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# Seroprevalence of Cytomegalovirus, Epstein-barr Virus and Herpes Simplex Viruses in Children Born HIV Positive at the Yaounde University Teaching Hospital, Cameroon

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors RESH and NBJ designed and set up the research project. Authors MMCA, SLB, MMM and MFA collected the samples, with NBJ and MDS, led the technical aspects at the microbiology laboratory. The analysis of the data and the writing of this article saw the collaboration of all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Cameroon is a country located in sub-Saharan Africa, which is an area endemic to *herpesviridae* family, there is very little data on the herpes virus infections epidemiology, especially associated with HIV infection.

**Aims:** The aim of our study was to determine the seroprevalence of four herpes viruses that are cytomegalovirus (CMV), Epstein-Barr virus (EBV), Herpes Simplex virus 1 (HSV-1) and 2 (HSV-2) in HIV-positive patients in Yaoundé University Teaching Hospital.

**Methodology:** the study was prospective cross-sectional and took place at Yaounde University Teaching Hospital and Department of Microbiology, Faculty of Sciences, University of Yaounde I, between November 2020 and April 2021. We included consecutively 74 on children living with HIV born HIV positive, on antiretroviral treatment (23 men, 51 women; age range 3-19 years), and whose medical file was complete and available within the ATC. IgG/IgM antibodies against HSV-1, HSV-2, CMV, and IgM against EBV were qualitatively determined by Rapid Diagnostic Tests, for the detection of these pathogens. The statistical analysis was done using IBM SPSS version 22.0, the Fisher exact and the Khi-sqaure tests to compare qualitative variables between groups, while on the other hand, we used the Mann-Whitney test to compare quantitative variables. All *P* values below 0.05 were considered significant.

**Results:** A total of 74 participants were enrolled in the study with a female predominance of 68.92% (n=51/74). The average age of our serie was 9.05±5.09 years, and a majority of participants was under 10 years old (56.76%, n=42/74). HSV-1, HSV-2, EBV and CMV Seroprevalences were 93.24%, 93.24%, 22.97% and 12.2% respectively. Other parameters such as sex, age, stage of disease, smoking and alcohol consumption were significantly associated with the seropositivity of these *herpesviridae*.

**Conclusion:** Despite the absence for most of the clinical manifestations related to HSV-1 and HSV-2, it was strong to note a high circulation of those virus in HIV infected patients, mainly in bi and tri co-infections.


*Keywords: Children; CMV; EBV; Herpesviridae; HIV; HSV-1; HSV-2; seroprevalence.*

## 1. INTRODUCTION

Human Immunodeficiency Virus Infection (HIV) remains a serious public health problem worldwide, affecting adults, teenagers and children of both sex [1] In 2016, 2.75% (2.1 million) of the global population of individuals living with HIV (estimated at 76.1 million) were children and adolescents under 15 years of age. About 200000 of this group were infected through Mother To Child Transmission (MTCT) [2] and according to the data of the National AIDS Control Committee (NACC), Cameroon in 2021, the prevalence of HIV infection was 3.1% [3]. HIV infection promotes exogenous infections or reactivation of solely controlled infections, with the LTCD4+ lymphopenia. With the weakening of the immune system, the reactivations concern more tuberculosis and the oncogenic viruses such as herpes virus [4]. A study done by Njimbam et al., In 2016 showing that there is very high prevalence of Herpes simplex virus (HSV) which was 88 % among PLHIV at the cite verte subdivisional Medical Centre of Yaounde [5]. An another study, done by Ouedraogo et al. in 2016 showing that there is very low prevalence of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) which was 5% respectively in pregnant women [6].

*Herpesviridae* are a family of managerial virus such as Kaposi Sarcoma and lymphomas, opportunistic infections of HIV/AIDS [7]. EBV and CMV are involved in infectious monocleosis and associated lymphomas, while the type 8 human herpes virus (HHV8) enters I the occurrence of Kaposi Sarcoma (KS) and associated lymphomas. The seroprevalence of HHV-8 varies across geographical regions and subpopulations [8,9]. This seroprevalence, while relatively low in northern countries, is high in Africa with more than 50% in some countries such as Cameroon [10-12]. A high prevalence of HHV-8 is observed in people with immunodeficiencies such as PLWHIV [13].

Herpesvirus EBV, CMV are viruses that evolve based on pandemic modeling and are responsible for congenital infections causing severe sequelae in infants. The immunosuppression of the individual, obesity, excessive alcohol and smoking considers as risk factors for the emergence of these viruses

[14,15]. Although Cameroon is an endemic zone for human herpes virus, and the work done by Njiki et al., In 2015 showing that there is very little information on the epidemiology of HHV8 infection particularly that associated with HIV infection [12], the finding remains with other viruses of the same family. Epidemiological data on the extent of opportunistic infections in immunocompromised people due to HIV are gradually postponed with little work highlighting the impact of the herpes virus on the HIV/AIDS field in sub-suharan Africa and especially in Cameroon where herpes virus simplex (HSV) promotes HIV infection and its pathological progression. The interaction between HSV and HIV would promote significant replication of HIV and progression to the AIDS stage [16]**.**

In this context of endemicity, in Cameroon very little data is available concerning the epidemiology of *herpesviridae* and an absence of systematic diagnosis of *herpesviridae* in people born with HIV, in order to fill this gap, the aim of our study was therefore to determine the seroprevalence of four HSV-1, HSV-2, EBV and CMV in patients born HIV positive at the Yaounde University Teaching Hospital (YUTH).

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Context

It was a prospective cross-sectional study, performed for a period of 6 months, from November 2020 and April 2021, at the YUTH, on patients who came for consultation or were followed at the Approved Treatment Center (ATC). For every participant who completed the inclusion criteria, a written informed consent was obtained from the parents, and a technical sheet had to be filled by each participants, providing the socio-demographic and clinical status as age, sex and HIV infection.

### 2.2 Data Collection Tool and Procedure

These data were completed and/ or confirmed by the patient's medical record. Then a sampling of venous blood into an EDTA tube of 5 ml was made and the samples taken then transported to the Microbiology Laboratory of the Faculty of Science. At the laboratory, after centrifugation (at 1300 tours/min for 10 min, between (18-24°C), the plasma obtained was kept in a freezer at -25°C, for the later serological research of the infectious agents [17]. According to the manufacturer's instructions, the kit and samples to be tested were first brought to room temperature. For the detection of IgM and IgG antibodies directed against CMV, HSV-1 and HSV-2 micro-organisms, the *One Step TORCH IgM/IgG Kit( TOX IgM/IgG, RV IgM/IgG, CMV IgM/IgG, HSV-1/-2 IgM/IgG (Bioneavan co.LTD., NO.18 Ke YuanLU, GongYeKaiFaQu, Huang Cun Zhen AaXing County, Beijing) was used, and for the detection of IgM antibodies directed against EBV, the kit Diagnostic Rapid Epstein-Barr(EB)-IgM antibodies was used according to the kit manufacturer's instructions (Bioneavan co.LTD., Beijing).* The test card was placed on a dry horizontal work surface, then 30 µl of sample plasma added on top. When the sample migration was found to be difficult, 20 µl of sample dilution solution was added immediately, and an additional 50 µl of the same solution 5 minutes later. 15 to 20 minutes after the addition of the plasma, the different results were observed [18].

### 2.3 Sample Size Calculation

The minimum sample size was 46.15 participants. The calculation of this sample size was made using the prevalence of HIV, which are 3.1 % in Cameroon [3]. We used the following formula [19]:

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

Z = the level of statistical significance with a 95% confidence interval (CI) of 1.96; i= the level of precision of 0.05; P =prevalence of outcomes.

### 2.4 Data Management and Analysis

For each participant, data on parameters of interest gathered through interviews and by blood analysis were recorded and processed using Excel 2016, and the statistical analysis was done using IBM Statistical Package for Social Science Version 22.0. on the one hand, we used the Fisher exact and the Khi-sqaure tests to compare qualitative variables between groups, while on the other hand, we used the Mann-Whitney test to compare quantitative variables. All *P* values below 0.05 were considered significant.

## 3. RESULTS

### 3.1 Sociodemographic and Clinical Parameters

The average age in the study was 9.05±65.09 years, the children were in majority (56.76%,

**Table 1. Baseline characteristics of the participants [20]**

| Characteristics | Number | Percentage(%) |
|---|---|---|
| **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 |
| III | 0 | 0 |
| IV | 0 | 0 |
| **Type of HIV** | | |
| HIV I | 70 | 94.6 |
| HIV II | 4 | 5.4 |
| HIV I +HIV II | 0 | 0 |
| **Protocol of treatment** | | |
| **Frist measure** | | |
| TDF/3TC/EFV | 59 | 79.73 |
| TDF/3TC/ATV | 5 | 6.76 |
| TDF/3TC/NVP | 1 | 1.35 |
| TDF/3TC/D | 0 | 0 |
| TDF/3TC/LPV | 0 | 0 |
| AZT/3TC/EFV | 4 | 5.4 |
| AZT/3TC/NVP | 5 | 6.76 |

**Table 2. Distribution of participants according to IgM/IgG antibodies against HSV-1, HSV-2, CMV and IgM against EBV results**

| Infections agent | Result | Number(n) | Percentage (%) | $IC_{95\%}$ | *P* Value |
|---|---|---|---|---|---|
| CMV(IgG | Positive | 9 | 12.2 | [5.6-22.6] | 0.8 |
| | negative | 65 | 87.84 | [78.16-94.29] | |
| EBV (IgM) | Positive | 17 | 22.97 | [13.99-34.21] | 0.6 |
| | Negative | 57 | 77.03 | [65.79-86.01] | |
| HSV-1(IgG) | Positive | 69 | 93.24 | [84.93-97.77] | 0.04 |
| | Negative | 5 | 6.67 | [2.23-25.07] | |
| HSV-2(IgG) | positive | 69 | 93.24 | [84.93-97.77] | 0.04 |
| | Negative | 5 | 6.76 | [2.23-15.07] | |

**Table 3. Distribution of participants according to *Herpesviridae* co-infections**

| Infections | N(%) | $IC_{95\%}$ |
|---|---|---|
| HIV-1 | 5(6.8) | [2.23–15.07] |
| HIV-1/CMV/EBV/HSV-1/HSV-2 | 2(2.70) | [0.33–9.42] |
| HIV-1/CMV/HSV-1/HSV-2 | 6(8.1) | [3.03–16.82] |
| HIV-1/CMV/RV/HSV-1/HSV-2 | 1(1.35) | [0.03–7.30] |
| HIV-1/EBV/HSV-1/HSV-2 | 11(14.86) | [7.66–25.04] |
| HIV-1/HSV-1/HSV-2 | 43(58.1) | [46.06–69.49] |
| HIV-1/RV/EBV/HSV-1/HSV-2 | 1(1.35) | [0.03–7.30] |
| HIV-1/RV/HSV-1/HSV-2 | 1(1.35) | [0.03–7.30] |
| HIV-2/CMV/HSV-1/HSV-2 | 1(1.35) | [0.03–7.30] |
| HIV-2/EBV/HSV-1/HSV-2 | 1(1.35) | [0.03–7.30] |
| HIV-2/HSV-1/HSV-2 | 1(1.35) | [0.03–7.30] |

*Legend : HSV-1/-2 : Herpès simplex Virus-1/-2 ;HIV-1: Human immunodeficiency virus type 1; HIV-2: Human immunodeficiency virus type 2 ; CMV : Cytomegalovirus ; EBV: Epstein-Barr virus, RV: Rubella virus*

**Table 4. Frequency of HIV infection according to age group and comorbidities**

| Age group (years) | Numbern (%) | Number of co-infection found | Alcohol consumption n (%) | Tobacco intake n(%) |
|---|---|---|---|---|
| ]0-5[ | 17 (22.97) | 17 | 0 | 0 |
| [5-10[ | 25 (33.78) | 24 | 0 | 0 |
| [10-15[ | 16 (21.62) | 16 | 3 (4.05) | 9 (12.16) |
| [15-20[ | 16 (21.62) | 16 | 14 (18.91) | 9 (12.16) |
| **Total 74 (100)** | | | | |

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. According to medical records, the most commonly used protocol was Tenofovir-Lamivudine-Efavirenz (TDF/3TC/EFV) with 79.73% of participants.

### 3.2 Seroprevalence of *Herpesviridae*

The seroprevalence of cytomegalovirus was 12.2% (n=9/74), that of HSV-1 at 93.24%(n=69/74), that of HSV-2 at 93.24% (n=69/74) that of EBV was 22.97%(n=17/74). Seropositivity for HSV-1 and HSV-2 was statically associated with HIV infection ($P$ <.05) (Table 1). Our participants presented multi-infections to *herpesviridae*. The majority of co-infections found was HSV-1/HSV-2 93.24 % (n=69) (Table 2). The co-infections found concerned all age groups (Table 3). Ages ranging from 0 to 15 years concentrate the de greatest number of co-infections and Ages ranging from 10 to 19 years concentrate the risk factors for the occurrence of these co-infections, and all the consumption of alcohol(n=17/74) and tobacco (n=18/74) (Table 3).

## 4. DISCUSSION

The main objective of our study was to determine the seroprevalence of four HSV-1, HSV-2, EBV and CMV in patients born HIV positive at the Yaounde University Teaching Hospital.

Our study revealed a female predominance (68.92%, n=51/74) with an average age of 9.05±5.09 years, the majority of participants were under 10 years. A study conducted by Njimbam et al., at the cite verte subdivisional Medical Centre of Yaounde in 2016, reported 66% of women [5]. This observation corroborates the feminization of the HIV pandemic, which is a major trend. Currently 50% of people living with HIV in the world are women, with this rate reaching 59% in sub-saharan Africa, the region most affected by the epidemic. Certainly, the risk of contamination during sexual intercourse is greater among theme, but they are also in a situation of greater social and economic vulnerability, and therefore greater exposure to risks, particularly in relation to AIDS [21]. The age groups obtained during our study follow the concerned population trend of PLHIV in Cameroon [16]. HIV-1-infected patients (94.6%) were predominant. These trends were also reported in 2014 by Sagna et al, who found in their study 92.7% (HIV-1) However, Karfo et al reported in 2018 a lower proportion of HIV-1-infected persons compared to our results (80.9%) [22]. Females were more representative among HIV-infected patients and this was also the bias reported by Ky-Zerbo et al. [23]. This same observation was made by WHO in 2017 and shows that women are the most affected by HIV-1 [24,25]. Most of our participants were under TDF/3TC/EFV treatment protocol (79.73%), a first-line regimen. This protocol is a preferred option in countries with limited resources, because it is simple, inexpensive, in combined form and can be used in HIV/HBV, HIV/BK and in pregnant and breastfeeding women [3].

In our study, IgG seroprevalence were 93.24% for HSV-1,93.24% for HSV-2, 12.20% for CMV, IgM seroprevalence was 22.97% for EBV. This high antibody seroprevalence demonstrates the widespread circulation of these viruses in the population, confirming the status of an endemic area for herpes viruses [12]. Also to date, population changes her sexual practices, such as oral sex associated with sociocultural changes, may explain the increasing trend of these infections [14]. The majority of co-infections between *herpesviridae* found concerned HSV-1, HSV-2 and EBV. EBV and CMV co-infections appear to be more frequent in the literature. A possible explanation is that most of the patients in this cohort, although hospitalized, were seen in outpatient care [26]. Testing for the presence of CMV infection is particularly important for HIV-positive patients to assess disease severity and monitor response to treatment. The influence of factors not investigated in this study such as genetics, nutritional status, socio-economic conditions could explain these a priori weight results concerning the prevalence of CMV [27].

Ages ranging from 0 to 15 years concentrate the greatest number of co-infections, and ages ranging from 10 to 19 years concentrate the risk factors for the occurrence of these co-infections (alcohol and tobacco consumption). If alcohol acts as a behavioral risk factor in acquisition of HIV, it also acts at the biological level through its immuosuppressive role increasing susceptibility to infections by reducing the inflammatory response [28], thus finding the smoking which is also associated with the metabolic complications of certain antiretroviral [2]. These data underline

the need to implement preventive actions, particularly with regard to the consumption of alcohol and increased dietary support [29].

## 5. CONCLUSION

Our study allowed us to evaluate the co-infection of four *herpesviridae* (EBV, CMV, HSV-1 and 2) in children born HIV positive at the YUTH. This study highlighted a high risk of herpesvirus infections among PLHIV born HIV positive, in ages ranging from 3 to 19 years, especially among women. The seroprevalences of these viruses were high and their association with children clinical profile highlights their risk factors. HSV-1, HSV-2 and EBV seroprevalences were lower in coinfection than in monoinfection. The prevalence of these viruses demonstrates their high endemicity in Cameroon.

## CONSENT

As per international standards or university standards, participants written consent has been collected and preserved by the authors.

## ETHICAL APPROVAL

The study was appoved by the Regional Ethics Committee for Research in Human Health (N°0082/CRERSHC/2023), and received authorization from the Yaounde University Teaching Hospital (N°494/AR/CHUY/DG/DGA/CAPRC).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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