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Differential of Antioxidant Ability, CD4+T Cells Count and Viral Load in HIV Infected Patients on cART in Yaounde, Cameroon

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Abstract

Background: Decreased antioxidant ability is one of the worsening conditions in AIDS. We aimed to evaluate total antioxidant ability among others, and their variation in HIV infected patients following their CD4+T cells count and viral load, in a context of new ART scarcity in most LMICs.

Material and Methods: We conducted a cross sectional study on 167 individuals (76 controls, 33 treatments naïve and 58 HIV-1 infected patients on ART). We assessed their plasma total antioxidant ability (FRAP), malondialdehyde (MDA) and thiol (SH) groups using standard spectrophotometric methods, then we calculated lipid peroxidation index (LPI). Statistical analysis was performed using GraphPad Prism 6. Data were analyzed by two-tailed unpaired t-test for two groups' comparison and ANOVA for more than two groups. Pearson correlation between CD4+T cells count, viral load and the above markers was determined; $P \leq 0.05$ was considered statistically significant.

Results: The following controls/naïve/treated subjects' values for FRAP(mM) (1.907±0.074/1.77±0.05/1.695±0.03); MDA(µM) (0.781±0.081/1.115±0.118/ 1.342±0.109); SH (µM) (2.747±0.130/1.582±0.197/1.498 ±0.140) and LPI (0.43±0.61/ 0.61±0.7/2.59±0.83) were all obtained with $P \leq 0.05$. The FRAP increased only with 3TC+TDF+EFV and 3TC+ABC+NVP cART while MDA decrease significantly with the later (p=0.027). MDA and LPI significantly increased in heavily treated patients with p<0.0014 and p=0.0001 respectively. overall, the patients showed an increase of viral loads following a decrease of CD4+T cells (r= -0.803, p=0.016) but 3TC+TDF+EFV seem to better manage the both. The only significant correlation was established between SH groups and CD4+Tcells count (r=0.447; p=0.0006);

Conclusion: Our study showed that thiol groups may be protective against CD4+Tcells count depletion and that the cART 3TC+TDF+EFV, 3TC+ABC+NVP may be helpful in fighting against free radical generation and particularly 3TC+TDF+EFV as controlling CD4+Tcells count and viral load in long term treated patients. The study particularly showed the implication of cART in increasing lipid peroxidation index following the treatment duration in heavily treated patients, which aggravated their conditions in an area where drug options are limited, calling for new drugs availability and personalized medicine.

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Introduction

About 36.9 million of people were currently living with HIV worldwide as of 2017, explained in part by a faster deaths decline compared to new HIV infections, with antiretroviral scale up (90-90-90 targets). Sub-Saharan Africa is home to 53% of the world's people living with HIV[1]. 21.7 million People are now on treatment all around the world, a net increase of 2.3 million people since the end of 2016. Cameroon, Cote d'Ivoire and Nigeria together accounted for approximately 71% of new HIV infections in Western and Central Africa in 2017[1]. In Cameroon, 51% of Adults aged 15 years and older, 25% of children aged 0 to 14 years old, are on treatment[1], and many remained on same antiretroviral therapy (ART) for years, strengthening the necessity for all these patients treated or not, to continue to be well managed in order to reduce HIV Mortality and morbidity.

Human plasma is endowed with an array of antioxidants [2, 3], acting as a coordinated and balanced system to protect tissues and body fluids from damage by reactive oxidants whether produced physiologically or as a response to inflammation, infection or disease. Thus, the protective effects of plasma may result from a concerted action of the numerous different antioxidants present in it [4, 5]. Under normal circumstances, the reactive oxidants produced in the course of metabolism [6-8] are scavenged or otherwise eliminated by the natural antioxidant system[9]. Reactive oxygen species (ROS) are free radicals of oxygen intermediates with high reactive capacity towards various biological molecules and the potential to cause significant biological damage [10]. Detrimental effects caused by reactive species occur as a consequence of an imbalance between the formation and inactivation of these species. Oxidative stress can be significant especially if individual is exposed to environmental challenges, for instance, infection. ROS are involved in HIV pathogenesis and disease progression [11]. HIV induces the generation of ROS through the regulatory protein Tat and the envelope glycoprotein gp120 [12, 13] and the oxidative stress induced, increases viral replication and a variety of biochemical and physiologic changes, and contributes to CD4+ T lymphocyte depletion by increasing their rate of apoptosis[10, 14]. Increased production of ROS during HIV-1 infection has been reported in some studies involving ART, indicating an increase of oxidative stress in response to treatment [15-18] whereas others described it to be more pronounced in naïve patients[19, 20]; but in Cameroun, a country located in Sub Saharan Africa, very little is known about, especially those who are heavily treated, and of our knowledge, no study evaluating this and the total antioxidant status in HIV infected patients on cART has been done. The overall antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual parameters, as it considers the cumulative effect of all antioxidants present in plasma and body fluids [21] and the effect of treatment may give an overview on the variation of oxidative stress markers. Therefore, we aimed to investigate some oxidative stress markers, the overall antioxidant ability using FRAP assay in HIV-1 infected patients and the effect of treatment on their variation, and then to find out their correlation to CD4+ T cells count and viral load, for the patients' better management.

Materials and Methods

Study Design, Population and Ethical Considerations

We conducted a cross sectional study on 167 individuals (76 matching HIV-1 negative controls, 33 HIV-1 infected treatments naïve and 58 HIV-1 infected patients on different combination therapy, all enrolled as an ongoing project on oxidative stress. Participants were

from the Day Hospital of Yaoundé Central Hospital and The Yaoundé University Teaching Hospital where the controls were recruited. This study was performed in accordance with guidelines of the Helsinki Declaration and was approved by the Cameroon National Ethics Committee, written informed consent was obtained from all participants and data were processed using unique identifiers to ensure confidentiality. (1) for control subjects, exclusion criteria were pregnancy, serological evidence of hepatitis B/C, diabetes, hypertension, current intake of antioxidant supplementation, alcohol, tobacco, malaria and other known parasitic infection and inclusion criteria were HIV negative with none of the above conditions, and be able to read and sign an informed consent; (2) for patients, the exclusion criteria were the same as for control subjects; in addition, HIV-positivity was confirmed. After informed consent, a questionnaire, including socio-demographic information, laboratory data (e.g, diagnosed with HIV/AIDS or without, CD4+T cells counts, viral loads and diagnosed HBV and HCV negative) and treatment history (naïve or on treatment, treatment combination, treatment duration), was provided to each study participant and completed by the clinician

HIV Serology, CD4+T Cell Counts and Viral Load Quantification

Sample collection and analyses were performed in the Hematology laboratory of the Yaoundé University Teaching Hospital, Cameroon. Venous blood samples, taken at a single time point, were collected and stored at room temperature in the Hematology laboratory, and analyses performed within 6 hours of blood collection. The HIV status of each participant was determined using the Alere Determine HIV-1/2 antigen/antibodies Combo (Jouy-En-Josas, France), and the Murex HIV antigen/ antibody Combination ELISA

(Abbott Diagnostics, Chicago, IL, USA), according to the manufacturer's instructions. Each batch of reagents was quality controlled with known samples before used. A participant was considered HIV-positive if he/she tested positive for the two tests, HIV-negative if non-reactive for both tests and discordant if positive for only one test.

No discordant result was observed in this study. CD4+ T-lymphocyte count was quantified by flow cytometry, using a Fluorescence Activated Cell Sorting (FACS) Count Instrumentation System, BD FACS Count, according to the manufacturer's instructions (BD Biosciences, San Jose, CA, USA). The FACS instrument was calibrated and quality control tested before each experiment. HIV RNA copies number in each plasma sample was quantified by reverse transcription polymerase chain reaction (RT-PCR), using ABBOTT m2000rt (Abbott GmbH & Co. KG, Wiesbaden, Germany), according to the manufacturer's protocol. The ABBOTT m2000rt detection limit was 40 viral copies/ml. Plasma samples were stored at −70 °C or lower.

Biochemical Analyses

A plasma aliquot obtained from Ethylene Diamine Tetra Acetate (EDTA) peripheral blood tube was directly used for oxidative stress markers assessment. Plasma was used to assess total antioxidant ability (FRAP) using Benzie and Strain method(1996) [22], malondialdehyde (MDA) using the method described by Lefevre (1998)[23], and thiol groups (SH) using Ellman method (1959)[24]. Lipid peroxidation indices (LPI) was determined using MDA level over FRAP level ratio [25].

ART Regimen

For those on treatment, antiretroviral therapy used was of first or second line based regimen consisting of 2NRTIs (lamivudine-3TC plus tenofovir-TDF or zidovudine or abacavir-ABC) plus 1NNRTI (efavirenz-EFV or nevirapine-NVP) for first-line therapy and 2 NRTIs (lamivudine-3TC plus zidovudine-AZT) plus one protease inhibitor (PI) (lopinavir boosted ritonavir-LPV/r) or 1NRTIs (lamivudine-3TC) plus one protease inhibitor (lopinavir boosted ritonavir-LPV/r) for Second-line therapy.

Statistical Analysis

All statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, LaJolla, CA, USA). Data were analyzed by two-tailed unpaired t-test for two groups' comparison and ANOVA (ordinary or non-parametric if the data did not follow the Gaussian distribution) when more than two groups were to compare; The Pearson correlation was used to find out the correlation between the studied parameters. *P*-value ≤ 0.05 was considered statistically significant.

Results

Descriptive Characteristics of Patients Included in the Study

Tables 1a and 1b summarized the characteristics of 167 participants eligible, including 82 males and 85 females. Seventy-six were controls recruited among blood donors and 91 were HIV infected (30 males and 61 females); among HIV infected participants 33 were naïve and 58 on ART. About 63.7% of patients were on first-line treatment and only 20% were on second line ART. All of them were submitted to the assessment of oxidative stress markers. Tables 1a and 1b.

CDC 1993 Classification[26]

Biochemical Analyses

Following the quantification of thiols groups (SH), plasma thiols did not differ between HIV infected naïve and treated patients (1.582±0.197 µM and 1.498±0.140 µM respectively, P = 0.721) but were significantly lower (P<0 .0001) in both groups compared to controls (2.747±0.130 µM) (Table 2). Plasma malondialdehyde (MDA) significantly increased (P = 0.0014) in treated patients (1.342±0.109 µM) and in naïve (1.115±0.118 µM) as compared to controls (0.781±0.081 µM). It showed no difference between naïve and treated patients (P = 0.185).

Plasma total antioxidant ability (FRAP) increased in controls (1.907±0.007 mM) but decreased in naive (1.774±0.05 mM) and treated patients (1.695±0.03 mM). It showed no difference between naïve and treated patients (P = 0.225). The decrease was statistically significant in treated patients as compared to controls (P = 0.017). Lipid peroxidation index (MDA/ FRAP) significantly increased in naïve (0.61±0.7) and treated patients (2.59±0.83) as compared to controls (0.43±0.61) (p<0.0001). It showed a statistically significant difference in naïve as compare to treated patients (P< 0.0001) (Table 2).

Comparing SH groups, MDA and FRAP concentrations in 3TC+TDF+EFV cART, we found a significant increase of FRAP and a decrease of SH groups (P=0.006) while there was an increase of FRAP following a decrease of MDA with 3TC+ABC+NVP (p=0.027) (Figure 1 a and Figure 1 b). Others cART did not show any statistically significant impact on the above cited markers, even though globally, there was an increase of MDA following a decrease of FRAP and SH groups in treated patients as compared to controls (Figure 1 a, Figure 1 b and Figure 1 c).

An analysis of the above studied parameters' variation according to the number of years of treatment, showed that MDA and the lipid peroxidation index MDA/ FRAP significantly increased (r=0.654, p<0.0001 and r=0.266, p=0.045 respectively) with the treatment duration while the total antioxidant ability (FRAP) and the thiol groups, did not show any statistically significant variation (r=0.239, p=0.07 and r=0.100, p=0.465 respectively) over time (Figure 2 a, Figure 2 b, Figure 2 c, Figure 2 d).

Immuno-Virological Status of Treated Patients.

CD4+T cells count (cells/m³) was significantly higher in patients on 3TC+TDF+EFV as compared to those on 3TC+ABC+NVP and 3TC+LPV/r, while it was lower for patients on 3TC+ABC+NVP as compared to those on AZT+3TC+EFV (p = 0.0012). The mean CD4+T cell count was around 200 cells/m³ or less for patients on different cART (figure 3-a). The viral load (log copies/ml) was significantly lower for patients on 3TC+TDF+EFV (4logcopies/ml) as compared to those on 3TC+ABC+NVP, AZT+3TC+EFV and 3TC+LPV/r (6logcopies/ml) (p=0.0002) even though all these viral loads remained higher than 1000 copies/ml, the WHO's viral load threshold for a successful treatment (Figure 3 b) outcome in low and middle income countries. Overall, these patients showed an increase of viral loads following a significant decrease of CD4+T cells count as the Pearson correlation highlighted (r = -0.803, p = 0.016) (Figure 3 c). No statistically significant correlation was found between CD4+T cells count and the studied markers in naïve patients but in treated patients, the only significant correlation was between the thiol groups and CD4+T cells count (r=0.447; p=0.0006). Also was not found any statistically significant correlation between viral loads and the stress markers but a borderline correlation between it and the lipid peroxidation index, MDA/FRAP (r=0.249; p=0.06) even though not significant.

Discussion

The descriptive characteristics of patients showed that there were more infected females than

Table 1a. Descriptive Characteristics of patients included in the study

Characteristics	Male	Female	*p-value*
Overall participants (n)	82	85	
Control (n)	52	24	
Age range (years)	[19-50]	[21-47]	
Mean age	30.88± 1.195	32.38±1.464	0.067
Distribution of patients relatively to HIV treatment			
Naïve (n)	12	21	
Age range (years)	[28-62]	[21-54]	
Mean age	42±3.224	33±2.286	0.042
CD4 cells range	[122-794]	[5-615]	
Mean CD4 cell count (cell/m³)	337.3±62.63	258.3±37.51	403
Viral load range (log copies/m)	[1.5-7.2]	[1.4-7.6]	
Mean viral load (log copies/ml)	4.41±1.1	4.06±1.40	0.23
Treated (n)	18	40	
Age range (years)	[29-59]	[20-58]	
Mean age	40.11±2.298	34.18±1.298	0.0286
Treatment duration range (years)	[1-7]	[1-7.3]	
Mean treatment duration (years)	3.300±1.69	3.763±1.50	0.311
CD4 cells range	[2-496]	[1-559]	
Mean CD4 cell count (cell/m³)	152.2±29.17	153.1±21.88	0.99
Viral load range (log copies/m)	[2.1-9]	[1.3-7.8]	
Mean viral load (log copies/ml)	6.5±1.4	6.33±1.3	0.381
Regimens (%)			
3TC+ABC+NVP	27.77	32.5	
3TC+TDF+EFV	27.77	25	
AZT+3TC+EFV	33.33	22.5	
AZT+3TC+LPV/r	5.55	5	
3TC+ LPV/r	5.55	15	

3TC, Lamivudine; ABC : Abacavir; NVP, Nevirapine; AZT, Zidovudine; EFV, Efavirenz; LPV/r, Lopinavir boosted ritonavir ; TDF : Tenofovir

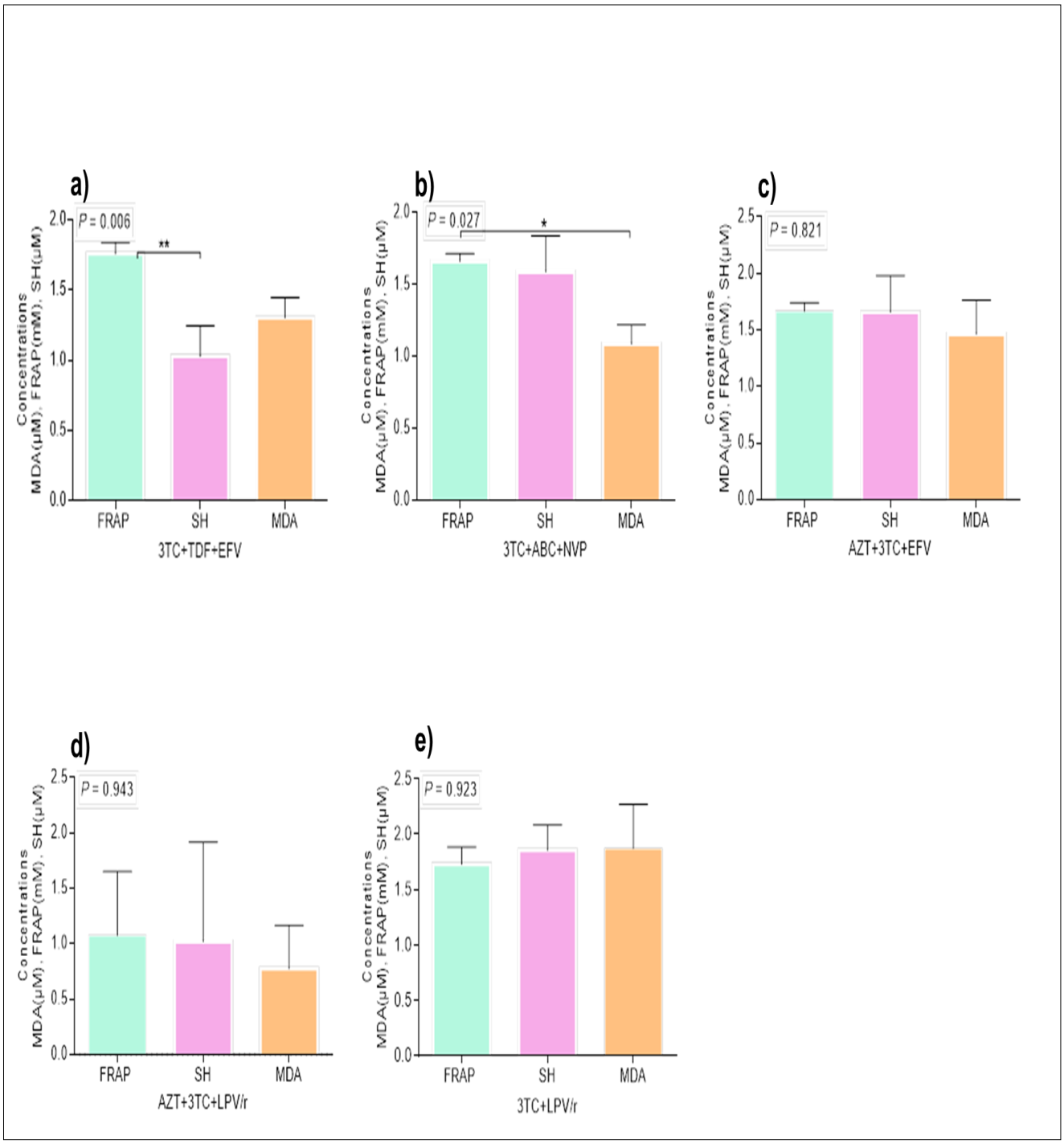

Figure 1. Variation of FRAP (mM), SH groups (µM) and MDA (µM) concentrations in patients' plasma for each treatment combination (a-3TC+TDF+EFV, b-3TC+ABC+NVP, c-AZT+3TC+EFV, d-AZT+3TC+LPV/r, e-3TC+LPV/r) in HIV-1 infected patients; * *P*-value ≤ 0.05.

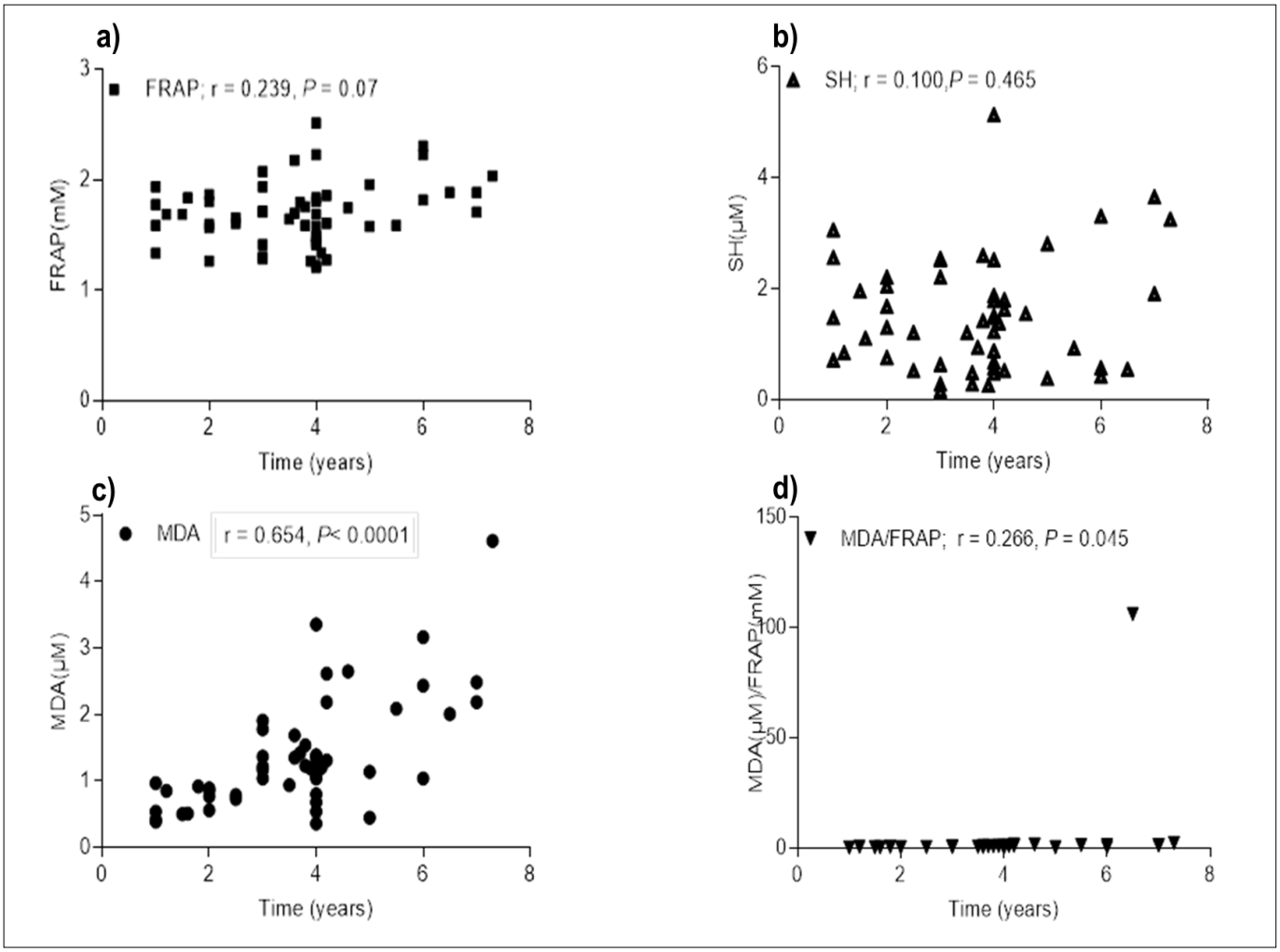

Figure 2. Variation of FRAP (mM) (a), SH groups (µM) (b), MDA (µM) (c) concentrations and MDA/FRAP (d) index in patients' plasma according to treatment duration; * *P*-value ≤ 0.05.

Table 1b. Distribution of patients following their clinical stage CDC 1993 Classification[26]

Clinical stage	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Naïve (%)	2(6)	4(12.1)		4(12.1)	8(24.2)	9(27.2)	4(12.1)	1(3)	1(3)
Treated (%)	/	/	/	/	1(1.7)	38(65.5)	3(5.1)	/	16(27.5)
Total (%)	2(2.1)	4(4.3)		4(4.3)	9(9.8)	47(51.6)	7(7.6)	1(1.09)	17(18.6)
***P*-value**	0.471								

CDC 1993 Classification[26]

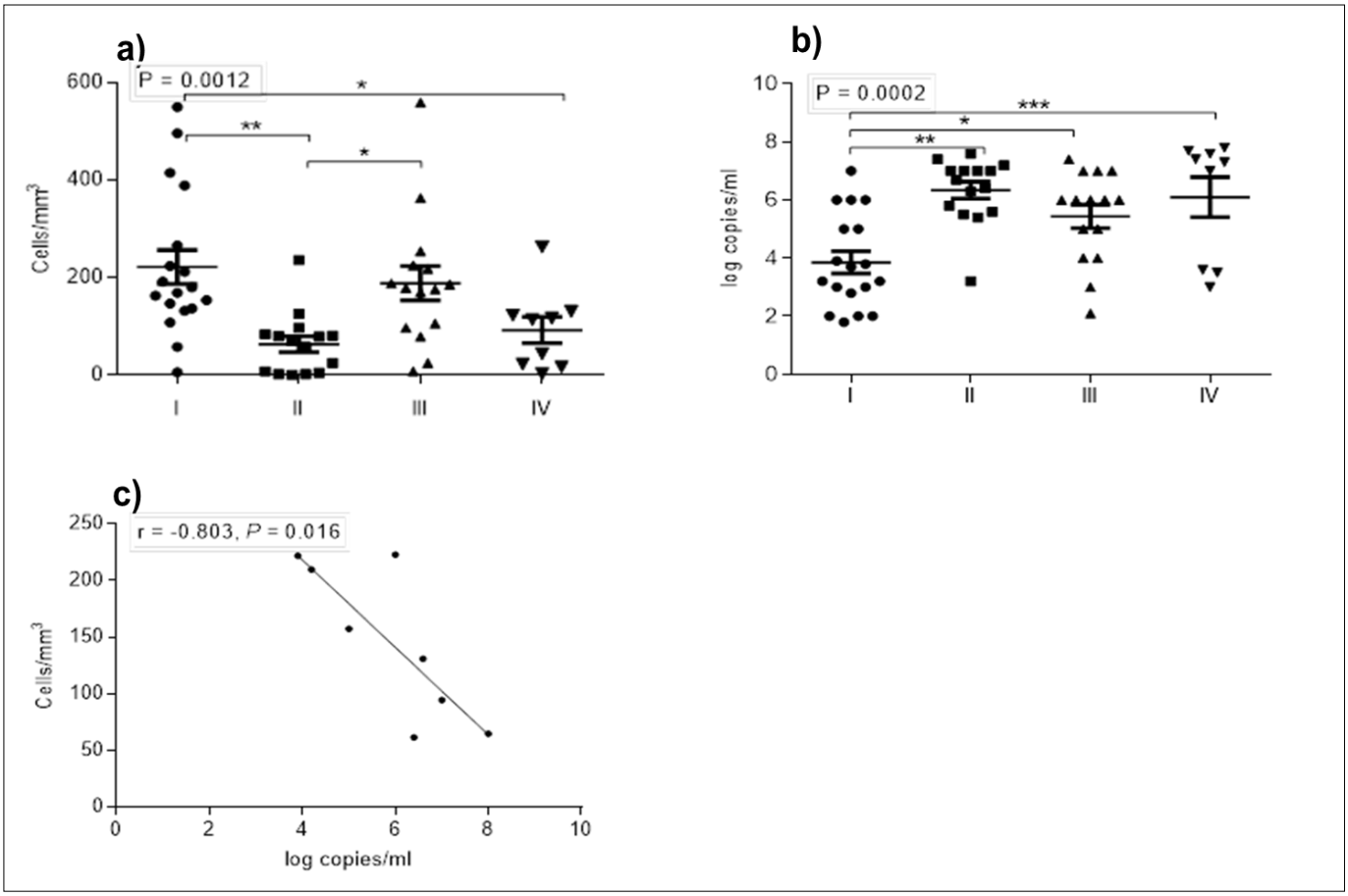

Figure 3. Quantification of patients' CD4+T cells count (Cells/mm^3) (a), viral loads (log copies/ml) (b) for each treatment combination (I = 3TC+TDF+EFV, II = 3TC+ABC+NVP, III = AZT+3TC+EFV, IV = 3TC+LPV/r) and the correlation between CD4+T cells count and viral loads in treated patients (c), * *P*-value ≤ 0.05.

Table 2. Variation of SH groups (µM), MDA (µM), FRAP (mM) concentrations and MDA/FRAP index in control, naïve and treated patients.

Biochemical parameters	**Control**	**Naive**	**Treated**	***P*-Value**
SH (µM)	2.747±0.130	1.582±0.197	1.498±0.140	< 0 .0001
		P = 0.721		
MDA (µM)	0.781±0.081	1.115±0.118	1.342±0.109	0.0014
		P = 0.185		
FRAP (mM)	1.907±0.007	1.774±0.05	1.695±0.03	0.017
		P = 0.225		
MDA/FRAP	0.43±0.61	0.61±0.70	2.59±0.83	< 0.0001
		P < 0.0001		

males, a phenomenon explained by socio-economic events and particularly women biological vulnerability[27, 28]. Following the WHO approach for HIV/AIDS treatment in Low and Middle Income Countries (LMICs)[29, 30], two third of subjects (63.7%) were on first line regimen, very few were on second line regimen; most of them have been on the same cART for several years (table 1a) and almost one third of patients were naïve (36.2%); this could be due to limited ART options for LMICs and would call for more drugs options to reduce HIV mortality and morbidity, and to consolidate the WHO's "Treat all" recommandations [31] in our country where only about 51% of adults aged 15 and older are on treatment [1] .

Following the assessment of oxidative stress markers namely malondialdehyde (MDA), thiol groups (SH), total antioxidant ability (FRAP) and lipid peroxidation index (MDA/FRAP); SH and FRAP significantly decreased in naïve and treated patients'plasma as compared to controls ($p<0.0001$ and $p=0.017$ respectively) while plasma MDA significantly increased in treated patients' plasma as compared to controls ($p=0.0014$) (Table 2). This shows the implication of the virus in the initiation and aggravation of oxidative stress. Lipid peroxidation significantly increased in treated patients as compared to naïve ($P < 0.0001$) (Table 2), showing the treatment as an increasing factor, as shown by previous studies [17, 25, 32, 33]. Similar results were found by Ngondi et *al* but their study did not assess the plasma total antioxidant ability and the lipid peroxidation index, they did not also consider the same drugs combinations included in our study [18]. Contrary to our findings, some studies have reported higher level of oxidative stress markers in naïve patients as compared to those on cART [19, 20], may be because in some circumstances, and perhaps due to cART's influence or the host genetics, HIV is a faster generator of free radicals than drugs.

As urate and α-tocopherol plasma sulfhydryl groups serve as antioxidants and are consumed by trapping the major proportion of the peroxyl radicals generated[3, 34], low thiol levels affect HIV progression both before and after AIDS diagnosis and may be magnified following treatment[34], this was observed in our study. Glutathione, a thiol tripeptide has been shown to decrease as HIV progresses; its deficiency contribute to oxidative stress and may play a key role in HIV pathogenesis[35]. Consistent with these results, it has been shown that antioxidant status progressively depleted in HIV infected persons as the disease progressed from asymptomatic state to AIDS[36]. This antioxidant deficiency in HIV-1 seropositive populations is probably due to depletion of antioxidant molecules consumed in the process of protecting cells against ROS[37].

The effect of different cARTs on the above studied markers showed that 3TC+TDF+EFV significantly increases the plasma FRAP concentration and decreases plasma SH groups as well as 3TC+ABC+NVP also increases the plasma FRAP concentration and decreases plasma MDA concentration ($p=0.006$ and $p=0.027$ respectively) (figure 1a and 1b); these two drugs combinations as compared to the others (figures 1c, 1d, 1e) may be helpful in fighting against antioxidative imbalance[38, 39], even though gobally, our study showed that all the patients were failing treatment and that the latter is an oxidative stress increasng factor, probably due to protease inhibitors[40-42] or AZT [40].

On another part, the increase of FRAP may be due to some endogenous antioxidants (enzymes) that may increase their activity in response to an increase production of free radicals [43, 44].

The study of the effect of heavy treatment on the variation of MDA, FRAP, SH concentrations and MDA/FRAP index, showed that plasma MDA concentration and lipid peroxidation index significantly increase with the number of years of treatment ($r = 0.654$, $p = 0.0001$ and $r = 0.266$, $p = 0.045$ respectively) (figure 2c and 2d); the unchanged treatment combination during several years for patients in advanced stage of desease (table 1b) and who are failing treatment as per WHO recommendations (viral load > 1000 copies/ml) [30, 45] may explain the worsening oxidative conditions, since as the virus replicates, there is an increased free radicals generation[46], leading to an increased lipid peroxidation, explained by the increasing MDA concentration[25].

Immuno-virologic analysis of treated patients showed that 3TC+TDF+EFV significantly increases CD4+Tcells count as compared to 3TC+ABC+NVP and 3TC+LPV/r ; furthermore, CD4+Tcells count was lower in 3TC+ABC+NVP treated patients as compared to those on AZT+3TC+EFV (P = 0.0012) (figure 3a). The viral load was significantly lower in 3TC+TDF+EFV treated patients as compared to those on 3TC+ABC+NVP, AZT+3TC+EFV and 3TC+PV/r (P = 0.0002) (figure 3b), even though all these patients were probably failing treatment [47] as established by the positive and significant Pearson correlation between CD4+Tcells count and viral load (r = -0.803, p=0.016). Several studies demonstrates the usefulness of ART as it increases CD4+Tcells count and stabilizes the immune status[48] while reducing the viral load [49] when the patients are well managed; 3TC+TDF+EFV cART could be useful in such conditions[50, 51]. The only significant correlation between the oxidative stress parameters and CD4+Tcells count or viral load, was the positive Pearson correlation between thiol groups and CD4+Tcells count (r=0.447; p=0.0006); thiol groups may be useful in protecting CD4+Tcells count against depletion but also, disulfide bonds are used for HIV to enter into CD4+Tcells through the glycoprotein gp 120; so as the thiol groups decrease, so are the CD4+Tcells [52-56].

Conclusion

Our study showed that thiol groups may be protective against CD4+Tcells count depletion or may explain this depletion due to their lack, and that the cART 3TC+TDF+EFV, 3TC+ABC+NVP may be helpful in fighting against free radical generation and particularly 3TC+TDF+EFV as controlling CD4+Tcells count and viral load in long term treated patients. The study particularly showed the implication of cART in increasing lipid peroxidation index following the treatment duration in heavily treated patients, which aggravated their conditions. Here we did not follow the patients and did not assess antioxidant enzymes activities, vitamins or minerals that would have brought an adding value to our study. A follow-up study including more patients, new drugs (if available) and assessing also the role of some HIV-genes in the generation of free radicals could help for a better management of HIV infected patients who are in need of new drugs in our context and for who personalized medicine is more than ever needed.

Declarations

Ethics Approval and Consent to Participate

This study was performed in accordance with guidelines of the Helsinki Declaration and was approved by the Cameroon National Ethics Committee, written informed consent was obtained from all participants and data were processed using unique identifiers to ensure confidentiality.

Consent for Publication

All the authors gave their consent for this work to be published.

Availability of Data and Material

The data used to support the findings of this study are available from the corresponding author upon request.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Funding

Not applicable.

Author Contributions

GT conceived and designed the study, carried out experiments, made figures and tables, did the analysis and interpretations, and participated in the writing of the manuscript. JKS carried out subject recruitment, collected demographic data from participating human subjects, participated in specimen collection, helped coordinate the clinical studies, did experiments, made tables, helped conceiving the study, participated in interpretations and in the writing of the manuscript. JF, FNN helped coordinate the clinical studies, made table and edited the manuscript. BD, DT, NMG, RDC helped coordinate the clinical studies and edited the manuscript. ACP and AN coordinated and supervised the study and edited the manuscript.

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