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Original Articles

EBV and HHV-6 Circulating Subtypes in People Living with HIV in Burkina Faso, Impact on CD4 T cell count and HIV Viral Load

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Abstract. Epstein Barr Virus (EBV) and Human Herpes Virus 6 (HHV-6) are responsible for severe diseases, particularly in immunocompromised persons. There is limited data of the infection of these opportunistic viruses in Burkina Faso.

The purpose of this study was to characterize EBV and HHV-6 subtypes and to assess their impact on CD4 T cell count, HIV-1 viral load and antiretroviral treatment in people living with HIV-1. The study population consisted of 238 HIV-positive patients with information on the CD4 T cell count, HIV-1 viral load and HAART. Venous blood samples collected in EDTA tubes were used for EBV and HHV-6 Real Time PCR subtyping.

An infection rate of 6.7% (16/238) and 7.1% (17/238) were found respectively for EBV and HHV-6 in the present study. Among EBV infections, similar prevalence was noted for both subtypes (3.9% (9/238) for EBV-1 vs 4.6% (11/238) for EBV-2) with 2.1% (5/238) of co-infection. HHV-6A infection represented 6.3% (15/238) of the study population against 5.0% (12/238) for HHV-6B. EBV-2 infection was significantly higher in patients with CD4 T cell count ≥ 500 compared to those with CD4 T cell count less than 500 cells (1.65% vs 8.56%, p = 0,011). The prevalence of EBV and HHV-6 infections was almost similar in HAART-naive and HAART-experienced patients.

The present study provides information on the prevalence of EBV and HHV-6 subtypes in people living with HIV-1 in Burkina Faso. The study also suggests that HAART treatment has no effect on infection with these opportunistic viruses in people living with HIV-1.

Keywords: EBV, HHV-6, HIV-1, subtype, CD4 T cell count, viral load, and treatment.

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Introduction. In the human species, there are eight (8) herpes viruses, including herpes simplex types 1 and 2 (HSV1 and HSV2), Varicella Zoster Virus (VZV), cytomegalovirus (CMV), Epstein-Barr Virus (EBV) and herpesviruses (HHV6, HHV7 and HHV8 associated with Kaposi's sarcoma).[1] They belong to the human herpesviridae family, and the high similarity

between these viruses shows that they have a common origin.[1]

EBV is one of the most common human viruses, and it is found all over the world. Recent studies have shown that EBV seroprevalence is estimated to be present in more than 90% of adults older than 35 years of age worldwide.[2] Each year, new infections are estimated at 200,000.[3] The Epstein-Barr virus is commonly acquired during childhood in developing countries (more than 90% of pre-school children). In developed countries, many people are not infected in childhood but are rather infected in adolescence or during adulthood. Variations in the EBV genome made it possible to distinguish two (02) subtypes of the virus: EBV-1 and EBV-2 (or EBV-A and EBV-B types).[4] Seroprevalence studies have shown that EBV-1 strain predominates in western countries, whereas EBV-2 strain is only common in some areas of Equatorial Africa and New Guinea.[4] Primary infection with EBV is often asymptomatic or is responsible for infectious mononucleosis[5] and generally with no serious complications.[6] On the other hand, chronic infection is reported in many cases of cancers (gastric carcinoma, Burkitt's lymphoma (BL to nasopharyngeal carcinoma, Hodgkin's classic lymphoma (LH), gastric carcinoma)[7-10] and oral hairy leukoplakia.[11] Furthermore, it is suggested that EBV is associated with brain cancer, salivary gland tumors, hepatocellular carcinoma[4] and also with certain autoimmune diseases such as multiple sclerosis,[12] especially in immunocompromised individuals.[13]

In most HIV-infected persons with progressive immunodeficiency, the number of EBV infected B cells increases in blood circulation[14] and can develop opportunistic lymphomas (Burkitt's lymphoma, lymphomas that diffuse to large cells and primitive cerebral lymphomas) all associated with EBV.

Human herpesvirus 6 (HHV-6) is a member of the beta-herpes virus family, genetically close to cytomegalovirus (CMV) and human herpesvirus 7 (HHV-7).[15] It is responsible for infection of the vast majority of children in the early years of life and persists like the majority of the others herpes viruses in the latent form after the primary infection. It is a ubiquitous virus that infects T lymphocytes, monocytes, macrophages, certain epithelial cells and central nervous system cells. It early appeared that there were two HHV-6 variants or subtypes, subtype A (HHV-6A) and subtype B (HHV-6B) defined according to antigenic, genetic and potential differences in their respective pathogenicity.[16] The seroprevalence of HHV-6 infection is estimated to be between 70 and 100% in the human population and varies according to geographic location.[17] In the United States and Japan, primary HHV-6 infection affects children between 6 and 12 months, and it is estimated to be between 97 and 100%. This primary infection is due to HHV-6B subtype,[18] and HHV-6B viral DNA is also frequently detected in children in parts of Sub-Saharan Africa where HIV-1 is endemic.[19] There are limited data available on HHV-6A prevalence in sub-Saharan Africa. Studies have shown that HHV-6A is acquired late in life with a primary infection, generally asymptomatic.[17] However, more recent studies have described symptomatic primary infection in American and African children including roseola and febrile diseases.[19] HHV-6B is the causative agent of a very young child benign disease, exanthema subitum, still called infantile roseola or sixth disease.

HHV-6A has mainly a neurological tropism. HHV-6A and HHV-6B also cause opportunistic infections in immunocompromised individuals, including systemic infections and organ disorders, particularly encephalitis, hepatitis, colitis, spinal cord insufficiency, pneumonia, interstitial pneumonitis.[20] It can also favor insurgency of acute lymphoblastic or non-lymphoblastic leukemias, cutaneous T-cell lymphoma, immunoblastic lymphoma, acute lymphoid leukemia[21] and Hodgkin's lymphoma.[19,22] Recently, HHV-6 has been described to be associated with Drug Rash with Eosinophilia and Systemic Symptoms.[23] The literature reported that HHV-6 and HIV-1 would act in concert by infecting and causing CD4+ T cell lysis, thereby accentuating immunosuppression and progression towards AIDS by accelerating the death of infected CD4+ T cells.[24-26] However, HHV-6 detection in the blood decreases with AIDS progression, since virus replication target cells, the CD4+ T cells, are reduced.[27] In HIV infected patients, HHV-6 reactivation are associated with encephalitis,[28] pneumonia[29] or retinitis.[30,31] It is currently unknown whether HHV-6 acts simply as an opportunistic pathogen or in synergy with HIV on the disease progression. Some of our previous studies have resulted in the detection of EBV, HHV-6, and CMV among blood donors[32,33] and

HIV-positive mothers.[34] These studies also made possible to determine the molecular epidemiology of these herpes viruses in the subpopulations concerned by the latter studies. To date, there are no studies that revealed information on the subtypes of circulating EBV and HHV-6 in Burkina Faso. Thus, the present study not only targets the characterization of EBV and HHV-6 subtypes; but also the impact of infection of both viruses on CD4 T cell count, HIV-1 viral load, and treatment in people living with HIV-1.

Materials and Methods.

Study setting. This prospective study was carried out in Ouagadougou, Burkina Faso from May 2016 to March 2017. The samples were collected at the Saint Camille Hospital of Ouagadougou (HOSCO), and the molecular analyses were carried out at the Molecular Biology and Genetics Laboratory (LABIOGENE) of the University Ouaga I Prof. Joseph KI-ZERBO and the Pietro Annigoni Biomolecular Research Center (CERBA).

Sampling. The study included 238 HIV-1 positive patients recruited during their routine visit at HOSCO. The samples consisted of 3 mL of venous blood collected in EDTA tubes. The whole blood was aliquoted and stored at -20 °C until DNA extraction for molecular analysis. Sociodemographic characteristics, CD4 T cell count and HIV-1 viral load results were collected in patient follow-up registries with their free and informed consent.

DNA extraction and qualitative diagnosis of HHV-6 and EBV by real-time PCR. Genomic DNA was extracted from whole blood using the standard salting-out method as previously described by Miller et *al*.[35] EBV and HHV-6 subtypes identification was carried out by real-time PCR using specific primers and probes previously described by Kwok et *al.* for EBV and Yavarian et *al*. for HHV-6.[36,37]

The amplification for two viruses subtyping was carried out at 95 °C for 5 minutes corresponding to initial denaturation, followed by 45 cycles of 95 °C for 15 seconds and 55 °C for 30 seconds.

*Statistical analysis***.** A database was compiled on Microsoft Excel 2013 and then analyzed using the software Epi Info™ 7 and Statistical Package for Social Sciences (SPSS) 21.0 (IBM, Armonk, NY, USA). The results were analyzed according to socio-demographic characteristics, clinical parameters, CD4 T cell count and HIV-1 plasmatic viral load. The chi-square test was used for the comparisons, and the difference was considered statistically significant for P value ≤ 0.05.

Ethical considerations. Our study was approved by the Ethics Committee on Health Research (CERS) of Burkina Faso (Ref: DELIBERATION N° 2014-9-113). Written informed consent was obtained from all the participants, and the results confidentiality was respected.

Results.

Socio-demographic characteristics. Our study involved 238 people living with HIV-1 (PLHIV-1). The study population consisted of 66.8% of women and 33.2% of men. Children under five years of age accounted for 9.7% of the study population while 90.3% of the individuals were over 15 years of age. The median age was 24.7 ± 18.9 years.

Prevalence of Herpes Virus Infections. Of the 238 patients tested in this study, 13.0% (31/238) were positive for at least one of the viruses (EBV or HHV-6). The prevalence of EBV, EBV-1 and EBV-2 was 6.7% (16/238); 3.9% (9/238) and 4.6% (11/238) respectively. EBV-1/EBV-2 co-infection was observed in 2.1% (5/238) patients of the study population. HHV-6 infections were detected in 7.1% (17/238) of the individuals with prevalence of 6.3% (15/238) and 5.0% (12/238) respectively for HHV- 6A and HHV-6B. HHV-6A/HHV-6B co-infection was observed in 10 patients or a prevalence of 4.2% (10/238). Two (2/238 or 0.8%) patients were co-infected with EBV/HHV-6. According to age, herpes infection was more observed in patients more than fifteen (15) years, except HHV-6B which was more observed in the group of more than 15 years old (**Table 1** and **Table 2**). However, it should be noted that these results were not statistically significant.

Analysis of the results by sex showed that infections were more common in men than in women, except for the higher prevalence of HHV-6A in males compared to females (**Table 1** and **2**).

Table 1. Prevalence of Herpes and EBV by sex, age, CD4 T cell count, viral load and treatment.

		Herpes Pos		EBV Pos		EBV-1 Pos		EBV-2 Pos		Total
		N (%)	P	N (%)	P	N (%)	P	N (%)	P	
Age	< 15 ans	10	0.490	5	0.570	3	0.776	3	0.334	90
		(11.1)		(5.6)		(3.3)		(3.3)		
	≥ 15 ans	21		11		6		9		148
		(14.2)		(7.4)		(4.1)		(6.1)		
Sex	F	19	0.489	7	0.050	5	0.475	6	0.218	159
		(11.9)		(4.4)		(3.1)		(3.8)		
	M	12		9		4		6		79
		(15.2)		(11.4)		(5.1)		(7.6)		
CD4	< 500	13	0.287	5	0.101	4	0.695	2	**0.011**	121
		(10.7)		(4.1)		(3.3)		(1.7)		
	≥ 500	18		11		5		10		117
		(15.4)		(9.4)		(4.3)		(8.5)		
VL	< 1000	22	0.594	11	0.864	5	0.475	9	0.527	159
		(13.8)		(6.9)		(3.1)		(5.7)		
	≥ 1000	9		5		4		3		79
		(11.4)		(6.3)		(5.1)		(3.8)		
Treatment	ARV	26	0.967	14	0.653	8	0.648	11	0.403	199
		(13.1)		(7.0)		(4.0)		(5.5)		
	NAIVES	5		2		1		1		39
		(12.8)		(5.1)		(2.6)		(2.6)		
Total		31		16		9		12		238

VL : Viral Load, **Pos**: Positive, **N:** Number, **%:** Percentage, **P**: P- Value

The difference between EBV prevalence in men and woman was slightly significant (P = 0.05).

Herpes virus infections and CD4 cell count. Depending on the CD4 T cell count, our results show that patients with high CD4 T cell counts (CD4 T cell count ≥ 500/mL) are the most infected with herpes virus, EBV, EBV-1, and EBV-2. (**Table 1**). This difference was very significant for EBV-2 (P = 0.011) but was not significant for the others. HHV-6, HHV-6A and HHV-6B infections were more common in patients with low CD4 counts (CD4 T cell count < 500/mL) or those with high CD4 counts (CD4 T cell count ≥ 500 / mL) (**Table 2**). It should be noted that this difference was not statistically significant.

Herpes viral infections and HIV viral load. Our results were also analyzed according to the patients HIV-1 plasma viral loads. This analysis showed that patients with a low viral load (VL < 1000 copies/mL) were the most infected except for EBV-1 and HHV-6A which were more common in patients with high viral loads (VL > 1000 copies/mL) (**Table 1** and **2**). However, these differences were not statistically significant.

Herpes virus infections and ARV treatment. The analysis of the results according to ARV treatment (**Table 1** and **2**), showed that patients on ARV treatment were the most infected with herpes viruses, EBV, EBV-1, EBV-2, and HHV-6A. Meanwhile, the individuals, naïve to the treatment were more infected by HHV-6A and HHV-6B (Note that these patients were all co-infected with HHV-6A/HHV-6B). These results were not statistically significant.

Discussion. The purpose of this study was to characterize EBV and HHV-6 subtypes and to assess their infections impact on CD4 T cell count, HIV-1 viral load and treatment in people living with HIV-1.

Table 2. Prevalence and HHV-6 by sex, age, CD4 T cell count, viral load and treatment.

		HHV-6 Pos		HHV-6A Pos		HHV-6B Pos		Total
		N (%)	P	N (%)	P	N (%)	P	
Age	< 15 ans	6 (6.7)	0.823	5 (5.6)	0.709	5 (5.6)	0.779	90
	≥ 15 ans	11 (7.4)		10 (6.8)		7 (4.7)		148
Sex	F	11 (6.9)	0.849	11 (6.9)	0.572	7 (4.4)	0.529	159
	M	6 (7.6)		4 (5.1)		5 (6.3)		79
CD4	< 500	9 (7.4)	0.875	7 (5.8)	0.738	6 (5.0)	0.952	121
	≥ 500	8 (6.8)		8 (6.8)		6 (5.1)		117
VL	< 1000	12 (7.5)	0.729	11 (6.9)	0.572	7 4.4)	0.529	159
	≥ 1000	5 (6.3)		4 (5.1)		5 (6.3)		79
Treatment	ARV	14 (7.0)	0.885	12 (6.0)	0.703	9 (4.5)	0.434	199
	NAIVES	3 (7.7)		3 (7.7)		3 (7.7)		39
Total		17		15		12		238

VL : Viral Load, **Pos**: Positive, **N:** Number, **%:** Percentage, **P**: P- Value

Our study focused on 238 PL-HIV and showed that the prevalence of EBV and HHV-6 was 6.7% and 7.1% respectively in our study population. These results are similar to those reported by Tao et al. with a prevalence of 5.4% for EBV among blood donors in Burkina Faso.[32] Our results are also consistent with those reported by Traoré et *al.* (5.1% and 6.0% respectively for EBV and HHV-6) in blood donors and Ouedraogo et *al.* (6.0% and 6.1% respectively for EBV and HHV-6) in pregnant women in Burkina Faso.[33,34] These findings suggest that the prevalence of herpes viruses determined by qualitative real-time PCR method are much lower compared to their seroprevalence. These results confirm that most people infected with herpes virus in the course of their lives are more likely to have a latent infection without viremia than a cleared infection.

This study has allowed us to characterize for the first time EBV and HHV-6 subtypes circulating in Burkina Faso. We found that EBV-1 (3.9%) and EBV-2 (4.6%) subtypes predominate with approximately the same proportions. Our results corroborate data from the literature showing that EBV-1 subtype is more prevalent in Europe, North America, and Asia; while a predominance of both subtypes is observed in Africa and New Guinea.[38-43] EBV-2 is also found in HIV-infected Europeans and Australians,[44-46] and approximately 20% of the healthy population in North America is co-infected with both subtypes.[47]

The subtyping of HHV-6 shows that HHV-6A and HHV-6B subtypes are both present in our study population with similar proportions of 6.3% and 5.0% respectively for HHV-6A and HHV-6B. The literature suggests that HHV-6B is found throughout the world while HHV-6A is less common in Asia, North America and Europe.[16] Primary HHV-6 infection is due to 86-100% of cases in subtype A (HHV-6A) in African children.[19] Our results are similar to those reported in the literature. Thus, in their study, Baillargeon et *al.* found a lower rates of HHV-6 shedding in the genital tract of pregnant, 7/297 (2.0%); non-pregnant, 8/214 (3.7%) women; of 14 samples subtyped, four (29%) were subtype A.[48] whereas our study shows a slight prevalence of HHV-6A compared to HHV-6B even if these differences are not significant.

The analysis of our data according to the different age groups did not give any significant results for neither EBV, HHV-6 nor for the corresponding sub-types. However, the highest prevalence of overall infection with these two

viruses was observed in the group of individuals who were less than five years old.

According to sex, men were more infected with both viruses as well as the corresponding subtypes. This result is especially significant for EBV infection.

Analysis of our data by CD4 T cell count and HIV-1 viral load shows that herpes infection is more common in patients with high CD4 T cell counts and those with a low viral load compared to patients with low CD4 T cell count and a high HIV viral load. This result is particularly significant for EBV. We also found that the prevalence of herpes viruses were high in HAART patients compared with those who were not under treatment. Our results corroborate those of Piriou et *al*. who have shown that although HAART treatment improves CD4 T cell restoration while contributing to lower HIV viral load, it did not affect EBV infection.[49] Thus, it is possible that herpes infection is more likely to be found in HAART patients with high CD4 T cell counts. This hypothesis is also supported by several studies which have shown that herpes/HIV co-infection further enhances CD4 T cell proliferation and thus broadens the types of target cells susceptible to HIV infection,[24,49-52] resulting in a high HIV viral load. Our results corroborate those of Erwan Piourou et al.,[49] Who also concluded that even in the long term HAART treatment does not reduce the herpes viral load, but above all, it makes it possible to fight against reactivation cases.

Conclusion. This study made it possible to characterize the subtypes of EBV and HHV-6. It showed the subtypes EBV-1, EBV-2, HHV-6A, and HHV-6B all circulating in Burkina Faso with almost identical proportions. We also support the hypothesis that HIV HAART treatment would not act on herpes virus infection but could prevent reactivation of these viruses.

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