VERY HIGH LIPID PEROXIDATION INDICES IN HIGHLY ACTIVE ANTIRETROVIRAL (HAART)-NAÏVE HIV-POSITIVE PATIENTS IN CAMEROON.

Teto G[1], Kanmogne D.G [4] , Torimiro J[2, 3], Alemnji G[3], Nguemaim Ngoufo F[1], Nana H[1], Tchejak R[1], Asonganyi T[3].

1. [1]*Faculty of Science, University of Yaoundé I*
2. [2]*CIRCB center*
3. [4]*Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center.*

Corresponding Author : Teto Georges .Email : ggteto@yahoo.fr . Cel : (237) 79 74 78 58

ABSTRACT

Introduction: *HIV infection is responsible for many biochemical disorders among which lipid peroxidation and antioxidant imbalance are of important consideration. HIV infection is a chronic disease which leads to chronic inflammation during which high amount of free radicals are produced. These free radicals lead to very high lipid peroxidation indices and to rapid disease progression due to CD4 cells apoptosis.*

Objectives: We determined lipid peroxidation indices (LPI), plasma malondialdehyde (MDA) concentration and total antioxidant ability of plasma (TAA) concentration in hiv-positive, treatment-naïve patients in order to contribute to the management of HIV-infected patients.

Methodology: *We measured MDA and TAA concentrations, and LPI indices in 285 individuals composed of 151 HIV-positive treatment-naïve patients and 134 controls that were recruited during a period of twelve months at the University Hospital Center in Yaoundé and in the South West region.*

Results: Values of the various parameters obtained were *We obtained the following patients/controls values for* MDA (0, 41±0, 10 µM patients vs 0, 20±0, 07µM for controls), TAA (*0*, 16±0, 16 mM *for patients vs* 0, 63±0, 17 mM for controls) *and* LPI *indices (*26, 02±74, 40 for patients vs 0, 34±0, 14 for controls). The differences between patients and controls *were statistically significant (p<0, 05) for all the parameters. There was a statistically significant decrease in TAA concentration in term of CD4 cell counts (p<0, 05) and a positive correlation between the both (r=0,199; p<0, 05). In term of sex, LPI and MDA were higher while TAA was lower in men compared to women (p<0, 05).*

Conclusion: *During HIV infection there is high lipid peroxidation, low plasma total antioxidant ability and very high LPI indices. This may be linked to chronic inflammation due to HIV infection which produces free radicals during HIV replication. These disorders contribute to CD4 cells apoptosis and disease progression.*

Keywords: *MDA, TAA, LPI, Free radicals, HAART, HIV infection*

[3]*Faculty of Medicine and Biomedical Sciences.*

RESUME

Introduction : L'infection VIH est responsable de plusieurs troubles biochimiques parmi lesquels la peroxydation lipidique et le déséquilibre antioxydant sont d'une grande importance. L'infection VIH est une maladie chronique qui induit une inflammation chronique dont l'issue est la génération des radicaux libres ; ces radicaux libres sont à l'origine d'un indice de peroxydation lipidique très élevée et d'une progression rapide de la maladie liée à l'apoptose des cellules CD4.

Objectif : Nous avons déterminé l'indice de peroxydation lipidique(LPI), la concentration plasmatique de malondialdehyde(MDA) et la capacité antioxydante totale du plasma(TAA) chez les patients VIH positifs naïfs de traitement antirétroviral dans l'optique de contribuer à l'amélioration de leur prise en charge.

Méthodologie : Nous avons mesuré les concentrations plasmatiques de MDA et de TAA et nous avons déterminé l'indice LPI chez 285 individus composés de 151 malades VIH positifs et de 134 témoins qui ont été recrutés durant une période de douze mois au Centre Hospitalier Universitaire de Yaoundé et dans la région du Sud-ouest.

Résultats : Les valeurs obtenues données sous forme de rapport patients/témoins pour les différents paramètres mesurés étaient pour le MDA (µM), la TAA (mM) *et* l'indice LPI respectivement: 0, 41±0, 10 /0, 20±0, 07*; 0*, 16±0, 16 /0, 63±0, 17 *and* 26, 02±74, 40/0, 34±0, 14. Les différences entre patients et témoins étaient statistiquement significatives pour tous les paramètres mesurés ; il y' avait une diminution statistiquement significative de la concentration de TAA en fonction du nombre de cellules CD4 ainsi qu'une corrélation positive entre les deux (r=0,199; p<0, 05).En fonction du sexe, l'indice LPI et le MDA étaient plus élevés et la TAA plus faible chez les hommes comparés aux femmes (p<0,05).

Conclusion : Au cours de l'infection VIH il y'a une augmentation de la peroxydation lipidique, une baisse de la capacité antioxydante totale du plasma et un très fort indice de peroxydation lipidique. Ceci serait lié à l'inflammation chronique due à l'infection VIH qui entraine la génération des radicaux libres. Tous ces désordres contribuent à l'apoptose des cellules CD4 et à la progression de la maladie.

Mots clés : MDA, TAA, LPI, radicaux libres, HAART, infection VIH

INTRODUCTION

The acquired immunodeficiency syndrome is one of the most mortal pandemic of the 21thcentury. It is a serious public health problem that is worldwide, especially in the African continent. About 60% of infected individuals live in sub Saharan Africa which the part of the world with the greatest poverty (6). UNAIDS (2012) has indicated that if African States do not make effort to produce their own drug, without the support of many International Organisation, more than half million of people would die of AIDS in that part of the world in 2014 (11). In Cameroon, it is estimated that 5,5% of individual live with AIDS (9). There is as yet no drug to cure the disease and no vaccine against the pandemic(17). HIV infection induces a chronic inflammation due to HIV replication and viral activation of macrophages and T cell lines; one of the consequences of this chronic inflammation is the high generation of free radicals which react with many biological molecules (lipids, proteins, carbohydrates, and nucleic acids) and affect cellular integrity. Malondiadehyde, a final product of lipid peroxidation, when associated to total antioxidant ability, helps to assess the extent of free radicals damage in the organism. Free radicals which are reactive oxygen species (ROS) (molecules or atoms that possess free electrons) have a life span of a few milliseconds only, and can be measured only through the product of their reaction (4) represented by malondialdehyde in case of lipid peroxidation. CD4 cells are important in immune system defense (16); and antioxidants are also important in free radicals neutralization. Since free radicals contribute to CD4 cell apoptosis in the presence of low antioxidant concentration (12), we measured or calculated MDA, TAA and LPI in HIV-infected patients in order to contribute to the better management of HIV-infected patients.

METHODOLOGY

After informed consent, we enrolled 285 individuals among which 151 patients and 134 controls(15). 5ml of blood was collected from the participants into labeled EDTA tubes after 12 hours of fasting. EDTA tubes were centrifuge at 1200g for 15 min to collect plasma which was aliquot and used for the biochemical analysis. All samples were stored at -20^{0}C and processed within three to four days. TAA concentration was determined by the method of Benzie and Strain, 1996(1) while MDA concentration was determined by the method of Kohn and
Liversedge, 1944 (8,). LPI was determined using the MDA/TAA ratio.

Statistical analysis

Data were analyzed using PASW STATISTICS version 18 software. We obtained means, standard deviation and percentages. The comparison was done using the Student t test and ANOVA was used when more than two series of data were compared. Kruskal Wallis test was used for quantitative variables while X^2 test was used for qualitative variables. Pearson Correlation was used to establish the correlation between the different parameters. Results were said to be statistically significant at p value less than 0, 05

RESULTS

Of the 151 patients, there were 55 men (36, 4%) and 96 women (63, 6%), while of the 134 controls there were 73 men (54, 5%) and 61(45, 5%) women. The mean age was 35, 5±9, 32 years for patients and 27, 5±7, 70 years for controls group.

Table 1 shows the means ± standards deviations of MDA, TAA and LPI between patients and controls; MDA and LPI are higher in patients while TAA is lower compared to controls ($p<0,05$); there was a negatively significant correlation between LPI and TAA ($r= -0,968$; $p<0,05$) and a positive correlation between MDA and LPI ($r=0,125$). Table 2 shows the means ± standard deviations of MDA, TAA and LPI between patients and controls in relation to CD4 cell counts; while TAA decreases with decreasing CD4 cell counts ($p<0,05$), MDA and LPI increased with CD4 cell counts although the differences were not statistically significant. There was a statistically significant positive correlation between TAA and CD4 cell counts ($r=0,199$; $p<0, 05$).

Table 3 shows means ± standard deviations of MDA, TAA and LPI between patients and controls by sex; while MDA and TAA are higher, LPI is lower in patients (men as well as women) compared to controls (men as well as women). MDA and LPI are higher while TAA is lower in men compared to women in the patient group ($p<0,05$). There was a negative correlation between MDA and TAA ($r= -0,022$) in the control group. Table 4 shows that MDA and LPI are higher while TAA is lower in patients compared to controls ($p<0,05$). Age seemed not to have an influence in these parameters in the control group.

DISCUSSION:

Malondialdehyde(MDA)

Generation of reactive oxygen species (ROS) increases during chronic inflammation due to HIV infection and replication (7).This increase is the consequence of T cell and phagocyte antigen activation, and lipid metabolism abnormalities (10). ROS constitute free radicals that are responsible for lipid peroxidation whose final product is malondialdehyde; higher MDA plasma concentration in patients compared to controls may be the consequence of aggravated lipid peroxidation by free radicals which often leads to oxidative stress and cell apoptosis, a major cause of CD4 cell count depletion particularly during primo-infection(12). In men, the higher MDA and LPI values compared to women among patients may be due to the infection, but also probably to the nature of male hormones, testosterone which has been established to be an oxidant and cannot protect men against free radical damage like estrogen, an antioxidant, does in women (2). MDA increased when CD4 cells count decreased although the differences are not statistically significant; this may be due to CD4 cells lyses because of cell membrane lipid peroxidation by free radicals

Total antioxidant ability (TAA)

An antioxidant is a molecule which, present in very low concentration compared to that of an oxidant, is able to limit significantly or to prevent the oxidation of the different biological substrates by the oxidant .In the presence of an antioxidant, the oxidant species reacts with it and never with the biological substrate which is hence protected (1,18). TAA was evaluated through the FRAP test which expresses the antioxidant potential of the organism; this means its capacity to neutralize through antioxidant molecules the oxidant (free radicals) damage of various substrates (proteins, lipids, carbohydrates, nucleic acids). Our results showed approximately a three times reduction in TAA plasma concentration in patients compared to controls.

This may be linked to the high free radical production due to the antigenic (virus) activation of lymphocytes, phagocytes and chronic inflammatory processes induced by viral replication (10). These ROS ($^{\cdot}OH$, $HO^{\cdot}$, $^{\cdot}O^{-}_{2}$, H_2O_2) produced during chronic inflammation react with antioxidant and contribute to greatly reduce their plasma concentration (14) with the consequence of turning the antioxidant/ pro-oxidant balance in favor of pro-oxidant; this leads to severe lipids peroxidation and to cells apoptosis as shown by high MDA concentration in patient plasma, and CD4 cell depletion (14). TAA decreases with CD4 cell counts ($p<0, 05$), probably due to free radical effects and to viral replication since it has been shown that HIV uses antioxidant during its replication (3); a high TAA decrease in men compare to women could be due to high free radical generation in men due to the testosterone oxidant function added to viral effects.

Lipid peroxidation indices (LPI)

Lipids peroxidation indices are the ratio MDA/TAA. It expresses the degree of free radical aggression due to HIV infection. When the plasma total antioxidant ability decreases or when the plasma MDA concentration increases, LPI increases accordingly and the patient will be subjected to the worst effects of oxidative stress (13). This study showed approximately a seventy six times increase of LPI in patients compare to the controls ($p<0,05$; Table 1) and a higher LPI in men compared to women due to hormonal differences($p<0,05$; Table 3). The results confirmed the high generation of free radicals and a considerable decrease of TAA during HIV infection with an elevated oxidative stress as consequence (10). It has been established that HIV-1 virus uses antioxidants for its replication and this phenomenon adds to the chronic inflammatory process that speeds up CD_4 cell apoptosis and disease progression (3).

The variation of MDA, TAA and LPI by age may be lined to duration of infection, rather than the patient's age.

Table1: Comparison of different parameters between patients and controls

Parameters	Controls	Patients	
TAA (mM)	0, 63±0, 17	0, 16±0, 16	*
MDA (µM)	0, 20±0, 07	0, 41±0, 10	*
LPI	0, 34±0, 14	26, 02±74, 40	*

CONCLUSION

Our results show high MDA plasma concentration, as well as very high LPI Indices, a low plasma TAA during HIV infection and progression. A sex dependant MDA, TAA concentration and LPI indices may be due to the nature of male and female hormones: antioxidant estrogen and oxidant testosterone (2). A decrease in TAA plasma concentration in relation to CD4 cell counts may be due to lipid peroxidation by free radicals. All these disorders may be the result of chronic inflammation and free radical generation due to the viral replication (5, 7).

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Table 2: Means ± standards deviations of MDA, TAA and LPI indices in relation to CD4 cell counts

Class of CD_4	≥500	200-499	<200
TAA **(mM)** *	0,27±0,26	0,17±0,14	0,13±0,13
MDA (µM)	0,39±0,10	0,41±0,11	0,42±0,10
LPI	17,53±32,83	30,83±96,87	31,41±90,51

*Difference statistically significant

Table 4: Comparison of different parameters between patients and controls following age range.

Tranches d'âge	16-25	26-30	36-45	>45
TAA Malade *	0,17±0,16	0,16±0,14	0,17±0,17	0,16±0,17
TAA Témoin	0,61±0,16	0,66±0,17	0,62±0,20	0,61±0,10
MDA*Malade	0,39±0,10	0,42±0,12	0,39±0,096	0,39±0,09
MDA Témoin	0,18±0,06	0,22±0,07	0,26±0,03	0,18±0,08
LPI Malade *	7,95±9,61	26,30±65,69	28,90±86,51	31,85±96,76
LPI Témoin	0,32±0,13	0,35±0,14	0,46±0,15	0,31±0,13

* statistically significant difference