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Research Article

Comorbidities in Children Born Hiv-Positive Followed at the Approved Treatment Center of the Yaoundé University Teaching Hospital

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Abstract

Introduction: The management of Human Immunodeficiency Virus (HIV) infection is now that of a chronic disease. Although patients carrying the HIV virus live longer, they more frequently, and often earlier, develop comorbidities called non- Acquired Immunodeficiency Syndrome (AIDS)-defining events compared to the general population. Despite the high prevalence of these comorbidities and overexposure to numerous risk factors, little data is available on comorbidity screening in PLHIV (People Living with HIV). This study aimed to determine the prevalence of comorbidities in children born HIV-positive to HIV-seropositive mothers with an undetectable viral load on antiretroviral therapy at the Yaound éUniversity Teaching Hospital (YUTH). *Methods:* Participants included in this study came for consultation at the Authorized Treatment Center of the YUTH and were selected based on their risk of developing a comorbidity (CD4:CD8 T-lymphocyte ratio < 1). The study was cross-sectional and lasted 1 year. After administering a questionnaire, a blood sample was obtained from each participant, then analyzed for immunophenotyping using Becton Dickinson equipment. After centrifugation, the extracted plasma was used with the Toxoplasma, Rubeola virus, Cytomegalovirus and Herpes Virus (TORCH) Kit, Epstein Barr-Virus (EBV) Kit, HBV (Hepatitis B Virus) kit, HCV (Hepatitis C Virus) kit to search for specific IgG antibodies or antigens. Statistical analysis was performed using Microsoft Excel 2019 and Statistical Package for the Social Sciences (SPSS) version 25. A p-value <0.05 was statistically significant at a 95% CI. *Results:* Of the 74 participants, 69% were girls versus 31% boys. The co-infection that most affected study participants was related to EBV at 22.97% (n=17), followed by CMV at 12.2% (n=9). The lowest positivity rate was found associated with the rubella virus (RV) at 5.41% (n=4). According to these results, only co-infections associated with HBV, HCV, RV, and Toxoplasma were found to be significant. The most frequently observed comorbidities were type I diabetes (4.05%), prostatitis (1.35%), and renal insufficiency (1.35%). A low CD4:CD8 ratio < 1 was significantly correlated with the number of comorbidities. *Conclusion:* It follows that people living with HIV presenting a low lymphocyte ratio are at risk of developing a comorbidity.

Keywords

EBV, HIV, PLHIV, YUTH

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1. Introduction

Since the discovery of the Human Immunodeficiency Virus (HIV) in 1983 [1], patient management has continuously seen improvements, and significant progress has been made. This is the case with antiretroviral triple therapy, which has considerably revolutionized the lives of people infected with HIV. Gradually, therapeutic efficacy has allowed for viral suppression and a considerable extension of their life expectancy [2, 3]. In 2023, 86% of all people living with HIV (PLHIV) knew their HIV status. Among people who knew their status, 89% had access to treatment. And among people with access to treatment, 93% had viral suppression. Among children aged 0-14, the 95-95-95 targets were 66%, 86%, 84%. Among women, the 95-95-95 targets were: 91%, 91%, 94%. Among men, the 95-95-95 targets were: 83% of HIV-positive adult men knew their HIV status, 86% had access to treatment, and 94% had an undetectable viral load [4]. Recently, several studies suggest that the expected life expectancy of a patient starting treatment with more than 350 CD4 cells/mm^3 and who is virologically controlled would be almost identical to that of the general population [5, 6]. But overall, even though mortality has dropped, it remains higher than that of the general population [7]. In Europe, more specifically in France, in 2010, pathologies related to Acquired Immunodeficiency Syndrome (AIDS) represented only ¼ of the causes of death of PLHIV compared to 36% in 2005 and 47% in 2000 [8]. The mortality rate due to comorbidities has therefore become higher than AIDS-related mortality. Non-AIDS-defining events are thus the leading cause of mortality in PLHIV, led by non-AIDS-defining cancers not related to viral hepatitis, followed by liver diseases, and in third place, cardiovascular pathologies. This is an aging population, with access to increasingly effective antiretroviral Treatment (ARVT), allowing better immuno-virological control and thus leaving less room for the AIDS stage in terms of morbidity and mortality [9]. But this observation only partially explains the excess mortality compared to the general population. Indeed, several studies have shown that PLHIV develop health problems earlier and more frequently compared to the general population, called non-AIDS-defining events [10]. In Cameroon, a lack of data on the prevalence of comorbidities in people born HIV-positive has been observed. The objective of this work was to determine the prevalence of comorbidities and risk factors for the occurrence of these comorbidities in people living with HIV and to compare this prevalence according to age and sex. To our knowledge, this study is the first to identify comorbidities associated or not with HIV in Cameroon.

2. Materials and Methods

The study was cross-sectional, conducted over a period of 12 months (November 2020 to October 2021) at the Approved Treatment Center (ATC) of the Yaoundé University Teaching Hospital (YUTH). Participants were patients born with HIV on ARVT with an undetectable viral load (viral load < 50 RNA copies/ml) managed at YUTH. Our sample size was calculated using the following formula:

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

α= 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 [11].

Each participant had to first sign a consent form before any enrollment. A questionnaire was then administered, and a blood sample was taken and sent to the Microbiology Laboratory of the University of Yaoundé I, Faculty of Science, located in the Centre region of Cameroon, which served as the biological analysis site for the samples. Missing demographic (age and sex) and clinical data (clinical manifestations, disease stage, and therapeutic protocol) from the interview could be completed using the medical record. The study was approved by the ethics committee of the Centre region of Cameroon under reference N/Ref: (N °0082/CR ERSHC/2023).

2.1. Study Subjects

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

In this work, for the analysis of comorbidity screening, we primarily used diagnostic algorithms and prescriptions for tests or consultations.

2.2. Sample Collection and Laboratory Analysis

Blood sampling was performed from the veins in the antecubital fossa, and blood samples were collected in dry tubes without anticoagulant. Subjects were invited to fast for at least 8 to 12 hours before collection [12].

Serology of HBV: HBsAg, HBeAg and Ab anti-HBs, Ab anti-HBe, Ab anti-HBc IgG antibody detection was performed on serum samples using the One Site HBV- 5 Rapid Test (HBV 5 in 1 Combtest (S/P) (Nantong, Diagnos Biotechnologyco., China) according to the manufacturer's instructions.

Serology of HCV: IgG rapid diagnostic test for Biolin-specific antibodies: HCV-specific IgG antibody detection was performed on serum samples using the One Step Rapid Test HCV AB Test Cassettes (S/P) (Hightop Boitech, China) according to the manufacturer's instructions.

Serology for the search for HIV virological status (IgG

anti-HIV-1 and IgG anti-HIV-2): The diagnosis was made by the Immunocomb II HIV-1-2 bispot (Orgenics), which is a rapid test for screening and differentiating antibodies against HIV-1-2, according to the kit manufacturer's instructions.

Serology for the search for EBV virological status: was performed in (plasma) samples by a qualitative method using Epstein Barr (EB)-IgM antibody Rapid Diagnostic Tests, according to the kit manufacturer's instructions (Bioneavan co.LTD., Beijing).

Serology for the search for TORCH: IgG and IgM rapid diagnostic test for TORCH-specific antibodies: HSV-specific IgM/IgG antibody detection was performed on serum samples using the One Step TORCH IgM/IgG kit (RV IgM/IgG, CMV IgM/IgG, HSV-I/2 IgM/IgG) (Bioneavan co.LTD., NO. 18 Ke YuanLu, GongYeKaiFaQu, Huang Cun Zhen AaXing County, Beijing) according to the manufacturer's instructions.

Lymphotyping of CD4+ T cells: CD4 LT measurement was carried out according to the principle of immunophenotyping [13] Fifty microliters (50 µL) of whole blood was used for analyses using the DB FACSCount reagent kit.*(Becton Dickinson Immunocytometry Systems)*, automated machine (BD Biosciences, San Jose, California, USA). Samples were analyzed based on the manufacturer's guidelines.

2.3. Diagnosis of Comorbidities

Tuberculosis: Tuberculosis is a chronic mycobacterial infection related, in most cases, to a pathogenic bacillus of the Mycobacterium tuberculosis complex (M. tuberculosis, M. bovis, M. africanum, and M. canettii). It is also called Koch's bacillus (KB). The M. tuberculosis bacillus is transmitted via the airborne route through suspended microdroplets containing the live bacillus. This transmission is sustained by sick individuals with respiratory tuberculosis who are excreting bacilli [14].

Type 2 Diabete: Diabetes is one of the most common diseases of the elderly, with almost 10% suffering overtly and probably another 10% unrecognized. Diabetes type 2 (T2DM) alone or associated with metabolic syndrome is related to chronic inflammation [15] which in turn is related to the pro-inflammatory activity of the dipose tissue, leading to some degree of insulin resistance and decreased insulin production by pancreatic Langerhans islet cells. It has also been shown that some inflammatory markers, such as F2 isoprostane in urine, IL-6, tumor necrosis factor (TNF), and C-reactive protein (CRP), are increased in T2DM [16]. These complications are further associated with an inflammatory process exaggerated by the presence of advanced glycation end products. Thus, diabetes is itself an inflammatory disease and, combined with other alterations and with immunosenescence, further alters the immune response.

Chronic kidney disease (CKD) is characterized by a progressive loss of the kidneys' ability to filter blood, resulting in the accumulation of uremic toxins in the blood and the appearance of hydroelectrolytic disorders. It is accompanied by the loss of certain hormonal functions leading to the appearance of calcium-phosphate disorders and anemia. The causes of chronic kidney disease are multiple, but the common complications include: anemia, edema, osteopathy, and an increased risk of mortality, particularly from infectious and cardiovascular origins. At the end-stage renal disease (ESRD) stage, two replacement therapies are available: extracorporeal purification, also called dialysis, and/or kidney transplantation. While dialysis is associated with high morbidity and mortality, kidney transplantation improves the quality and life expectancy of patients and remains the treatment of choice for end-stage renal disease to date [17].

2.4. Statistical Analyses

Comparisons of mean results between HIV-positive subjects on ARVT, HIV-positive subjects without ARVT (newly diagnosed), and control subjects were performed using Student's t-test. The threshold for statistical significance was set at $P \leq 0.05$.

3. Results

Sociodemographic and Clinical Parameters

The average age in the study was 9.05±65.09 years, the children were in majority (56.76%) n=42/74) under 10 years old and female gender (68.92%, n=51/74).18/74 children (24.32%) were smoke and 17/74 concerned by alcohol consumption (22.97%). HIV-1 infection was the most encountered in our cohort, with 94.60% of participants at WHO stage I of the disease.

Table 1. *Baseline characteristics of the participants.*

Characteristics	Number (%)	Median
Sex		
Male	23 (31.08)	
Female	51 (68.92)	
Ages (Years)		
[0-5]	17 (22.97)	
[5-10]	25 (33.78)	
[10-15]	16 (21.62)	
[15-20]	16 (21.62)	
WHO stage		
I	70 (94.6)	
II	4 (5.4)	
Type of HIV		
HIV I	70 (94.6)	

Characteristics	Number (%)	Median
HIV II	4 (5.4)	
Protocol of treatment		
Frist measure		
TDF/3TC/EFV	59 (79.73)	
TDF/3TC/ATV	5 (6.76)	
TDF/3TC/NVP	1 (1.35)	
AZT/3TC/EFV	4 (5.4)	
AZT/3TC/NVP	5 (6.76)	
CD4 level		
[0-200]	3 (4.35)	
[200-349]	23 (95.83)	
[350-499]	2 (2.7)	
[500-1600]	41 (91.11)	
Ratio Lymphocytaire		
Ratio CD4:CD8 < 1	66 (89.02)	0.850
Ratio CD4:CD8 ≥1	8 (10.08)	1.160
Style of live		
Tabagisme	18	24.32
Alcoolisme	17	22.96
Risks Factors		
Coinfection HBV	14 (18.92)	
Coinfection HCV	7 (9.46)	
Total 74 (100)		

Table 2. *Frequency of HIV infection according to age group and alcohol consumption, Tabacco intake.*

Age group (years)	n (%)	Alcohol consumption n (%)	Tobacco intake n(%)
[0-5]	17 (22.97)	0	0
[5-10]	25 (33.78)	0	0
[10-15]	16 (21.62)	3 (4.05)	9 (12.16)
[15-20]	16 (21.62)	14 (18.91)	9 (12.16)
Total	74 (100)		

The co-infection that most affected study participants was related to HSV-1/-2 at 93.24% (n=69), followed by EBV at 22.97% (n=17) and CMV at 12.2% (n=9). According to these results, only co-infections associated with HBV and HCV were found to be significant.

Table 3. *Distribution of participants based on the different infections tested.*

Infectious Agent	n (%)	95% CI
HCV	7 (9.46)	[3.89-18.52]
HBV	14 (18.92)	[10.75-29.70]
CMV	9 (12.2)	[5.6-22.6]
EBV	17 (22.97)	[13.99-34.21]
Total 74 (100)		

Legend: n: effectif of participants, IC $_{95\%:}$ Intervalle of confiance; HCV: Hépatite C Virus; HBV: Hépatite B Virus; CMV: Cytomégalovirus; EBV : Epstein-Barr Virus

Non-HIV-related Comorbidities

In this study, comorbidities were reported in 32.43% of the 74 participants, including 3 participants (4.05%) who presented with type I diabetes, 1 participant (1.35%) presented with prostatitis, 1 participant (1.35%) presented with renal insufficiency, and 50 participants (67.57%) did not present any comorbidities.

Table 4. *Prevalence of comorbidities in at-risk HIV-positive individuals.*

Ratio lymphocytaire T CD4:CD8 < 1	
Presence of Comorbidities	**Absence of Comorbidities**
24 (32.43%)	50 (67.57%)
Comorbidities	
Type of comorbidities	n (%)
Malaria	17 (70.83)
Prostatitis	1 (4.17)
Type I diabetes	2 (8.33)
renal insufficiency	1 (4.17)
Typhoide	1 (4.17)
Type I diabetes + Malaria	1 (4.17)
Tuberculosis+Malaria	1 (4.17)

4. Discussion

This is the first study to investigate the prevalence of chronic comorbidities and the use of comedications in PLHIV in Cameroon taking antiretrovirals. Immunosenescence sug-

gests that the immune system as a whole is aging, not in one block, but with certain parts aging more than others. The immune response is composed by two distinct, but closely interrelated parts: the innate and the adaptive parts [18]. The research found that chronic comorbidities were common in PLHIV in Cameroon, particularly diabetes and Kidney failure among younger patients. In terms of burden by age group, this study revealed that younger patients had greater numbers of chronic comorbidities and also used greater numbers of co-medications than younger patients did.

In our present work, comorbidities were observed in children born HIV-positive to seropositive mothers; other studies have reported the presence of comorbidities in PLHIV (People Living with HIV) [19-21]. The results of this study confirm the facts already presented by Guaraldi et al. (2011) in terms of prevalence and precocity [22]. Regarding comorbidities, the study revealed a significant association between the duration of treatment and the occurrence of comorbidities in PLHIV ($p<0.05$). Furthermore, the prevalence of comorbidities increased from 43.24% to 50% during follow-up. Although these figures are lower than those reported in other studies [19-23], they underscore the importance of comprehensive care for PLHIV, taking into account traditional risk factors and immunosenescence. Diabetes and tuberculosis are the most frequently observed comorbidities in this study. The prevalence of diabetes (10.81%) is lower than that reported by Mbaga et al. (2022), but nevertheless justifies systematic screening in PLHIV on antiretroviral therapy. The prevalence of tuberculosis (1.35%) is also lower than that observed by Keita et al. (2022), but requires close monitoring due to the increased risk of complications in PLHIV. These results suggest that PLHIV, even with an undetectable viral load, have an increased risk of non-HIV-related comorbidities compared to the general population, which could be linked to accelerated aging [10]. Chronic inflammation and persistent immune activation, even under effective antiretroviral therapy, could play a role in the development of these comorbidities (Markowitz et al., 2010). Our clinical data reveal a predominance of participants at WHO stage I (70 out of 74), with only 4 participants at stage II. This observation is consistent with the results of a descriptive prospective study conducted in Conakry by Keita et al. (2022), which reported a majority of participants in stage I (21 out of 45). This distribution could be attributable to the test and treat policy implemented in Cameroon since 2015, favoring early management of the infection. Furthermore, the prevalence of HIV-1 (93.24%) observed in the present study is consistent with the global trend, where this serotype is the most widespread. HIV-2, on the other hand, is more common in West Africa [24]. Regarding antiretroviral treatment (ARVT), the results of this study indicate that two-thirds of the children (79.72% at the first sampling and 66.21% at the second) were receiving a Tenofovir-Lamivudine-Efavirenz (TDF/3TC/EFV) based regimen. This predominance of TDF in the therapeutic regimens (more than half of the ongoing therapies) aligns with the observations of Penda et al. (2018) and reflects the rigorous application of national and WHO (2013) guidelines for ART management in adolescents. The median duration of viral undetectability (4 years) and the median treatment duration (6 years) that we observed are slightly higher than the results reported by Han et al. (2020). It is important to emphasize that these data are encouraging and suggest sustained effectiveness of ARVT in our population. It is relevant to recall that several studies have demonstrated the association between early initiation of ARVT and a better CD4:CD8 T-lymphocyte ratio in children [25, 26]. These results highlight the importance of early screening and management of HIV infection to optimize the immune status of patients.

The study reveals a significant increase in CD4+ and CD8+ T lymphocytes after the initiation of antiretroviral therapy, indicating immune restoration. However, despite viral suppression, a persistent immune imbalance (CD4+ < CD8+) was observed in the majority of patients, with an average CD4:CD8 ratio below 1 throughout the follow-up. This result is consistent with other studies [27] and suggests that normalization of the CD4:CD8 ratio may be difficult to achieve, even with effective viral suppression. Several factors could explain this imbalance, including late initiation of antiretroviral therapy, as suggested by Thornhill et al. (2016). Studies have also shown that an inverted CD4:CD8 ratio is associated with chronic immune activation and an increased risk of non-HIV-related comorbidities [28, 29]. The CD4:CD8 ratio could therefore be a more relevant marker of immunological dysfunction and morbidity risk than the CD4+ cell count alone. Regarding the seroprevalence of infectious agents, the study reveals a strong association between treatment duration and the incidence of infectious agents, particularly cytomegalovirus (CMV) and herpes simplex virus (HSV). The prevalence of CMV increased considerably during follow-up, reaching a high level comparable to that reported by Mazeron (2009) [30]. This high prevalence underscores the need for careful monitoring of PLHIV, as CMV infection can be associated with premature aging and co-infections with other pathogens [31]. Indeed, the high prevalence of Herpes Simple Virus type-1 (HSV-1) and Herpes Simple Virus type-2 (HSV-2) observed in this study is also concerning. Cameroon being an endemic area for herpesviruses [32], this high prevalence could be explained by geographical and behavioral factors [33, 34]. HSV-2, in particular, is associated with serious complications, including vertical transmission to the fetus [20, 35] and facilitation of HIV transmission [36]. The prevalence of Epstein Barr-Virus (EBV), although lower than that of CMV and HSV, remains concerning and underscores the need for systematic screening for Herpesviridae in PLHIV.

The present study was able to highlight the presence of comorbidities in PLHIV, although we were unable to determine the exact cause of these comorbidities. This work demonstrates a number of limitations such as the small sample size and the short duration of the study, which do not provide enough statistical power to generalize the results of this study to all children born with HIV at the Yaoundé University Teaching Hospital. Comorbidities and treatments that were not evaluated before diagnosis. The true causes of these

comorbidities were not established. The absence of certain information.

5. Conclusion

In conclusion, comorbidities and chronic complications are common among PLHIV in Cameroon. This suggests the need to strengthen the follow-up of people born with HIV who are on ARVT. Application of our results can support the development of optimal healthcare strategies for this growing population.

Abbreviations

AIDS	Acquired Immunodefciency Syndrome
ATC	Approved Treatment Center
ARVT	Antiretroviral Treatment
CKD	Chronic Kidney Disease
CMV	Cytomegalovirus
CRP	C-Reactive Protein
EBV	Epstein Barr-Virus
ESRD	End-stage Renal Disease
HIV	Human Immunodeficiency Virus
HSV-1	Herpes Simple Virus Type-1
HSV-2	Herpes Simple Virus Type-2
YUTH	Yaounde University Teaching Hospital
RI	Renal Insufficiency
KB	Koch's Bacillus
PLHIV	People Living With HIV
TNF	Tumor Necrosis Factor

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Author Contributions

Mbongue-Mikangue Chris André is the sole author. The author read and approved the final manuscript.

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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 author declares no conflict of interest.

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