

**EVALUATION OF OXIDANT AND ENZYMATIC ANTIOXIDANT STATUS IN ASYMPTOMATIC SUBJECTS WITH HIV-1.****Adeoti Mansour Franck<sup>\*1</sup>, Camara-Cisse Massara<sup>2</sup>, Monteomo Gnate Francois<sup>1</sup>, Koffi Gervais<sup>2</sup>, Kolia Innocent<sup>1</sup>, Djaman Allico Joseph<sup>3</sup> and Dosso Mireille<sup>4</sup>**<sup>1</sup>Laboratory of Medical Biochemistry, University Teaching Hospital of Yopougon-Abidjan, Côte d'Ivoire.<sup>2</sup>Laboratory of Medical Biochemistry, UFR-Medical Sciences-University Felix Houphouët-Boigny, Abidjan, Côte d'Ivoire.<sup>3</sup>Laboratory of Medical and Basic Biochemistry, Pasteur Institut of Abidjan, Côte d'Ivoire.<sup>4</sup>Pasteur Institut, Côte d'Ivoire.**\*Corresponding Author: Adeoti Mansour Franck**

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Article Received on 20/09/2016

Article Revised on 10/10/2016

Article Accepted on 30/10/2016

**ABSTRACT**

Chronic viral infection with HIV is characterized by progressive and irreversible destruction of CD4 lymphocytes causing oxidative stress and antioxidant reaction. This study aimed to evaluate the immune responses, the balance between oxidant status and antioxidant capacity after in the Acquired Immunodeficiency Syndrome. The authors conducted a prospective study of 60 subjects aged 18 to 65 both sexes, including 30 people with VIH1et 30 healthy control subjects HIV-. In these patients and witnesses, it was made HIV status with an Elisa test and discriminative test (Immunocomb II HIV 1 & 2 BiSpot / PBS Organics) and counts as lymphocyte subsets (CD4+ and T-CD8+) is performed by the direct immunofluorescence technique. GPx was assayed by Elisa, SOD and catalase using the PLC Cobas Mira® (Roche Diagnostics) using Randox reagents. There was a significant reduction ( $p < 0.05$ ) in the number of CD4 cells ( $373.13 \pm 69.90$  vs  $750 \pm 30$ ) and CD8 ( $759.28 \pm 131.58$  versus  $1450 \pm 46$ ) patients HIV-1 compared to control subjects. However, a significant increase ( $p < 0.05$ ) markers peroxidation, TBARS ( $0.99 \pm 0.26$  versus  $3.32 \pm 0.40$  nmol/MDA) and proteins, OAPP ( $39, 49 \pm 21.20$  versus  $62.28 \pm 13.70$   $\mu$ mol) which reflect an important oxidative stress. The low concentrations significantly ( $p < 0.05$ ) of GPx ( $40 \pm 11$  versus  $82 \pm 15$  IU/gHb), SOD ( $1095 \pm 20$  vs  $1313 \pm 41$  IU/gHb) and catalase ( $125 \pm 18$  versus  $210 \pm 13$  IU/gHb) respectively related to chronic oxidative stress, are more pronounced among the HIV-1 patients. **Conclusion:** Considering the fall in activity of GPx, SOD and catalase in subjects with HIV-1 infection, they are exposed to oxidative stress and long term consequences.

**KEYWORDS:** TBARS, OAPP, Superoxide dismutase, Glutathione peroxidase, Catalase, HIV.**INTRODUCTION**

At the time of the discovery of HIV, the fact that the CD4<sup>+</sup> molecule is the viral receptor, left suggest that the disappearance of cells expressing this receptor in patients was due solely to their subsequent destruction to viral infection.

A better understanding of molecular and cellular mechanisms provided a better understanding today pathophysiology and tif replica cycle of HIV causes AIDS.<sup>[8,22]</sup>

It has been observed in fact in the lymph nodes of patients infected with HIV, a high rate of early cell death by apoptosis, affecting much the T-CD4<sup>+</sup> uninfected T cells -CD8<sup>+</sup>, B cells, NK (Natural Killer) cells and dendritic cells.<sup>[5,13]</sup> However, B cells and T-CD8<sup>+</sup> not carrying HIV binding sites are not the main targets and would therefore not be infected with HIV.

Thus, the destruction of these cells is not due to a phenomenon related directly to viral infection, but is rather due to metabolic disorders caused by inflammatory processes, which involve indirect mechanisms of cell killing which oxidative stress.<sup>[24,26]</sup>

Oxidative stress participates in amplifying the impact of adverse effects of HIV infection, premature death of immune cells, the rational use of antioxidants could offset.<sup>[1,20]</sup>

The antioxidant defense system is the set of measures implemented to control oxidation and its negative effects.

It comprises on the one hand antioxidants produced by the cells (enzymes, metabolites) and other antioxidants from vegetable intake. The antioxidant defense system is intended to prevent the formation of free radicals and

neutralize them when they are already formed, repair their damage or prevent the conditions for their education, for example, blocking the iron atoms, which act as catalysts.<sup>[10,19]</sup>

Defense systems enzymatic antioxidants against free radicals are formed by superoxide dismutase, glutathione and catalases.

Also, this study aimed to evaluate the immune responses, the balance between oxidant status and enzymatic antioxidant capacity during the Acquired Immunodeficiency Syndrome.

## I. MATERIAL AND METHODS

### patients

This cross-sectional study included 30 patients selected bioclinical integrated research center of Abidjan (CIRBA) among people living with HIV (PLHIV). Were included in the study subjects infected with HIV-1, clinically asymptomatic with a CD4 count greater than 200 copies per mm<sup>3</sup> plasma, aged 18 to 50 years naïve antiretroviral (ARV).

The subjects infected with HIV-2 and those HIV1, but who developed opportunistic infections were excluded from this study. 30 healthy subjects, aged 18 to 50, HIV negative, served as controls.

All participants gave informed consent. The study protocol was approved by the Faculty of Medical Sciences of the University Félix Houphouët-Boigny in Abidjan Cocody, Côte d'Ivoire and was explained to the subjects before they gave their well informed consent.

### Blood samples

Blood samples were taken from subjects fasted from the evening (12 hours) by venipuncture in the elbow and collected in heparinized tubes vacutainer. After centrifugation at 3000 rev/min for 5 min and the plasma was separated and aliquoted.

The aliquots were stored at -80°C in lab CIRBA before being shipped by air in dry ice to the laboratory of biochemistry CHU Kremlin Bicetre, Paris, France. Aliquots there were stored at -80°C until analysis.

### CD4 and CD8

Counts in lymphocyte subsets (T-CD4<sup>+</sup> and T-CD8<sup>+</sup>) is performed by the direct immunofluorescence using the FACS Calibur PLC Becton Dickinson, with monoclonal antibodies.<sup>[2,15]</sup> This technique uses three fluorochromes of different colors.

Sample preparation involved the use of a Falcon tube. 10µl of reagent (CD3/ CD4 / CD45) were added to the tube 50 µl whole blood of the patient. After homogenization with a vortex, the tube was incubated in the dark for 15 min. 500 µl of lysis solution (diluted to 10%) are then added to the tube and the mixture is

incubated for 15 min for complete lysis of red blood cells. Cytometer after setting and after the tube vortexed, it passed through the cytometer.

### TBARS assay

This is a technique for indirect determination of the free radicals by the TBARS assay (substances reacting with thiobarbituric acid) according to spectro-fluorimetric method Kunio acted modified Sess.<sup>[23]</sup> This method measures the substances from lipid peroxidation as malondialdehyde (MDA) and alkanals alkénals, reacting with thiobarbituric acid (TBA).

The principle is based on the reaction of two molecules of thiobarbituric acid with an acetic medium malondialdehyde molecule acid at a temperature of 95°C leading to the formation of a pink complex read by a spectrofluorimeter P 450 with a length excitation wavelength of 513 nm and an emission wavelength of 553 nm. The results are expressed in nmoles of MDA/ml.

### Determination of the oxidation and products advanced protein (Advanced oxidation protein and product) (AOPP)

The dosage of OAPP is performed by the method spectrophotometric.<sup>[27]</sup> The product assay kit of Advanced oxidation protein and product (AOPP) is a direct bioassay tool for quantitative measurements of AOPP in biological samples. The unknown AOPP is searched in samples where chloramine standards are first mixed with a reaction initiator. After a short incubation, stop solution is added and the samples can be read with a standard spectrophotometer.

Absorbance of Chloramine-T at 340 nm is linear in the range of 0 to 100 µmol / liter, the AOPP concentrations are expressed in micromoles per liter equivalents chloramine-T.

### Determining the activity of superoxide dismutase (SOD)

The dosage of SOD was performed using the PLC Cobas Mira® (Roche Diagnostics) using Randox reagents (Randox Laboratories, France) following the same manipulations, the difference being in assay kits.

Concerning the expression of results, almost entering patient data, the selection of parameter to be measured is performed on the request input mask, the parameter is analyzed on the automated random Cobas Mira®, the result expressed as IU/g Hb is printed.

### Determination the activity of glutathione peroxidase (GPx)

The activity of glutathione peroxidase (GPx) was measured by the method of Paglia.<sup>[16]</sup> The assay reagent used is presented with two kits (Randox (Randox Laboratories, France): a first kit consisting of 20 ml of K<sub>2</sub>PO<sub>4</sub>/ DETAPAC, 2.5 ml of glutathione 19.5 mM, 2.5

ml of NADPH 0.55 mM and finally 64 µl of glutathione reductase.

The second Kit consists a solution of tert-butyl hydroperoxide diluted 1/20 in methanol.

#### Determining the activity of catalase (Cat)

The dosage of catalase was performed using the PLC Cobas Mira® (Roche Diagnostics) using Randox reagents (Randox Laboratories, France) following the same manipulations, the difference being in assay kits.

Concerning the expression of results, almost entering patient data, the selection of parameter to be measured is performed on the request input mask, the parameter is analyzed on the automated random Cobas Mira®, the result expressed as IU/gHb is printed.

#### Statistical Analyses

The treatment of the collected data was performed using the Epi-Info version 6.0 software. Results were expressed as mean ± standard deviation. The comparison of means was performed by parametric Student test. The test is considered significant when  $p < 0.05$ .

## II. RESULTS

**Table I: Average values of the concentrations of cellular immunity markers**

| Settings (SI Units)                         | Control subjects HIV- (n = 30) | HIV-1 (n = 30)  | P    |
|---|--------------------------------|-----------------|------|
| T-CD4 <sup>+</sup> (cells/mm <sup>3</sup> ) | 750 ± 30                       | 373.13 ± 69.90  | 0.05 |
| T-CD8 <sup>+</sup> (cells/mm <sup>3</sup> ) | 1450 ± 46                      | 759.28 ± 131.58 | 0.05 |
| T-CD4 <sup>+</sup> /T-CD8 <sup>+</sup>      | 0.51 ± 1.5                     | 0.46 ± 0.12     | 0.05 |

The National algorithm requiring ARV patients at a rate of T-CD4<sup>+</sup> less than 250 cells/mm<sup>3</sup>.

**Table II: Peroxidation makers of lipids and proteins in HIV-1 asymptomatic subjects**

| Settings (SI units) | Control subjects HIV- (n = 30) | HIV-1 (n = 30) | P    |
|---------------------|--------------------------------|----------------|------|
| TBARS (nmol/l MDA)  | 0.99 ± 0.26                    | 3.32 ± 0.40    | 0.05 |
| OAPP (mmol/l)       | 39.49 ± 21.20                  | 62.49 ± 13.70  | 0.05 |

The values of these markers increased for TBARS (nmol/l MDA) and OAPP (mg/dL), respectively 235% and 58.9% among HIV-1 patients.

**Table III: Mean values of markers of antioxidant capacity in the subject HIV 1**

| Settings (SI units) | Control subjects HIV- (n = 30) | HIV-1 (n = 30) | P    |
|---------------------|--------------------------------|----------------|------|
| SOD (IU/gHb)        | 1313 ± 41                      | 1095 ± 20      | 0.05 |
| GPx (IU/gHb)        | 82 ± 15                        | 40 ± 11        | 0.05 |
| CAT (IU/gHb)        | 292 ± 13                       | 135 ± 18       | 0.05 |

Free radicals have contributed to reducing the average rates markers of antioxidant capacity, SOD (IU/gHb), GPx (IU/gHb) and CAT (IU/gHb) in HIV-1 patients respectively 16,60%, 51,21% and 53,75%.

Normal values of SOD, GPX and CAT in HIV negative patients are ranging respectively between 1211- 1415 IU/gHb, 227- 383 (IU/gHb) and 62-102 IU/gHb.

## III. DISCUSSION

This study has highlighted the scale of the oxidizing capacity and the enzymatic reaction antioxidant in patients infected with HIV 1 whose immune status was determined for the control of free radicals and the proper functioning of cells.

T lymphocyte CD4<sup>+</sup> is the main target of HIV. Clinical and laboratory manifestations observed in HIV infection is due to a massive destruction of CD4<sup>+</sup> lymphocytes. Several mechanisms are implemented to result in the depletion of this cell population.

These include the cytopathogenic effect of the virus, cytotoxic immune responses, apoptosis, but also the loss of regenerative capacity of these cells by the lymphoid system. Indeed, CD4<sup>+</sup> lymphocyte activated is not only necessary to amplify a humoral response, but also essential to induce the differentiation and proliferation of T-CD8<sup>+</sup> lymphocytes' cytotoxicity effectors. These T-CD8<sup>+</sup> lymphocytes are essential to the defense of the body against infected cells with HIV.<sup>[5]</sup>

We determined the plasma concentrations of T-CD4<sup>+</sup> and T-CD8<sup>+</sup> and T-CD4<sup>+</sup>/T-CD8<sup>+</sup> patients to assess their immune capacity.

As normal values were established by Ho and Bissagné,<sup>[2,9]</sup> as follows TC<sup>+</sup> = D4 [400-1200 cells/mm<sup>3</sup>], T-CD8<sup>+</sup> = [300-1000 cells/mm<sup>3</sup>] and CD4<sup>+</sup>/CD8<sup>+</sup> T ≥ 1.

Referring to this classification, our results have shown a deficiency of CD4<sup>+</sup> to all patients with an average plasma levels of CD4<sup>+</sup> = [300 ± 6.98 8 cells/mm<sup>3</sup>].

Despite this deficit, these subjects were not eligible for treatment according to the National ARV algorithm that determined the national rate to 250 cells of T-CD4<sup>+</sup>.<sup>[2]</sup>

Regarding the plasma level of lymphocytes T-CD8<sup>+</sup> with a mean of 629,06 ± 131,58 cells/mm<sup>3</sup>, our results did not reveal a major deficiency. Which will result in a T-CD4<sup>+</sup>/T-CD8<sup>+</sup> less than 1 for all patients.

These observations are consistent with those of some authors that showed that not only were dying apoptotic cells but also all subpopulations (T-CD4<sup>+</sup>, T-CD8<sup>+</sup>, B-Lymphocytes, monocytes) were affected.<sup>[7]</sup>

Indeed, an increased release of free radicals has been shown in non-activated leukocytes from HIV-positive patients in the early stage of infection.

The isolated analysis of the evolution of markers oxidative stress and antioxidant capacity showed the following results s.

The average concentrations of MDA (2.91 ± 0.40 nmol/ml) and AOPP (62.24 ± 13,7 mmol/ml) obtained showed the occurrence of stress in PHAs. Indeed, the values of the indices of lipid peroxidation (MDA) and protein oxidation (AOPP) are significantly elevated by compared to healthy patients presumed as confirmed by the authors.<sup>[3,25]</sup>

Nos results are consistent with those of Edeas and Pasupathi which revealed a decline in the activity of antioxidant enzymes in HIV patients with asymptomatic with a CD4 count > 250 cells/mm<sup>3</sup>.

The main mechanisms of natural defenses against free radicals are formed by the antioxidants enzymes: superoxide dismutase, the glutathione peroxidases, glutathione reductase and catalase, which each have their place and their mode of action, while acting in a complementary way.

Thus, the activities of the three enzymes (SOD, GPx, Cat) were measured to evaluate the antioxidant capacity to HIV1 patients.

SOD ensures the elimination of superoxide anion, the first toxic species formed from oxygen. It thus provides the first line of defense against oxidative stress, therefore, would be used in HIV+ and Hiv-Aids patients. An activity of this enzyme lower than 1211 IU/g Hg is considered low.<sup>[20]</sup> and expose subjects to oxidative stress, one of the consequences would be immune deficiency. Our results thus demonstrate very low activity SOD in patients HIV-1 compared to control patients, healthy and produce enough to limit the oxidation reactions.

These results were agreed with those of Edeas and Pasupathi<sup>[4,18]</sup> which showed a decrease in SOD activity

in people living with HIV/AIDS. This could be explained by the nature of these protein enzymes which would target free radicals.

Indeed, a Tat protein of HIV is able to decrease gene expression of SOD, glutathione peroxidase and facilitating T cell death by apoptosis.<sup>[5]</sup>

This decrease in the expression of the SOD gene correlated with that of glutathione peroxidase which explained in our results, the significant drop in the activity of that enzyme in our patients HIV 1 compared to controls. Indeed, the activities of GPx which was below 68 IU/g are low.<sup>[20]</sup>

The main role of glutathione peroxidase which is a selenoenzyme is to eliminate lipid peroxides resulting from the effect of oxidative stress on the polyunsaturated fatty acids.<sup>[14]</sup> This drop is positively correlated with selenium deficiency found in this population. Our results are consistent with the hypothesis that a long-term selenium deficiency contribute to the fall in activity of glutathione peroxidase in people living with HIV.<sup>[12]</sup> Like the SOD, the Tat protein HIV is also capable of decreasing the expression of glutathione peroxidase.<sup>[5]</sup>

Catalase meanwhile, enzyme peroxisome cells and red blood cells, participates in the metabolism of hydrogen peroxide into oxygen and water.<sup>[18,21]</sup> A catalase activity of less than 227 IU/g Hg is considered low<sup>[20]</sup> and in these conditions favor the proliferation of superoxide radicals.

In fact, our results are in agreement with those of Edeas and Pasupathi that revealed a decrease in antioxidant enzyme activity to asymptomatic HIV positive patients with a CD4 count > 250 copies/mm<sup>3</sup>. This decrease would result in the occurrence of oxidative stress and apoptosis observed a s respectively in the table 2 and 1.<sup>[4,18]</sup>

In addition, these three enzymes (SOD, GPx, Cat) representing different lines of defense are inter-dependent. The quantitation of each of these elements helps to have information about their individual capacity defense. However, such dosage does not reflect the complexity of the system and the interactions of all its members. This is the main reason for performing an overall measure of antioxidant activity which is closest to the biological reality.

## CONCLUSION

The oxidant capacity of HIV patients without HAART with CD4<sup>+</sup> > 250 confirmed the involvement of oxidative stress in lymphocyte destruction HIV1 in these subjects. This oxidative stress and amplify the impact of the adverse effects of HIV infection in premature death of immune cells.

The effectivity of the oxidant capacity was revealed by a high plasma concentration of biomarkers that are AOPP and TBARS as well as low activity of antioxidant enzymes that are Gpx, has the catalase and SOD.

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