

Research Article

Seroprevalence of IgG Antibodies Against Herpes Simplex Virus 1 and 2 in Children Born HIV Positive at the Yaounde University Teaching Hospital

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Abstract

Background: Infection with the Human Immunodeficiency Virus (HIV) is responsible for the terminal stage of Acquired Immunodeficiency Syndrome (AIDS), which was a latent infection until the introduction of antiretroviral treatment. The progression to chronicity of this infection leads to chronic inflammation that facilitates the occurrence of opportunistic infections such as herpes simplex virus types 1 and 2 (Herpes Simplex Virus-1 (HSV-1) and Herpes Simplex Virus-2 (HSV-2)). The lack of data on these viruses has led to the present study. **Objective:** aimed to determine the seroprevalence of IgG antibodies against herpes simplex virus types 1 and 2 in children born HIV-positive to HIV-positive mothers with an undetectable viral load and receiving antiretroviral treatment at the Yaounde University Teaching Hospital (YUTH). **Method:** The study was cross-sectional conducted after 12 months of follow up. After administering a questionnaire, a blood sample was obtained from each participant in an EDTA tube and analyzed using rapid diagnostic tests for the detection of specific IgM/IgG antibodies. Statistical analysis was performed using Microsoft Excel 2019 and SPSS version 25. A P-value <0.05 was considered statistically significant at a 95% confidence interval. **Result:** Among the 74 participants, 51 were female, resulting in a sex ratio of 0.45. In the population of children born HIV-positive, the seroprevalence was 93.24% (n=69) for IgG anti-HSV-1, 93.24% (n=69) for IgG anti-HSV-2, and 93.24% (n=69) for IgG anti-HSV-1/-2. The seroprevalence of IgM anti-HSV was found to be zero; however, the prevalence of IgG anti-HSV was 93.24%. The seroprevalence of HSV-1/-2 was associated with the age of the participants. **Conclusion:** It can be concluded that the herpes simplex virus circulates in Cameroon, with a high presence of anti-HSV-1/-2 antibodies in the population born HIV-positive to HIV-positive mothers. It becomes important to implement systematic diagnostic measures for children born HIV-positive to HIV-positive mothers, and its transmission is associated with various risk factors.

Keywords

YUTH, HSV-1, HSV-2, HIV, AIDS

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1. Introduction

43 years after the discovery of the Human Immunodeficiency Virus (HIV) worldwide, it has become certain over the years that its transmission and progression lead to Acquired Immunodeficiency Syndrome (AIDS), relying on several biological factors, among which are opportunistic infections [1, 2]. These opportunistic infections are frequently encountered in developing countries [3]. Opportunistic infections present differently depending on their origin (bacterial, viral, parasitic, and fungal); those of viral origin, particularly infections caused by the Herpes Simplex Virus (HSV), are responsible for a contagious disease affecting the skin and mucous membranes, characterized by a vesicular eruption of grouped lesions [4]. This disease, long considered benign in immunocompetent individuals, can prove to be very severe in subjects with immune deficiencies [5-8]. This is all the more important as infections caused by Herpes Simplex Virus types 1 and 2 (HSV-1 and HSV-2) would be frequent in contexts of limited resources, although generally asymptomatic notably in Cameroon, where HSV may facilitate HIV infection and its pathological progression [9]. HIV infection is a significant public health issue [10].

Cameroon remains in a generalized HIV epidemic situation (prevalence: 2.7%) [11]. Still neglected in our context of endemic herpes viruses [12] and generalized HIV epidemic, herpes would be emerging among PLHIV (People Living with HIV), especially in sub-Saharan African countries, thus suggesting the need for factual evidence. This is all the more important as infections caused by Herpes Simplex Virus types 1 and 2 (HSV-1 and HSV-2) would be frequent in contexts of limited resources, although generally asymptomatic [9]. In cases of viral reactivation with virus excretion, transmissibility is significant (even from an inactive carrier of the virus), and cellular immunity (T lymphocytes) plays an essential role in controlling this infection and in the replicative activity of the virus. The interaction between HSV and HIV would promote significant HIV replication and progression to AIDS stage. Furthermore, genital infection with HSV-2 would be a risk factor for acquiring HIV, facilitated by the ulcerative and inflammatory nature of the genital infection caused by HSV. Given its complete incurability and latent character in immunocompetent individuals in general, it is imperative to monitor this viral infection in individuals born HIV-positive from HIV-positive mothers for better clinical-biological management and improved prevention of HSV consequences in the context of HIV in routine clinical practice in Cameroon. It is also essential to understand potential risk factors as well as the general level of knowledge among patients about this opportunistic infection. The results arising from such studies would contribute to reducing morbidity and mortality among PLHIV in the Cameroonian context. In immunocompromised individuals (pregnant women, transplant recipients, or those infected with HIV), HSV-1 and HSV-2 infections can manifest as extensive chronic and debilitating lesions: oral or gen-

ital ulcers that are excavating and persistent, tracheitis, and painful esophagitis. Infections can also disseminate, affecting organs (hepatitis, pneumonia, encephalitis). Herpetic hepatitis is a rare manifestation but is associated with high mortality. It primarily occurs in immunocompromised patients or pregnant women [13]. In 2016, Cameroon implemented the "test and treat" policy [14].

Furthermore, according to several studies, the diagnosis of HSV is a significant public health challenge in developing countries [15, 16]. Hence, there is a need to update data concerning the epidemiology of herpes simplex virus. In Cameroon, there has been a noted lack of data on the seroprevalence of herpes simplex virus-1/-2 infection among individuals born HIV-positive. Additionally, although herpes simplex virus serology is not part of routine examinations for people living with HIV (PLHIV), it is not systematically conducted among PLHIV. The neglect of these tests, due to a lack of knowledge about the diseases and the cost of examinations in the facilities where they are conducted, often results in inadequate follow-up.

In light of this observation, in the present manuscript, we reported the seroprevalence of anti-IgM/IgG herpes simplex virus-1/-2 antibodies among children born HIV-positive to mothers with an undetectable viral load who are on antiretroviral therapy at the Yaoundé University Teaching Hospital.

2. Materials and Methods

2.1. Study Design

The study was a cross-sectional type conducted after a period of 12 months (November 2020 to October 2021) at the Treatment Center of the Yaoundé University Teaching Hospital (YUTH).

2.2. Sample Size Calculation

The minimum sample size was 162 participants. The minimum sample size was calculated using the prevalence of Herpes Simplex Virus 1 and 2 that was reported in Cameroon, which was 88% [17]. We used the following formula [18]:

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

Z = the level of statistical significance with a 95% confidence interval (CI) of 1.96; i = the level of precision of 0.05; P = The proportion of patients estimated to be infected by HSV-1/-2.

Given that the study was conducted during the COVID pandemic, during which patients were hesitant to come to the

hospital or even participate in a study, we were unable to reach the calculated sample size. Consequently, we decided to conduct a non-probabilistic sampling and consecutive.

2.3. Data Collection

Each participant had to sign a consent form before enrollment. A questionnaire was then administered, and a blood sample was collected and sent to the Microbiology Laboratory of the Faculty of Science, located in the Central Region of Cameroon, at the University of Yaounde I which served as the site for biological analysis of the samples. Demographic data (age and sex) and clinical information were collected. Missing information during the interview could be completed using the medical record. The study was approved by the ethics committee of the Central Region of Cameroon under reference N/Ref: (N°0082/CRERSHC/2023).

2.4. Participation Criteria for the Study

A total of 74 participants were included in the study. Exclusion criteria included being born HIV-positive with an HIV-positive mother on ART; having an undetectable viral load at the time of inclusion in the study (< 50 copies of RNA/ μ l); giving assent for adolescents aged 12 to 20 years; obtaining parental consent for children aged 0 to 20 years; giving informed consent for individuals aged 21 years and older.

2.5. Sample Collection

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.

2.6. Analyses de Laboratoire

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 (TOX IgM/IgG, 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.

The study was conducted during the COVID pandemic, when more specific diagnostic kits were in short supply and very expensive. To facilitate HSV screening, we were forced to find a kit that was easy to use and accessible to everyone. This test is a reference diagnostic kit to facilitate screening in remote areas. It has excellent specificity and medium sensitivity.

CD4 examination: CD4 counts were measured using the BD FACSCOUNT system (Becton Dickinson and Company, CA, USA) on all participants after whole blood samples were collected. It is a reference technique in the field of immunology, hence its use in this work.

2.7. Statistical Analysis

Data curation was performed using Excel 2019 and analyses were conducted with the statistical software SPSS, version 25 (SPSS, Chicago, Illinois, USA). On one hand, we used the Fischer exact and the Chi-square tests to compare qualitative variables between groups, while on the other hand, we used the Mann-Whitney test to compare quantitative variables. The threshold for statistical significance was set at $P \leq 0.05$.

3. Results

3.1. Sociodemographic and Clinical Parameters

A total of 74 participants were included in the study regarding demographic data, where females represented the vast majority of participants infected with HIV at 68.92% ($n=51$), while males accounted for only 31.08% ($n=23$), resulting in a sex ratio of 0.45. (Figure 1).

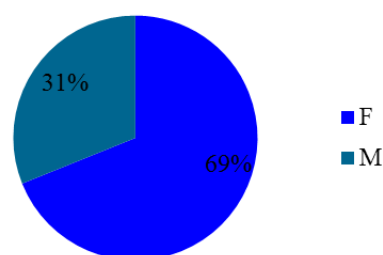


Figure 1. Distribution of participants by sex.

The 74 participants included had ages ranging from 3 to 19 years. The average age within the study was 9.054 ± 5.096 years. Figure 2 below shows that the most represented age group was [5-10], accounting for 33.78% ($n=25$). Based on the classification proposed by the WHO (2014) [19], which states that a person is considered a child if they are between 0 to 14 years old and an adolescent if they are between 15 to 19 years old, it appears that children were the most affected at 56.76% ($n=42$ children) with ages ranging from 0 to 9 years, compared to 43.24% of adolescents ($n=32$) whose ages ranged from 10 to 19 years. (Figure 2)

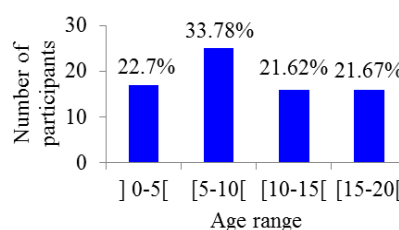


Figure 2. Distribution of participants by age groups.

3.2. Serological Parameters

Prevalence of herpes simplex virus in the study population

The prevalence of co-infection with HSV-1/-2 was 93.24%. In total, it was found that out of the 74 participants included, 69 were infected with HSV, resulting in an overall prevalence of

93.24% (n=69) (Table 1). Among the 51 women and 23 men enrolled, there was a feminization of the infection (69.57%) compared to men (30.43%); $p < 0.05$ (Table 2). Whether for HSV-1 or HSV-2, the vast majority of the infected population (78.26%) falls within the age range of 3 to 14 years (Table 3).

Table 1. Distribution of patients by type.

Type of HSV	Size (n=74)	IC ₉₅	Percentage (%)
HSV-1	69	[84.93-97.77]	93.24
HSV-2	69	[84.93-97.77]	93.24
HSV-1/-2	69	[84.93-97.77]	93.24
Absence of HSV-1/-2	5	[2.23-15.07]	6.76
74 (100%)			

Table 2. Gender wise distribution of HSV.

Number	Gender		HSV-1 only	HSV-2 only	HSV-1+HSV-2	Absence of HSV-1/-2
			HIV infected persons			
1	Male sex (n=23/74, 31.1%)	N	21	21	21	2
		%	91.30	91.30	91.30	8.7
2	Female sex (n=51/74, 68.92)	N	48	48	48	3
		%	94.12	94.12	94.12	5.9

Table 3. Years wise distribution of HSV and Risks factors.

Age group (years)	n (%)	Alcohol consumption n (%)	HSV-1/-2	Tobacco intake n (%)
[0-5]	17 (22.97)	0	17 (24.64)	0
[5-10]	25 (33.78)	0	21 (30.43)	0
[10-15]	16 (21.62)	3 (4.05)	16 (23.19)	9 (12.16)
[15-20]	16 (21.62)	14 (18.91)	15 (21.74)	9 (12.16)
Total 74 (100%)				

HSV infection based on immune status

When evaluating the association between infection and immune status, no significant difference was observed ($p > 0.05$). Thus, immune status in terms of CD4⁺ counts did not have a significant impact on the occurrence of HSV-1 and/or HSV-2 infection.

However, the distribution of infected patients based on clinical signs and CD4⁺ T-cell counts shows that the vast

majority of patients presenting clinical signs are those with competent immune systems, with 59.42% (n=41) having CD4⁺ T-cell counts ranging from [500-1600], with a statistically significant difference ($p < 0.05$) compared to those with CD4⁺ T-cell counts < 500 cells/mm³. Despite the subjective nature of patient history, the most common clinical sign is cold sores (14.54%). (Table 4)

Table 4. Distribution of HSV-1 and HSV-2 seropositive patients by the rate of CD4⁺ count.

Groups of CD4 ⁺ LT count (cellules/mm ³)	HSV-1 n(%)	HSV-2 n(%)	HSV-1/-2 n(%)	Size (%)	P-Value
[500-1600]	41 (91.11)	41 (91.11)	41 (91.11)	45 (60.8)	0.002
[350-499]	2 (2.7)	2 (2.7)	2 (2.7)	2 (2.7)	0.05
[200-349]	23 (95.83)	23 (95.83)	23 (95.83)	24 (32.4)	0.002
[0-200]	3 (4.05)	3 (4.35)	3 (4.35)	3 (4.1)	0.05

4. Discussion

The present study aimed to determine the seroprevalence of herpes simplex virus types 1 and 2 among individuals born HIV positive followed at the Yaoundé Central Hospital and University to fill the gap in epidemiological data regarding herpes viruses 1 and 2. Generally, the prevalence of HSV was associated with the female sex in the results of this study, which is consistent with findings by Nwadike et al., [20] but contradicts the work of Karad et al. [21]. The seroprevalence of HSV-1 is very high among people living with HIV (93.24%), and our results are higher than those reported by Njimban et al., which was 32% [17] and of Onu et al. which was 76.8% [22]. This high prevalence of HSV could be explained by the fact that Cameroon is located in an area of high endemicity for herpes viruses [12].

The seroprevalence of HSV-2 was 93.24% in our study, which is higher than the results obtained by Onu et al. in Nigeria and by Remis et al. in Canada, which were 56.2% and 86%, respectively [22, 23]. These differences in prevalence may be due to variations in sampling methods, study populations, geographical regions, as well as behaviors of the study population [17, 22, 24]. Herpes simplex virus type 2 (HSV-2), the primary cause of genital herpes, is also responsible for genital ulcers [4, 25]. It can be transmitted vertically to the fetus and is associated with severe consequences for its development, leading to mortality rates reaching 60% in some countries, while the other 40% who survive this infection suffer from very serious damage. [26].

In the case of HSV-2, seroprevalence varies significantly by geographical location and is higher in Sub-Saharan Africa (80% seropositive for HSV-2). The prevalence of HSV-2 is consistently higher among individuals who are HIV positive, sex workers, and individuals with other sexually transmitted infections. Indeed, due to its ulcerative nature, genital HSV-2 infection seems to facilitate HIV transmission [27]. Moreover, the impact of genital herpes is major on the emotional, sexual, and social lives of affected individuals, and it is one of the most common STIs and the leading cause of infectious genital ulcers worldwide [28]. Furthermore, awareness of the signs and symptoms associated with HSV is low in this study, at

only 34%, which is higher than the result obtained in the study by Njimban et al. [17]. Although knowledge about herpes viruses is becoming increasingly widespread, it remains necessary to continue raising awareness among HIV-positive individuals about prevention methods for herpes viruses, as emphasized by Wald et al. [14].

This similar prevalence of HSV-1 and HSV-2 in our study population can be attributed to the similar transmission routes but also to the endemic nature of the sub-saharan region. [13].

CD4 T lymphocytes do not have a significant impact on the emergence of HSV-1 and/or HSV-2 infections; however, immunosuppression is associated with the appearance of clinical signs related to HSV. This result confirms the benign nature of HSV in immunocompetent individuals and its severity in immunodeficient individuals [29]. The immunodeficiency caused by HIV infection is primarily responsible for the changes observed in individuals co-infected with HSV and HIV. In this regard, clinical manifestations of HSV-2 are indeed more severe and persistent even with CD4⁺ T lymphocytes above 500 cells/mm³, more closely linked to the clinical signs encountered in this study (cold sores, acute gingivostomatitis, herpetic whitlow, and genital herpes). The ulcerative lesions are large, very painful, necrotic, and persistent. Additionally, HIV-1 and HSV-2 co-infection promotes HSV-2 replication more than in those mono-infected with HSV-2 [28].

Limitations: the monocentric nature of the study. The small sample size and the short duration of the study do not provide enough statistical power to generalize the results of this study to all children born HIV positive at the Yaoundé Central Hospital and University. The seroprevalence obtained using molecular techniques. The limited number of risk factors considered in this work. The use of CD4⁺ cell count as the sole marker of immunosuppression.

5. Conclusions

It should be noted that the seroprevalence of Herpes simplex virus -1/-2 is 93.24%. This indeed reflects the circulation of this pathogen in Yaoundé. The risk factors associated with the infection are age and drug use. It would be essential to integrate the diagnosis of these infections into the follow-up of people living with HIV born to HIV-positive mothers and

to raise awareness among them regarding knowledge related to the herpes virus.

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
HIV	Human Immunodeficiency Virus
HSV-1	Herpes Simplex Virus Type 1
HSV-2	Herpes Simplex Virus Type 2
HSV	Herpes Simplex Virus
PLHIV	People Living with HIV
YUTH	Yaoundé University Teaching Hospital

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Author Contributions

Mbongu é Mikangu é Chris André Conceptualization, Resources, Writing – review & editing, Formal Analysis, Investigation.

Dikabo-Mikangu é Grace Emmanuelle: Investigation, Visualization, Methodology

Riwom Essama Sara Honorine: Software, Supervision, Validation

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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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