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Research Article

Seroprevalence and Association of Cytomegalovirus, Eptein-Barr Virus, Herpes Simplex Virus Type 1 with Childhood Hematological Malignancies in Yaoundé, Cameroon: A Cross-sectional Study

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Abstract

Background: Hematological malignancies include all cancers that originate in blood cells, such as leukemia and lymphoma. Some viruses have an implication, it is the case of viruses of the family of *herpesviridae*, which are associated with certain cancers. The objective of our study was to determine the seroprevalence of four *herpesviridae* and their association with pediatric hematologic malignancies.

Methods: From August 2021 to January 2022, a cross-sectional study on leukemia and lymphoma children ≤15 years old was done at the Chantal Biya Foundation in Yaoundé. Sociodemographic and clinical data were collected using a predefined form. HSV1, EBV, CMV IgG/IgM were assessed using rapid diagnosis tests (RDTs) analysis.

Results: During the study period, we recruited 52 participants aged 0 to 15 years, 36 cases of lymphoma were recorded against 16 cases of acute leukemia. The acute leukemias were lymphoblastic (62.50%) and myeloblastic (37.50%). A male predominance was observed with a sex ratio of 3.3. Hematological malignancies were more frequent in the 5 to 10-year age group and the majority of patients were children with Burkitt's lymphoma in advanced stages of the disease. Among the viruses assessed, CMV (96.15%) and HSV-1 (92.30%) antibodies rates were high compared to EBV (23.07%). A co-infection was observed CMV+HSV-1 (69.23%) and CMV+EBV+HSV-1 (19.23%) but not significant. Of the 52 cases we observed an association between Burkitt's lymphoma, Hodgkin's disease and these *herpesviridae*, and EBV-CMV-HSV1 tri-infection gives 6.7 times more chance of developing Hodgkin lymphoma and 6.1 times for Burkitt lymphoma.

Conclusion: An association was seen between some haematological malignancies and certain viruses of the *herpesviridae* family.

Keywords: EBV; CMV; HSV1; Lymphoma; Leukemia; Children

Abbreviations

EBV: Eptein-Barr Virus; CMV: Cytomegalovirus; HSV: Herpes Simplex Virus; RDT: Rapid Diagnosis Tests; IgG: Immunoglobulin G; IgM: Immunoglobulin M; EDTA: ÉthylèneDiamineTétraAcétique

Introduction

Herpesviridae are known for their ability to induce certain cancers, and under special conditions, this is the case of hematological malignancies [1]. Haematopoietic malignancy (leukemia and lymphoma) is a cancer of hematopoietic tissue characterized by a disorder of multiplication and differentiation of cells of a blood line. They are the leading cause of cancer in children and account for about 40% of all pediatric cancers [2]. While significant progress has been made in the prognosis and treatment of pediatric hematological malignancies, these protocols induce immunosuppression that could result in a new infection or reactivation of a latent infection, as in the case of viruses of the human *herpesviridae* family [3]. It has been reported that co-infection between herpes viruses is an important cofactor of severity and lethality of these cancers in children [4]. It therefore seems important for us to study the presence of *herpesviridae* in children from 0 to 15 years, treated for a malignant blood disease at the Mother and Child Center of the Chantal Biya Foundation of Yaoundé. The purpose of this study was to determine the seroprevalence of 4 viruses in this family, namely HS1 and 2, CMV and EBV and the possible influence of their co-infection during hematologic malignancies.

Methods

- **Study design and settings:** It was a cross-sectional hospital-based study conducted from 1st August 2021 to 30th January 2022 in Yaoundé – Cameroon. Mother and Child Center of the Chantal Biya Fondation was selected as the study site based on the availability of the reference oncopaediatric care service.
- **Ethics approval and consent to participate:** An administrative authorization for data collection at the Chantal Biya Foundation Mother and Child Centre in Yaoundé was delivered by the Director in date of 8 July 2021. An ethical clearance was obtained by the Joint Institutional Board for Animals and Human Bioethics (JIRB) of the University of Yaoundé 1 (Ref N° BTC-JIRB2022-015). Prior to enrolment of each participant, the study information sheet was given to the respective mother or guardian. A written informed consent was then provided prior to the child enrolment. Confidentiality was ensured throughout the data collection and processing by using specific identification numbers. All experiments were performed in accordance with relevant guidelines and regulations.
- **Participants enrolment:** Study participants were enrolled during their routine clinic attendance at the Mother and Child Center. Inclusion criteria were: (a) have a hematological malignancy; (b) be aged ≤15 years old; (c) be registered for monitoring at this study site; (d) have maternal/guardian informed consent for participation. Any child fulfilling the aforementioned criteria was excluded if unable to provide a blood sample.
- **Data collection:** After the parental ascent, data performed on all eligible children covering demographic and clinical informations from a standard questionnaire was administered to the respective mother or guardian (sex, age, region, clinical features…). These data were verified using the medical records available at the health facility.
- **Laboratory methods:** A total volume of whole blood comprised between 2,5 mL et 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 HSV1/2, CMV and EBV, serological testing targeting Anti-HSV-1 and 2, 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 and anti-EBV from (EB)-IgM antibody Bioneavan co.LTD., Beijing. Each sample was tested following the manufacturer's instructions. Results were reported either as positive, negative or invalid.
- **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 X^2 test or Fisher exact test wherever applicable, Odds ratio is used to highlight the strength of an association between herpes virus as risk factors and hematological malignacies as clinical outcomes. P-value <0.05 was considered as statistically significant.

Results

Characteristics of study participants

Over a period of 4 months, a total of 52 children with hematological malignancies were recruited. Preponderance of males was observed (76.92% male vs 23.08% female) with a *sex ratio* of 3.33. The mean age was 8.65 (± 3.95) years, ranged from 2 to 15 years, and the most common age group affected was between 5 to 10 years of age (n = 28, 53.84%). The type of hematological malignancy at the time of presentation are shown in figure 1.

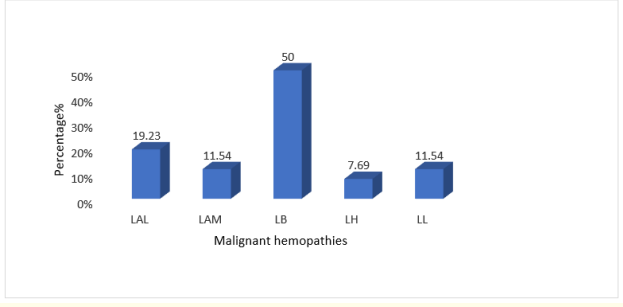

Figure 1: Malignant hemopathies profile in children.

LAM: Acute Myeloblastic Leukemia; LAL: Acute Lymphoblastic Leukemia; LL: Lymphoblastic Lymphoma; LH: Hodgkin Lymphoma; LB: Burkitt Lymphoma.

We have 30.76% (n = 16) of leukemia with 62.50% acute lymphoblastic leukemia and 69.24% (n = 36) of lymphoma which 72.22% Burkitt lymphoma.

Burkitt's lymphoma was the most represented blood disease in all age groups but absent between 11 and 13 years (P = 0.086). Although the male sex predominated in all blood diseases, Hodgkin's lymphoma and acute myeloid leukemia were observed to be absent in girls (P = 0.1). Most children were recruited with advanced malignant hematology depending on the specific stage of each type of cancer (stages III and IV for lymphomas, and high risk for leukemia), with a P = 0.05.

Herpesviridae seroprevalence

The *Herpesviridae* profiles at the time of presentation are shown in figure 2.

Of a total of 52 children tested for the presence of IgG and IgM, seroprevalence with cytomegalovirus CMV-IgG was 96.15% (n = 50), herpes simplex 1 virus (HSV1-IgG 92.30% (n = 48),

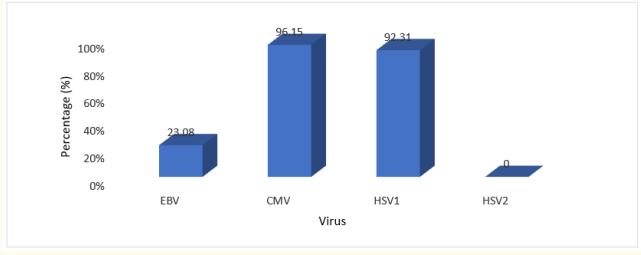

Figure 2: Virus seroprevalence.

Epstein Barr virus (EBV) positive EBV-IgG 23.07% (n = 12). Only the cytomegalovirus CMV-IgM was 7.69%. The presence of anti-virus herpes simplex 2 (HSV2) antibodies was not found in these patients.

The seroprevalence CMV and HSV1, defined as the presence of IgG was respectively 96.15% and 92.30% in the entire study population, indicating a highly prevalence of these viruses.

EBV was absent only in female children (P = 0.01), but was with a high frequency in the age group [14-16[(P = 0.1) and in patients with lymphoma especially Burkitt lymphoma (P = 0.9), while CMV was majority (P = 0.1) in patients with acute lymphoblastic leukemia.

Co-infection between *Herpesviridae*

Our participants introduced mono-, bi- and tri-infections to human herpes viruses. 46/52 children had a *herpesviridae* co-infection with CMV+HSV1 bi-infection (69.23%, n = 36) leading the way, followed by EBV+CMV+HSV1 tri-infection (19.23%, n = 10) (Table 2).

Herpesviridae	**Effective (%)**
EBV	0 (0.0)
CMV	2 (3.85)
HSV1	2 (3.85)
HSV2	0 (0.0)
EBV+CMV	2 (3.85)
EBV+HSV1	0 (0.0)
CMV+HSV1	36 (69.23)
EBV+CMV+HSV1	10 (19.23)

Table 1: Seroprevalence of *herpesviridae* found in mono and co-infections.

EBV = Epstein Barr Virus; CMV = Cytomégalovirus; HSV 1/2 = Herpes Simplex Virus ½.

CMV+HSV-1 co-infection was most prevalent in patients under 10 years of age, 66.66% (n = 24), in 65.38% of hematological malignancies of which 70.58% were associated with lymphomas (P = 0.8). We have had an association between herpes viruses and Hodgkin/Burkitt lymphomas, and EBV-CMV-HSV1 tri-infection gives 6.7 times more chance of developing Hodgkin lymphoma and 6.1 times for Burkitt lymphoma (Table 2).

Virus	LAL	LAM	LL	LH	LB
	OR [CI 95%], p	OR [CI 95%], p	OR [CI 95%], p	OR [CI 95%], p	OR [CI 95%], p
CMV	0.2 [0.0-0.1], 0.4	0.8 [0.0-7.1], 0.7	0.5 [0.0-1.5], 0.07	2.6 [0.3-24.0], 0.3	0.5 [0.0-4.0], 0.4
HSV1	0.2 [0.0-0.1], 0.4	0.8 [0.0-7.1], 0.7	0.5 [0.0-1.5] , 0.07	2.6 [0.3-24.0], 0.3	0.5 [0.0-4.0] , 0.4
CMV-HSV1	0.8 [0.0-26.5] 0.9	0.3 [0.0-4.5], 0.4	0.3 [0.0-9.4], 0.05	1.3 [0.1-13.2], 0.8	1.1 [0.1-10.2], 0.8
EBV-CMV	0.2 [0.0-6.1], 0.4	0.8 [0.0-7.1], 0.8	0.4 [0.0-1.5], 0.08	2.7 [0.3-24.0], 0.3	0.4 [0.0-4.0], 0.4
EBV-CMV-HSV1	0.1 [0.0-5.1], 0.2	0.4 [0.0-8.1], 0.6	0.8 [0.0-28.7], 0.9	6.7 [0.2-17.8], 0.2	6.1 [0.3-97.4], 0.1

Table 2: Herpesviridae infections and risk of childhood hematological malignancies.

OR = Odds ratio, [IC 95%] = Confidence Interval at 95%, p = p value.

LAM: Acute Myeloblastic Leukemia; LAL: Acute Lymphoblastic Leukemia; LL: Lymphoblastic Lymphoma; LH: Hodgkin Lymphoma; LB: Burkitt Lymphoma.

EBV = Epstein Barr Virus; CMV = Cytomégalovirus; HSV 1/2 = Herpes Simplex Virus ½.

Discussion

Herpesviridae are known for their ability to induce malignant blood diseases. It has been reported that a co-infection between herpes viruses is an important cofactor of severity and lethality of these cancers in children. The purpose of this study was to determine the seroprevalence of CMV, EBV and HSV1/2, and the possible influence of their co-infection during hematological malignancies.

The average age of our participants was 8.65±3.95 years (a majority of children between 5 and 10 years old, 53.84%) with a male predominance 76.92% (n = 40), or a sex ratio of 3.33. Another study conducted in Cameroon in 2018 in the same center, reported the same predominance (63%, sex ratio 1.7), with an average age of 7.5 years and a majority of children aged between 5 and 10 years (40%) [5]. Since the risk factors for cancer development are similar between boys and girls, we should observe a similar incidence. These rates also correspond to social levels of gender equality. Thus, the most likely reason for this discrepancy is that girls are less likely than boys to be referred to a doctor when they become ill. Thus, the high sex ratios in developing countries reflect more the socio-economic level of society than the nature and etiology of the disease [6]. Most of our patients had lymphoma (69.23%) and a large part of it had Burkitt's lymphoma (72.22%) with a peak in the age range of 5 to 7 years. A multicentric study conducted in Mali, Zambia and Ivory Coast in 2017 also reported a high rate of Burkitt lymphoma [7]. Pondy., *et al.* in 2018, in Cameroon, had also shown that Burkitt lymphoma incidence was the highest (28%) [5]. This could be explained by the fact that Burkitt's lymphoma is relatively easy to diagnose clinically compared to other tumors [8]. Another study done in 2014 in South Africa showed that black children had Burkitt lymphoma at a much younger age (5-6 years) than the white population (8-9 years) [9]. Most of the children in our study arrived with advanced malignant blood disease. This is a primary consequence of the lack of information on pediatric cancers by the community. The children are only brought to hospital after traditional treatment failure. Indeed, in developing countries like ours, the ignorance and neglect of parents to show up at the hospital at the first symptoms on the one hand, the use of traditional healers and self-medication on the other hand explain the long development delays before the consultation [10]. The difficulty of late diagnosis of childhood cancer is common to many african countries. These findings from our work were similar in a study on the state of paediatric oncology care in 10 low- and middle-income countries, including two countries in sub-Saharan Africa (Tanzania and Senegal) [11] and Malawi [12].

Cytomegalovirus and EBV infections have always been widespread, with high rates of infection in Third World Countries (up to 100%) [13]. In our study, the positive CMV-IgG/IgM rates were 96.15%, the positive VHS1-IgG rates were 92.30%, and the positive EBV-IgG rates were 23.07%. Seroprevalence being dominated by the presence of IgG, this demonstrates past infections, however detection of 02 CMV-IgM demonstrates a primary infection or re-infection. This high rate linked to CMV infections was also observed in a retrospective study (2008-2014) investigating the incidence of CMV infection in a total of 271 patients with malignant hematological diseases. The authors reported the presence of CMV IgG antibodies in 154 patients (75.5%) [14].

HSV infection was only determined with HSV-1. The presence of circulating HSV1-IgG is an indication of the potential risk of recurrent infection. Since during an HSV1 infection the infected person remains a source of contamination all his life, therefore is a carrier of the virus. Its presence in patients with hematological malignancies justifies this reasoning.

In our study, while 15.38% of Burkitt lymphomas and 100% of patients with Hodgkin's disease had an association with EBV, which is contrary to what Radji had to show in 2020 where EBV infection in endemic areas was associated with 96% Burkitt lymphoma and 40% Hodgkin's disease [1] CMV is more common in patients with leukemia than in those with lymphoma. One study showed that children with acute lymphoblastic leukemia were 3.71 times more likely to be diagnosed with CMV at birth and this risk is 5.6 times higher in children of Hispanic origins [15]. Our study found that CMV+HSV1 co-infection was 69.23% and EBV+CMV+HSV1 co-infection was 19.23%, with the majority of hematological malignancies associated with CMV and HSV1, especially Burkitt lymphoma. Our results also showed an association between herpes viruses and Hodgkin/Burkitt lymphomas, and EBV-CMV-HSV1 triinfection gives 6.7 times more chance of developing Hodgkin lymphoma and 6.1 times for Burkitt lymphoma (Table 2). The high rate of CMV+HSV1 infection can be due to the fact that CMV and HSV infection can occur simultaneously at distinct sites. In fact, the long term effects of CMV infection may contribute to immunosenescence and therefore predispose patients to the reactivation of other herpesviruses, such as HSV [16]. An important finding of the study lead by Delaney., *et al.* in 2015 at USA was that children who were EBV antibody positive were 10.29 times more likely to have both CMV and HSV-1 antibodies. This can be explained by the fact that here were several risk factors in common for acquiring EBV, CMV, and HSV-1 antibodies. This is plausible because these 3 viruses are presumably spread through close contact with infected individuals [17].

Conclusion

Lymphomas, especially Burkitt's lymphoma, were more common, with an age variation between 5 and 10 years. The male sex was more observed as well as the advanced stage of the disease. Out of 52 serologies, CMV and HSV 1 serprevalences were low in mono-infection than in co-infection. The majority of infections associated with hematological malignancies were in favour of CMV/HSV-1. These data indicated a high exposure of these viruses in childhood, but also reactivations for CMV (presence of IgM), but do not allow to conclude an association between *herpesviridae* and hematologic malignancies.

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Conflict of Interest

The authors declare that they have no competing interests.

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