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Seroprevalence of Cytomegalovirus Among Children Born Hiv Positive at the Yaounde University Teaching Hospital, After 12 Months of Follow-up: A Cross Sectional Study

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Abstract:

Background: Cameroon is a country located in Sub-Saharan Africa, an endemic area for the *Herpesviridae* family. There is very little data on the epidemiology of herpes virus infections, particularly those associated with Human Immunodeficiency Virus (HIV) infection in people living with HIV(PLHIV), HIV-positive infants, and those with undetectable viral loads. The objective of our study was to determine the seroprevalence of four herpes viruses: cytomegalovirus (CMV) in children living with HIV who were born HIV-positive in Yaoundé. **Methods:** This was a prospective cross-sectional study conducted at Yaounde University Teaching Hospital after 12 months of follow-up among children living with HIV who were born HIV-positive, under antiretroviral treatment, and whose medical records were complete and available at the Approved Treatment Center. IgG/IgM antibodies against CMV were qualitatively determined using rapid diagnostic tests for the detection of these pathogens. Data entry and analysis were performed using the Statistical Package for Social Sciences (SPSS) v 22.0, with Fisher's exact test, Chi-square test, and Mann-Whitney test. P-values less than 0.05 were considered statistically significant. **Results:** Seventy-four participants were included in the study, with a female predominance of 68.92% (n=51/74). The mean age of our cohort was 9.05±5.09 years, and most participants were under 10 years old (56.76%, n=42/74). The seroprevalence of CMV was 95.95%, and the prevalence of alcohol and tobacco use was 22.97% and 24.32%, respectively. Other parameters such as sex, age, disease stage, smoking, and alcohol consumption were significantly associated with seropositivity for these herpesviruses. **Conclusion:** Despite the absence of most clinical manifestations related to CMV, HSV-1, and HSV-2, it is important to note a high circulation of these viruses among HIV-infected patients, mainly in bi- and tri-infections.

Keywords: Children; CMV; *Herpesviridae*; HIV; Seroprevalence.

Introduction

Herpesviridae constitute a family organized into three subfamilies [1]. Cytomegalovirus (CMV) is a member of this family, also known as human herpesvirus 5, isolated by Weller, Smith, and Rowe respectively in 1956 and 1957 [2]. It is a DNA virus, ubiquitous and responsible for widespread infections worldwide. It causes a primary infection after which a state of latency occurs, allowing the virus to evade the immune system and explaining its persistence throughout the life of the infected individual. This state of viral latency is periodically broken by reactivations [3]. CMV infection, whose frequency increases with age, is asymptomatic in 90% of cases in immunocompetent subjects and is well tolerated when symptomatic [4].

In immunocompromised patients, CMV is the most commonly found opportunistic pathogen. Clinical manifestations appear during a primary infection, reinfection, or reactivation of the latent virus [5]. It can be transmitted through the airways, sexually, in utero, or at birth, as well as through allografts from multiple sources such as saliva, pharyngeal secretions, tears, urine, cervical-vaginal secretions, sperm, breast milk, and grafts [6]. CMV has a very broad cell tropism and is particularly known for strongly activating T lymphocytes. This dysregulated activation of lymphocytes leads to synergistic phenomena between this virus and the Human Immunodeficiency Virus (HIV), which mutually facilitates their replication [2]. The CMV virus primarily establishes itself in endothelial cells of blood vessels, in stem cells of the bone marrow, and in monocytes of peripheral blood [7]. It is often responsible for mortality associated with immunodepression [8] and can lead to serious complications such as chorioretinitis [9]. Since the implementation of the "test and treat" policy, there is a lack of data on the prevalence of CMV [10]. Furthermore, there is very little data on the management of CMV among people living with HIV (PLHIV) in our context of resource-limited countries [11] especially given that Cameroon is located in a region of high endemicity [12]. Given this situation, the objective of this study was to determine the seroprevalence of Cytomegalovirus and the associated risk factors.

2. Materials and Methods

2.1. Study design and settings

It was a cross-sectional study conducted from November 2020 to October 2021 in Yaounde-Cameroon. The choice of this hospital was motivated by the availability of a care unit for PLHIV with a large number of patients followed, hence the probability of having the sampling required for our work.

2.2. 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

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mother on antiretroviral therapy (ART); having an undetectable viral load at the time of inclusion in the study (< 50 copies of RNA/µl); 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.

2.3. Data collection

After obtaining parental assent, data were collected on all eligible children covering demographic and clinical information from a standard questionnaire administered to the respective mother or guardian (sex, age, region, alcohol consumption, tobacco intake, etc.). These data were verified using the medical records available at the health facility.

2.4. Laboratory methods

A total volume of whole blood of 5 mL, depending of the child weight, was collected in EDTA tubes by venipuncture. Samples were transported and tested at the Laboratory of Microbiology, at the University of Yaoundé 1. After centrifugation, plasma samples were obtained and stored at - 20°C aliquots until examined. For the determination of the prevalence's of CMV, serological testing targeting anti-CMV IgM and IgG antibodies, were carried out using the two lateral flow immuno-chromatographic assays from One Step TORCH IgM/IgG (TOX IgM/IgG, RV IgM/ IgG, CMV IgM/IgG, HSV-I IgM/IgG, HSV-II IgM/IgG antibody) Bioneavan co.LTD., Beijing. Each sample was tested following the manufacturer's instructions. Results were reported either as positive, negative or invalid.

2.5. Statistical analysis

Data were entered into an excel spreadsheet, double-checked for accuracy and cleaning, then closed for data analysis. The cleaned dataset was then transferred into Statistical package for social sciences version 22.0 was used for statistical analyses. Categorical variables and their comparison were done using a Fisher exact test wherever applicable, Odds ratio is used to highlight the strength of an association between herpes virus. P-value <0.05 was considered as statistically significant.

2.6. Ethical Considerations

The study was approved by the Regional Ethics Committee for Research in Human Health (N°0082/CRE RSHC/2023) and received authorization from the Yaounde University Teaching Hospital (N°494/ AR/CHUY/DG/DGA/CAPRC). The Microbiology Laboratory allowed the laboratory analyses. Patients signed consent forms regarding the use of their plasma and the collection of their medical data.

3. Results

3.1. Sociodemographic and Clinical Parameters

Distribution of participants by sex.

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. (Table 1).

Distribution of participants by age groups.

The 74 participants included had ages ranging from 3 to 19 years. The average age within the study was 9.054 ± 5.096 years. Table 1 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) [13], 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. (Table 1)

Table 1. Sociodemographic and Clinical Parameters

Sociodemographic and Clinical Parameters of HIV infected persons		
Sex		
Girls	51	69%
Boys	23	31%
Years		
[0-5]	17	23
[5-10]	25	33.8
[10-15]	16	21.6
[15-20]	16	21.6

3.2. Serological Parameters

Prevalence of herpes simplex virus in the study population

The seroprevalence of co-infection with Cytomegalovirus was 95.95 %. In total, it was found that out of the 74 participants included, 71 were infected with CMV, resulting in an overall prevalence of 95.95 % (n=71) (Table 2). Among the 51 women and 23 men enrolled, there was a feminization of the infection (98.04 %) compared to men (91.3%); $p < 0.05$ (Table 2). For Cytomegalovirus, the vast majority of the infected population (78.26%) falls within the age range of 3 to 14 years (Table 3).

Table 2. Distribution of patients by type of infecction.

Type of infection	HIV infected persons		Percentage (%)	IC_{95}
	IgM-anti CMV	IgG-anti CMV		
CMV	0	71	95.95	[88.61-99.16]
Absence of CMV	0	3	4.05	[2.23-15.07]
74 (100%)				

CMV: Cytomégalovirus

Table 3. Gender wise distribution of cytomegalovirus.

Number	Gender		Cytomegalovirus	Absence of cytomegalovirus
			HIV infected persons	
1	**Male sex**	**N**	21	2
	(n=23/74, 31.1%)	%	91.30	8.7
2	**Female sex**	**N**	50	1
	(n=51/74, 68.92)	%	98.04	1.9
Total :74(100%)				

HIV: Human Immunodeficiency Virus

Table 4. Years wise distribution of cytomegalovirus and Risks factors.

Age group (years)	n (%)	Alcohol consumption n (%)	CMV	Tobacco intake n (%)
[0-5]	17 (22.97)	0	16(94.12)	0
[5-10]	25 (33.78)	0	23(92.0)	0
[10-15]	16 (21.62)	3 (4.05)	16(94.12)	9 (12.16)
[15-20]	16 (21.62)	14 (18.91)	16(94.12)	9 (12.16)
Total 74 (100%)				

CMV: Cytomégalovirus

HSV infection based on immune status

When evaluating the association between infection and immune status, a significant difference was observed (p**<0.01**). Thus, immune status in terms of $CD4^+$ counts did not have a significant impact on the occurrence of cytomegalovirus infection.

However, the distribution of infected patients based on clinical signs and $CD4^+$ T-cell counts shows that all patients presenting clinical signs are those with competent immune systems, with 95.95 % (n=71) 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/mm^3. Despite the subjective nature of patient history, the most common clinical sign is cold sores (14.54%). (Table 5)

Table 5. Distribution of Cytomegalovirus seropositive patients by CD4+ count.

Groups of $CD4^+$ LT count *(cellules/mm^3)*	CMV	Size *(%)*	P-Value
[500-1600]	71(95.95)	74 (100)	*<0.01*
[350-499]	//	//	
[200-349]	//	//	
[0-200]	//	//	

4. Discussion

Cameroon is located in an area of high endemicity for herpesviruses *herpesviridae* [12]. The objective of this study was to determine the seroprevalence of CMV in children born to HIV-positive mothers and the associated risk factors. The prevalence obtained in our study was 95.95%. Our results are higher than the data in the literature on the prevalence of cytomegalovirus in Africa, which is around 60% [14]. This high prevalence could be explained by the heterogeneity of the characteristics of the HIV-infected children included in the study, as well as by the endemic nature of the study area [12]. The transmission of cytomegalovirus likely occurred vertically, which is one of the most common routes of transmission [8,15]. At birth, the child's immune system is immature and fragile; therefore, where immune defenses are weak, the consequences of acquiring CMV very early, particularly through breastfeeding, could manifest in the long term. The duration of CMV infection is likely significant, as chronic activation induced by CMV persists throughout life from the moment of virus acquisition. Until recently, CMV was considered a virus that it was ultimately preferable to be infected with early in life to eliminate it and avoid the risk of primary infection during pregnancy. However, it remains a pathogenic virus at the cellular level [16]. In children, CMV is highly replicative and correlated with greater mortality compared to those without CMV [15]. This high prevalence of cytomegalovirus suggests a need for systematic screening in all healthcare facilities and the provision of funding by the WHO. This study revealed a high prevalence of CMV in Cameroon (95.95%) as well as risk factors among children born to HIV-positive mothers, providing important information for clinicians, researchers, and policymakers. However, this work presented several limitations, such as the lack of documentation for certain data, the absence of diagnosis for immune reconstitution inflammatory syndrome (IRIS), the cross-sectional nature of the study, the small sample size which does not allow for generalization of our results, a limited number of risk factors, the absence of antibody titration which does not allow for proper assessment, and a lack of serological data regarding the diagnosis of cytomegalovirus in mothers. Additionally, there was no molecular diagnosis performed.

Conclusion:

The present study aimed to determine the seroprevalence of cytomegalovirus in children born to HIV-positive mothers at the Yaoundé University Teaching Hospital. The seroprevalence of cytomegalovirus in this study was 95.95%, highlighting the significant burden of cytomegalovirus in Cameroon. This emphasizes the need to focus efforts on the diagnosis of cytomegalovirus and the prevention of its transmission.

Abbreviations

ART: Antiretroviral Therapy; **ATC**: Approved Treatment Center; **CMV**: Cytomegalovirus; **HIV:** Human Immunodeficiency Virus; **IRIS**: immune reconstitution inflammatory syndrome; **PLHIV**: People Living with HIV; **SPSS**: Statistical Package for Social Sciences; **YUTH**: Yaoundé University Teaching Hospital;

Conflicts of Interest

The authors declare no conflicts of interest

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