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HEMATOLOGICAL PROFILE OF PATIENTS BORN WITH HIV AT THE YAOUNDE UNIVERSITY TEACHING HOSPITAL: CROSS-SECTIONAL STUDY

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Abstract:

Background and rationale: Human immunodeficiency virus (HIV) infection is a major public health problem; however, sub-Saharan Africa is the most affected by it. The advent of antiretroviral treatment has caused the infection to become chronic, leading to biological abnormalities.

The objective of this study was to determine the hematological profile and biological abnormalities of children undergoing antiretroviral treatment at the Yaoundé University Hospital Center (YUTH) in Cameroon.

Methodology: A prospective, cross-sectional, single-center study was conducted from November 2020 to October 2021 in 74 young people aged 3 to 19 years. After obtaining ethical clearance, sociodemographic and clinical data were collected; samples were also collected and analyzed by immunophenotyping and blood count (CBC) for the quantification of $CD4^+$, CD8+ T cells and blood cells such as: Total leukocytes, Monocytes, Neutrophils, Platelets, Red blood cells. Statistical analysis was performed using Microsoft Excel 2019, SPSS version 22 software. Any value of $P<0.05$ was considered statistically significant.

Results: A total of 74 children were included in the study. The median age was 9 years, 68.92% of the children were female. HIV-1 infection was predominant (94.59%). The mean $CD4^+$ count was 536.75 cells/µl (SD = 216.25 cells/mm^3), and the median CD4+ count was 600 cells/µl. Among these patients, 4.1% had a CD4 count$^+$< 200 cells/mm^3, 32.4% had a $CD4^+$ count between 200 and 349 cells/µl, 2.7% had a CD4 count$^+$ between 350-499 cells/µl and 60.8% had a $CD4^+$ count ≥ 500 cells/µl. The average hemoglobin level was 11 g/dl (standard deviation = 2.3 g/dl) with extremes of 4 and 14 g/dl.

Conclusion: The prevalence of biological abnormalities in people born with HIV under antiretroviral treatment suggests systematic performance of blood counts and lymphocyte phenotyping.

Keywords: HIV; YUTH; CBC.

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1. Introduction

Human Immunodefiency Virus (HIV) infection is a public health [1]. According to UNAIDS estimates, worldwide 39.9 million people [36.1-44.6 million] were living with HIV worldwide in 2023, including 1.4 million [1.1-1.7 million] children (0-14 years), 53% of people living with HIV were women and girls [2].

HIV (retrovirus) causes a slow but progressive alteration of the host's immune system. Hematological abnormalities occur in almost all patients during the course of the disease. They result from the consequences of immunodeficiency and/or dysregulation of the immune system, complications of bacterial, viral or fungal infections, side effects of multiple treatments and the direct role of the virus on certain hematopoietic progenitors and stromal cells [3,4]. However, infection of bone marrow progenitors in vitro has rarely been demonstrated with certainty outside of megakaryocytes and stromal cells (macrophages/monocytes, fibroblasts, endothelial cells). These disorders are explored by the blood count [5]. The blood count is the most prescribed paraclinical examination since it is part of the assessment carried out in the face of any clinical symptoms, any alteration of the general condition, any anomalies noted during the clinical examination. Like any complementary examination, it must correspond to a question asked by the clinician and the biologist, the answer to which would be provided by the examination report.

The biological assessment constitutes a real-time indicator of the effectiveness and tolerance of the treatment and of the development of HIV infection. [6]. In Cameroon, HIV-positive patients undergoing ARV treatment face a problem of biological monitoring. Given all these difficulties in the care of PLHIV, we set out to evaluate the impact of treatment on the hematological parameters of HIV-positive patients at the Yaounde University Hospital.

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2. Materials and Methods

2.1. Study design:

The study was cross-sectional and monocentric, carried out over a period of 12 months (November 2020 to October 2021) at the Approved Treatment Center of the Yaoundé University Hospital Center (YUTH).

2.2. Sampling size:

Participants were HIV-positive patients on ART with an undetectable viral load treated at YUTH. Our sample size was calculated using the following formula:

$$N = \frac{P(1-P)(Z_{1-\alpha})^2}{i^2}$$

alpha*=0.05→ Z1-α= 1.96 according to the normal law; P: Prevalence of subjects presenting the studied variable; Z1-α: constant sampling error; i : operational error or margin of error*[7].

2.3. Study subjects

Each participant was required to sign a consent form prior to enrollment. A questionnaire was then administered and a blood sample was collected and sent to the Hematology Laboratory/Blood Bank of the Yaoundé University Teaching Hospital and Control of Communicable Diseases of the Faculty of Medicine and Biomedical Sciences of Yaoundé. Immunology, located in the Central Region of Cameroon, which served as the site for biological analysis of the samples. Demographic data (age and sex) and missing information from the interview could be completed using the medical record.

A total of 74 participants were included in the study. Exclusion criteria included: Being born HIV positive to an HIV-positive mother and on ART; Having an undetectable viral load at the time of study inclusion (< 50 RNA copies/µl); Providing assent for adolescents aged 12–20 years; Obtaining parental consent for children aged 0–20 years; Providing informed consent for those aged 21 years and older.

2.4. Sample collection

Blood sampling was performed from the veins in the flexor of the elbow, and blood samples were collected in tubes with anticoagulant. Subjects were asked to fast for at least 8 to 12 hours before collection. They were also advised to avoid smoking or exercising before the appointment to minimize potential disruptive factors.

2.5. Laboratory Analyses

Determination of blood cell population: The blood counts were performed in the hematology laboratory/Blood Bank using a Mindray 30C type machine.

Determination of T+ cell counts: Lymphocyte phenotyping at the Center for the Study and Control of Communicable Diseases of the Faculty of Medicine and Biomedical Sciences of Yaoundé. Immunology

Laboratory of the said institute was carried out with a FACScount type automaton from Becton-Dickinson.

Fifty ml of whole blood was collected in a vacutainer EDTA tube using standard blood collection methods. Samples were analyzed based on the principle of Immunophenotyping. In brief, 50µl of well mixed whole blood was added to perforated CD4/CD3 and CD8/CD3 reagent tubes containing monoclonal CD4/CD8 antibodies fixed on beads. They were incubated for 80 minutes, after which a 50µl of fixative (5% formaldehyde) was added, vortexed and analyzed using the Fluorescence Activated Cell Sorting (FACS) Count Analyzer (BD FACS Count tri CD4/CD8/CD3 reagent kit) (BD Biosciences, San Jose, California, USA). This procedure was maintained for all 50 blood samples stored at the two different temperatures. All the samples were analyzed strictly following the manufacturers' guidelines.

2.6. Ethical consideration:

The study received approval from the Human Health Research Ethics Committee of the Central

Region of Cameroon under reference N/Ref: (No. 0082/CRERSHC/2023)

2.7. Statistical analyses

Excel version 2019 software was used for spreadsheets and to set up the database. Analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 22. Means were calculated with a 95% confidence interval. Correlations were performed using the Fisher exact test. The significance threshold was set at a P value (probability of error) less than 0.05.

3. Results

3.1 Sociodemographic and Clinical Parameters

The samples were taken from 74 patients.

We obtained a predominance of female sex 68.92% (n=51) compared to male sex which represents only 31.08 % (n=23). The average age was 9.09 years with extremes ranging from 3 to 19 years. Table I represents the main characteristics of the study population, HIV-1 was identified in 70 cases (94.6%) compared to 4 cases of HIV-2 (5.4%) and 0 cases of HIV 1+2 co-infections (0 %). The distribution of participants living with HIV at different stages of the disease progression was as follows: 70 (94.6%) at stage 1, 4 (5.4 %) at stage 2.

CD4+ counts were reported in 74 participants (100 %). The mean $CD4^+$ count was 536.75 cells/µl (SD = 216.25 cells/mm3), and the median $CD4^+$ count was 600 cells/µl. Among these patients,4.1% had a $CD4^+$ count$^+$< 200 cells/mm3,32.4% had a $CD4^+$ count between 200 and 349 cells/µl, 2.7% had a $CD4^+$ count between 350-499 cells/µl and 60.8% had a $CD4^+$ count ≥ 500 cells/µl. According to the participants' gender, the mean $CD4^+$ count in girls (536.76 cells/µl) was higher than in boys (543.67 cells/µl); and this difference was significant (ANOVA parametric test; p = 0.0001). (Figure 1)

Hemoglobin levels were recorded in the records for all 74 patients (100%). The mean hemoglobin level was 11 g/dl (standard deviation = 2.3 g/dl) with extremes of 4 and 14 g/dl. The median hemoglobin level was11g/dl. Considering the sex of the patients, the mean hemoglobin level was significantly higher in boys (10.11 g/dl) than in girls

(10.05 g/dl): Chi test^2(Kruskal-Wallis H) = 101.9; p< 0.0001. More than half of the patients (74 %) were anemic in both sexes, with a hemoglobin level below 12 g/dl in patients (Table 3)

Mean values of laboratory parameters in HIV-infected patients compared with normal values are shown in Table 2. White blood cells, neutrophilic granulocytes, and eosinophils had lower mean values in PLWH than in HIV patients, unlike the mean hematocrit value. The hematological profile of PLWH compared with normal values is shown in Table 2.

At the level of the different cell lines, it was observed that the HIV-positive patients had more marked leukopenia and neutropenia, while the HIV patients presented leukocytosis, neutrophilia and thrombocytosis.

On the leukocyte line: 62% of patients had a WBC greater than 10,000/mm³, 68% had eosinophilia and 58% had basophilia.

The table below shows that 32.43% of our series had an abnormal total leukocyte count, 74.0% had an abnormal hemoglobin count, and 56.0% had a platelet count below normal.

Table1: Sociodemographic and clinical parameters

Settings	**N^(%)**
Sex	
Girls	51(69)
Boys	31(31)
Years	
] 0-5 [	17 (22.97)
[5-10[	25 (33.78)
[10-15[	16 (21.62)
[15-20[	16 (21.67)
Stage of infection	
I	70(94.59)
II	4(5.40)
III	//
IV	//
Number of opportunistic diseases	
Diabetes	3(4.05)
Renal Failure	1(1.35)
Malaria	25(33.78)
Prostatitis	1(1.35)
Tuberculosis	1(1.35)
Typhoid fever	1(1.35)
No disease	42(56.76)
Type of HIV	
HIV-1	70(94.59)
HIV-2	4(5.40)
HIV-1+HIV-2	//

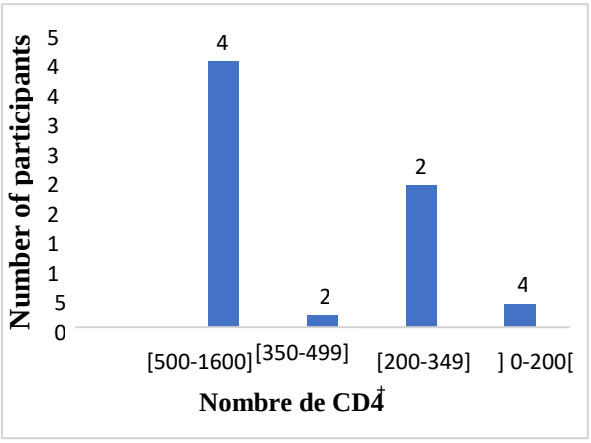

Figure1: Distribution of patients according to their $CD4^+$ count in cells/µl

Table2: Average values of laboratory parameters of HIV-positive people born with HIV

Laboratory Settings	**HIV-positive people born HIV**	
Blood cell type ($\times 10^3$)	**Mean ± Standard deviation (SD)**	**Normal cell values**
Total leukocytes	4.3903 ±1.98	4 – 10
Monocytes	0.3812 ±0.27	0.1 – 1
Platelets	309.28 ±70.84	150-300
Neutrophils	41.4068 ±13.34	1.7-7
$CD4^+$ cells/L lymphocytes	536.76±216.25	500-1600
$CD8^+$ cells/L lymphocytes	754±115.05	100-1200
CD4/CD8 ratio	0.73±0.32	1-2

*Legend: leukocytosis:White blood cell count > 10/mm3; Leukocytopenia:White blood cell count < 4/mm3;Thrombocytopenia:Platelet count < 150 cells/mm3;Thrombocytosis: Platelet count > 400/mm3;*Neutropenia:Neutrophil count < 1.7/mm3; Polynucleosis Neutrophils: Neutrophil count>7/mm3; Monocytosis: Monocyte count> 1/mm3; Monocytopenia: Monocyte count < 0.1/mm3

Table3: Main hematological abnormalities observed in HIV-positive people born with HIV

Laboratory Parameters	**Constants**	**HIV-positive people born HIV**	
		N (%)	**95% CI**
Total leukocytes (10^3/µl)	<4	**24**(32.43)	[38.0-63.3]
	≥ 4	50 (68.57)	[36.7-62.0]
Hemoglobin	<12	**55 (74.00)**	[62.8-3.78]
(g/dl)	≥ 12	19 (26.00)	[16.2-7.16]
Platelets	<150	**42 (56.0)**	[38.0-70.0]
(10^3/µl)	≥150	32 (44.0)	[30.0-62.0]

Legend: leukocytosis: White blood cell count > 10,000/mm^3; Leukocytopenia: White blood cell count < 4,000/mm^3; Thrombocytopenia:Platelet count < 150 cells/mm^3;Thrombocytosis: Platelet count > 400,000/mm^3

4. Discussion

The present work aimed to determine the hematological profile of HIV-positive children born at the

Yaoundé University Hospital. Regarding hemoglobin, the study revealed an increase in the number of people with anemia between the first and second assessments. This result contrasts with the data of Karfo et al. [6] who report a decrease in anemia over the same period. This difference could be explained by the immaturity of the immune system of our participants at the time of HIV infection. Anemia, defined by insufficient production of red blood cells [8], can have various causes, including kidney disease, HIV infection, nutritional deficiencies, chronic illnesses, and blood loss.

Concerning the anomalies found in the white bloodcell lineage, Throughout the follow-up, a constant prevalence of 32.43% of participants with leukopenia emerged. This result is lower than that obtained by the work of Aka and the work of Nacoulmaye et al. in Ivory Coast and Burkina Faso respectively. [14,15]. According to the work of Talom (2005) in Mali on adults living with HIV [9], it is a clinical manifestation frequently encountered in people infected with HIV, 60 % of people at the AIDS stage and approximately 40 % of HIV-positive people. This sign is generally observed in people with CD4$^+$ lymphopenia. These hematological abnormalities can be explained by the various inflammatory mechanisms due to HIV infection, or by iatrogenic causes, notably antiretroviral and anti-infective treatments. The results obtained suggest performing CBC systematically in its participants born HIV positive.

More than half of the participants were at WHO stage I. Our result is similar to that reported by Fatimata [10]. Concerning CD4 T lymphocytes, the average rate of 500 cells/L observed in our study is higher than those found by Loua in Guinea, Mouhari-Touré in Togo and Elira Dokekias in Brazzaville in Congo [11-13].

This study has limitations such as small sample size, single-center nature, and missing data. However, this study showed an increased prevalence of hematological abnormalities in children born HIV-positive.

5. Conclusions

The study of the hematological profile is an interesting factor in the diagnosis and prognosis of HIV infection. In the present study, various hematological abnormalities were observed. At the level of the erythrocyte lineage, a predominance of anemia is noted. Concerning the white blood cell lineage, leukopenia and neutropenia are the major abnormalities found. An immune deficiency characterized by a decrease in CD4$^+$ T lymphocytes is common. These various abnormalities are likely due to the terrain of HIV-infected subjects but also potentiated by the intensity of immune activation.

Consideration of clinical and therapeutic data of HIV-infected patients should be considered to better clarify their impact on hematological abnormalities. However, this work indicates the need for adequate biological monitoring for better management of people born HIV-positive in Cameroon.

Abbreviations

HIV: Human Immunodeficiency Virus

PLWH: People Living With HIV

SD: Standard Deviation

YUTH: Yaounde University Teaching Hospital

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Author Contributions

Mbongué-Mikangué. C. André, designed and set up the research project., collected the samples, led the technical aspects at the Hematology Laboratory/Blood Bank Laboratory. The analysis of the data and the writing of this article saw the collaboration of all authors.

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Data Availability Statement

The data supporting the results of this study are available on request from the corresponding author. The data is not publicly available because it contains information that could compromise the confidentiality of research participants.

Conflicts of Interest

The authors declare no conflicts of interest.

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