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Research Article

High Levels of Oxidative Stress in Ivorian People Living with Human Immunodeficiency Virus Undergoing Antiretroviral Treatment

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Abstract

Introduction: During HIV infection, the virus and antiretroviral (ARV) treatment generate oxidative stress. The aim of this study is to determine the level of oxidative stress based on changes in CD4+ T-cell counts and the age of people living with HIV in Côte d'Ivoire using the copper/zinc (Cu/Zn) ratio. *Methodology*: 254 people (127 HIV-infected individuals on ARV and 127 controls) were recruited for this study. CD4 counts were performed by flow cytometry (FacsCalibur). The COBAS Integra 400 Plus was used to measure biochemical parameters. Serum copper and zinc levels were determined by air/acetylene flame atomic absorption spectrophotometry (Varian AA20 Pattern®, France). The Cu/Zn ratio was obtained by dividing the copper concentration by the zinc concentration. *Results*: Among HIV+ patients, the average age was 32 ±0.50 years, with Cu/Zn ratios greater than 2 in 31.4% of females and 80.7% of males. A positive correlation (r = 0.190; p = 0.032) was found between the Cu/Zn ratio and age. For CD4+ counts below 200 cells/mm^3, high Cu/Zn ratios were observed in females (1.75 ±0.49) and males (1.91 ±0.19). *Conclusion*: High levels of oxidative stress associated with progression of the inflammatory process with age were observed in patients undergoing antiretroviral therapy, particularly those with CD4+ T-cell counts < 200 cells/mm^3. It would be interesting to consider the Cu/Zn ratio, oxidative stress levels, age and CD4+ T lymphocyte counts when treating people living with HIV in Côte d'Ivoire. However, this study should be supplemented by an assessment of the Cu/Zn ratio in relation to the different types of ARVs prescribed to patients.

Keywords

Copper, Côte d'Ivoire, Cu/Zn Ratio, HIV, Zinc

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1. Introduction

The widespread use of antiretroviral therapy and the ongoing commitment to counter the human immunodeficiency virus (HIV) pandemic have led to a 31% decrease in new HIV infections and a 47% reduction in HIV-associated mortality worldwide between 2010 and 2020 [1]. However, the antiretroviral regimen is involved in the increased generation of circulating reactive oxygen species (ROS), probably by producing more oxidized metabolites derived from the interaction between ROS and the biomolecules of infected cells such as proteins, lipids, carbohydrates and nucleic acids [2]. When the balance between biosynthesis and the elimination of EROs cannot be maintained, and the production of free radicals exceeds the activity of antioxidant responses, the cell enters a pathological state known as oxidative stress (OS) [3, 4]. This state, exacerbated by HIV-1 infection alone or in combination with antiretroviral therapy (ARV), can further increase the pathogenesis of HIV-1 [5, 6] and cause complications [7, 8] by activation and stimulation of the transcription factor NF-κβ, which in turn causes the synthesis of a number of pro-inflammatory cytokines (NF- κβ), which Allegra et al. [9]. Recent data show that chronic inflammation, and more particularly oxidative stress, plays a key role in the pathogenesis of HIV infection; hence the need to control it. These EROs are naturally produced during mitochondrial oxidative metabolism in response to various cellular processes and are naturally supported by trapping enzymes such as Superoxide dismutase (SOD), Catalase, Glutathione Peroxidase and Glutathione S-Transferase, and by antioxidants such as vitamins A, C and E, β-carotene, polyphenols, selenium, zinc and copper [10].

Several methods and approaches have been developed to evaluate oxidative stress in biological samples. These methods and approaches use either the direct chemical imbalance to detect the oxidative state (direct measurement of reactive oxygen species, evaluation of the antioxidant status from enzymatic antioxidants, non-enzymatic antioxidants and total antioxidant capacity), or the downstream consequences of the oxidative state on proteins, lipids, DNA or cellular processes [11]. Alongside these methods, studies have shown that the Copper/Zinc ratio (Cu/Zn) is a good indicator of the level of oxidative stress during infectious diseases [12-15]. Indeed, the calculation of the ratio between serum copper and zinc concentrations provides valuable information on oxidative stress and inflammatory responses [12, 16]. This Cu/Zn ratio would be clinically more important than the individual concentrations of copper and zinc, because better than the absolute quantities of these trace elements in the body, it makes it possible to maintain the normal activity of the enzymes [17]. It has been shown to be a better predictive value of disease severity and/or mortality than copper levels [12, 18].

The relationship between HIV infection, antiretroviral therapy and oxidative stress represents a complex obstacle in the management of HIV/AIDS. The investigation of the Cu/Zn ratio in the context of HIV infection can provide valuable information on oxidative stress and the immune status of patients. In Côte d'Ivoire, where the prevalence of HIV infection was 1.82% in 2024 [19], little data exist on the Copper/Zinc ratio and oxidative stress in the context of the HIV pandemic. The objective of this study is to determine the level of oxidative stress according to the evolution of the CD4+ T lymphocyte level and the age of people living with HIV in Côte d'Ivoire.

2. Material and Methods

2.1. Type and Period of Study

The collection of samples for this work was carried out from November 2012 to January 2014 at the Pasteur Institute of Côte d'Ivoire (IPCI). The analytical phase of this study took place from December 2014 to November 2016 at the department of Medical and fundamental Biochemistry of the Institut Pasteur of Côte d'Ivoire (IPCI). This cross-sectional descriptive study included 254 adult subjects (127 people living with HIV and 127 HIV-negative people) in the age range of 15 to 49 years. All HIV-positive subjects had been receiving antiretroviral treatment for 6 to 8 years. Pregnant women and subjects receiving trace element supplements or consuming alcohol, tobacco or psychoactive substances were not included in this study. Similarly, individuals carrying or having been in contact with hepatitis B, hepatitis C and syphilis were not included.

2.2. Serological Tests and Collection of Blood Samples

For the detection of HIV seropositivity, a rapid ELISA analysis was performed on blood taken with a finger prick using the Alere Determine™ HIV-1/2 kit (Alere Medical Co., Japan) and the GENIE II HIV-1 / HIV-2 kit (BIO-RAD, France). The principle of both test methods is based on the antigen-antibody reaction revealed by staining. Samples with a staining bend are considered positive while negative samples do not have a staining spot.

The sera obtained after centrifugation of the tube without anticoagulant at 1500 rpm/minute for 15 minutes for the determination of trace elements and at 3000 rpm/minute for 15 minutes for the determination of biochemical parameters (Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT), Creatinine, Urea), were stored at - 20 ℃. until the analysis. The plasma collected in the tube containing potassium oxalate and sodium fluoride after centrifugation at 3000 rpm for 15 minutes was used to assay the blood glucose.

2.3. Evaluation of Biochemical Parameters

The determination of the serum concentrations of creatinine, urea, glycaemia, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), was carried out on the COBAS Integra 400 Plus following the manufacturer's instructions. The COBAS Integra 400 Plus (Roche Diagnostics, Germany) is a spectrophotometer whose principle is based on the realization of manual dosages in a closed circuit, without human intervention. The biochemical parameters are determined after calibration of the kit and quality control. A volume of 500 µL of serum is used for the quantitative analysis of the biochemical parameters after calibration of the kit and validation of the results of the quality control. After conversion of the absorbances from the equations of the calibration lines integrated into the automaton, the results are obtained directly in concentration.

2.4. CD4+ T-Cell Count

The CD4+ T lymphocyte count was carried out by Flow Cytometry (FACSCalibur) by distributing a volume of 20 µL of TriTEST (CD 3, CD 4, CD 45) in each Trucount tube. After adding a volume of 50 µL of whole blood, a diluted lysis solution (1/10), at a rate of 500 µL, was added to each tube. The tubes were then homogenized and incubated for 15 min in the dark. This operation (homogenization and incubation of the tubes in the dark) was repeated a second time, then the Trucount tubes were placed on the grid of the FACSCalibur device after a third homogenization. The mixture was sucked into the flow cytometric counter and the result was displayed electronically.

2.5. Determination of Serum Concentrations of Trace Elements

The air/acetylene flame atomic absorption spectrophotometry (Varian AA20 Pattern®, France) made it possible to determine the serum levels of copper and zinc. All the glassware used was soaked the day before (12 hours) in a 10% (v/v) nitric acid (HNO_3) solution, followed by washing with a 10% (v/v) hydrochloric acid (HCl) solution. This glassware was then rinsed twice with distilled water and dried. The precipitation of the proteins was carried out by diluting 1 mL of serum in 4 mL of a hydrochloric acid solution (2 M). After homogenization, each sample was left to rest. The clear supernatant obtained was sucked directly into the atomic absorption spectrophotometer in flame mode [20] at the wavelength of 324.8 nm and 213.9 nm respectively for copper and zinc. A standard multi-element solution of 1000 ppm (Merck), previously diluted to 1/250 with nitric acid - deionized water (0.03 M), was used to prepare the calibration range (0; 0,5; 1,5; 2,0; 4 ppm) and the concentration measurements were carried out in triples and adjusted against the white (2 M HCl solution). The normal reference values for serum copper and zinc levels were respectively 601 - 1803 ng/mL and 587 - 1215 ng/mL [21]. According to Gaier et al. [22], the reference value of the Cu/Zn ratio was 1.12 - 1.27. A Cu/Zn ratio < 0.93 was low and a Cu/Zn ratio >1.51 was high.

2.6. Statistical Analyses

The GraphPad Prism 9.0.0 (121) software made it possible to calculate the averages. The Kolmogorov-Smirnov test was performed to identify Gaussian and non-Gaussian distributions. The student's t-test was used to compare the quantitative variables if the distribution was normal (Gaussian), and the Pearson test was used to determine the correlations. If the distribution was not Gaussian, the Spearman test was used for the correlation and the nonparametric Mann-Whitney-U test was used to compare means. The degree of significance has been set at 5%.

2.7. Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki 2000 on HIV and AIDS research conducted in poor countries and in accordance with local legislation on the national care program for people living with HIV / AIDS (Decree no 411 of December 23, 2001). The blood samples were taken from HIV-positive patients at the Pasteur Institute of Côte d'Ivoire (IPCI) which is a reference center and support for public health programs in Côte d'Ivoire in accordance with the global HIV / AIDS / malaria / tuberculosis fund. In addition, consent was obtained from the participants for the use of their blood for research purposes during biological monitoring.

3. Results

3.1. Epidemiology of Infection in the Study Population

This study involved 254 individuals (127 HIV-positive infected and 127 HIV-negative controls). The overall average age of the infected subjects (HIV+) was 32 ± 0.50 years compared to 37 ± 0.52 years for the HIV-negative controls (HIV-). The average age of males (HIV+) (34 ± 0.79 years) and females (HIV+) (30 ± 0.55 years) was significantly ($P < 0.0001$) higher than that of controls (Table 1).

3.2. Biochemical Profile of the Study Population

The mean values of the biochemical parameters (glycaemia, creatinine, urea, transaminases) analyzed in the PLHIV and the controls were respectively in the range of the usual standard reference values and without significant difference compared to the controls (Table 1).

Table 1. *Average ages and overall average concentrations of biochemical parameters in controls and PLHIV.*

Parameters	PLHIV	Controls	*P*-value
Ages (years)			
All	32 ±0.50	37 ±0.52	< 0.0001
Females	30 ±0.55	35 ±0.62	< 0.0001
Males	34 ±0.79	40 ±0.75	< 0.0001
Biochemical parameters			
Glycaemia (0.75 - 1.10 g/L)	0.96 ±0.09	0.8 ±0.08	0.531
Creatinine (6 - 12 mg/L)	10 ±0.91	09 ±0.47	0.476
Urea (0.10 - 0.35 g/L)	0.37 ±0.04	0.39 ±0.05	0.782
ASAT (8 - 49 UI/L)	45 ±0.89	30 ±0.42	0.531
ALAT (7 - 48 UI/L)	34 ±0.69	29 ±0.41	0.659

PLHIV = people living with HIV; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase. Mann-Whitney U-test: p significant for $P < 0.05$.

3.3. Serum Concentrations of Copper, Zinc and Cu/Zn Ratio in the Study Population by Gender

In the females in this study, serum copper concentration was significantly higher in controls than in HIV+ females (P = 0.0019). In contrast, in males, serum copper concentration was significantly higher in HIV+ patients than in controls (P < 0.0001). In terms of zinc, HIV+ patients had higher serum concentrations than controls. This increase was highly significant (P < 0.0001) in females only. The mean Cu/Zn ratio values were above the reference values (1.12 - 1.27) without being high (Table 2).

Table 2. *Average serum concentrations of copper, zinc and Cu/Zn ratio in women and men according to serological status.*

Parameters	PLHIV	Controls	P-value
Copper (ng/mL)			
Females	2434 ±194	3115 ±116	0.0019
Males	3304 ±139	1282 ±168	< 0.0001
Zinc (ng/mL)			
Females	2376 ±101	1071 ±184	< 0.0001
Males	2034 ±99	1662 ±163	0.0557
Cu/Zn ratio			
Females	1.46 ±0.08	1.37 ±0.06	0.3615
Males	1.38 ±0.05	1.48 ±0.08	0.2774

PLHIV: people living with HIV; Low Cu/Zn ratio (< 0.93); Reference Cu/Zn ratio (1.12-1.27); High Cu/Zn ratio (>1.51); Copper: 601-1803 ng/mL; Zinc: 587-1215 ng/mL. Mann-Whitney U-test: P significant for P < 0.05.

3.4. Distribution of Patients Living with HIV According to Cu/Zn Ratio Reference Values and Age

In this study, 64.3% (45/70) of females and 12.3% (7/57) of males had Cu/Zn ratios below 1.12. Furthermore, normal Cu/Zn ratios were observed in 4.3% of females and 7.0% of males. Finally, Cu/Zn ratios above 1.27 were observed in 31.4% (22/70) of females and 80.7% (46/57) of males. In this study, for Cu/Zn ratios below 1.12, males had mean ratio values (0.82 ±0.02) that were not significantly (P = 0.0954) higher than those of females (0.62 ±0.05). For normal Cu/Zn ratios, both females and males had virtually identical mean ratio values (1.16 ±0.00 and 1.15 ±0.00, respectively) (P = 0.8000). Finally, for Cu/Zn ratios greater than 1.27, females had significantly (P = 0.0121) higher mean Cu/Zn ratios (2.43 ±0.12) than males (2.05 ±0.09) (Table 3).

This study was characterized by high Cu/Zn ratios in 24.7% (22/70) of females under 30 years of age (1.59 ±0.33) and in 92.3% (54/57) of males over 30 years of age (1.91 ±0.15). Among patients under 30 years of age, females (1.59 ±0.33) had higher Cu/Zn ratios than males (1.25 ±0.43). However, this increase was not significant (P = 0.9577). In contrast, in patients over 30 years of age, males (1.91 ±0.15) had significantly higher Cu/Zn ratios (P < 0.0001) compared to females (1.05 ±0.12) (Table 3).

Table 3. *Distribution of HIV-positive patients according to average Cu/Zn ratio values and age.*

Parameters	Females (N = 70)		Males (N = 57)		*P*-value
	Population (%)	Means	Population (%)	Means	
Cu/Zn ratio					
< 1.12	45 (64.3%)	0.62 ±0.05	7 (12.3%)	0.82 ±0.02	0.0954
1.12 - 1.27	3 (4.3%)	1.16 ±0.00	4 (7.0%)	1.15 ±0.00	0.8000
> 1.27	22 (31.4%)	2.43 ±0.12	46 (80.7%)	2.05 ±0.09	0.0121
Age (years)					
15 - 30	22 (24.7%)	1.59 ±0.33	3 (7.7%)	1.25 ±0.43	0.9577
30 - 49	48 (75.3%)	1.05 ±0.12	54 (92.3%)	1.91 ±0.15	< 0.0001

Low Cu/Zn ratio (< 0.93), reference Cu/Zn ratio (1.12 - 1.27), high Cu/Zn ratio (>1.51), Mann-Whitney U-test: *P* significant for $P < 0.05$.

3.5. Cu/Zn Ratio According to CD4+ T Lymphocyte Count and Gender in HIV-Positive Patients

The results showed relatively normal Cu/Zn ratio values in females, except for patients with CD4+ counts below 200 cells/mm^3, who had high Cu/Zn ratios with an average of 1.75 ±0.49. In contrast, in males, all Cu/Zn ratio values were high, with an average of 1.91 ±0.19 (Table 4).

Table 4. *Average Cu/Zn ratio values and distribution of the population living with HIV according to TCD4+ lymphocyte count.*

CD4+ T cell count (cells/mm^3)	Females (N = 70)		Males (N = 57)		*P*-value
	Population (%)	Means	Population (%)	Means	
≤ 200	14 (20.0%)	1.75 ±0.49	12 (21.0%)	1.91 ±0.19	0.4965
> 200	56 (80.0%)	1.10 ±0.12	45 (79.0%)	1.85 ±0.12	< 0.0001

Low Cu/Zn ratio (< 0.93), reference Cu/Zn ratio (1.12 - 1.27), high Cu/Zn ratio (>1.51), Mann-Whitney U-test: P significant for P < 0.05.

3.6. Study of the Correlation Between the Cu/Zn Ratio and Age and CD4+ T Lymphocyte Counts in Patients Living with HIV

A positive association (r = 0.190; p = 0.032) was found between the increase in the Cu/Zn ratio and the age of HIV-positive patients (Figure 1). The results also showed a negative but non-significant correlation (r = - 0.07; P = 0.434) between decreased CD4+ T lymphocyte counts and increased Cu/Zn ratio values (Figure 2).

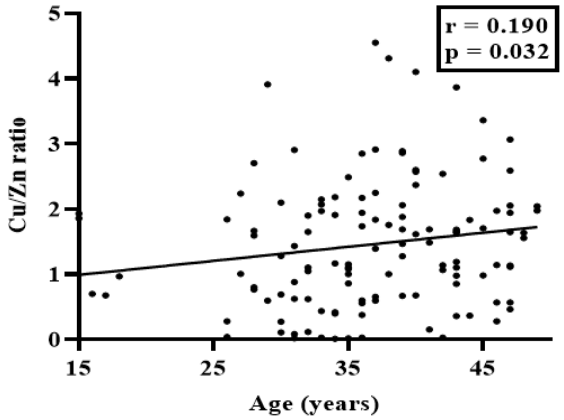

Figure 1. *Correlation between the Cu/Zn ratio and the age of HIV-positive patients.*

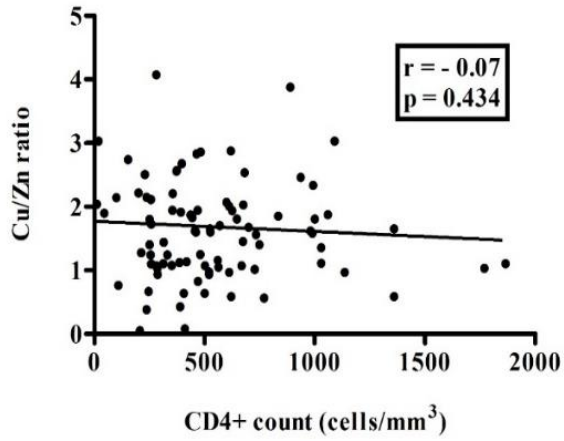

Figure 2. *Correlation between the Cu/Zn ratio and the CD4+ T lymphocyte count in HIV-positive patients.*

4. Discussion

This study was characterized by high levels of copper and zinc in HIV positive patients compared to HIV negative controls as reported by Bilbis et al. [23] in Nigeria and Elatif et al. [24] in Sudan. These high levels of zinc, in parallel with copper excesses, could indicate an imbalance in the interaction between these two elements [25, 26].

Also in this study, high values of the Cu/Zn ratio were found in 31.4% of females and 80.7% of males living with HIV. According to Ide et al. [27], this difference in proportion is due to the fact that males suffered oxidative stress more frequently than females. Indeed, lower levels of antioxidants such as GSH, catalase and SOD, have been demonstrated in males compared to females [28]. This would mean that differences in the molecular processes that regulate resistance to oxidative stress could be responsible for the longer life expectancy observed in females compared to males [29-31].

These high Cu/Zn ratio values also applied to females and males with CD4+ T cell counts below 200 cells/mm^3. In these patients, however, Cu/Zn ratio values increased independently of the decrease in CD4+ T cell counts (r = - 0.07; P = 0.434). It is true that at this stage of infection, persistent immune activation in treated patients contributes to the onset and maintenance of chronic inflammation [32, 33], but it is mainly the age factor that could explain this situation. Indeed, in this study, the population is relatively young, with an average age of 32 ±0.50 years, and the Cu/Zn ratio increased significantly with age. According to Baudry et al. [34], with a Cu/Zn ratio greater than 2, a significant increase in the percentage of individuals with age would be expected. This situation would thus indicate the presence of progressive inflammation associated with increased oxidative stress [35], exacerbated by HIV-1 infection. This would affect cellular metabolism and, over time with ageing, promote disease in people living with HIV, particularly those on antiretroviral therapy (ART) [36, 37]. Indeed, studies on the underlying mechanisms of ageing have highlighted an age-related increase in oxidative stress leading to an accumulation of oxidized proteins, lipids and DNA damage, while stress response and repair pathways are thought to gradually decline with age. The transcription factor Nuclear Factor Erythroid 2-like 2 (Nrf2), the main regulator of cellular redox homeostasis, is one such pathway. However, with ageing, the transcriptional induction of Nrf2-regulated genes involved in antioxidant defense, redox homeostasis, DNA repair and iron metabolism is reduced [38]. Oxidative stress (OS) is implicated in the development of human diseases [7, 8] such as neurodegenerative diseases [39], cancer [4], cardiovascular diseases [3, 40], autoimmune diseases [41, 42] and diabetes [43].

5. Conclusions

In this study, high Cu/Zn ratios were observed in people living with HIV on antiretroviral therapy. This Cu/Zn ratio increased significantly with age, indicating the presence of a progression of the inflammatory process associated with increased oxidative stress especially in patients with CD4+ T-cell counts below 200 cells/cm^3. This study highlighted the importance of considering the Cu/Zn ratio, oxidative stress levels, age and CD4+ T lymphocyte counts when treating people living with HIV in Côte d'Ivoire. However, it would be interesting to evaluate the Cu/Zn ratio in relation to the different types of ARVs prescribed to patients in order to supplement this study.

Abbreviations

PLHIV	People Living with HIV
ARV	Antiretroviral
ROS	Reactive Oxygen Species
OS	Oxidative Stress
NF- κβ	Nuclear Factor-kappa B
SOD	Superoxide Dismutase
Nrf2	Nuclear Factor Erythroid 2-like 2

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Author Contributions

Séri Kipré Laurent: Methodology, Writing – original draft, Writing – review & editing

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Siransy Kouabla Liliane: Formal Analysis

Sékongo Yassongui Mamadou: Supervision

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Field

Séri Kipré Laurent: Biochemistry, Functional and molecular Biology, Functional nutrition, Toxicology, Pharmacology

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Bahi Gnogbo Alexis: Biochemistry, Hematology, Pharmacology, Proteomic, Metabolomics

Siransy Kouabla Liliane: Immunology, Allergology, Biochemistry, Molecular Biology, Histocompatibility

Sékongo Yassongui Mamadou: Hematology, Immunology, Blood transfusion, Hemoglobinopathies

Djaman Allico Joseph: Biochemistry, Parasitology, Molecular Biology, Microbiology, Hematology, Pharmacology