Lipid Peroxidation and Total Cholesterol in HAART-Naïve Patients Infected with Circulating Recombinant Forms of Human Immunodeficiency Virus Type-1 in Cameroon

Georges Teto¹,³*, Georgette D. Kanmogne⁴, Judith N. Torimiro²,³, George Alemnji¹,³, Flore N.Nguemaim¹,³, Désiré Takou², Aubin Nanfack², Asonganyi Tazoacha¹,³

¹Laboratory of Immunology, Biochemistry and Biotechnology, University of Yaoundé I, Yaoundé, Cameroon, ²CIRCB (Chantal Biya International Research Center), Yaoundé, Cameroon, ³Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon, ⁴Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, Nebraska, United States of America

Abstract

Background: HIV infection has commonly been found to affect lipid profile and antioxidant defense.

Objectives: To determine the effects of Human Immunodeficiency Virus (HIV) infection and viral subtype on patient’s cholesterol and oxidative stress markers, and determine whether in the absence of Highly Active Antiretroviral Therapy (HAART), these biochemical parameters could be useful in patient’s management and monitoring disease progression in Cameroon. For this purpose, we measured total cholesterol (TC), LDL cholesterol (LDLC), HDL cholesterol (HDLC), total antioxidant ability (TAA), lipid peroxidation indices (LPI), and malondialdehyde (MDA) in HIV negative persons and HIV positive HAART-naïve patients infected with HIV-1 group M subtypes.

Methods: We measured serum TC, LDLC, HDLC, plasma MDA, and TAA concentrations, and calculated LPI indices in 151 HIV-positive HAART-naïve patients and 134 seronegative controls. We also performed gene sequence analysis on samples from 30 patients to determine the effect of viral genotypes on these biochemical parameters. We also determined the correlation between CD4 cell count and the above biochemical parameters.

Results: We obtained the following controls/patients values for TC (1.96 ± 0.54/1.12 ± 0.48 g/l), LDLC (0.67 ± 0.46/0.43 ± 0.36 g/l), HDLC (105.51 ± 28.10/46.54 ± 23.36 mg/dl) TAA (0.63 ± 0.17/0.16 ± 0.16 mM), MDA (0.20 ± 0.07/0.41 ± 0.10 µM) and LPI (0.34 ± 0.14/26.02 ± 74.40). In each case, the difference between the controls and patients was statistically significant (p < 0.05). There was a positive and statistically significant Pearson correlation between CD4 cell count and HDLC (r = +0.272; p < 0.01), TAA (r = +0.199; p < 0.05) and a negative and statistically significant Pearson correlation between CD4 cell count and LPI (r = −0.166; p < 0.05). Pearson correlation between CD4 cell count and TC, CD4 cell count and HDLC was positive but not statistically significant while it was negative but not statistically significant with MDA. The different subtypes obtained after sequencing were CRF02_AG (43.3%), CRF01_AE (20%), A1 (23.3%), H (6.7%), and G (6.7%). None of the HIV-1 subtypes significantly influenced the levels of the biochemical parameters, but by grouping them as pure subtypes and circulating recombinant forms (CRFs), the CRF significantly influenced TC levels. TC was significantly lower in patients infected with pure HIV-1 subtypes (1.32 ± 0.68 g/l) compared to patients infected with CRF02_AG (0.38 ± 0.08 µM) (p < 0.018).

Conclusion: These results show that HIV infection in Cameroon is associated with significant decrease in TAA, LDLC, HDLC and TC, and increased MDA concentration and LPI indices which seem to be linked to the severity of HIV infection as assessed by CD4 cell count. The data suggests increased oxidative stress and lipid peroxidation in HIV-infected patients in Cameroon, and an influence of CRFs on TC and MDA levels.

Citation: Teto G, Kanmogne GD, Torimiro JN, Alemnji G, Nguemaim FN, et al. (2013) Lipid Peroxidation and Total Cholesterol in HAART-Naïve Patients Infected with Circulating Recombinant Forms of Human Immunodeficiency Virus Type-1 in Cameroon. PLoS ONE 8(6): e65126. doi:10.1371/journal.pone.0065126

Editor: Rui Medeiros, IPO, Inst Port Oncology, Portugal

Received December 1, 2012; Accepted April 23, 2013; Published June 7, 2013

Copyright: © 2013 Teto et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: CIRCB for the facilities put at our disposal for this project. Dr. Georgette Kanmogne is partially supported by the United States National Institute of Health grant R01 MH094160. No additional external funding received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: ggteto@yahoo.fr
Introduction

Human immunodeficiency virus type 1 (HIV-1) is the pathogen responsible for acquired immunodeficiency syndrome, a disease which has spread throughout the world and which affects immune cells, especially CD4+ lymphocytes and macrophages [1]. About 68% of HIV-infected individuals live in sub Saharan Africa [2], one of the most impoverished regions of the world; this represents two third of 34 millions individuals currently living with HIV/AIDS [3,2]. In Cameroon the prevalence of HIV infection is estimated at 5.5% [4], while antiretroviral therapy (ART) coverage is below 40% [2], suggesting that about 60% of HIV-infected Cameroonian in need of treatment do not have access to ART. For these patients, monitoring of biochemical parameters such as nutritional status and oxidative stress markers could help in the management of HIV/AIDS patients.

HIV-1 is divided into four groups: M for major, O for outlier, N for non M non O [6,7], and P [5]. HIV-1 group M viruses are further divided into nine pure subtypes and about 54 circulating recombinant forms (CRF) [8,9]; CRF02_AG subtypes are predominant in West and Central Africa while CRF01_AE subtypes are present in Central Africa, Thailand and other Asian countries [10,11]. All these groups and subtypes are present in countries where HIV-1 has been implicated in many biochemical disorders among which dyslipidemia and antioxidant imbalance [12,13].

Dyslipidemia is a clinical condition which often leads to alterations in lipid profile: total cholesterol (TC), low density lipoprotein cholesterol (LDLC) and high density lipoprotein cholesterol (HDL) [14,15]. Antioxidant imbalance which is assessed through plasma malondialdehyde concentration and plasma total antioxidant ability, is a condition which can contribute to increased destruction of CD4+ T cells and disease progression if the balance is in favor of pro-oxidant (free radicals) generation [16,17]. Deficiency in antioxidants in many HIV/AIDS patients may also potentiate the harmful effects of free radical action and accelerate disease progression [18,12].

Cameroon is a country with a high diversity of HIV-1 subtypes [19,20] but the clinical implications of this multitude of HIV-1 subtypes has not previously been investigated. The objective of this study was to determine the effects of HIV-1 infection and viral subtype on patient’s cholesterol (TC, LDLC, HDLC), lipid peroxidation indices (LPI), and oxidative stress markers [total antioxidant ability (TAA) and malondialdehyde (MDA)], to determine whether in the absence of HAART, these biochemical parameters could be useful in patient’s management and monitoring disease progression.

Subjects and Methods

Subjects

Informed consent was obtained from all subjects according to the guidelines of the Cameroon National Ethics Committee that approved the study. After obtaining informed consent, we enrolled 285 individuals who met our inclusion criteria: (1) for control subjects, exclusion criteria were pregnancy, serological evidence of hepatitis B/C, diabetes, hypertension, current intake of drugs, 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.

The 285 individuals included 151 patients (thirty were taken for genotypic studies) and 134 control subjects.

Sample Collection

Following a 10 hour fast, 10 ml of blood was collected from each participant into a labeled dry tube (5 ml) and a tube containing EDTA anticoagulant (5 ml). Following clotting, the dry tubes were centrifuged at 1200 g for 15 min to collect serum which was aliquoted and used for the assay of the different biochemical parameters while EDTA tubes were centrifuged at the same conditions to collect plasma which was also aliquoted and used for RNA extraction and measurement of oxidative stress markers. All samples were stored at −20°C and processed within four days after collection.

1) Quantification of biochemical parameters. Serum TC, HDLC, LDLC concentrations were determined using commercially available kits (Human Gesellschaft für Biochemica und DiagnosticambH Kit, Max-Planck-Ring 21-Wiesbaden-Germany). Plasma TAA was determined using the method of Benzie and Strain [21], while MDA was determined using the method of Kohn and Liversedge [22] as described by Lefèvre [23]. MDA measurement is based on a reaction between malondialdehyde and thiobarbituric acid which forms a pink pigment that absorbs at 532 nm at 90°C – 100°C at pH 2, while TAA is measured using the ferric reducing ability of plasma (FRAP) based on the ability of the antioxidants present in plasma to reduce Fe3+ to Fe2+ at pH 3.6 in the presence of 2,4,6 tri (−pyridyl-s-triazine); the reaction produces an intense blue color that absorbs at 593 nm. LPI was obtained by calculating the ratio MDA/TAA.

TC concentration was determined using colorimetric enzymatic techniques based on the successive action of cholesterol oxidase and peroxidase; HDLC concentration in the serum supernatant was determined by the same process after the precipitation of VLDL cholesterol, LDL cholesterol and chylomicrons in the presence of phosphotungstic acid and MgCl2. Results were calculated using the formula:

\[ \text{TC (g/l) or HDLC (mg/dl) concentrations} = \text{OD500nm sample/OD500nm standard} \times \text{Concentration of standard (essentially as recommended by the manufacturer in the kits).} \]

LDLC concentration was determined using the formula of Friedewald et al. [24]:

\[ \text{LDLC (mg/dl)} = \text{TC (mg/dl)} - \text{HDLC (mg/dl)} - \text{Triglycerides (mg/dl)/5}. \]

2) RNA extraction and PCR amplification. RNA was extracted from patient’s plasma using the QiAmp Viral RNA kit ([Qiagen, Hilden, Germany]), according to the manufacturer’s instructions.

RNA and cDNA samples were then amplified in a one tube, two steps RT-PCR using a thermal cycler (TECHNE TC 412[TECHNE Inc, Burlington, New Jersey, USA]) with the following primers: H1G777 (TCACCTAGAAGTTTTGAAATG-CATGG) sense (nucleotide 777 to 801 of HIV-1 genome) and H1P202 (CTTAATCTGTTATCATCTGCTGTCCTG) antisense (nucleotide 1874 to 1896 of HIV-1 genome). Nested PCR was performed using H1Ggag1584 (AAAGATGGA-TAATCTGAG) sense and g17 (TTCAACATTTCAACACGCC) antisense primers to enable amplification of the 460 bp encoding amino acid 132 of p24 to amino acid 40 of p7 from the gag gene. The amplification conditions of the RT-PCR were as followed: 50 min at 42°C (cDNA reaction) followed by 5 min at 94°C and the addition of 0, 2 μl Taq polymerase (5U/ μl); then the reaction continued with 40 cycles of 30s, 30s, and 90s at 94°C, 50°C, and 72°C respectively, and 1 cycle of 7 min at 72°C. For Nested PCR, cycling conditions were 1 cycle of 2 min at 94°C; 35 cycles of 30s, 30s, and 60s at 94°C, 50°C, and 72°C respectively; and 1 cycle of 7 min at 72°C. The PCR amplification

Lipid Peroxidation and HIV-1 Infection

radius

radius

radius

radius
products were detected by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining under UV light.

3) DNA sequencing. The 460 bp fragments obtained were sequenced using the previously described primers H1Gag 1584 and g17 with the same PCR amplification program [11]. Nucleotide sequences were obtained by direct sequencing of the PCR products. The amplified DNA was purified using an AmiconMicrocon Ultra pure kit (centrifugal filters devices-Millipore) and directly sequenced using Big-Dye chemistry (Perkin-Elmer). Electrophoresis and data collection were done on an Applied Biosystems 3130 XL automatic DNA sequencer. Nucleotide sequences were aligned using CLUSTAL W [25], with minor manual adjustments as appropriate for the DNA sequences. Regions that could not be aligned unambiguously, due to sequence variability or length, were omitted from the analysis. The phylogenetic tree (Figure 1) was generated by the neighbor-joining method [26] and reliability of the branching orders determined by the bootstrap approach [27]. The CLUSTAL W. Genetic distances were calculated using the Kimura’s two-parameter method [28].

Statistical Analysis
Data were analyzed using PASW STATISTICS version 18 software. We obtained means, standard deviation and percentages. Two-group comparisons were done with the parametric Student t test or the non parametric Mann Whitney test, and ANOVA was used when more than two series of data were compared. Kruskal Wallis test was used for quantitative variables while X² test was used for qualitative variables. Pearson (parametric) or Spearman (non parametric) correlations were used to establish the correlation between the different parameters. Logistic regression and ANOVA were used to study the association of the different subtypes with biochemical parameters.

Results
Participants’ Demographics and Clinical Characteristics
Participant’s demographics characteristics are summarized in Table 1. A total of 285 subjects (151 HIV+ and 134 seronegative controls) were evaluated in this study. Of the HIV+ group, 55 (36.4%) were male and 96 (63.6%) were female. Of the 134 subjects in the control group, 73 (54.5%) were male and 61 (45.5%) were female. The average ages were 35.5±9.32 years for HIV+ group and 27.5±7.70 years for the control group. Of the 151 HIV+ cases, 15 (10%) were asymptomatic, while 136 (90%) had experienced at least one AIDS event based on the occurrence of opportunistic infections (prurigo in 43 cases, cryptococcosis in 8 cases, Kaposi sarcoma in 8 cases, cytomegalovirus infection in 10 cases, toxoplasmosis in 10 cases, pneumocystosis in 24 cases, and tuberculosis in 33 cases). Based on the Centers for Disease Control (CDC) AIDS classification criteria [29], the patients belonged to category A (10%), category B (51.65%) and category C (38. 41%).

Increase in LPI and MDA and decrease in TC, HDLC, LDLC, TAA are linked to reduction in CD4 cell counts in a statistically significant manner (Table 2). There was a positive and statistically significant Pearson correlation between CD4 cell count and HDLC ($r = +0.272; p<0.01$) and TAA ($r = +0.199; p<0.05$) and a negative and statistically significant Pearson correlation between

Figure 1. Phylogenetic tree of the different subtypes of HIV-1 group M included in the study (460 bp encoding amino acid 132 of p24 to amino acid 40 of p7 from the gag gene). Cons = reference sequences; G = sample. doi:10.1371/journal.pone.0065126.g001
CD4 cell count and LPI ($r = -0.166; p<0.05$). Pearson correlation between CD4 cell count and TC and LDLc was positive but not statistically significant while it was negative and not statistically significant with MDA (Table 3).

**HIV Genotyping**

Samples from 50 HIV+ patients were used in genotypic studies, and we successfully sequenced the viral genome in samples from 30 patients, all of which belonged to the CDC category B [29]. Results indicated that 43.3% were HIV-1 CRF02_AG, 20% CRF01_AE; 23.3% subtype A1, 6.7% subtype H, and 6.7% subtype G (Table 4).

Results for the effect of HIV subtype on TC are summarized in Table 6. There was a statistically significant difference in the level of TC in patients infected with CRFs (CRF02_AG and CRF01_AE) and pure HIV-1 subtypes (G, H and A1) ($p = 0.017$); there was a lower mean value in CRFs patient group (0.87 ± 0.27 g/l) compared to patients carrying pure subtypes group (1.32 ± 0.68 g/l). Patients carrying CRFs had lower LDLc, HDLc, TAA mean values compared to patients carrying the pure subtypes although the results were not statistically significant (Table 6).

Further, the results for the effect of HIV subtypes on MDA, TC, HDLc, LDLc, TAA, and LPI were summarized in Table 6. There was a statistically significant difference in MDA levels in patients with the CRF01_AE subtype (1.32 ± 0.68 μM) compared to patients infected with CRF01_AG subtype (0.38 ± 0.08 μM) ($p = 0.018$).

Levels of TC, LDLc, HDLc and LPI in patients infected with the CRF01_AE subtype were higher compared to patients infected with TC and LDLc ($r = 0.530$); TC and HDLc ($r = 0.583$) and a statistically significant Pearson negative correlation ($p<0.01$ at a bilateral level) between TAA and LPI ($r = -0.968$). The Pearson correlation between TC and MDA was negative and non significant ($r = -0.035$).

**Biochemical Parameters and HIV-1 Subtypes Effects**

Results in Table 4 show that CRF02_AG subtype is the most frequent (43.3%) followed by A1 (23.3%), CRF01_AE (20%), G (6, 7%) and H (6, 7%) subtypes. CRF02_AG and CRF01_AE subtypes were the most frequent in women compared to men; every HIV-1 subtype represented here is implicated in at least one class of CD4 cells count in men as well as in women.

Results for TC, LDLc, HDLc, TAA, MDA, and LPI are summarized in Table 5. There was a statistically significant difference ($p<0.05$) between patients and controls for TC, LDLc, HDLc, TAA, MDA, and LPI. MDA (an oxidative stress marker), and LPI mean values are higher in patients compared to controls while TC, LDLc, HDLc, TAA, mean values are lower in patients compared to controls (Table 5); there was a statistically significant Pearson positive correlation ($p<0.01$ at a bilateral level) between every HIV-1 subtype and HDLc, TC, LDLc, TAA, MDA, and LPI. MDA is a marker mean value. (A1), (B2), (C3): Clinical categories.

Table 1. Demographics and clinical characteristics of participants.

| Characteristics | HIV+ Patients | HIV-Controls | $P$ |
|-----------------|---------------|--------------|-----|
| Total number    | (N = 151)     | (N = 134)    |     |
| Sex (% female)  | 63.6          | 45.5         | 0.0001 |
| Age (mean ± SD) | 35.58 ± 9.32  | 27.65 ± 7.70 | 0.0001 |
| Age range       | 16–56         | 16–56        |     |
| Education (mean years ± SD) | 12.20±1.68 | 12.50±1.57 | 0.71 |
| AIDS (%)        | 38.41         |             |     |
|                 | doi:10.1371/journal.pone.0065126.t001 |

Table 2. Biochemical parameters in HIV-infected patients, stratified according to CD4 cell count, compared with control subjects.

| Parameters | HIV-Controls | HIV+ $\geq$500 | <200 | (Cell/μL) | $P$ |
|------------|--------------|----------------|------|------------|-----|
| N          | 134          | 15             | 78   | 58         |     |
| TC (g/l)   | 1.96±0.54    | 1.18±0.55      | 1.07±0.38 | 0.97±0.36 | 0.0001 |
| LDLc (g/l) | 0.67±0.46    | 0.29±0.21      | 0.50±0.42 | 0.37±0.26 | 0.0001 |
| HDLc (mg/dl)| 105, 51±28, 10 | 46.91±25.22 | 46.51±21.56 | 45.27±26.45 | 0.0001 |
| TAA (mM)  | 0.63±0.17    | 0.27±0.16      | 0.17±0.14 | 0.13±0.13 | 0.0001 |
| MDA (μM)  | 0.20±0.07    | 0.39±0.10      | 0.41±0.11 | 0.42±0.10 | 0.0001 |
| LPI        | 0.34±0.14    | 17.53±32.83    | 30.83±96.87 | 31.41±90.51 | 0.0001 |

*Every value is the mean ± standard deviation. P value: statistically significant difference between each clinical category and HIV-controls group for each biochemical marker mean value. (A1), (B2), (C3): Clinical categories.*

doii:10.1371/journal.pone.0065126.t002

Table 3. Biochemical parameters in HIV-infected patients, correlated with CD4 using Pearson correlation coefficient.

| CD4 | TC | HDLc | LDLc | TAA | MDA | LPI |
|-----|----|------|------|-----|-----|-----|
| 1   | 1  | 0.97 | 1    |     |     |     |
| 0.037| 1  | 0.583| 1    |     |     |     |
| 0.065| 0.142| 1  |     |     |     |     |
| 0.019| 0.032| 0.018| 1  |     |     |     |
| 0.059| 0.035| 0.022| 1  |     |     |     |
| 0.166| 0.079| 0.022| 1  |     |     |     |

*Significant Pearson correlation ($P<0.05$ at a bilateral level).
**Significant Pearson correlation ($P<0.01$ at a bilateral level). doi:10.1371/journal.pone.0065126.t003
Discussion

Transport of cholesterol in the organism is by low density lipoproteins (LDL, 70%), high density lipoproteins (HDL, 20 to 35%) and by very low density lipoproteins (VLDL, 5 to 12%) [30]. LDL-cholesterol is implicated in the genesis of atherosclerosis, while HDL-cholesterol facilitates the elimination of excess lipids from cells to liver; high LDL-cholesterol or low HDL cholesterol is associated with coronary heart disease [31].

Age is a major factor in the amount of cholesterol in blood for men over 50 years and women older than 55 years [32]. Our current study show a 76-fold increase in LPI in patients with the CRF01_AE subtype, although the differences were not statistically significant. In general, the CRF01_AE subtype seemed to induce higher lipid peroxidation. We performed additional analyses to determine whether HIV-1 subtypes A1, G, and H influenced the levels of the different biochemical parameters, but results showed no statistically significant difference (data not shown).

Table 4. Distribution of HIV-1 subtypes in patients by sex and CD4 cell counts.

| SUBTYPES     | Men | Women | Total (%) |
|--------------|-----|-------|-----------|
| CD4 cells count/µl | ≥500 | 200–499 | <200 |
|              |     |       |           |       |       |
|              |     |       |           |       |       |
| SUBTYPES     |     |       |           |       |       |
| CRF01_AE     | 0   | 2     | 0         | 0     | 4     | 0     | 6 (20.0%) |
| CRF02_AG     | 1   | 3     | 2         | 5     | 2     | 13 (43.3%) |
| A1           | 0   | 4     | 2         | 0     | 0     | 1     | 7 (23.3%) |
| G            | 0   | 0     | 1         | 1     | 0     | 2     | 6 (6.7%) |
| H            | 0   | 1     | 0         | 0     | 0     | 2     | 6 (6.7%) |
| CRFs         | 1   | 5     | 2         | 9     | 2     | 19 (63.3%) |
| Pure         | 0   | 5     | 3         | 0     | 2     | 1     | 11 (36.6%) |
| Total number of subjects | 10 | 5 | 3 | 0 | 11 | 3 |

Table 5. Comparison of different biochemical parameters between patients and controls.

| Parameters     | Controls±SD | Patients±SD | P     |
|----------------|-------------|-------------|-------|
| TC (g/l)       | 1.96±0.54   | 1.12±0.48   | 0.0001|
| LDLC (mg/dl)   | 0.67±0.46   | 0.43±0.36   | 0.0002|
| HDL (mg/dl)    | 105.51±28.10| 46.54±23.36 | 0.0001|
| TAA (mM)       | 0.63±0.17   | 0.16±0.16   | 0.0001|
| MDA (µM)       | 0.20±0.07   | 0.41±0.10   | 0.0002|
| LPI            | 0.34±0.14   | 0.26±0.40   | 0.0001|

In our study is directly due to HIV infection or viral-induced lipodystrophy, as lipodystrophy in HIV infection is associated with dyslipidemia [34,35]. Our data are in agreement with previous studies that showed lower TC, LDLC and HDLC in HIV-1 infected patients [36], and demonstrated that HIV-induced dyslipidemia was associated with lower HDLC and LDLC [37].

TAA was evaluated using the FRAP test which expresses the antioxidant potential of the organism. It measures its capacity to neutralize through antioxidant molecules the oxidant (free radicals) damage on various substrates (proteins, lipids, carbohydrates, nucleic acids). Our results showed about a threefold reduction of TAA plasma concentration in patients compared to controls. This may be linked to the high level of free radicals production due to the antigenic (virus) activation of lymphocytes, phagocytes and chronic inflammatory processes induced by viral replication [38]. The reactive oxygen species (O2•-,HO•,O2, H2O2) produced during chronic inflammation react with antioxidants and contribute to the reduction of their plasma concentration [13]. This results in the antioxidant/pro-oxidant balance altered in favor of pro-oxidant; which leads to severe lipids peroxidation and cells apoptosis as reflected by the high concentration of MDA in HIV-infected patients (our results and refs [39,13]).

Chronic inflammation in HIV infection increase free radicals formation; these free radicals induce lipid peroxidation which leads to MDA formation [40]. Our results also showed high plasma MDA concentration in patients compared to controls (Table 7); these results are in agreement with those reported by Djinji and collaborators [41]. The high plasma MDA concentrations we report here are probably the consequence of the effects of free radicals on polyunsaturated lipids which induce oxidative stress, and produces destructive effects such as cells apoptosis, a major cause of CD4 cell depletion during HIV infection, particularly in the early stage of the infection [39,42]. We established a negative Pearson correlation between MDA and TC (r = -0.035), meaning that MDA plasma concentration increases with the decrease of total cholesterol due to lipids peroxidation.

Lipid peroxidation index (LPI) is the ratio MDA/TAA; it estimates 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 and this is associated with increased oxidative stress in patients [38]. Our current study show a 76-fold increase in LPI in patients compared to the controls, which is in agreement with previous
could be an indication of an elevated free radicals generation in LDLC in the CRFs group than in the pure subtype group. This also shows high plasma MDA concentration and higher levels of subtypes like D were established to be more implicated than others subtypes are implicated in disease progression, although some subtypes, circulating recombinant forms (CRFs), as well as pure determined by the sequencing experiments (Table 4). All HIV-1 [43,41,14].

LDLC, HDLC and TC and the increase of MDA and LPI (TNFα) are thought to be implicated in the decrease of TAA, and increased oxidative stress. It has also been associated with increased production of free radicals, considerable studies [38] and show that in HIV infection in Cameroon is associated with increased production of free radicals, considerable decrease in TAA, and increased oxidative stress. It has also been established that HIV-1 uses available antioxidants for its replication process that speeds up CD4 cells apoptosis and disease progression [36]. Generation of free radicals and certain cytokines (TNFα, IL1) are thought to be implicated in the decrease of TAA, LDLC, HDLC and TC and the increase of MDA and LPI [43,41,14].

Our patients were infected by different HIV-1 subtypes, as determined by the sequencing experiments (Table 4). All HIV-1 subtypes, circulating recombinant forms (CRFs), as well as pure subtypes are implicated in disease progression, although some subtypes like D were established to be more implicated than others due to their dual tropism [44].

Our results (Table 6), in spite of the small sample size, seem to indicate that CRFs may have aggravating effects on lipodystrophy since they lead to dyslipidemia in HIV infected patients [45]. We also show high plasma MDA concentration and higher levels of LDLC in the CRFs group than in the pure subtype group. This could be an indication of an elevated free radicals generation in CRFs infected patients, thus explaining the low serum TC, HDLC and LDLC concentrations due to lipid peroxidation. These results are in conformity with those of other authors [45,39,13,15].

The CRF01_AE subtype seems to induce high lipid peroxidation (Table 6), probably due to its replication velocity, since high concentrations of free radicals are produced during HIV-1 replication process [46]. Free radicals formation may also enhance HIV replication in T cells and macrophages by acting on the transcription factor NF-κB [46,17]; and HIV replication stimulates cytokine production, particularly the tumor necrosis factor alpha (TNFα) which in turn stimulates free radical generation [41]. It has also been established that during HIV replication, HIV infected cells express different proteins (kinases, transport proteins, receptors, chaperons molecules), some of which were identified to be responsible for free fatty acids synthesis, lipids oxidation, alteration in lipid metabolism, and lipid transport deregulation [37]. Our future studies will determine whether any of these viral-induced kinases, receptors or chaperons is responsible for the high lipid peroxidation and increased oxidative stress in our HIV-infected population.

### Conclusion

These results, in spite of some limitations like mean age and sex distribution differences, and small sample size for genotyping studies, show a significant reduction in TAA, LDLC, HDLC, TC and an elevated MDA concentration and LPI in HIV-positive patients compared to serologically negative controls. This may be due to chronic inflammation caused by HIV replication which produces free radicals. These free radicals may be responsible for the lipids peroxidation, CD4 cell reduction, low TAA, and high LPI and MDA observed in our study. The differences in biochemical parameters in patients infected with different HIV subtypes may be due to their replication velocities as HIV-1 CRF01_AE has been shown to have a faster replication velocity [46].

### Acknowledgments

We thank all the individuals who gave their informed consent to participate in this study.
Author Contributions
Conceived and designed the experiments: GT. Performed the experiments: GT DT. Analyzed the data: FNN DT AN AT. Contributed reagents/

References

1. Levy JA (2007) HIV and the pathogenesis of AIDS. American society for microbiology 3: 87–88.
2. UNAIDS (2011): Journée mondiale sida/2011. Rapport ONUSIDA.
3. UNAIDS (2010) Rapport ONUSIDA sur l'épidémie mondiale de sida.
4. National AIDS Control Committee/National Statistique Institute (2006) Preliminary Report of Cameroon Demographic and Health survey 2005.
5. Plantier JC, Leoa Marie, Dickerson EJ, Oliveira De Fabienne, Cordonnier francois, et al. (2009)A new human immunodeficiency virus derived from gorillas. Nature medicine; Brief communication:1–2. Available: http://www. nature.com/naturemedecine/. Accessed 12 May 2012.
6. Heyndrickx L, Janssens W, Ndembe P, Verrecken K, Coppens S, et al. (2000) HIV-1 gene variability in Cameroon. Research Letters: 1862–1864.
7. Robertson DL, Anderson JP, Brauds JA, Carr JK, Foley B, et al. (2000). HIV-1 nomenclature proposal. A reference guide to HIV-1 classification. Science 280: 55–63.
8. Aerts SM, Robbins KE, Omalu I, Saacke A, Zeh G, et al. (2001) Development of an env g1 g2-based heteroduplex mobility assay for rapid human immunodeficiency virus type 1 typing. J Clin Microbiol 39: 2110–2114.
9. HIV sequence data base (2012) Available: www. Hiv. lanl. Gov/content/ sequence/hiv/uclsf/uclsf. Html?m=en&hl=fr. Accessed 20 July 2012.
10. Adje C, Cheingsong R, Roels HT, Maurice C, Djomand G, et al (2001) High prevalence of genotypic and phenotypic HIV-1 drug-resistance strains among patients receiving antiretroviral therapy in Abidjan, Côte d’Ivoire. J Acquir Immune Defic Syndr 26: 501–506.
11. Heyndrickx L, Auwera GV (2002). Heteroduplex mobility analysis, une technique de mobilité électrophorétique des héte`roduplexes. Kit, version 5.
12. Marston B (2004). Multivitamins, nutrition, and antiretroviral therapy for HIV infection. New England Journal of Medicine 351: 78–80.
13. Halliwell B, Cross CE (1991) Reactive oxygen species, antioxidants, and human disease progression and mortality. New England Journal of Medicine 351: 23–32.
14. Safrin S, Grunfeld C (1999) Fat distribution and metabolic changes in patients with HIV infection. AIDS 13: 2493–505.
15. Lopez O, Bonnefont-Rousselot D, Mollereau M (1996) Increased plasma Lipid Peroxidation and HIV-1 Infection thiobarbituric acid-reactive substances (TBARS) before opportunistic infection among HIV-infected patients from Cameroon: a case control study. HIV Medicine 11: 335–339.
16. Delaugerre C, Ghosn J (2007) Les sous-types du VIH-1: épidémiologie et impact. Lett Infect 22(2): 56–60.
17. Falutz Julian, Potvin Diane, Mamputu Jean-Claude, Assaad Hani, Zoltowska Anna-Lise, et al. (2010) Effects of tesamorelin, a growth hormone-releasing factor, in HIV-infected patients with abdominal fat accumulation: a randomised placebo-controlled trial with a safety extension. J Acquir Immune Defic Syndr advance online publication.
18. Belec L (2007) Transmission sexuelle de l’infection par le VIH. John Libbey Eurotext 301–309.
19. Marston B (2004). Multivitamins, nutrition, and antiretroviral therapy for HIV infection. New England Journal of Medicine 351: 78–80.
20. Creixell P, Forns X, Vives X, de la Torre J, Plana J, et al. (2008) Immune Defic Syndr 26: 501–506.
21. Martin Zabell (2011) Normal Cholesterol Levels by Age. Available: http://www. livestrong.com/article/275091-normal-cholesterol-levels-by-age/. Accessed 23 August 2012.
22. Nguemaı¨m NF, Mbouagbaw J, Teto G, Nkoa T, Same ´-Ekobo A, et al. (2012) Apolipoprotein and lipid composition of low-density lipoproteins and high-density lipoproteins in Cameroonians: A very low atherogenic risk. Ann.Univ.Sci.Santé 5(3–4): 753–763.
23. Hamsten A (1990) Hypertriglyceridemia, triglyceride rich lipoproteins and coronary heart disease. In Betteridge J Ed. Lipid and Lipoprotein disorders. Lipid and lipoprotein disorders. 16: 16–61.
24. Kimura M (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotides sequences. J Mol Evol 16: 1650.
25. Saitou N and Nei M (1987). The neighbour joining method: a new method for constructing phylogenetic trees. Mol Biol Evol 4: 406–425.
26. Belec L (1993) Thérapeutique pratique du SIDA. Médecine. 9–40.
27. Hellenstein J (1983) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 36: 783–791.
28. Buonomo PF, Seibert B, Bresolin N, et al. (2001) Effect of tesamorelin, a growth hormone-releasing factor, in HIV-infected patients with abdominal fat accumulation: a randomised placebo-controlled trial with a safety extension. J Acquir Immune Defic Syndr advance online publication.
29. Belec L. (2003) Apports de la recherche dans la lutte contre le Sida en Afrique. 3 déce mber 2003; Rapport.
30. Jerri L, Lemorre Mc, Peggy Beeley, Karla Thorton, Kevin Morrisroe, et al. (1997) Rapid automated determination of lipid hydroperoxide concentrations in plasma, without use the preparative TLC. J Clin Chem 43: 1641–1650.
31. Benze E, Keating M, Surridge N, Kelleher AD, et al. (2000) Determination of HIV Type 1 in Rural Eastern Cameroon. JAIDS 37: 1641–1650.
32. Friedewald WT, Levy RI and Lees RS (1972) Estimation of concentration of low density lipoprotein cholesterol in plasma, without use the preparative ultracentrifuge. Clin Chem 18: 499–502.
33. Benze E, Keating M, Surridge N, Kelleher AD, et al. (2000) Determination of HIV Type 1 in Rural Eastern Cameroon. JAIDS 37: 1641–1650.
34. Raisonnier A, Tagny JP, Kamso-Tchakounte J, Sile HJ, Muna W (1988) Apolipoprotein and lipid composition of low-density lipoproteins and high-density lipoproteins in Cameroonians: A very low atherogenic risk. Ann.Univ.Sci.Santé 5(3–4): 753–763.
35. Nguemaı¨m NF, Mbuagbaw J, Teto G, Nkoa T, Same ´-Ekobo A, et al. (2012) Apolipoprotein and lipid composition of low-density lipoproteins and high-density lipoproteins in Cameroonians: A very low atherogenic risk. Ann.Univ.Sci.Santé 5(3–4): 753–763.
36. Safrin S, Grunfeld C (1999) Fat distribution and metabolic changes in patients with HIV infection. AIDS 13: 2493–505.
37. Faluwzi AW (2004) A randomized trial of multivitamin supplements and HIV disease progression and mortality. New England Journal of Medicine 351: 23–32.
38. Nguemaı¨m NF, Mbuagbaw J, Teto G, Nkoa T, Same ´-Ekobo A, et al. (2012) Apolipoprotein and lipid composition of low-density lipoproteins and high-density lipoproteins in Cameroonians: A very low atherogenic risk. Ann.Univ.Sci.Santé 5(3–4): 753–763.
39. Lopez O, Bonnefont-Rousselot D, Mollereau M (1996) Increased plasma thiobarbituric acid-reactive substances (TBARS) before opportunistic infection among HIV-infected individuals. J Clin Immunol 16: 16–61.
40. Belec L (1993) Thérapeutique pratique du SIDA. Médecine. 9–40.
41. Martin Zabell (2011) Normal Cholesterol Levels by Age. Available: http://www. livestrong.com/article/275091-normal-cholesterol-levels-by-age/. Accessed 23 August 2012.
42. Kimura M (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotides sequences. J Mol Evol 16: 1650.
43. Saitou N and Nei M (1987). The neighbour joining method: a new method for constructing phylogenetic trees. Mol Biol Evol 4: 406–425.
44. Buonomo PF, Seibert B, Bresolin N, et al. (2001) Effect of tesamorelin, a growth hormone-releasing factor, in HIV-infected patients with abdominal fat accumulation: a randomised placebo-controlled trial with a safety extension. J Acquir Immune Defic Syndr advance online publication.
45. Friedewald WT, Levy RI and Lees RS (1972) Estimation of concentration of low density lipoprotein cholesterol in plasma, without use the preparative ultracentrifuge. Clin Chem 18: 499–502.
46. Buonomo PF, Seibert B, Bresolin N, et al. (2001) Effect of tesamorelin, a growth hormone-releasing factor, in HIV-infected patients with abdominal fat accumulation: a randomised placebo-controlled trial with a safety extension. J Acquir Immune Defic Syndr advance online publication.
47. Belec L (2007) Transmission sexuelle de l’infection par le VIH. John Libbey Eurotext 301–309.
48. Buonomo PF, Seibert B, Bresolin N, et al. (2001) Effect of tesamorelin, a growth hormone-releasing factor, in HIV-infected patients with abdominal fat accumulation: a randomised placebo-controlled trial with a safety extension. J Acquir Immune Defic Syndr advance online publication.