Macrocytosis anemia, Dyslipidemia and Hyperamylasemia in patients living with HIV in Cote d’Ivoire

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ABSTRACT
The destruction of the immune system, especially lymphocyte cells (or CD4), by HIV is responsible for hematopoietic and metabolic disorders (lipid disorders, amylase and lipodystrophy) caused by HIV-persistent viral replication and tritherapy increasing cardiovascular risk. Few studies were conducted in Côte d’Ivoire related to these disorders, while the country is the most affected by HIV/AIDS in West Africa (prevalence rate 3.4%). The aim of our study is to evaluate the hematological and biochemical profile (blood count, lipids, amylase) in Patients infected with HIV monitored at the Institute Pasteur of Cote D’Ivoire. This is an experimental prospective study to determine the biological parameters in the whole blood and serum samples of a cohort of 173 HIV positive adult subjects versus HIV negative the control subjects. After confirmation of HIV status (through a rapid screening test) of subjects included, CD4 lymphocytes, total cholesterol and its fractions and blood count were determined respectively by flow cytometry on FacsCalibur, on Cobas Integra 400 Plus and Sysmex XT-1800i. Anemia was significantly more common in PLHIV (72.26%) than in the controls population (26.59%) (P <0.0001). However, the high presence of macrocytosis anemia (18.50%) and isolated macrocytosis were observed (11.56%) versus (0% and 1.73%) in controls population respectively. They are lower HDL cholesterol (P <0.0001) in infected patients with a consequent increase in atherogenic index (AI) (P <0.0001) and hyperglycemia (P <0.05) compared to controls subjects. The obtained results suggest a biological monitoring before and during antiretroviral therapy to prevent complications.

Keywords: Amylase, HDL Cholesterol, HIV-infected patients, Macrocytosis anemia

INTRODUCTION
The HIV / AIDS affect 25 million people in sub-Saharan Africa, which recorded 1.3 million deaths in 2012 [1]. Unfortunately, Côte d’Ivoire is among the most affected countries in West Africa with a prevalence rate of 3.4% in 2009[2]. Therefore, HIV/AIDS is a real public health problem, with a negative impact on all sectors of the country’s economy.

HIV virus infection[3] attacks various systems of the body including the metabolic and hematopoietic system[4]. These hematological and metabolic disorders caused by persistent viral replication and tritherapy, consisted of anemia, lipid disorders, amylase, lipodystrophy and dyslipidemia which increase cardiovascular risk leading to cardiovascular diseases (heart attack, stroke, etc.) in some patients[5]. In Côte d’Ivoire, few studies[6] were conducted concerning these disorders in adults living with HIV. The research on hematological and metabolic status of adults'patients living with HIV (PLHIV) is justified, in view of the high rate of anemia,
dyslipidemia and especially due to the hematological and metabolic toxicity of some antiretroviral drugs, in order to ensure good medical care and improve quality of life in patients.

The main objective of this study is to evaluate the hematological and biochemical profile of patients living with HIV being monitored at the Institute Pasteur of Côte d’Ivoire.

The specific objectives are to determine the Complete Blood Count, the concentrations levels of biochemical substrates Glucose, Creatinine, Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol, Triglycerides, serum iron and enzymes AST, ALT, Amylase in PLHIV. Secondly describe the hematological abnormalities and the influence of anemia on the level of CD4 T cells and the biochemical markers of PLHIV with or without antiretroviral treatment in PLHIV.

**MATERIALS AND METHODS**

**Study period, Type of study and population**

The study was conducted between August 2011 and December 2012 in the Department of clinical and Fundamental Biochemistry of the Institute Pasteur of Côte d’Ivoire.

This is an experimental study, based on the determination of the blood parameters on a cohort of 173 HIV-positive patients versus 173 uninfected persons (HIV-negative). Were included, only adult subjects (males and females) HIV positive and HIV negative subjects as control subjects confirmed after serological tests. Pregnant women and children either HIV-positive or HIV-negative were not included in this study. Were excluded, all HIV positive patients whose basic biological profile are missing (CD4, CBC, glucose, creatinine, ALT, AST) or uncompleted and all unwilling HIV negative patients.

**Data collection**

One hundred and seventy-three (173) blood samples of patients living with HIV (PLHIV) were collected in 9 voluntary testing centers and medical care centers (Abobo-Doume, Agban, Attécoubé, Centre Plus, 220 housing estate, Locodjro, Yopougon and Williamsville) in the district of Abidjan. Blood samples from 173 HIV-negative subjects from the Community Based Health Center (FSU-COM) Yopougon Toit Rouge were collected, used as control. And then forwarded to the IPCI to perform the basic analysis recommended by the National Program of Medical Management for people living with HIV/AIDS for the biological monitoring of PLHIV.

**Biological materials and reagents used**

The whole blood samples from HIV positive and negative subjects were collected in EDTA tube, non-anticoagulant tubes (dry tubes) and the tubes containing potassium oxalate and sodium fluoride (gray tops tubes)[7]. EDTA tubes were used to carry out the CD4 T cells count and blood count.

Sera obtained after centrifugation at 3000 rev / min for 5 min in the dry tubes were used for HIV screening and biochemical diagnosis (creatinine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, serum iron, amylase, transaminases) and plasmas obtained under the same conditions of centrifugation in gray tops tubes were used for blood glucose assay.

**Methods**

The CD4 cell count was carried out using flow cytometry technology (Facs Calibur) as follows: to 20µL of the trittest (CD3, CD4, CD45) distributed into each tube, 50µL of whole blood were added. Five hundred microliters of the Lysing solution (lysing red blood cells) diluted 1:10 was added to each tube prior homogenized and incubated for 15 min in the dark. This operation (homogenization and incubation of tubes in the dark) was repeated a second time, then the trucount tubes were placed on the rack of the Facs Calibur device after a third homogenization for CD4 lymphocytes count [8]. Normal CD4 reference values according to the WHO are: 600-1750 cells / mm$^3$ (31-60%).

Leukocyte count, erythrocytes, platelets and white blood cell count were performed using electronic hematology analyses Sysmex (XT-1800i), combining the principle of the impedance variation and flow cytometry[9]. Samples were placed on the rack and a standard sample collected on the previous day added as an internal control, was placed first on the rack. The numbers of samples previously recorded on the work list displayed on the screen were saved by pressing "save" button of the machine. After passing the control sample and the result validated, the other samples were placed on the right tray of the passing rack, one after the other according to the work list. For each sample to be analyzed, it is necessary to press "Auto" to proceed with the recording of the next sample, on "passing" to check the identification numbers of the sample on the rack and position of the tube to be analyzed and press start.
"passing" then finally OK "to confirm starting of the analysis. The printing of the results was made automatically taking into account the number of red blood cells per unit volume, hemoglobin level, hematocrit, red cell constant, the level of platelets and leukocytes per unit volume and leukocyte formula [polymorph nuclear cells (neutrophils, eosinophils, and basophils), lymphocytes, and mononuclear cells]. After their validation, Hemoglobin, hematocrit, platelets, neutrophils, and erythrocyte constants and the mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) are the parameters used in interpreting the results of this study.

According to the WHO classification, anemia is said to be severe when Hb less than 8g / dL; moderate when hemoglobin is between 8-10 g / dL, and light when Hb greater than 10 g / dL[10,11].

The following biochemical parameters: Glucose, creatinine, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, serum Iron, amylase, transaminases were performed on the fully automated Cobas 400 Plts. The principle is based on the reaction of TRINDER (enzymatic method plus colorimetric using a chromogen)[12]. The deepness of the coloration is directly proportional to the concentration.

After switching on the automated Cobas and entering the identification code, Surname followed by first name on the worksheet on the screen, the parameters to be determined were entered and validated by pressing "Save". Racks "sample rack" "cassette door Control", "cassette door calibrator", and "cassette door reagents" were confirmed in their positions, and then installed in their respective compartments. The assay of the samples was performed after the exact value of the control serum assay and calibration performed. The results were validated.

**Statistical analysis**
Stata software was used for data analysis. Quantitative variables were compared with Student's t test or Wilcoxon where appropriate and the variables with the chi2 test or Fisher's exact test. The analysis was performed at the 5% threshold. The obtained hematological and biochemical results were compared to the usual standard values of reference[13, 14].

**Ethical considerations**
Consent was obtained from the patients for the use of their blood for research purpose.

**RESULTS AND DISCUSSION**

**Epidemiology of HIV infection in the study population**
Among the 173 PLHIV subjects biologically monitored, there are more women infected with HIV (140; 80.92%) than men (33; 19.08%), a sex ratio of (M / F) 0.24 and the age group of 26-49 years (132/173, 76.3%) is the most exposed to HIV infection (Figure 1).

The average age of the study population was 37 ± 9.9 years for PLHIV, and 29 ± 8.9 years for HIV negative control population (P <0, 0001).

There is a predominance of HIV1 in the population of PLHIV (165/173; 95.38%) compared to HIV2 which represents only 1.73% (3/173).

**Hematological and CD4 profile of PLHIV**
Hemoglobin (Hb) level in HIV Positive Patients is significantly low 10.52 ± 0.12 g/dL compared to Hb in HIV Negative Controls subjects 12.53 ± 0.12 g/dL. P <0.0001. Severe anemia is absent in controls (0%) but present in HIV positive patients (6.94%) (Table1).According to the level of lymphocytes CD4, Severe anemia (7/12, 58.33%) and moderate anemia (19/38; 50%) were predominate in patients with CD4 <200 cells /μL. Light anemia 35/75 (46.66%) was more frequent in patients with CD4 200-349 cellules/μL.

The normocytic and normochromic anemias (NNA) 71/173 (41.03%) prevail in PLHIV and The microcytic hypochromic anemias (MHA) 25/173 (14.45%) recorded in HIV negative subjects. Macrocytosis anemia cases 32/173(18.50%) and Isolated macrocytosis cases 20/173 (11.56%) were observed in HIV+ patients against 0%; 1.73% (3/173) respectively in HIV negative control subjects (Figure 2).
Figure 1: Distribution of with HIV-infected patients according to age groups

- 132 (76.30%) patients
- 22 (12.72%) patients
- 19 (10.98%) patients

16-25 years
26 - 49 years
≥ 50 years

Figure 2: Type of anemia and isolated macrocytosis in HIV-infected patients and HIV negative controls

- AMH = Hypochromic microcytic anemia
- ANN = Normochromic Normocytic Anemia
- AMA = Microcytic Anemia
- MAI = Macrocytic Anemia

- Absence d’anémie
- Macrocystose isolé

Number of Patients

- 22 (12.72%) AMH
- 25 (14.45%) ANN
- 21 (12.14%) AMA
- 20 (11.56%) Macrocystose isolé
- 28 (16.18%) Absence d’anémie
- 71 (41.04%) VIH(+) (41.04%)
- 124 (71.68%) VIH(-) (71.68%)
Table 1. Distribution of anaemia level in HIV-infected patients and in Control population

<table>
<thead>
<tr>
<th>Level of Anaemia</th>
<th>HIV-infected patients (n = 173)</th>
<th>Control population (n = 173)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients MeanHb</td>
<td>Control MeanHb</td>
</tr>
<tr>
<td>Severe</td>
<td>12(6.94%) 6.41 ± 0.94</td>
<td>0 0</td>
</tr>
<tr>
<td>Moderate</td>
<td>38(21.97%) 9.18 ± 0.6</td>
<td>38(21.97%) 9.43 ± 0.51</td>
</tr>
<tr>
<td>Light</td>
<td>75(43.35%) 10.70 ± 0.41</td>
<td>127(73.41%) 13.21 ± 1.26</td>
</tr>
<tr>
<td>Absence of anaemia</td>
<td>48(27.74%) 12.34 ± 0.79</td>
<td></td>
</tr>
</tbody>
</table>

Anaemia was classified as follow:
- HB < 8 g/dL = Severe
- HB between 8 - 10 g/dL = Moderate
- HB > 10 g/dL = Light
- HB ≥ 11.5 g/dL = Absence

Table 2. Average values of HDL cholesterol, triglycerides and amylase observed in PLHIV and control

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Men (n=33)</th>
<th>Women (n=140)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-Cholesterol (mmol/L)</td>
<td>0.59 ±0.03 1.47±0.04</td>
<td>0.57±0.02 1.45±0.02</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>Atherogenic index(TC/HDL) (Ref. H &lt;4.4 ; F &lt;3.3)</td>
<td>13.92±4.2 3.69±0.81</td>
<td>11.87±1.41 3.53±0.18</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>Triglycerides (mmol/L) (Ref. 0.34-1.53)</td>
<td>1.23±0.18 0.89±0.11</td>
<td>1.12±0.06 0.76±0.03</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>α amylase (UI/L) (Ref. &lt; 100)</td>
<td>110±9.81 103±6.73</td>
<td>109±6.23 93±3.30</td>
<td>0.0117*</td>
</tr>
</tbody>
</table>

Ref = References values
HDL-Cholesterol = high density lipoprotein-cholesterol;TC/HDL = Total Cholesterol / high density lipoprotein-cholesterol
* The difference is significant for p<0.05 between the two groups
** The difference is significant for p<0.001 between the two groups
*** The difference is significant for p< 0.0001 between the two groups

Biochemical profile of PLHIV

The average values of biochemical substrates (in Men / Women), such as blood glucose (P = 0.9116 / P = 0.2215), creatinine (P = 0.2755 / P = 0.1913), LDL cholesterol (P = 0.8288 /F = 0.8084) and serum iron (P = 0.4908 / P = 0.1752) and the transaminases (AST, ALT) (P = 0.8797/ P = 0.5744) were within the range of the usual standard values of reference and showed no significant difference in HIV positive compared to control subjects.

However, only HDL-cholesterol in Men (0.59 ± 0.03 /1.47 ± 0.04) (P<0.0001) Women 0.57 ±0.02 /1.45 ± 0.02 ( P <0.0001) and atherogenic index values in Men (13.92 ± 4.2 /3.69 ± 0.81 (P <0.05) Women (11.87 ±1.41 /3.53 ± 0.18)/(P <0.0001) experienced significant decrease and increase respectively in PLHIV compared to controls (Table 2).

The average values of alpha-amylase were high in PLHIV men (110 ± 9.81) and women (109 ± 6.23). However, the difference is not significant (p = 0.6049) in men, but it is in women (p = 0.0117) (Table 2).

Influence of the ARVs treatment on CD4 and biochemical parameters:

In general, according toCD4lymphocytes counts and prescribed treatments: 122PLHIVhaveCD4 cell counts > 200 cells /µL and 51 PLHIVhaveCD4 count< 200cells /µL. 78of 122(63.93%) PLHIV were on cotrimoxazole(CTX) against21/51(41.18%) of PLHIV on antiretroviral treatment (ART).

The mean values of HDL cholesterol(0.36 ± 0.03 mmol/L; 8.28 ± 0.72 mmol/L), triglycerides (1.59±0.20 mmol/L and0.95±0.05 mmol/L), and atherogenic index(21.31 ±4.31; 8.14±1.32) in PLHIV without ART were below and above the reference values, respectively. Under ART, HDL-Cholesterol is higher and the atherogenic index is lowered. Triglycerides are lowered (Table 3).

The mean values of the alpha-amylase serum(73 ± 8.98 IU / L; 68 ± 10.78 IU/L)decreased not significantly in PLHIV on ART, p = 0.7375 and p = 0.7154 respectively (Table 3).
### Table 3. Average values of HDL cholesterol, triglycerides and amylase in PLHIV with and without antiretroviral therapy (ART)

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Lymphocytes CD4 &lt; 200</th>
<th>ART (n=21)</th>
<th>NO ART (n=30)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-Cholesterol (mmol/L)</td>
<td>8.28±0.72</td>
<td>0.36±0.03</td>
<td></td>
<td>0.0008</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>8.14±1.32</td>
<td>21.31±4.31</td>
<td></td>
<td>0.0138</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.95±0.05</td>
<td>1.59±0.20</td>
<td></td>
<td>0.0237</td>
</tr>
<tr>
<td>Amylase (UI/L)</td>
<td>68 ±10.78</td>
<td>73 ±8.98</td>
<td></td>
<td>0.7154</td>
</tr>
</tbody>
</table>

*The difference is significant for P < 0.05.

**DISCUSSION**

This higher prevalence of anemia in PLHIV (72.26%) than HIV-negative controls subject (26.59%) in this study was reported in the work of Diallo et al. (2003) [15] in Africa and Cosby et al. (2000) [16] in the United States who obtained a prevalence of 78.9% and 85% respectively. Anemia is the most frequently encountered anomaly in PLHIV. The causes of anemia in HIV/AIDS are numerous and multifactorial, from associated pathologies encountered in immunocompromised health to anemic side effects of certain drugs such as AZT and Trimethoprim-Sulfamethoxazole administered to patients [17]. The severity of anemia, significantly higher in patients PLHIV than in controls subjects, increases with the decrease in CD4 lymphocytes. This suggests that the incidence of HIV/AIDS is a contributing factor to the occurrence of severe anemia [18,19].

The prevalence of anemia normocytic normochromic among PLHIV observed in our study was highlighted in other works [20]. Macrocytic anemia and isolated macrocytosis encountered at a higher frequency in our study, and identified different CD4 count rates depending on whether the patient is on ART or not, is in contrary to other studies that showed a lower frequency of macrocytic anemia [20]. This can’t be due to either antiretroviral (ART) treatment, or Trimethoprim-Sulfamethoxazole or cotrimoxazole (CTX), because only patients with a CD4 count <200 cells / µL were subjected to triple therapy in Côte d’Ivoire as at the time of our study.

In Africa, the multiple and intricate etiologies of anemia associated [21], with HIV being particularly difficult because of inadequate diagnostic facilities, the assay of vitamin B12 is needed in the advent of macrocytic anemia and isolated macrocytosis [22]. The reticulocytes count is also necessary to examine whether the anemia is regenerative or non-regenerative.

Moreover, the risk of anemia associated with taking Trimethoprim-Sulfamethoxazole [23,24] in preventing opportunistic infections in Africa [25], should be better evaluated.

No abnormal value of glucose, creatinine, total cholesterol and transaminases were observed in this work. However, the study highlighted a decrease in HDL cholesterol in PLHIV with the consequence of an increase in the atherogenic index (AI) calculated according to TC / HDL-C. These results are consistent with those of [26, 27] confirming that there is an increase in atherogenic risk in the course of HIV infection. This decrease in HDL cholesterol is related to the decrease in the synthesis of apolipoprotein A1 [28] which is the major constituent of HDL [29].

Enzymatic analysis result showed hyperamylasemia in patients living with HIV. This hyperamylasemia observed could be related to the virus itself, which represents an independent factor of inflammation of the pancreas [30].

**CONCLUSION**

The occurrence of anemia in PLHIV imposes closer monitoring of hematological parameters in order to reduce the prescription of hematotoxic molecules for PLHIV patients. This will ensure better biological stability of infected individuals.

For properbiological monitoring of HIV Positive Patients, it would be important to include in the basic laboratory tests, the semestrial or annual assay of vitamin B12 and systematize the counting of reticulocytes.
The biochemical abnormalities observed in PLHIV were: a low HDL cholesterol with a consequent increase in atherogenic index (AI) and hyperamylasemia. These lipids disorder increases cardiovascular risk[31]. Given the atherogenic risk in people living with HIV, it is important to study the mechanism of action of cytokines that are immune system mediators and involved in lipid disorder.

Finally, for better management and proper monitoring of HIV Positive Patients, biological monitoring is required before and during antiretroviral treatment in order to prevent complications that could arise.

Acknowledgement
- Dr Soumahoro-Agbo Man Koumba, Researcher and head of Epidemiology Department and Clinical Research in Institut Pasteur of Cote d’Ivoire for the statistical analysis.
- The District of Abidjan for their assistance in the acquisition of certain equipment.

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