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Seroprevalence of human herpesvirus type 8 (HHV-8) in blood donors at Laquintinie Hospital of Douala (LHD), Cameroon

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Abstract

Background: The Human Herpesvirus type 8 (HHV-8), also called Kaposi sarcoma-associated herpesvirus (KSHV) is an oncogenic virus. Its prevalence is high in many sub-Saharan African countries and some Asian areas. HHV-8 is transmitted through saliva and is acquired in early childhood. HHV-8 can also be transmitted sexually and by organ transplant or blood transfusion. Cameroon is an endemic area. That is why, for improving transfusion safety purpose, we assessed the HHV-8 infection rate in blood donors since the risk of transmission by blood transfusion was shown.

Material and methods: Blood samples were collected from blood donors at Laquintinie Hospital of Douala (Cameroon) blood bank for 5 months. Participants were from different ages. Collected samples were tested for detection of antibodies against HHV-8 using enzyme-linked immunosorbent assay (ELISA). Data analysis was perform and P-value of 0.05 was statistical significance threshold.

Results: Our result showed 2.2 % (2/91) seroprevalence rate of HHV-8 in blood donors. The two positive results were single and male-gendered. These observations were not statistically significant (P=0.6959 and P=0.851 respectively). Males were the most represented of our participants (92.31%). The mean age of our subject was 29±7 years. None of the studied population were at risk of HHV-8 infection. However, young adults aged between 18 to 28 years (54.94%) were the most represented. No evident association with socio-demographic data such as age, sex, level of education, and marital status was observed. Though, the high the educational level was the fewer we had volunteers for blood donation. Also, the number of blood donors decreased in an age-depending manner.

Conclusion: Thus in our study, the HHV-8 infection rate was low in blood donors. Even though the potential risk of transmission might be low, we should consider HHV-8 infection check in some special cases such as immunodeficiency.

Keywords: Human herpesvirus type 8 (HHV-8); Seroprevalence; Blood donors; Blood transfusion; HHV-8 transmission risk

1. Introduction

Human herpesvirus type 8 (HHV-8) belongs to the same family as Ebstein-Barr Virus (EBV), the *herpesviridae*, *Gammaherpesvirinae* subfamily, and *Rhadinovirus* genus [1]. HHV-8 is responsible for Kaposi's Sarcoma, Multicentric Castleman's disease, and primary effusion lymphoma (or body cavity-based lymphoma) [2, 3]. It is then called Kaposi's sarcoma-associated herpesvirus (KSHV). This virus is transmitted by contact through saliva acquired in early childhood [4]. HHV-8 can also be transmitted sexually and by organ transplant or blood transfusion [3, 5]. HHV-8 is endemic in some areas, especially in many regions in Africa and some countries in Asia (China, Iraq) [6-8]. The seroprevalence is not significantly different amongst African regions [9]. HHV-8 infection is associated with other medical conditions

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leading to immunodeficiency such as HIV-AIDS which is considered the major risk factor [5, 10-12]. The prevalence of KSHV is high in many African countries, including Uganda (14-86%), Botswana (76-87%), Cameroon (28-62%), and Gambia (29-84%) [6]. Children are the most affected. Central African children are most infected than those in other African regions (2% to 69%). Besides, the prevalence of HHV-8 infection increases when those kids are HIV-positive (in southern Africa) [9]. Generally in Africa, the prevalence increases also with age [13-18]. For example, it is higher in the population older than 50 years in Malawi, an endemic area [19] and in Mediterranean regions, the prevalence ranged from 9.7% in children to 26.3% in adults older than 59 years [13]. In an endemic area such as Uganda, it was demonstrated that blood transfusion recipients were at risk of being contaminated by HHV-8 since the prevalence of HHV-8 was high in the blood donors population (36.2%). The transmission rate is higher when fresh whole blood (stored for less than 4 days: short stored) of ≤4 days was used [20]. It was shown an increased risk of transmission with the number of transfusions in patients with sickle cell anemia in Uganda [21]. In a non-endemic area like the US where HHV-8 seroprevalence is not too high in blood donors (3-3.5%; 5.1%), the transmission risk by transfusion remains present [22, 23]. In a country like China, the seroprevalence of HHV-8 among blood donors varied from 18.6 to 36.8% in different ethnic groups living in endemic areas [7]. Another Asian country Iraq exhibited elevated seroprevalence of HHV-8 in blood donors (86.6%) [8]. Death rates associated with blood-borne HHV-8 posttransfusion contamination range from 10 to 17% depending on the storage duration of the blood. The mortality rate is high when fresh blood is used (≤4 days of storage than long-stored) and also when the recipient is immunocompromised (HIV-positive). Even seronegative blood transfusion is related to some risk of death post-transfusion [24]. That is why in this study we aimed to assess the HHV-8 infection rate among blood donors in one of our blood banks.

2. Material and methods

Five months of descriptive cross-sectional study was carried out at Laquintinie Hospital of Douala (LHD). Our subjects were blood donors (males and females) whose ages ranged from 18 to 60 years with no apparent health conditions, who agreed to be enrolled in our investigation within the period from January to May 2015. A questionnaire was filled out during an interview, before blood collection. About 3 ml of blood was collected according to standard technic using EDTA (Ethylenediaminetetraacetique) tubes. The samples were centrifuged at 3000 rpm for 10 to 15 minutes. Plasma was separated from the pellet, then 300 to 500 µL was stored at -20 °C for further analysis.

Samples were analyzed in search of IgG antibodies against LANA (latency-associated nuclear antigen), using Enzyme-Linked ImmunoSorbent Assay (ELISA HHV-8 IgG Shanghai YuanMu Biotechnology Co.Ltd) as recommended by the manufacturer. This test was carried out using ninety-six (96) well plates. The adsorbances were read using a spectrophotometer at 450 nm wavelength and optical densities (OD) were recorded for analysis and interpretation.

Data were analyzed using EPI Info 7.1.3.0 after processing the information collected in Excel. Descriptive statistical values like means and frequencies, plus the Chi^2 test were used to compare the different populations.

Informed consent was given by each of the enrolled subjects. Confidentiality of the identities, data collected and results of the participants have complied with rules and regulations on human health research at Laquintinie hospital of Douala.

3. Results

3.1. Socio-demographic data description

To assess the prevalence and potential risk of HHV-8 transmission through blood transfusion, we conducted a study on 91 subjects aged between 18 to 60 years. Both males and females were recruited. Our analysis showed that 84 blood donors (92.31%) were males. Female-gendered were represented by only 7 (7.69%) participants (Table 1). The mean age was 29±7 years. The majority of the subjects were young adults aged between 18 to 28 years (54.94%). The number of candidates for blood donation during our data collection period decreased in age-depending manner (table 1). At 50 years plus not many people were able or willing to donate their blood. According to the familial situation, 76 (83.52%) of the participant were single opposite to 15 (16.48%) married. Educational level was included as a descriptive feature of participants. Our results exhibited 39 (42.85%) subjects followed by 31 participants (34.06) who just finished junior high school and high school respectively. There was no clear pattern of participants according to educational background. However, the high the degree was the less subject were willing to donate blood. Many of the graduated and post-graduated were less likely to donate blood (7/91 and 3/91 participants respectively) (Table 1).

3.2. HHV-8 IgG antibody frequency

ELISA test was carried out to detect the presence of IgG directed against LANA HHV-8 protein. Different OD was analyzed and we found 2 positive samples (2.2%) versus 89 negatives (97.80%) (Table 2). Only a few of the participants expressed IgG anti-LANA in their blood. To assess potential HHV-8 infection risk exposure associated factors, we analyzed the relationship between positive results with socio-demographic description. Our results showed that all of the positive IgG anti-LANA donors were single and male-gendered with no statistical significance (P=0.6959 and P=0.851 respectively) (Table 2). None of the enrolled candidates were HIV-positive or positive for Hepatitis B and C, and for Syphilis according to blood donation screening test standard. So there was no evidence of an association between HIV infection and seropositivity for HHV-8 in our study.

Table 1 Participant socio-demographic characteristics

	Characteristics	**Number of subjects (N)**	**Percentages (%)**
Sexes	Female	7	7.69
	Male	84	92.31
Age range (years)	[18-28[	50	54.94
	[28-38[	33	36.3
	[38-48]	7	7.7
	]+50]	1	1.1
Marital status	Single	76	83.52
	Married	15	16.48
Level of education	Master	3	3.3
	Bachelor	7	7.7
	High school	31	34.06
	Junior high school	39	42.85
	Primary school	11	12.1

Table 2 IgG anti-LANA1 frequencies in blood donors

	Characteristics	**Positive N (%)**	**Negative N (%)**	**Records**
IgG anti-LANA	Frequencies	2 (2.20 %)	89 (97.80%)	91
Marital status	Single	2 (2.20 %)	74 (81.32%)	
	Married	0 (0%)	15 (16.50%)	
Sexes	Female	0 (0%)	7 (7.70%)	
	Male	2 (2.2%)	82 (90.10)	

4. Discussion

We found in our study carried out in the HLD blood bank that 2.2% (2/91) of the subjects recruited to donate blood were seropositive for HHV-8. Like many African countries, Cameroon (central Africa) is an endemic area for HHV-8. A previous study showed high seroprevalences, 43.2% in Bantu and 27.6% in Pygmy populations from rural areas of southern, central, and eastern Cameroon [18]. In the literature, it was proven that the risk of seroconversion after receiving HHV-8 seropositive blood was about 4.1 % (with 2.8% excess risk) in a study completed in Uganda during 24 weeks of follow-up [20]. This risk increased with the number of transfusions [21]. In most cases, HHV-8 infection is diagnosed using a serological test such as ELISA to detect the presence of HHV-8 antibodies against LANA or immunofluorescence. Though those serological tests are not always correlated with the active presence of the virus in

ELISA-positive patient samples. A study on healthy ELISA-positive HHV-8 individuals from the Brasilian population showed no presence of detectable DNA in their blood samples. Brasil is an HHV-8 low prevalence area [25]. This finding highlight the fact that the risk of HHV-8 blood-borne transmission might not be that elevated, especially in a non-endemic area. There was no evidence of the same observation in high prevalence areas like Africa. This can also explain the low transmission rate (4.1%) of the virus in the endemic area of Uganda [20].

We found no apparent association between sociodemographic factors of studied population and HHV-8 seropositivity. The 2 positive samples were from male participants. That is because in most of blood banks in Cameroon, blood donors used to be males, including in LHD where male subjects were 82 over 91 (90.10%). According to blood donation requirements and recommendations, many women are excluded if they are anemic, pregnant, on their period or if they are breastfeeding. This is why in our study female participants were few, just 7 over 91 (7.70%). According to the educational background, most of our subjet was done with high school (70/91), whether junior (39/91) or high school (31/91). On the other hand, subjects who attended university were less likely to donate blood. This goes along the same line as the observation that seroprevalence decreased with increasing education level or years of education [17]. A higher university degree increases awareness of potential blood donation consequences like anemia with all the discomfort associated, and the diet one should be on in case of anemia post-donation. These reasons may explain why the higher the educational level was, the less the willingness to give blood is. No participants aged above 50 years donated blood during our study period. It may be because in our country at that age people are facing some health issues and are cautious about giving blood. Single subjects were 76 (83.52%) and the most represented. Not having a family lead to minimizing the "supposed risk" of blood donation. A study carried out in Nairobi Kenya showed a similar result, no association between HHV-8 seroprevalence and socio-demographic data that included age, sex, marital status, level of education, and socioeconomic status [26].

In blood banks, some transmissible infections are exclusion criteria for blood donation. That is why no link between routinely screened Transfusion-Transmissible Infections (TTIs: HIV, Hepatitis B, and C viruses and syphilis) and HHV-8 seroprevalence was highlighted. It is known that HIV infection is a major risk factor for HHV-8 infection [5, 10-12]. In Cameroon, It was shown highly significant association between HIV infection and HHV-8 seropositivity (90% at the study timepoint to 74% 12 months later) [12]. Likewise, Nigerian HIV-positive patients harbored high seropositivity of HHV-8, 62% [27]. In contrast, in the country of Indonesia, only 7.7% of HIV-positive patients were seropositive for HHV-8 [28].

We found 2.2% of HHV-8 seropositive participants in our study. It was shown that the seroprevalence was higher in rural than in urban regions. Douala is an urban area. In Southern and East Africa, in Kampala, the prevalence was 18.1%, in an urban area, and 33.6% in children from the West Nile District [16]. While the opposite observation was made in West Africa [9]. We cannot compare those previous results in children with ours because we just focused on adults whose ages were above 18 years.

Even though the prevalence of HHV-8 was not elevated in our study (2.2%) it should be wise to take some precautionary measures according to blood donation. Furthermore, we may have found a higher rate of seroprevalence if our sample size was larger than 91 and the recruitment period was broader. Leucoreduction and long-stored blood transfusion might be options [20, 29]. Leuroreduction filtration is a procedure that reduces HHV-8 cell-associated viral load by more than 60% in HHV-8 positive whole blood. In that study, seven subjects happened to be HHV-8 negative (no HHV-8 detectable in their blood) out of 12 HHV-8 positives before leukoreduction filtration [29].

5. Conclusion

Our investigation on HHV-8 seroprevalence in blood donors at the HLD blood bank highlighted 2.2% of positive participants. It was shown that HHV-8 prevalence is not that high in Cameroon which is supposed to be an endemic area. This rate is low compared to other results found in endemic regions. Nevertheless, blood-borne HHV-8 transmission risk is not zero in our population. Introducing leukoreduction of short-stored HHV-8 seropositive blood should be considered especially for immunocompromised recipients.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors declared nonexistence of conflict of interest.

Statement of ethical approval

The authorization N°CEI-UD/257/02/2015/M of Institutional ethic committee for research on human health of University of Douala was given to carried out this study. Besides the clearance N°848/AR/MINSANTE/DHL/CM was granted by the Director Laquintinie Hospital of Douala.

Statement of informed consent

All the participants agreed to be enrolled in this study by signing the informed consent.

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