23 patients (81%) of the therapy II group. When redox indexes (PP, MDA and DNAF%), showing significantly beneficial changes, were also factored into a global analysis, an overall positive change in 7 patients (23% of the total patients of study) was evidenced for therapy I, and in 4 patients (12%) for therapy II (Table 3).

Discussion

Elevated lipid peroxidation products, damage to DNA and depleted antioxidant capacity have been documented previously in HIV, HTLV-1 dengue-infected individuals and also in DM patients [1, 3, 9]. The current studies provide additional evidences about altered redox status in these individuals in relation to progression indexes of each clinical condition. These responses could be associated with cell damage, metabolic abnormalities and immune dysfunctions all of which are observed in patients. Also the evidences of the studies contribute to show the influence of alternative or therapeutic interventions on redox status with beneficial or negative impact.

Healthy individuals with increased age

In view of the correlation between MDA and 4-Hydroxy-2-Nonenal (a fatty acid peroxidation product), we suggest that aging is a process directly related to systemic oxidized-lipid accumulation. Both MDA and 4-Hydroxy-2-Nonenal strongly correlates with GSH decrease during aging suggesting that consumption of this endogenous metabolite is directly related to aging too.

There are several hypotheses to explain how aging occurs, considering complex physiological alteration in the organism evolution described as: mitochondrial changes, accumulation of aberrant proteins in the cytosol, chemical damage to macromolecules, and somatic mutations. No theory has been generally accepted. The OS hypothesis offers one of the best mechanistic elucidations of the aging process and other age-related phenomena such as age-related diseases. Antioxidant enzymes levels are sensitive to OS. Both increased and decreased levels have been reported in different disease states in which an enhancement of ROS is a cause or a consequence of the disease. The possible explanation for this phenomenon could be a compensatory mechanism by the body to prevent tissue damage [1, 4].

These findings are a valuable contribution as reference values for studies in age-related diseases and infections and for the evaluation and interpretation of OS in an integral fashion too. Differences in the range of concentration respect to the parameters evaluated...
in others reports may be related to diverse population which means the influence of nutrition, habits and life styles. Another important point is that those samples come from a general population [3, 6].

**Diabetes mellitus type I**

Evidences of lipid peroxidation have also been reported in a number of diabetic complications. This is consistent with the finding of Santini et al. [18], and Atalay et al.[19], suggesting that redox metabolism is altered in DM type 1 [1, 20].

The prospectively observations during transition process from porcine to human insulin contribute to both evidences, it can improve redox status of diabetic patients and could be beneficial to DM type 1 patients, and an integral overview about metabolic events involved could be valid to evaluate treatment effects, follow the effect of nutritional regimens or control the degenerative damage associated to OS.

**HTLV-1, dengue and HIV/AIDS**

Redox equilibrium is an important factor for the well function of several cell species, concerning different functions as activation, maturation, cell signalling and death [7]. During viral infection the host inflammatory response appears to be an important contributor to the pathogenesis of human illness [2]. Virus-induced OS could be mediated by an early phase of liberation of pro-inflammatory cytokines.

Human T cell lymphotropic virus I (HTLV-1) is a retrovirus that causes adult cell leukemia and the neurological disorder known as tropical spastic paraparesis. Pathology apparently results from the expression of viral and cellular genes which might lead to cell transformation, immortalization and disease. The results reveal both the presence of a pro-oxidant state with the activation of the cytotoxic cellular response and the activation of cellular gene expression. These determinations may be of interest for knowledge of physiopathology of the infection and for possible preventive therapeutic interventions.

Taking into account the results of the investigation, it is possible to consider that dengue virus could induce apoptosis through the alteration of the redox status. The findings suggest the relationship of *in vivo* OS with the pathogenesis of dengue infection, indicated by high levels of sensitive markers of lipid peroxidation. Lipid peroxidation levels and endothelial dysfunction could be related and would acutely enhance local or systemic vascular leakage. However, it is necessary to study the kinetic of redox markers in a more representative number of Dengue hemorrhagic fever serum samples as well as to introduce other markers as NO synthesized by endothelial cells and close related with vascular diseases [21].

OS condition has been documented previously in HIV populations. The current studies provide evidences in relation to moderate to severe micronutrients intake deficiency and that deficiency could be related to their redox status [1, 8]. After 3 months of increased micronutrient intake but unaltered energy and macronutrient intake, we noted a significance increase in antioxidant status related to an increase in PP and GSH and a decrease in SOD and CD38+/CD8+ counts. Also during Vimang® supplementation, HIV patients showed evidences of improved antioxidant status and reduced oxidative damage with a tendency towards lower CD95 and stabilization of CD4+ T lymphocyte relative counts without dietary interference. These findings are worthy of larger clinical trials; especially both in HIV-positive infected persons who cannot afford antiviral therapies and also in those that took it. Also these results indicate the necessity for larger controlled studies in order to confirm the present observations and to clarify the underlying mechanisms.

The OS effects occurring as a consequence of phosphorylated-NRTI, which means HAART, may amplify some of the pathophysiological and phenotypic event in infection. This aspect results in a new tissue target as treatment is prolonged with increased longevity of AIDS patients, resistance to NRTI appears and AIDS become a relatively manageable chronic illness [8].

Some groups have been worked with different strategies to attenuate the OS during HIV infection, resulting in a tentative option the combination of modified diet and antioxidant supplementation, or combination of both.

The indicators evaluated as redox indexes could contribute to an integral overview in patients. The methods used are relatively cheap, easily performed, non time-consuming procedures and possess sufficient precision to be extrapolated for routine clinical analysis.

OS underlying diseases evolution causes a very wide spectrum of genetic, metabolic, and cellular response from diverse tissues overwhelms the organism [3, 4]. These determinations may be of interest for knowledge of infection physiopathology. The alteration resounds on many malignancies associated. The OS evaluations will therefore become potential useful factors to follow not only therapeutically effects, but also antioxidant and alternative therapies effects on the disease evolution. The counteracting actions’ strategy to diminish the impact of oxidative damage may contribute to both restoration of immune response and also may attenuate its toxic effects which would influence on individuals’ quality of life.

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