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Original Research

# Multivariate discriminant analysis of redox and progression indexes in Cuban acquired immunodeficiency syndrome patients with Kaposi's sarcoma

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## ABSTRACT

**Objectives:** Infection by human immunodeficiency virus (HIV) generates sustained reactive oxygen species production. An increasing number of studies underline the pathogenic impact of high-grade local and systemic oxidative stress in the activation of Herpesvirus 8 producing Kaposi's sarcoma (KS) co-infection in acquired immunodeficiency syndrome (AIDS) patients. This study aimed to determine the redox status in AIDS-KS Cuban individuals and to explore the relation between redox and progression variables. **Methods:** Blood samples were drawn from 99 individuals divided into three groups (33 each one; age range 30-50 years): AIDS, AIDS-KS, and presumable healthy subjects. Total peroxide, malondialdehyde, and advanced oxidation protein products as damage indexes and antioxidant responses (glutathione, peroxidation potential, superoxide dismutase, and catalase) were determined from the blood samples. Furthermore, hematological and hemochemical indexes, progression indexes (viral load, $CD4^+$ T lymphocyte absolute count), and tumoral progression indexes were assessed. Different statistical analyses were done. **Results:** Compared to healthy subjects, both groups of AIDS patients had significant differences in global indices of damage and antioxidant status. The comparison between the groups revealed that AIDS-KS group had a significantly higher damage and lower antioxidant status compared to the healthy control and AIDS groups. Multivariate statistical analysis clearly separated groups according progression indexes and redox profile, obtaining two canonical functions. **Conclusions:** These results corroborate that substantial oxidative stress occurs in AIDS condition and also during KS-HIV co-infection with different molecular extension and interdependence to progression indexes. Redox indexes diagnosis should be considered in early diagnostics, prevention and treatment of KS-HIV coinfection, which would be worthwhile to conduct a more comprehensive study and manage of infection.

KEY WORDS: Acquired immunodeficiency syndrome, early diagnosis, human immunodeficiency virus, Kaposi's sarcoma

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## INTRODUCTION

Human herpesvirus 8 (HHV-8) can infect a variety of cells including endothelial lineage, monocytes, and B cells. HHV-8 encodes many oncogenic viral homologs of host proteins with the potential to drive cell survival, proliferation, immune evasion, and angiogenesis [1]. Acquired immunodeficiency syndrome (AIDS)-related Kaposi's sarcoma (KS) is a systemic disease, a vascular malignancy of endothelial cells with multifactorial pathogenesis including induction of latent HHV-8 and intense immune stimulation [2-4]. The common physiological trigger that reactivates HHV-8 from latency in patients remains unclear. Host phagocytes in conjunction with high circulating levels of pro-inflammatory mediators promoting phagocytic activation which result in high tissue levels of reactive species generated during antimicrobial oxidative burst are factors suggested as implied on induction of HHV-8 and intense immune stimulation. Another complicating variable is the fact that human immunodeficiency virus (HIV) infection is often accompanied by significant suppression in antioxidant status [5-8].

Diverse authors have been contributed to the observation that oxidative stress in HIV could drive disulfide CD4 modification, necessary for HIV entry on host, CD4 T lymphocyte depletion and also viral replication increase influencing both aspects on the HIV immunodeficiency condition with unfavorable evolution [9-14]. Furthermore, antiretroviral therapy could influence on oxidative stress in HIV infection [15].

Patients with AIDS-KS could present any of four recognized clinical forms. All forms of KS are characterized by high levels of inflammation which could mediate oxidative stress as a result of host responses to HIV infection and HHV-8. Those events imply that due to their impaired antioxidant scavenging capacities and thiol redox perturbations, AIDS-KS cells are inherently susceptible to the deleterious, tumorigenic consequences of oxidative stimulus [16,17].

Data that support the contribution of oxidative stress in AIDS-KS pathogenesis has also been accrued at the cellular biochemical level [16-19]. *In vitro* studies conducted by Ye *et al.* showed that AIDS-KS strains demonstrate enhanced susceptibility to oxidative stress as induced by either physiologically relevant levels of hydrogen peroxide [18] or conducted by Li *et al.* who show activation through a drug capable of undergoing redox cycling (doxorubicin) [19]. AIDS-KS cells also show an acute hyperresponsiveness to a pro-inflammatory cytokine that signals by redox-mediated pathways (interleukin 6) [20] and possess an inherent perturbation in their thiol redox status characterized by significantly lower levels of both glutathione (GSH) and the GSH reductase co-factor, nicotinamide adenine dinucleotide phosphate [21].

Inflammatory reactions combined with the disruption of the organism's control mechanism could lead to a persistent pro-inflammatory state as evidenced in a wide range of diseases that involve no-resolving or re-occurring reactivities [7,8]. Consistent changes in redox responsive cascades and in the expressions of corresponding target genes may have a similar or even greater impact on senescence as the direct radical inflicted damage of cellular constituents [9,16].

HHV-8 lytic replication also contributes to KS pathogenesis evolution [4]. Both viral lytic products and *de novo* infection promote cell proliferation, invasion, angiogenesis, inflammation, and vascular permeability; all processes related to oxidative stimulus [7,16].

The better comprehension about the interrelation of viral and host factors in KS carcinogenesis is necessary for the rational development of interdisciplinary clinical interpretation and effective intervention [1,21,22]. The aim of this study was to determine the redox status in AIDS-KS individuals. In addition, progression and follow-up clinical biomarkers were evaluated; redox and progression data were analyzed by a multivariate statistical model exploring relation between these variables.

## MATERIALS AND METHODS

### Study Design, Standard Protocol Approvals and Patient Consents

A case and control study was designed enrolling non-HIV and HIV positive individuals. All the patients were attended at the outpatient clinic at the Institute "Pedro Kourí" (IPK) Hospital for HIV. 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 were previously reviewed and approved by the IPK Committee for Research on Human Subjects. The study is in accordance with the principle of the Declaration of Helsinki concerning the Ethical Principles for Medical Research Involving Human Subjects. The protocol was also approved by Determinant Program of Cuban Ministry of Health (Code 151068).

### Patients

A non-probabilistic convenient sample of patients attending the specialized consult of the tertiary hospital was consecutively enrolled in an open prospective study. At study entry, a complete medical history was obtained, and the patients underwent a physical examination, including the evaluation of their medical record, diet and supplemental intake history, anthropometrics data (weight and height) and review of clinical lab results (such as complete blood count, glucose, creatinine, urea, and liver enzymes). Subjects were eligible if they had recently confirmed HIV/AIDS or co-infected with HIV/HHV-8 infection (delayed diagnosis). Exclusion criteria were: Pregnancy, smoke habits, initiation of antioxidant vitamin therapy before the study, hyperlipidemia, diabetes, or kidney/liver dysfunction. Furthermore, both not family history of coronary heart disease or previous antiretroviral use were considered for exclusion criteria. They were recruited sequentially. Antiretroviral therapy doses prescriptions were in accordance of the World Health Organization guidelines [23]. Considering, this data recruitment was closed, and the blood extraction for redox and progression indexes evaluation was indicated before antiretroviral or other treatment consumption. 99 subjects ranging from 30 to 50 years of age were enrolled. All subjects were assessed at the clinical visit. 66 subjects were HIV+, 33 were classified as AIDS, and the other 33 were AIDS-KS with confirmed HHV-8 and HIV test [24]. 33 subjects were randomly selected age-matched healthy individuals with serological evidence for non-HIV and/or non-HCV infection and no abnormal clinical laboratory values.

Demographics and age data were processed by SIDATRAT (Software Package 2008). Subjects were classified according to gender, age, skin color, viral load, and $CD4^+$ T lymphocyte subset count.

### Treatments

The antiretroviral regimen prescribed consist of a triple-drug combination allocated free on pharmacy, including two

nucleoside reverse-transcriptase inhibitors (RTI) and one protease inhibitors (PI), according to current guidelines. The antiretroviral drugs used in the different combinations were indicated for daily consumption at the recommended doses for RTIs (zidovudine, lamivudine, and nevirapine) and PIs (indinavir, ritonavir, saquinavir, and nelfinavir). Patients also were recommended to use concomitant prophylaxis for HHV-8/SK and/or opportunistic infections if needed [23].

### Tumoral Progression Indexes

At study entry, a physical examination was performed including measurement of all cutaneous and mucosal lesions. In addition, clinical suspicion of visceral KS underwent gastrointestinal/bronchial endoscopy and chest tomography. The patients were clinically staged according to the AIDS Clinical Trial Group criteria based on tumor extent (T) and other systemic HIV-1 associated diseases (S) [25-27].

### Flow Cytometry Analysis

A study of T lymphocytes subsets $CD3^+/CD4^+$ in total blood was conducted. These analyses were performed on a CyFlow® Space cytometer (PARTEC GmbH, Münster, Germany).

### HIV-RNA Plasma Viremia (Viral Load)

Viral load was determined with the bioMérieux polymerase chain reaction nucleic acid sequence-based amplification ultrasensitive assay with the lower limit of quantification of 50 IU.

### Oxidative Stress Parameters

After informed consent was signed, venous blood samples were taken from each fasted patient between 08:00 and 10:00 h in the morning. Blood samples were collected by venipuncture into heparin-treated tubes and centrifuged to obtain serum. For almost of analyzes serums were employed except for hematological test and subset of $CD4^+$ lymphocytes. Serum samples were frozen at −70°C and protected from light exposure until analyzes were carried out. All redox parameters were determined by spectrophotometric methods.

Reduced GSH (Sigma, St. Louis, MO, USA) was used to generate standard curves. Serum GSH concentrations were measured by the kinetic assay using the GSH reductase reaction [28]. Autoxidation of GSH to GSH disulfide (oxidized GSH) was prevented by addition of N-Ethylmaleimide to the samples.

Malondialdehyde (MDA), a marker of lipid peroxidation, concentrations were analyzed with 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 spectrophotometrically. To ensure that no lipid oxidation occurs during the assay, butylated hydroxytoluene (0.01% [v/v] of a 2% stock solution in ethanol) and ethylenediaminetetraacetic acid (1 mM final concentration) were added to the sample before assay develops. Freshly prepared solutions of MDA 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 nanomoles per gram hemoglobin (Hb) [29].

For the determination of the susceptibility to lipid peroxidation, serum 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 [29-31].

Total hydroperoxide (HPO) was measured based on the oxidation of ferrous ions to ferric ions by HPOs under acidic conditions. Ferric ions bind 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 [32].

Superoxide dismutase (SOD) activities were assayed in plasma by a modified pyrogallol autoxidation method [33].

Catalase (CAT) activity was measured according with the method of Clairborne [34]. 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 unites per milligrams of protein.

Serum advanced oxidation protein products (AOPP) were measured according to the methods of Witko-Sarsat *et al.* [35]. The values were expressed in chloramine T equivalents and corrected by serum albumin concentrations.

### Biochemical Indexes

Blood parameters such as Hb, lymphocytes, and erythrocyte sedimentation rate (ESR) were screened by a hematological counter. Others as, uric acid, cholesterol and alanine aminotransferase (ALAT) activity were performed by standard procedures in a specialized laboratory of IPK Hospital.

### Statistical Analyses

For descriptive statistics of continuous variables, means and standard deviations were calculated, whereas categorical variables were expressed as proportions. The normality of variables was evaluated by the Kolmogorov–Smirnov test. Comparisons between the cases and control groups were assessed using repeated measures of ANOVA followed by *post-hoc* Newman–Keuls methods. Statistical significance was defined as $P < 0.05$. A canonical discriminate analysis was performed combining redox and progression indexes with other markers evaluated. The SPSS software (version 20) was used for all statistical analyses.

## RESULTS

The baseline characteristics of the 99 subjects are shown in Table 1. There were no statistical significant differences between the groups at baseline according to demographics, gender, body mass index, and a number of patients ($P > 0.05$). Major percent of the patients in three groups were older than 38 years, with white color of skin and were on normal-weight.

Accordingly, progression tumoral extensions 54.5% of studied patients were classified as T1 and 45.5% as T0. For systemic status, 39% of studied patients were classified as S1 and 61% as S0. The characterization of T1 classified patients is showed in Figure 1. The presences of more than 26 skin lesions were observed in 57.6% of studied patients, and gastrointestinal affectation was diagnosed in 54.5% of patients. Other progression markers were less manifested.

Among patients none reported a previous history of opportunistic infections, except 4 patients co-infected who previously presented wasting syndrome (loss of 10% of weight in about 2 months); for 29 patients, KS was the AIDS-defining illness without previous HIV diagnosis.

The mean value of all redox indexes, HIV progression markers and biochemical indices evaluated for control and AIDS groups are shown in Tables 2 and 3, respectively.

MDA, SOD, HPO, and AOPP serum concentrations were significantly higher ($P < 0.05$) in AIDS groups with respect to control group. Serum GSH, CAT, and PP levels were significantly different in AIDS and AIDS-KS individuals compared to control value with significant differences between groups ($P < 0.05$). GSH and PP mean values of AIDS-KS group showed a higher value than AIDS group. In contrast, CAT mean values of AIDS-KS group showed a lower value than AIDS group [Table 2].

Kaposi's sarcoma progression markers with respect to the HIV/AIDS patients group with antiretroviral therapy were analysed identifying 15 patients with PP, CAT and GSH alterations. Others simultaneous modifications were presented in fewer patients considering other indexes (Figure 2).

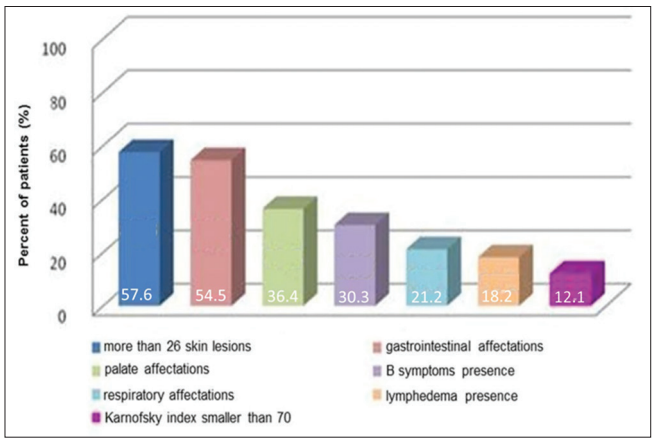


**Figure 1:** Percent of patients with bad prognosis according to Kaposi's sarcoma progression markers

The immunologic and virological indices showed significant differences between groups ($P < 0.05$). AIDS-KS group showed significant lower CD4 T-lymphocyte value than AIDS and control group ($P < 0.05$). Viral load mean values of AIDS-KS group showed significant higher value than AIDS group ($P < 0.05$).

Hb from AIDS-KS group showed significant differences ($P < 0.05$) compared to other groups but remained on interval considered as physiological reference [Table 3]. The ESR mean value of AIDS-KS group was significantly higher ($P < 0.05$) with respect to AIDS and control group and it was out of the interval considered as physiological reference; also mean value of the AIDS group was out of the interval considered as physiological

**Table 1: Demographic data of study participants**

| Variables | HIV seronegative (healthy control) | AIDS | AIDS-KS |
|---|---|---|---|
| Age, years (median±SD) | 40±4.83 | 41.55±6.59 | 38.45±11.65 |
| Gender | | | |
| Male | 31 | 29 | 32 |
| Female | 2 | 4 | 1 |
| Ethnicity | | | |
| White | 19 | 23 | 19 |
| Mixed race | 8 | 5 | 9 |
| Black | 6 | 5 | 7 |
| BMI (kg/m$^2$) | 23.41±1.15 | 23.65±3.88 | 21.7±3.01 |

No significant differences were detected in comparison between above variables for different groups ($P<0.05$). $n=33$ for each group. Source: Clinical history deposed in medical register department (IPK 2015; period of consults in institute was from January to August 2015). SD: Standard deviation, BMI: Body mass index, HIV: Human immunodeficiency virus, AIDS: Acquired immunodeficiency syndrome, KS: Kaposi's sarcoma

**Table 2: Redox indexes and HIV progression markers data in different study groups**

| Variables | HIV seronegative (healthy control) | AIDS | AIDS-KS |
|---|---|---|---|
| MDA (nmol/g Hb) | 2.28±0.35 | 9.06±1.3[a] | 11.01±1.16[a] |
| HPO ($\mu$M) | 116.7±3.45 | 164.4±21.72[a] | 168.1±4.08[a] |
| AOPP ($\mu$M Chloramine T) | 13.70±2.51 | 26.88±7.87[a] | 28.95±1.46[a] |
| GSH ($\mu$M/g Hb) | 1215±207.4 | 333.9±35.05[a] | 405.4±41.54[ab] |
| CAT (U/mg protein) | 144.5±22.29 | 480.0±67.92[a] | 308.2±99.58[ab] |
| Superoxide dismutase (U/mg protein) | 2.82±0.69 | 3.9±1.6[a] | 5.02±1.95[a] |
| PP ($\mu$M) | 6.81±0.29 | 11.94±1.35[a] | 16.90±1.08[ab] |
| CD4$^+$T lymphocytes (cel/mm$^3$) | 1312±248 | 501.1±241.3[a] | 205.1±177.1[ab] |
| Viral load (IU) | - | 239.2±149.9 | 395464±50334.5[b] |

All values are given as "mean±standard deviation of the mean." Different letters represent significant differences at $P<0.05$; [a]compared to control group, [b]compared to AIDS group. MDA: Malondialdehyde, HPO: Hydroperoxide, AOPP: Advanced oxidation protein products, GSH: Glutathione, CAT: Catalase, PP: Peroxidation potential, HIV: Human immunodeficiency virus, AIDS: Acquired immunodeficiency syndrome, KS: Kaposi's sarcoma

Table 3: Hematologic and hemochemical indexes of different study groups

| Variables | HIV seronegative (healthy control) | AIDS | AIDS-KS |
|---|---|---|---|
| Hb (g/L) (RI: 11-16) | 13.45±0.64 | 12.65±1.02 | 11.27±2.33a |
| ESR (mm/h) (RI: M<15, F<20) | 13.52±1.6 | 32.03±17.07*a | 65.82±20.66*ab |
| Lymphocytes (%) (RI: 17-48) | 38.42±3.1 | 37.97±5.2 | 35.55 15.97 |
| Alanine aminotransferase (U/L) (RI: M<41, F<33) | 37.48±2.18 | 26.27±17.86a | 23.51±11.71a |
| Cholesterol (mmol/L) (RI: <5.18) | 3.86±1.38 | 4.29±1.23 | 3.31±1.01 |
| Uric acid (μmol/L) (RI: M=202-416, F=142-339) | 361.5±17.27 | 346.6±37.63 | 403.2±45.2 |

All values are given as "mean±standard deviation of the mean." Different letters represent significant differences at $P<0.05$; [a]Compared to control group, [b]compared to AIDS group. M - Male; F - Female; RI - Reference interval; *indicates out of reference value. ESR: Erythrocyte sedimentation rate, AIDS: Acquired immunodeficiency syndrome, KS: Kaposi's sarcoma

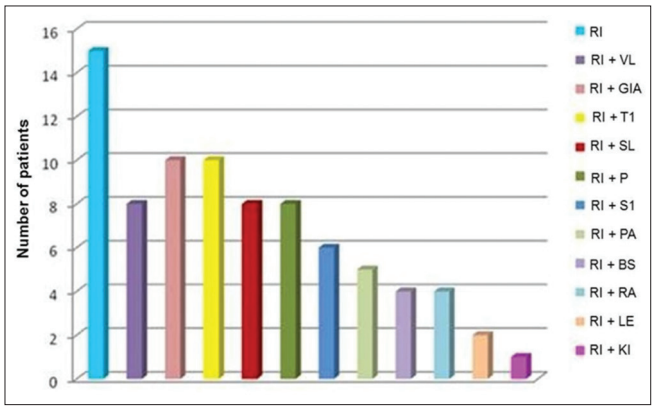


**Figure 2:** Number of human immunodeficiency virus (HIV)/AIDS patients with Kaposi's sarcoma that modify simultaneously redox indexes (peroxidation potential, catalase, and glutathione) and some Kaposi's sarcoma progression markers with respect to the HIV/AIDS patients group with antiretroviral therapy (RI - Redox indexes; VL - Viral load [>55,000 IU], GIA - Gastrointestinal affectations; T1 - Extensive oral commitment, edema presence or tumoral ulcer or visceral illness; SL - Skin lesion (more than 26); P - Anatomopathologic classification (proliferative or tumoral); S1 - Opportunistic infection history; PA - Palate affectation; BS - B symptoms presence; RA - Respiratory affectation; LE - Lymphedema; KI - Karnofsky index ≤70; PP - Peroxidation potential; CAT - Catalase; GSH - Glutathione)

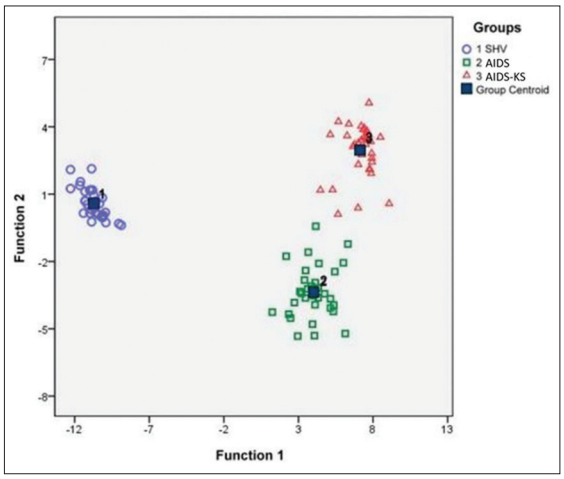


**Figure 3:** Canonical discriminate analysis representation related to redox indexes and progression indexes in different studied groups. (1 SHV - Seronegative human immunodeficiency virus -presumable healthy subjects; 2 acquired immunodeficiency syndrome [AIDS] - Subjects with acquired immunodeficiency syndrome; 3 AIDS-Kaposi's sarcoma - Function 1 0.608 MDA + 0.453 PP – 0.003 GSH + 0.003 CAT + 0.042 PAOP + 0.041 HPO + 0.103 Superoxide dismutase [SOD] – 0.002 CD4 – 15.093, Lambda de Wilks = 0.002 [P < 0.05]; Function 2, –0.044 MDA + 0.781 PP + 0.003 GSH – 0.012 CAT + 0.034 PAOP + 0.027 SOD + 0.001 CD4 – 9.009, Lambda de Wilks = 0.125 [$P < 0.05$])

reference and showed significant differences compared to control group ($P < 0.05$).

For lymphocytes nonsignificant differences were found ($P > 0.05$) comparing AIDS-KS and AIDS groups to control group; both were lower than the control group, but in the interval considered as physiological reference. ALAT mean values of AIDS and AIDS-KS groups were significantly difference with respect to control, but both remained on the physiological reference interval.

Cholesterol mean values of AIDS and AIDS-KS groups showed only nonsignificant differences ($P > 0.05$) compared to control group; the mean value of AIDS groups was higher with respect to the control group but remained in the physiological reference interval. The uric acid mean value of the AIDS group was nonsignificantly lower ($P > 0.05$) than AIDS-KS and control group, but all were in reference ranges.

Multivariate discriminate analysis considering oxidative stress indexes, HIV progression markers of infection and clinical condition are showed in Figure 3. Discriminant analysis using redox characterization and HIV progression markers revealed that 99.6% of the variation between groups was accounted by the two first discriminant functions ($P < 0.001$) [Figure 3]. The biochemical redox indexes and HIV progression markers were related in these two functions with discriminant loadings of 0.982 and 0.814, respectively. Furthermore, 100% of original grouped cases were correctly classified according function 1.

## DISCUSSION

Actually, there are controversial data that substantiate the association of oxidative stress and persistent inflammation in several human diseases including HIV infection and neoplastic conditions [8,36-40]. It has been previously shown that the HIV-infected populations have significantly lower antioxidant concentrations than non-HIV individuals [9,10,13]. Lower non-enzymatic antioxidant concentrations occurred also in HIV patients with diverse neoplastic manifestation [41,42].

In this study, redox indexes evaluated in AIDS patients showed significant differences with respect to healthy individuals

similar to the previous reports with high values of biomolecules' damage (MDA, HPO, and AOPP) and lower antioxidants' concentration (PP, GSH, and CAT). In the case of AIDS-KS also antioxidants' concentration differs with respect to AIDS condition. Abnormally high levels of reactive oxygen species (ROS) as a consequence of chronic immune system activation by HIV infections could lead to a decline of antioxidant defense molecules leading to cumulative damage of cellular components generating augmented lipid peroxidation products, oxidized proteins and altered DNA sequences [43]. Almost all redox implicated enzymes and molecules are endogenously generated and involved in detoxification mechanisms and general metabolism [44,45].

The reliable redox markers altered in AIDS-KS group compared to non-HIV and AIDS conditions were PP, CAT, and GSH. CAT alterations could indicate altered peroxide generation which has been associated to KS lytic replication through both paracrine and autocrine mechanisms [18]. Depletion of GSH and antioxidant capacity evaluated as PP could be related to consumption by increased chronic generation of ROS. These modifications not result on biomolecule oxidative damage evaluated at this period, but sustained oxidative modification could be related to chronic activation of inflammation on HIV condition and progressively modify immune cellular and microenvironment and also influence viral replication. Between AIDS and HIV HVV-8 groups, differences in redox indexes arise similar alteration with the previous studies related to coinfection [46,47]. Except the findings regarding to SOD activity, the previous reports found lower activity in different HIV conditions, but in this study, the mean value was higher than the control group; those data have to be explored in future research. With respect to hematologic and hemochemical indexes, only ESR showed alteration compared to AIDS patients; however, this is an unspecific index and thus is not attributable to the poly symptoms observed in KS patients.

The involved AIDS patients have to use antiretroviral treatment to avoid infection evolution. The multidrug antiretroviral regimens intervention based on a combination of reverse transcriptase and PIs have improved the clinical outcome of HIV-1 infection indicated by an important decline in AIDS incidence and mortality, but, on the other hand, they almost contribute to the oxidative metabolism which adds risk of molecular damage and replication of diverse virus contributing to polyp pathology condition [2,48,49]. These findings could be explained in part by several mechanisms such as low intake of antioxidants or their precursors, malabsorption and, in peripheral tissue, enhanced cysteine metabolism with a consequent loss of sulfur group which may account for GSH and antioxidant deficiency during HIV infection which persists after antiretroviral therapy [14,15,50,51]. Considering those aspects the exploration of redox status before antiretroviral therapy is encouraged in different conditions.

Previous reports address that oxidative stress could be related to viral replication in HIV infection and also implicated on $CD4^+$ T-cell apoptosis. Through ROS the system could modulate and activate nuclear transcription factors, which ultimately lead to viral gene expression of HIV, HHV-8, and concomitant related opportunistic infections [52-54].

In this study, all redox indexes evaluated in AIDS groups showed differences with respect to healthy control. Alterations of lipid and protein metabolism are common complications of HIV disease related to the inflammatory response to chronic HIV infection, which is probably mediated by cytokines [40,42]. Chronic inflammation has been linked to those findings with controversial results considering biological fluids for determination [16,53].

In the case of KS, as malignancy it was early reported in HIV-infected patients since the first cases in 1982; oxidative stress is suggested to interact with all multistage process of this coinfection, i.e., initiation, promotion, and progression [17]. How oxidative stress is involved in these various steps is the object of revision and investigation. Furthermore, ROS may augment tumor invasion and metastasis by increasing the rates of cell migration [55,56].

The relation of redox indexes as an underlying factor with viral load and $CD4^+$ T-cells in those conditions have been elucidated as nonlinear. In this study, simultaneous modification analyses of redox status permit to identify 15 patients with PP, CAT, and GSH alterations which represent 45% of the group; 8 of these 15 patients present also simultaneous viral load (VL) alteration (>55,000 IU). 10 patients presented alteration of gastrointestinal affectations and T1 affectation involving extensive oral commitment, edema presence or tumoral ulcer or visceral illness simultaneously with redox indexes modifications. Risk analyses indicate an association of antioxidant depletion and oxidative damage to be significantly related as HIV progression markers and influencing the major manifestations of KS.

Discriminant analysis considering progression and redox indexes works by conforming one or more linear combinations of detected predictors and creating a new latent variable for each function. These functions called discriminant functions could express accumulated variance contemning on data.

The analyses offer an integral observation and synthetic representation of multiple indicators and also summarize possible complex correlations. Redox metabolites and species interact very quickly with biomolecules in cellular and fluid microenvironment and also with antioxidants; so, the relation between different redox indicators could be multifactorial and nonlinear. In this study, discriminant functions observed clearly separate groups according to progression indexes and redox profile. Scarce previous report about that possible relation has been found.

Taking into account that causes of poly pathologies are complex and multifaceted, the recognition of molecular and cellular concert involved are crucial. A causal relationship between some elements such as oxidative macromolecules modifications, immunological status, and viral load has emerged but the

mechanism by which these molecular and biochemical events occur remain to be established. The oxidative stress evaluations and multivariate analyses considering immunological and virological status will, therefore, become potential useful to characterize infection, also antiviral combinations effects, as well as the usefulness of antioxidant and alternative therapies for counteracting oxidative damage [57,58].

Substantial progress has been made toward an integrative understanding to delineate mechanisms considering oxidative stress and ROS as potential key participants in cancer development [59]. In this sense, significant difference evidenced in some redox indexes related to progression markers and also to clinical condition evaluated by discriminant analysis support association to HIV infection evolution. Gaining in knowledge of specific redox pathway and their relation to other factors, investigators will be provided with additional opportunities to impact both on the quality of life and related diseases in humans and others species.

Although huge impact of antiretroviral treatment on morbidity and mortality associated to this cause, a significant percentage of AIDS-KS patients never achieve total remission [60-62]. Understanding the interplay of viral and host factors in KS carcinogenesis is critical for the rational effective intervention in future.

Several previous studies have been shown that also antiretroviral treatments have additional impact on pre-existing oxidative stress related to HIV condition and other diseases. Considering that some authors have been suggested the use of antioxidants as agents which might reduce the incidence of oxidative stress as a consequence of infection or treatment and also it could impact on the development of other related diseases [63-65].

In conclusion, this study contributes to evidences that oxidative stress evaluated in plasma by several parameters could increase during AIDS-KS coinfection. It is possible that the concentration of this cumulative damage reported has a direct impact on efficiency and cell functioning. Metabolic abnormalities as altered redox indexes remain an important part of complications in HIV infection. Their etiology, including roles for both non-HIV and HIV viral-related effects and treatment-associated factors requires ongoing investigation. These complications could be implicated in patients' active clinical status and long-term consequences. Management options are encouraged, and therapeutic interventions may provide substantial benefits to patients. These conclusions are also methodologically important for the follow-up and management of infected individuals.

A clear understanding of the pathways most critically involved in KS progression and the consequences of altered cell behavior in the tissue micro-environments will provide nuggets of information which will help us in formulating better therapeutic approaches. It is likely that a combination of therapeutic agents targeting multiple signal transduction pathways would be needed for maximum therapeutic benefits.

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