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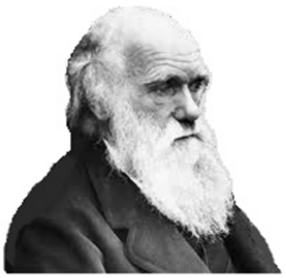

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Improvement in CD4 cells counts in newly diagnosed HIV infected Cameroonian patients under HARRT is counterbalanced by the mild deleterious effects of antiretroviral therapy on liver function: a longitudinal study

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ABSTRACT

Human immunodeficiency virus (HIV) infection still is a worldwide public health concern, but especially so in Cameroon. Still, the health of HIV-infected people has greatly improved following the introduction of highly active antiretroviral therapy (HAART). Conversely, some authors reported the occurrence of side effects. Unfortunately, data on the topic are scarce in Cameroon. This study aimed at investigating the impact of HAART on liver function in newly diagnosed HIV-infected people. An 18-month longitudinal study was carried out in a health facility in the town of Douala, Cameroon. Investigative methods relied on a questionnaire approach, clinical and biological analyses and medical records exploitation. A total of 107 patients aged 18-45 years old were included in the study. Most participants were females (94, 87.9%) and aged 30-40 years (60, 56.1%). A higher significant fraction of patients had CD4 lymphocytes counts less than 200 cells/mm^3 (42, 39.3%) at baseline. We recorded

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a 1.5-fold increment in the CD4 lymphocytes counts over the follow up period (P-value < 0.0001). Indeed, globally, a significant increasing in ALT level was recorded over follow up. There is a critical need for paying attention to liver function in people living with HIV/AIDS and under therapy.

***Keywords*:** HIV infection, HAART, CD4 cells count, transaminases levels, Douala

1. INTRODUCTION

Human immunodeficiency virus (HIV) infection is still a major public health concern worldwide. The matter is particularly obvious in sub-Saharan Africa (SSA) countries where in 2017 this disease is responsible for about 25.7 million disease cases [1]. Nevertheless, the burden of this disease in terms of morbidity and mortality is counterbalanced by the introduction of highly active antiretroviral therapy (HAART) for treating HIV-infected people. HAART consists in administration of highly active drugs which belong to many therapeutic classes. More than 20 drugs for HIV treatment have licensed throughout the world [2] and their number is increasingly larger every year. The mainly prescribed HAART regimens vary from country to country and the commonest prescribed initial regimens include Nucleos(t)ide reverse transcriptase inhibitors (NRTIs) and non-reverse transcriptase inhibitors (NNRTIs) [2, 3]. Besides, some reports outlined the fact that HAART was harmful to many organs as liver and kidney and can be consequently induce disorders referred to as antiretroviral drug-related liver injury (ARLI) and HIV-associated nephropathy (HIVAN) [4,5]. These conditions are important in terms of public health as they can be responsible for treatment discontinuation in HIV-infected people [6]. Therefore, their diagnosis and management are both critical with the aim to improve the quality of life and life expectancy of the population. In Cameroon, HIV infection is a cause for concern where the prevalence of the viral infection is estimated at 5.6% and a rate of 141 newly diagnosed infections per day is recorded [7]. To be noted, HIVAN and ARLI are very little known in Sub-Saharan Africa (SSA) and especially in Cameroon. Some authors have addressed the issue in the country [4, 5] but unfortunately data on the topic are deeply missing in the town of Douala. We present here the first results of a research project having as final aims at improving the health and prognosis of people living with HIV infection (PLWHIV). This study aimed at investigating the evolution in CD4 lymphocytes blood count and transaminases enzymes in newly diagnosed people for HIV and under HAART over an 18-month period.

2. MATERIALS AND METHODS

2. 1. Study site

This study took place at the Catholic Hospital Saint Albert Le Grand (Douala, Littoral region, Cameroon). This hospital is one of the main health facilities for management of people living with HIV infection (PLWHIV) given it owns a specialized management center for these people. The hospital is located at the third Division of the town of Douala, Littoral Region of Cameroon. Douala is the main business one of the country and is ranked sixth in terms of HIV prevalence rate [7].

2. 2. Study population

The study population consisted of patients newly diagnosed for HIV infection and under HAART less than 2 years old. Patients were aged between 18 and 45 years, of both gender and followed up at the Catholic Hospital Saint Albert Le Grand.

2. 3. Study design

The study was designed as longitudinal and prospective study. Any HIV-infected patient, under HAART since less than 2 years old, having a complete medical record and signed an informed consent form was included in the study. Conversely, patients did not meet any of these aforementioned criteria were excluded from the study. Furthermore, patients are not being under HAART, pregnant women and presenting any infection other HIV were not also included. Investigative methods have only relied on a questionnaire approach, clinical and biological analyses and exploitation of medical records. Clinical and biological analyses were performed as recommended by the facility where the study was conducted.

2. 4. Blood collection

A volume of about 4 mL was collected from each participant by venipuncture and then transferred into sterile EDTA and dry tubes for determination of CD4 and transaminases respectively. All tubes were labeled with the patients' barcode and pathology number. Blood sample was centrifuged at 3000 rpm for 5 minutes and obtained sera were stored at 2°C until determination of transaminases levels.

3. LABORATORY PROCEDURES

3. 1. CD4 lymphocytes count

These immune cells were counted with a flow cytometer CyFlow® (Sysmex-Partec Görlitz, Germany) according to the manufacturer's instructions. Briefly, 20 µL of phycoerythrine-conjugated monoclonal antibody to human CD4 were slightly mixed with 20 µL of whole blood into a test tube and incubated for 15 minutes at room temperature under the lee of light. 800 µL of no-lyse buffer were added to the mixture and after homogenizing the content, tube was introduced into the CyFlow® automatic Counter [8]. CD4 counts were classified into 4 categories namely < 200; 200-350; 350-500 and > 500 cells/µL [9].

3. 2. Determination of transaminases

Measurement of transaminases was done following a method described by the 2002 International Federation of Clinical Chemistry (IFFC) protocol [9]. Commercial kits produced by HOSPITEX DIAGNOSTICS Ltd were used and the test was performed using the monoreagent procedure. Briefly, the working reagent was prepared by mixing 4 volumes of reagent one (R1) in 1 volume of reagent 2 (R2). R1 and R2 were gently mixed and stored far from light sources at 2-8 °C. The spectrophotometer was calibrated for transaminases measurement and readings made at 340 nm wavelength. A series of labeled test tubes, i.e. Blank, normal control and patients from 1, 2…. n^{th} according to the number of samples to be analyzed. The preparation was mixed and the first reading of absorbance was executed after 90 seconds. Incubating at 37 °C, 3 other readings were performed at 60 seconds interval. The

change in absorbance per minute was then calculated. The activities were obtained from the following calculations:

340 nm: Activity (U/L) = change in absorbance/ min. x 1769.
Normal values for ALT (SGPT) at 37 °C: Women up to 34U/L. Men up to 45U/L. Normal values for AST (SGOT): Women up to 31U/L and Men up to 35U/L).

3. 3. Ethical considerations

This study was carried out according to the guidelines for human experimental models in clinical research as stated by the Cameroon Ministry of Public Health. Besides, the ethical and administrative clearances for this study were sought.

3. 4. Statistical analyses

All data were verified for consistency, coded, and keyed in an Excel sheet (Microsoft Office, USA). Thereafter, statistical analyses were performed with GraphPad 5.0 (San Diego, California, USA) for Windows. Data were summarized in table mean ± standard deviation (SD) for quantitative. Box-and-Whisker plots and percentage were used to depict qualitative variables where appropriate. Repeated measures analysis of variance (ANOVA) was used to study the influence of confounders on CD4 blood counts and serum liver enzymes titers over time. Goodness-of-fit and independent Chi-square tests (x^2) were computed to compare categorical variables. Unpaired and paired Student's t tests were used to compare means between two groups. Significance was set at p-value < 0.05.

4. RESULTS

4. 1. Baseline characteristics of the participants

A total of 107 patients were included in the study. The mean age of the study cohort was 34 ± 6 years old. No difference was found between the both gender regarding age (34 ± 6 and 37 ± 6 for female and male respectively, P-value = 0.0854). Most participants were females (87.9%; P-value < 0.0001) and aged 30-40 years old (56.1%; P-value < 0.0001) as depicted in Table 1.

Table 1. Baseline sociodemographic characteristics of the study population.

Variables	Categories	Frequency	Percentage	P-value
Gender	Female	94	87.9	< 0.0001
	Males	13	12.1	
Age (years)	< 30	25	23.4	< 0.0001
	[30-40]	60	56.1	
	≥ 40	22	20.5	

Baseline HAART	AZT + 3TC + NVP	52	48.6	< 0.0001
	TDF + 3TC + EFV	47	43.9	
	TDF + 3TC + NVP	7	6.5	
	AZT + 3TC + EFV	1	0.9	
HAART Switch	No	101	94.4	< 0.0001
	Yes	6	5.6	

Data are presented as frequency (percentage).
Goodness-of-fit chi square was used to compare proportions. P-value < 0.05 was considered as significant.
HAART = Highly active Antiretroviral therapy; AZT = Zidovudine; 3TC = Lamivudine; NVP = Nevirapine; EFV = Efavirenz.

All patients were under HIV therapy and treatment regimen based on AZT + 3TC + NVP (52, 48.6%) and TDF + 3TC + EFV (47, 43.9%) were mainly represented (p-value < 0.0001). It should be noted that six patients switched their treatment because of side effects (Table 1). Five of them have switched to AZT + 3TC + NVP and the sixth patient to TDF + 3TC + NVP regimen.

4. 2. Baseline biological variables of the participants

Table 2 presents data on CD4 lymphocytes counts and liver enzymes levels of patients at baseline. A higher fraction of patients had CD4 lymphocytes counts less than 200 cells/mm^3 (42, 39.3%, P-value < 0.0001). As regards transaminases, a significant fraction (P-value < 0.0001) of patients was presenting high levels of AST (10, 9.7%) and ALT (5, 4.8%).

Table 2. Baseline CD4 lymphocytes Blood count and serum liver enzymes of the study population.

Variables	Frequency	Percentage	Mean ± SD	P-value
CD4 count (cells/mm^3)				
< 200	42	39.3	144 ± 36	< 0.0001
[200-349]	33	30.8	264 ± 44	
[350-499]	22	20.6	408 ± 49	
≥ 500	10	9.3	617 ± 114	

AST (IU/L)				
< 40	97	90.3	13.89 ± 8.37	< 0.0001
≥ 40	10	9.7	57.13 ± 24.28	
ALT (IU/L)				
< 40	102	95.2	17.89 ± 6.76	< 0.0001
≥ 40	5	4.8	48.57 ± 6.91	

Data are presented as frequency (percentage) and mean ± standard deviation.
Goodness-of-fit chi square was used to compare proportions. P-value < 0.05 was considered as significant.
CD = cluster of differentiation; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; IU = International unity

4. 3. Evolution of CD4 lymphocytes counts and associated factors

We recorded a 1.5-fold increment in the CD4 lymphocytes counts over the follow up period passing from 279.486 ± 155.535 cells/mm^3 to 408.208 ± 169.491 cells/mm^3 at the baseline and after 18 months respectively (paired Student's test, t = -6.123, P-value < 0.0001) as depicted in Table 3. Gender and age were not associated with variation of CD4 cells counts (P-value > 0.05).

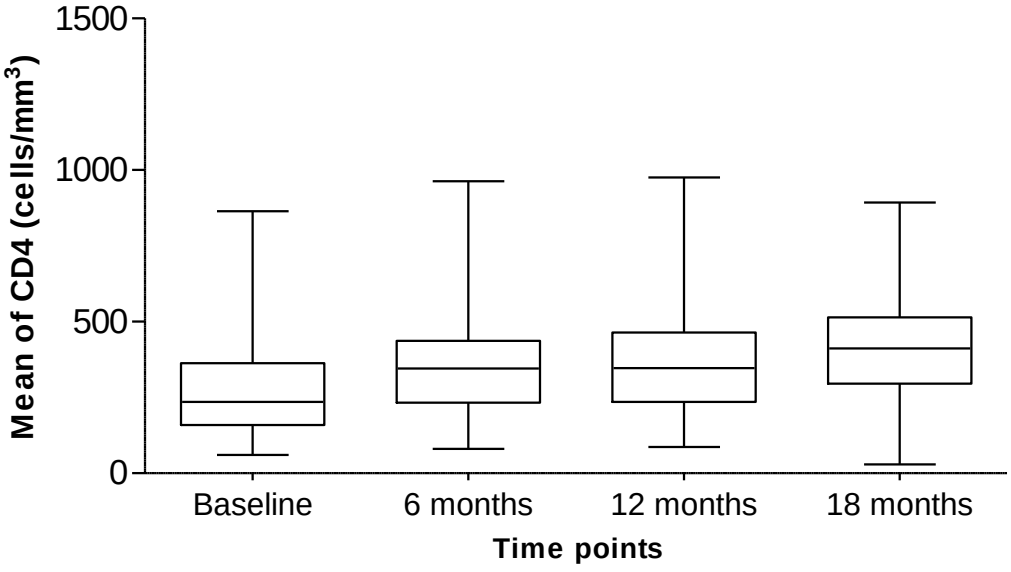

Figure 1. Evolution of CD4 blood count during follow up

4. 4. Evolution of serum liver enzymes levels and associated factors

Globally, a significant increasing in ALT level was recorded over follow up as depicted in Table 3. Indeed, ALT level slightly increased by 1.04 and 1.05 after 6 months and 12 months respectively compared to baseline. AST level slightly increased by 1.08 after 6 months follow up as well and then decreased by 0.97 and 0.94 after 12 months and 18 months respectively compared to baseline. Besides, none factors (gender, age) were found associated with variation of transaminases (P-value > 0.05).

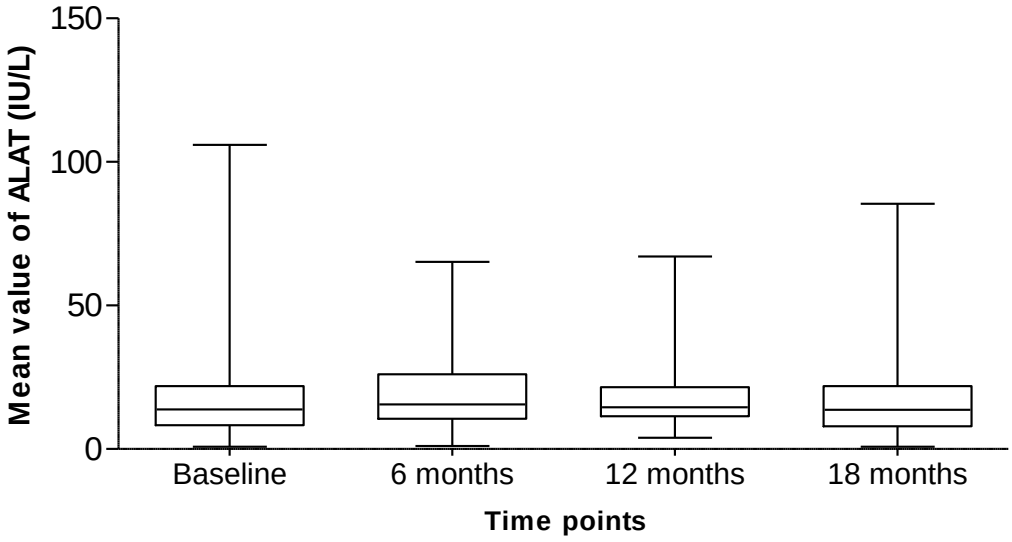

Figure 2. Evolution of ALT during follow up

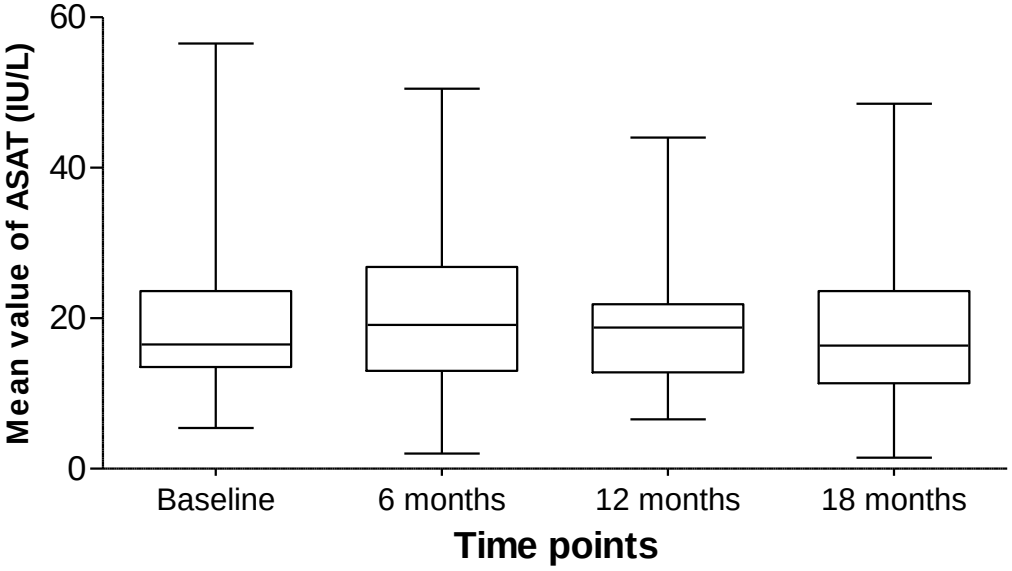

Figure 3. Evolution of AST during follow up

5. DISCUSSION

HIV/AIDS remains a cause for concern in Cameroon in terms of morbidity and mortality. This study was designed to investigate the evolution in CD4 lymphocytes blood count and liver enzymes in newly diagnosed people for HIV and under HAART over an 18-month period.

Most participants were females translating into a female-to-male ratio of 7.23. This is line with some studies conducted in Cameroon [10] and outside [11, 12] which outlined the existence of a gender-related differential risk of HIV infection. This is consistent with some reports across the world along with Cameroon where the same trend was reported [9]. This female predominance might be a sample bias and thus might mean a more pronounced health-seeking behavior in women population than their male counterparts. In addition, we also have previously pointed out males are more skeptical to know about their serological status and also would be prone to attend health facilities (KLP, Personal communication).

The age of participants ranged between 18 et 45 years (34 ± 6 years). This is line with nationwide data that outline people aged 15-49 years old are the most at risk people to get HIV infection. Furthermore, our result is consistent with that recorded by Nkoghe *et al*. [13] and Nadembaego *et al*. [14] who found had mean age of 39 ± 10 years and 35.87 ± 7.55 years in Belgium and Burkina Faso respectively.

As regards viral therapy, four treatment regimens were recorded in this study. AZT + 3TC + NVP (48.6%) and TDF + 3TC + EFV (43.9%) were both regimen the most administered for treating patients. Dimodi and colleagues [15] found similar results in Yaoundé: AZT + 3TC + NVP (31.0%), TDF + 3TC + EFV (24.6%), AZT + 3TC + EFV (27.6%) and TDF + 3TC + NVP (10.1%). This study outlines that the Catholic Hospital Saint Albert Le Grand applies national treatment guidelines recommended by the World Health Organization [16].

More than one third (39.3%) of patients were having CD4 cells counts less than 200 cells/mm^3 and the mean value was 144 ± 36 cells/mm^3. This can be explained by the fact that HIV infection is responsible for impairment of immune response by decreasing CD4 cells which are mainly targeted by the virus during its life cycle.

Importantly, we observed an improving in CD4 cells counts over time following starting on treatment. Some studies reported the same finding elsewhere [17-19]. Conversely, we also reported in this study, a HAART-induced deleterious effect in liver function translating into elevation in ALT levels even though less than 40 IU. Furthermore, we recorded some cases of treatment switch due to the occurrence of side effects. This HAART-induced hepatotoxicity, and increasing over time, has been well documented in both animal and human models [20]. This low level hepatotoxicity recorded following starting on HAART therapy was also observed in others settings in Cameroon [4, 5]. Over time, antiretroviral drug-related injury (ARLI) can occur and then responsible for discontinuance or arrest of treatment in patients.

This can obviously jeopardize the quality of life and consequently life expectancy of HIV-infected patients. Thus, it is critical to routinely check the blood transaminases level during follow up of patients and adequately supply them with HAART. The biological mechanism involved in the antiretroviral treatment-related hepatotoxicity is still elusive. Nevertheless, direct toxicity, mitochondrial damage and hypersensitivity reactions have been proposed [3, 21]. Importantly, confounders such as alcohol ingestion, viral hepatitis B and C or other medicines can also deteriorate liver function [22, 23].

These factors are worth mentioning but were considered as exclusion criteria in this study. Thus, we fairly think the increasing in AST and ALT levels reported in this study was induced by HAART.

6. CONCLUSION

This study aimed at investigating the evolution in CD4 lymphocytes blood count and liver enzymes in newly diagnosed people for HIV and under HAART less than 2 years. This study outlines the positive effect in patients' immune status related to antiretroviral therapy. This also points out a concomitant deterioration of liver function of these. Thus, there is a critical need for paying attention to liver function in people living with HIV/AIDS and under therapy. All this, put together can allow improving quality of life and increasing consequently the life expectancy of these people.

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AUTHORS' CONTRIBUTIONS

Authors **KLP** and **AEV** designed the study, participated in review literature, helped in data interpretation and corrected the manuscript. Author **KLP** performed statistical analysis. Author **TTSK** provided the material, collected field data and wrote the first version of the manuscript. Author **KML** revised the first version of the manuscript and supervised the work at all stages. All authors read and approved the final version of the manuscript.

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