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Molecular detection and genotyping of human herpes virus 8 in blood donors in Congo

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Abstract

Objectives. Human herpesvirus 8 (HHV8) is rarely studied in Congo, despite its prevalence in Africa. Among healthy individuals, HHV-8 does not always lead to a life-threatening infection; however, in immunocompromised individuals, it could lead to more severe disease. The distribution of HHV-8 genotypes varies depending on ethnicity and geographic region.

Method. A prospective cross-sectional study included 265 samples from healthy blood donors from the National Blood Transfusion Center in Brazzaville, with an average age of 35 years, with extremes ranging from 18 to 60 years. After DNA extraction, a nested PCR was carried out for molecular detection, followed by genotyping by amplification of specific primers.

Result. In this study, 4.9% were positive for molecular detection of HHV-8 DNA. All HHV-8 positive DNA samples that were subjected to genotyping by amplification with specific primers allowing discrimination of two major genotypes (A and B). Genotype A was identified in 5 (1.9%) samples and genotype B in 2 (0.7%) samples, indicating that both genotypes were predominant. The remaining viral DNA samples not identified as the major genotypes were classified as «indeterminate» and consisted of 6 (2.3%) samples.

Conclusion: The results of the study suggest that Congo is an area where HHV-8 infection is endemic.

Keywords: *Congo; HHV-8; KSHV; Genotypes; Blood donors*

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Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

Ethics approval. The study was conducted with the informed consent of the patients. This study was carried out in compliance with the ethical standards for research in health sciences (N0: 62/UMNG.FSSA.V-DOY) and with the agreement of the Internal Ethics Committee of the CNTS.

ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ
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Обнаружение и генотипирование вируса герпеса человека 8-го типа молекулярными методами у доноров крови в Конго

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Резюме

Введение. Вирус герпеса человека 8-го типа (HHV-8) мало изучен в Конго, несмотря на его распространенность в Африке. Среди здоровых людей HHV-8 не всегда приводит к опасной для жизни инфекции; однако у лиц с ослабленным иммунитетом он может приводить к более тяжелому течению заболевания. Распределение генотипов HHV-8 варьирует в зависимости от этнической принадлежности и географического региона.

Методы. Проспективное поперечное исследование включало 265 образцов от здоровых доноров крови из Национального центра переливания крови в Браззавиле, средний возраст которых составлял 35 лет, с предельными значениями от 18 до 60 лет. После выделения ДНК из образцов была проведена гнездовая ПЦР для выявления HHV-8 с последующим генотипированием методом амплификации с генотип-специфичными праймерами.

Результаты. ДНК HHV-8 была выявлена в 4,9% образцов. Все образцы ДНК HHV-8, которые были подвергнуты генотипированию путем амплификации с генотип-специфичными праймерами, принадлежали к двум основным генотипам: A и B. Генотип A был выявлен в 5 (1,9%) образцах, а генотип B – в 2 (0,7%) образцах, что подтвердило встречаемость обоих генотипов. Остальные образцы вирусной ДНК, не идентифицированные как принадлежащие основным генотипам, были классифицированы как «неопределенные» и включали 6 (2,3%) образцов.

Заключение. Полученные результаты свидетельствуют о том, что Конго является областью, где инфекция HHV-8 является эндемичной.

Ключевые слова: *Конго; HHV-8; KSHV; генотипы; доноры крови*

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Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.
Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациентов. Данное исследование было проведено в соответствии с этическими стандартами исследований в области медицинских наук и с согласия Внутреннего этического комитета CNTS (N0: 62/UMNG.FSSA.V-DOY).

Introduction

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV), causes several neoplastic diseases and is responsible for all forms of Kaposi's sarcoma (KS). HHV-8 infection is not ubiquitous, but endemic in sub-Saharan Africa, parts of Eastern Europe, and the Mediterranean. It is the etiological agent of KS, primary effusion lymphoma (PEL) and multicentric Castleman disease (MCD) [1]. KS is today the most common cancer in people living with HIV (PLHIV), as well as in people living with HIV who have progressed to acquired immunodeficiency syndrome (AIDS). The prevalence of HHV-8 varies among different populations and is commonly found in human immunodeficiency

virus (HIV)-positive people and men who have sex with men [2, 3].

HHV-8 is characterized by its high genetic variability across the entire genome. Sequence analysis of the highly variable K1 open reading frame (ORF) allowed the identification of six major genotypes of HHV-8, named A–F. In addition, several subtypes have been described for genotypes A to E [4–6]. Geographically, HHV-8 subtypes exhibit distinct distribution patterns linked to the coevolution of viruses with humans during migrations throughout history [7]. Furthermore, evidence for a relationship between clinical progression of KS and HHV-8 genotypes has also been proposed. In this regard, genotype A was significantly associated with rapid progression of classic KS and high viral load, while genotype C was linked to low viral load and slow progression of KS, and genotype B was associated with a better prognosis of this disease [8–10].

Previous reports indicated that HHV-8 can be transmitted through saliva, sexual and non-sexual transmission, through blood or blood components, and during organ transplantation [11]. However, the exact route of transmission is still very controversial. Some studies report that saliva is implicated as the main vehicle of transmission in sub-Saharan children; others, on the other hand, support the hypothesis of horizontal transmission in adulthood [3, 12].

It should be noted that HHV-8 can be transmitted by blood transfusion. Ensuring good hemovigilance remains a major public health problem in sub-Saharan Africa and more particularly in low-income countries on the African continent [13].

Several studies support the hypothesis of a clear probability of transmission of HHV-8 by blood transfusion in sub-Saharan Africa. A study carried out in Uganda on transfused patients tested before and after transfusion showed that 43% of patients received HHV-8 seropositive blood and that the risk of seroconversion was significantly higher in recipients of HHV-8 seropositive blood [14]. Some countries in the subregion have reported prevalences for HHV-8 among blood donors of 14, 22 and 57% in Burkina Faso, Central African Republic and Tanzania, respectively [3, 15, 16]. A recent study carried out among Malian blood donors reports a prevalence of 10.4% [17].

The HHV-8 genome, whose size is estimated between 160–170 kb, with at least 87 reading frames (ORFs), is characterized by significant heterogeneity. Molecular epidemiology studies of HHV-8 are mainly based on the ORF-K1 (870 bp), the analysis of this region has made it possible to identify seven major molecular subtypes or genotypes (A, B, C, D, E, F and Z) [10, 18]. Some HHV-8 genotypes are associated with rapid progression of certain forms of Kaposi's sarcoma. The distribution of HHV-8 genotypes varies by geography and ethnicity. Genotypes A and C are found in Europe, North America, the Middle East and North Asia [10, 19, 20]; genotypes B and A5 are characteristic of Africa [21]; genotype D is found in the Pacific Islands and Taiwan [5]; genotype E is reported in Native Americans and Brazilians [22]; genotype F was first identified in Uganda [4] and recently reported in France [2], and genotype Z was found in a small cohort of Zambian children [23].

HHV-8 studies in blood donors are almost absent while the most feared post-transfusion infections are of viral origin. We studied the prevalence of HHV-8 among blood donors in Congo.

Material and methods

Study setting and participants

This is a prospective cross-sectional study including 265 blood samples from apparently healthy blood donors collected at the National Blood Transfusion Center in Brazzaville.

Inclusion criteria:

Age between 18 and 60 years old;
Physically fit for clinical examination;
Weight greater than or equal to 55 kg.

Non-inclusion criteria:

To take pills;
Have a chronic illness;
Having received a blood transfusion;
Behavior at risk of sexually transmitted diseases (STDs);
Menstruating, lactating or pregnant women;

We analyzed 265 samples from blood donors of both sexes aged 18 to 60 years in accordance with national guidelines on blood donation, from the blood bank of the National Transfusion Center of Congo-Brazzaville.

Viral DNA extraction

After routine blood bank screening, high molecular weight DNA was extracted from peripheral blood leukocytes of all samples selected by the genomic DNA kits. The protocol was adopted in accordance with the manufacturer's instructions and laboratory practices.

The purity and concentration of viral DNA were measured using a nucleic acid measuring instrument (nanodrop), the quality of which was tested by amplifying the human beta globin gene (268 Pb) with primers GH20: 5' GAA GAG CCA AGG ACA GGT AC 3' and PC04: 5' CAA CTT CAT CCA CGT TCA CC 3' in order to check the quality of our samples. After amplification on a thermal cycler, the detection was carried out on 2% agarose gel. All samples were suitable for viral DNA amplification.

Positive and Negative Controls

As a positive control we used samples identified as positive for HHV-8 during a study carried out in people living with HIV in Brazzaville [14], and as a negative control we used Ultra Pure Water for PCR.

Detection of HHV8 viral DNA and genotyping

All blood samples were amplified with a negative control and the human gene β-globin. PCR products were detected by 2% agarose gel electrophoresis with ethidium bromide staining.

HHV-8 infection was determined by nested PCR with primers to the K1 gene (ORF-K1). In the 1st PCR round, sense primer 5'-GGC CCT TGT GTA AAC CTG T -3' (51–69) and antisense primer 5'-AGT ATC CGA CCT CAT AAA ATG-3' (1081–1061) were used. In the 2nd PCR round, sense primer 5'-GAC CTT GTT GGA CAT CCT GTA 3' (76–96) and antisense primer 5'-ACT GGT TGC GTA TAG TCT TCC-3' (961–941) were used. The reaction mix included 2 µl of DNA in a full volume of 25 µl containing 12.5 µl of Green Taq Mix, 6.5 µl of ultra pure water, 2 µl of the sense primer and 2 µl of the antisense primer.

Both reactions were carried out under the following cycle conditions:

For the 1st round of nested PCR, initial denaturation at 94 °C for 120 s followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 60 s, elongation at 72 °C for 120 s that followed by the final step which is the extension at 72 °C for 5 minutes.

For the 2nd round of nested PCR, the conditions were the same, with the exception of the annealing step which this time took place at 50 °C for 45s.

After amplification with a thermal cycler amplicons were visualized on a 2% agarose gel.

All HHV-8 positive DNA samples were subjected to genotyping by amplification with specific primers (**Table 1**).

Statistical analysis

In blood donors, the correlation between HHV-8 positivity and independent variables such as sex, age, marital status, blood donor types and genotypes was analyzed using a Pearson's chi-square test or a Fisher's exact test with RStudio software 2023.06.0+421 «Mountain Hydrangea» Release with version 4.2.2 of R.

The study was conducted with the informed consent of the patients. This study was carried out in compliance with the ethical standards for research in health sciences (N0: 62/UMNG.FSSA.V-DOY) and with the agreement of the Internal Ethics Committee of the CNTS.

Result

Sociodemographic characteristics of donors

A sociodemographic analysis of the donors revealed a male predominance, with a sex ratio (M/F) of 1.4. The age group of 31 to 45 was the most represented. The average age was 35 years, with extremes ranging from 18 to 60 years. Voluntary donors made up the majority of the donor group. According to marital status, the most represented status was that of single.

Epidemiological characteristics of the population studied according to HHV-8 infection

Of 265 DNA samples, 4.9% were positive for HHV-8 DNA. All HHV-8 positive DNA samples that were subjected to genotyping by amplification with specific primers belonged to two major genotypes (A and B), Table 1. Genotype A was identified in 5 (1.9%) samples and genotype B – in 2 (0.7%) samples, indicating that both genotypes were predominant. The remaining viral DNA samples not identified as the major genotypes were classified as «indeterminate» and consisted of 6 (2.3%) samples (**Table 2**).

The presence of HHV-8 was higher in men than in women (4.1% versus 0.8%) with a positive correlation (p = 0.08), in regular donors with the prevalence of 2.3%, with a higher prevalence among donors aged between 18 and 30 years (2.3%), and singles – 4.2% (Table 2).

Description of sociodemographic characteristics according to genotype

Among the genotypes (A and B) identified during this study, genotype A was predominant in men. The study group ranged in age from 18 to 60 years old with an average of 35 years old. The highest frequency of predominant genotype A was observed in the age group (31–45) years, as well as in singles (**Table 3**).

Discussion

Tremendous efforts have been made since the discovery of HHV-8 more than two decades ago and until recently to demonstrate the potential risk of HHV-8 transmission through blood transfusion. Many routine tests have been carried out in blood donors around the world to ensure that immunologically safe blood is provided to patients in response to the increasing demand for blood transfusion in our country for various medical or surgical conditions. So far, HHV-8 testing is not routinely recom-

Table 1. Primers for HHV-8 genotyping
Таблица 1. Праймеры для генотипирования HHV-8

Gene Ген	Primers Праймеры	Position Позиция	Reference Ссылка
Type A (AF133038.1)	5′-ATACTCGGCTTTTCCGACCG-3′ 5′-GCTCTGTCCGATGCCCAGATT-3′	265–284 359–340	Zainab B. Mohammed, Shatha F. Abdullah
Type B (AF133040.1)	5′-CTGGAGTGATTTCCACGCCT-3′ 5′-AGTCCCGTTGCAATACCAGG-3′	190–209 269–250	Zainab B. Mohammed, Shatha F. Abdullah
Type C (AF133041.1)	5′-CAACGCCTTACACGTTGACC-3′ 5′-CATGCGTCAGTCGGAAAAGC-3′	202–221 291–272	Zainab B. Mohammed, Shatha F. Abdullah
Type D (EF589758.1)	5′-GGCCCTTGTGTAAACCTGT-3′ 5′-AGTATCCGACCTCTAAAATG-3′	159–178 239–220	Zainab B. Mohammed, Shatha F. Abdullah
β-globin / GH20 PCO4	5'-GAA GAG CCA AGG ACA GGT AC-3' 5'-CAA CTT CAT CCA CGT TCA CC-3'	268Pb	

Table 2. Epidemiological characteristics of the population studied according to HHV-8 infection status
Таблица 2. Эпидемиологическая характеристика обследованной популяции в соответствии со статусом по инфицированию вирусом герпеса человека 8-го типа

Variables Параметры	N/265 (%)	Negative (%) Негативный (%)	Positive (%) Позитивный (%)	*p*
Gender Пол				0.08
Male Мужчины	155 (58.5)	145 (54.7)	10 (4.1)	
Female Женщины	110 (41.5)	107 (40.4)	3 (0.8)	
Age Возраст				0.1
18–30	113 (42.7)	108 (40.8)	6 (2.3)	
1–45	127 (47.9)	119 (44.9)	5 (1.9)	
46–60	25 (9.4)	25 (9.4)	2 (0.7)	
Marital status Семейное положение				0.3
Single Одинокие	141 (53.2)	134 (50.6)	11 (4.2)	
Married В браке	43 (16.2)	43 (16.2)	2 (0.7)	
Concubine В сожительстве	81 (30.6)	75 (28.3)	0 (0.0)	
Blood donors Доноры крови				0.1
Voluntary Безвозмездные	125 (47.2)	121 (45.7)	5 (1.9)	
Family Родственники	59 (22.2)	57 (21.5)	2 (0.7)	
Regular Постоянные	81 (30.6)	74 (27.9)	6 (2.3)	
Genotypes Генотипы				
A	5 (1.9)	0 (0.0)	5 (1.9)	–
B	2 (0.7)	0 (0.0)	2 (0.7)	–
Indefinite Неопределенный	6 (2.3)	0 (0.0)	6 (2.3)	–
Negative Негативный	252 (95.1)	252 (95.1)	0 (0.0)	–

mended, except in an endemic area, because several facts suggest a potential role of HHV-8 in the pathogenesis of many diseases. More importantly, most blood recipients have immune problems that promote HHV-8 activation. The increasing spread of HHV-8 around the world and neighboring countries has led to the decision to test blood donors [17]. Apart from the recent study by Malonga G et al, there were no such studies or statistics in Congo that would allow further interpretation [24].

Among the 13 positives, 7 samples were typeable, and genotype A was the most predominant (1.9%), followed by genotype B (0.7%). The major genotypes (A to Z) of the virus have been identified as having distinct distributions among different geographic and ethnic groups that are influenced by population migration [10, 19]. Our results showed that the majority of blood donors had both genotypes A and B, which coincided with a recent study conducted in Congo in people living with HIV, and are consistent with data from the study by Betsem et al. which found genotype A and genotype B circulating mainly in the Cameroonian population [25]. It should be noted that genotypes A and B were also found among KS patients in Zimbabwe [26], in the Central African Republic [27, 28], in South Africa and Uganda [4, 21]. In contrast, a study conducted by Lacoste et al. in a specific region described genotype B as the only one circulating in Congo in a patient with MCD. On the other hand, Varmazyar et al. reported that genotype A was more fre-

Table 3. Description of sociodemographic characteristics according to HHV-8 genotype
Таблица 3. Описание социально-демографических характеристик в зависимости от генотипа HHV-8

Variables Параметры	HHV-8 genotype Генотип HHV-8			
	N/265 (%)	A (%)	B (%)	Indefinite Неопределенный (%)
Gender Пол				
Male Мужчины	155 (58.5)	4 (1.51)	2 (0.76)	4 (1.51)
Female Женщины	110 (41.5)	1 (0.38)	0 (0.0)	2 (0.76)
Age Возраст				
18–30	113 (42.7)	2 (0.76)	1 (0.38)	3 (1.13)
31–45	127 (47.9)	3 (1.13)	1 (0.38)	1 (0.38)
46–60	25 (9.4)	0 (0.0)	0 (0.0)	2 (0.76)
Marital status Семейное положение				
Single Одинокие	141 (53.2)	4 (1.51)	1 (0.38)	6 (2.27)
Married В браке	43 (16.2)	1 (0.38)	1 (0.38)	0 (0.0)
Concubine В сожительстве	81 (30.6)	0 (0.0)	0 (0.0)	0 (0.0)
Blood donors Доноры крови				
Voluntary Безвозмездные	125 (47.2)	2 (0.76)	1 (0.38)	2 (0.76)
Family Родственники	59 (22.2)	1 (0.38)	0 (0.0)	1 (0.38)
Regular Постоянные	81 (30.6)	2 (0.76)	1 (0.38)	3 (1.13)

quently detected in HIV-infected patients, with or without KS, than in HIV-negative individuals [20]. Unlike other studies where genotypes A and C have the largest area of prevalence, including Africa, Europe, Middle East, Asia [29, 30], genotype A was mainly identified in our study and this genotype was mainly detected in Africa [21].

Conclusion

In conclusion, this study provides additional evidence of the potential risk of HHV-8 transmission through blood transfusion. Genotyping results showed the predominance of genotype A. Further research is needed to explain the possible presence of HHV-8 infection in the population, which could help clarify many aspects of HHV-8 epidemiology and viral transmission factors.

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