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Molecular Detection of *Herpesviridae* (HSV 1/2, VZV, EBV, CMV, HHV-6) among Patients with Encephalitis in Côte d'Ivoire

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Abstract

Background: Encephalitis is a serious neurological syndrome that can be caused by various infectious agents, particularly in immunocompromised individuals. Among these agents, viruses from the *Herpesviridae* family play a major role, although their diagnosis is often underestimated and their involvement remains poorly understood, particularly in developing countries such as those in West Africa. **Aim**: This cross-sectional, descriptive, multicenter study was conducted in hospitals in two regions of Côte d'Ivoire, namely Abidjan and Bouaké, between March 2024 and April 2025 to assess the prevalence of herpesviruses. **Methods**: CSF samples were collected from patients aged ≥ 15 years who presented with clinical signs of encephalitis and were subjected to molecular analysis. Viral DNA extraction and PCR tests were used to detect *Herpes simplex virus* 1 and 2 (HSV 1/2), *Varicella-Zoster virus* (VZV), *Epstein-Barr virus* (EBV), *Cytomegalovirus* (CMV), and *Human herpesvirus* 6 (HHV6). Clinical data and molecular analysis results were recorded using Excel software, and statistical analysis was performed using RStudio 4.2.1 software. Results are presented as mean and standard deviation for quantitative

variables and as percentages for qualitative variables. **Results**: A total of 388 patients were included in this study (222 women and 166 men; age range 15 - 79 years; mean age 45.36 ± 13.55 years), and 60 (15.46%) were positive for one or more herpesvirus. *Epstein-Barr* virus was most frequently detected in 33 (55.00%) of positive cases, followed by *Cytomegalovirus* with 20 (33.33%) and *Varicella-Zoster virus* and *Human herpes virus* 6 with respective rates of 20.00% (12) for VZV and 11.67% (07) for HHV6. A single *Herpes simplex virus* (1.67%) was detected. Among the patients with a positive PCR, 10 (16.67%) were co-infected with herpesviruses. All co-infected patients were HIV seropositive. The chi-square test showed a significant association between HIV infection status and the molecular detection of herpesvirus in patients with encephalitis (OR 3.9; 95% CI: [1.4 - 9.3]; $P < 0.0015$). **Conclusion**: Herpesviruses are a significant cause of encephalitis, particularly in immunocompromised and immunocompetent patients. In our study, EBV and CMV were the most common. Therefore, the rapid identification of these viruses in the CSF and the initiation of appropriate antiviral treatment are essential to reduce mortality and improve the management of patients with encephalitis. Furthermore, the development of new antiviral molecules, optimization of therapeutic strategies, and improvement of diagnostic techniques would provide prospects for better control of these infections.

Keywords

Viral Encephalitis, *Herpesviridae*, Côte d'Ivoire, HIV Infection, PCR

1. Introduction

Encephalitis is a severe neurological syndrome associated with high morbidity and mortality, long-term neurological sequelae, and a significant burden on the healthcare system [1]. It is a relatively common disease, with a global incidence of 1.5 - 14 cases per 100,000 people annually [2]. In Africa, the annual prevalence of encephalitis is between 10% and 20% [3]. Data on the causes of encephalitis in West Africa are sparse, with infectious pathogens of the central nervous system typically identified through isolated case reports [4] [5]. Information specific to Côte d'Ivoire is particularly lacking. Nonetheless, regional studies suggest a rising incidence of viral encephalitis, especially linked to herpesviruses, with cases documented in various hospitals. Recent studies in urban areas estimate a prevalence of 5 - 8 cases per 100,000 people annually, although this figure can fluctuate depending on the diagnostic capabilities of local laboratories [3].

Encephalitis can result from a wide range of infectious and non-infectious etiologies. Bacterial, fungal, and parasitic agents, such as *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Cryptococcus neoformans*, and *Toxoplasma gondii*, remain important, particularly in individuals with compromised immune systems. Autoimmune encephalitis and paraneoplastic syndromes have been increasingly recognized in clinical practice [6]. However, viruses remain the predomi-

nant cause worldwide, with herpesviruses responsible for most cases of encephalitis [7] [8].

Among herpesviruses, HSV-1, HSV-2, VZV, EBV, CMV, and HHV-6 are the most frequently implicated, particularly in high-resource settings where their diagnosis is well established [9]. In contrast, in low- and middle-income countries, including those in West Africa, the burden is unknown because of limited access to advanced diagnostic tools and treatments.

Real-time PCR has emerged as a powerful tool for the rapid, sensitive, and simultaneous detection of multiple pathogens directly from cerebrospinal fluid [8] [10] [11]. This technique significantly improves the diagnostic yield, especially in settings where empirical treatment is common and laboratory infrastructure is limited. The implementation of molecular methods in routine diagnostic processes could significantly improve the early detection and management of herpes virus-associated encephalitis in Côte d'Ivoire and other similar settings with limited resources.

This study aimed to determine the prevalence of *Herpesviridae* in patients with encephalitis who were hospitalized in tertiary care centers in Côte d'Ivoire. The findings of this study are expected to highlight the role of herpesviruses in encephalitis cases and support the implementation of molecular tests, such as real-time PCR, as routine diagnostic tools in the national surveillance and management of encephalitis.

2. Material and Methods

2.1. Study Design and Participants

It is a cross-sectional, multicenter study carried out between March 2024 and April 2025 in teaching hospital centers in two regions of Côte d'Ivoire (Bouaké and Abidjan), that receive the majority of patients from different areas in Côte d'Ivoire with signs of encephalitis (fever, convulsions, altered consciousness). Informed consent was obtained from all participants in this study, either directly from the patient when conscious or from a legal representative if the patient was unable to provide consent.

Socioepidemiological and clinical data were collected from the patients' medical records using a data collection form. Participants' sociodemographic data, such as age and sex, were collected using a survey form. The clinical data were extracted from the patient files. The questionnaire included exposure history, symptoms, and clinical signs of encephalitis.

2.2. Inclusion/Exclusion Criteria

This study focused on patients aged 15 years or older who were admitted to the hospital with clinical signs consistent with encephalitis, such as fever, confusion, convulsions, or neurological deficits, and who provided their informed consent directly or indirectly through a legal representative. Patients with a confirmed non-infectious diagnosis and those with contraindications for lumbar puncture

were also excluded from the study.

2.3. Sample Collection

A volume of 500 µL of cerebrospinal fluid (CSF) was collected from all patients included in the study under strict sterile conditions by lumbar puncture performed by trained medical staff. Samples were collected in sterile tubes and immediately transported to the Molecular Biology Unit of the CHU Treichville Central Laboratory for further analyses.

In the laboratory, samples were stored at −20˚C until molecular analysis was performed.

2.4. Laboratory Analysis

Cerebrospinal fluid samples were thawed at room temperature and gently homogenized. Viral DNA was extracted from 200 µL of CSF using a commercial extraction kit (PureLink™ Genomic DNA Mini Kit, Invitrogen) according to the manufacturer's protocol. The extracted DNA was immediately used for amplification or stored at −20˚C until analysis.

The extracted DNA was used as template for PCR reactions targeting 6 viruses of the Herpesviridae family, namely, *Cytomegalovirus*, *Varicella-zoster virus*, *Herpes simplex* type 1 and 2, *Epstein Barr virus* and *Human Herpesvirus* type 6. Table 1 shows the nucleotide sequences of the primers and probes used for the target genes of each of the viruses.

Table 1. Probes, primers and target genes for the viruses tested.

Herpesvirus	Target	Primers and hydrolysis probe TaqMan (5'→3')	Reference
HSV 1/2	gB	F CCGTCAGCACCTTCATCGA R CGCTGGACCTCCGTGTAGTC P (FAM) CCACGAGATCAAGGACAGCGGCC (TAMRA)	[12]
CMV	US8	F ACCAACATAAGGACTTTTCACACTTTT R GAATACAGACACTTAGAGCTCGGGGT P (FAM) CTGGCCAGCACGTATCCCAACAGCA (TAMRA)	[13]
EBV	BXLF1	F GGGGCAAAATACTGTGTTAG R CGGGGGACACCATAGT P (FAM) CGGCGCATGTTCTCCTCCAC (TAMRA)	[14]
HHV6	U65_U66	F GACAATCACATGCCTGGATAATG R TGTAAGCGTGTGGTAATGGACTAA P (FAM) AGCAGCTGGCGAAAAGTGCTGTGC (TAMRA)	[15]
VZV	ORF63	F CGCGTTTTGTACTCCGGG R ACGGTTGATGTCCTCAACGAG P (FAM) TGGGAGATCCACCCGGCCAG (TAMRA)	[16]

F = forward; R = reverse; P = probe.

PCR was performed in a total volume of 20 µL, consisting of 15 µL of reaction mixture (4 µL of HOT FIREPol ® Plus qPCR master mix (without ROX), 0.5 µl of primers and probe corresponding to each virus target gene, and 9.5 µL of sterile nuclease-free water) and 5 µL of extracted DNA.

The cycling conditions for the reaction were as follows: initial denaturation at 95˚C for 15 min, followed by a cyclic phase repeated 40 times, including a denaturation step at 95˚C for 15 s and a hybridization-elongation step at 60˚C for 1 min. The Bio-Rad CFX96 C1000 thermal cycler (Bio-Rad Laboratories Inc., USA) was used for cycling and analysis of PCR results. Negative and positive samples for the different targets of interest were included in the tests as negative and positive controls, respectively, to validate the results. Samples with cycle threshold (Ct) values lower than 38 were considered positive. Samples with a clear amplification curve but Ct values greater than 38 were considered negative.

2.5. Data Management and Statistical Analysis

Data collected from the form were recorded using Excel version 2019 (Microsoft Corporation, Washington, USA), and analysed with statistical software R version 4.2.1 (The R Foundation, Auckland, United States). Statistical analysis was used to assess the distribution of demographic and clinical characteristics and the associations between the various parameters.

A descriptive analysis was performed to summarize the data collected. Summary frequencies and proportions were used to describe all nominal characteristics, including sex and patient symptoms. The mean and standard deviation were used to describe quantitative variables. Comparisons between groups were made using the Chi2 test (or Fisher's exact test when numbers were small) for categorical variables. For all analyses, a value ($P < 0.05$) was considered statistically significant.

2.6. Ethics

This study was conducted after administrative authorization was granted by the management of the various participating teaching hospitals, and approval was obtained from the Comité National d'Éthique des Sciences de la Vie et de la Santé (CNESVS), which examined and approved the study protocol with reference number 00211 24/MSHPCMU/CNESVS-km. Written informed consent was obtained from all the patients. For patients under the age of 18 years and those with altered consciousness, consent was obtained from their parents or legal representatives.

In addition, the study was conducted in accordance with the principles governing the conduct of research involving human subjects as set out in the Declaration of Helsinki [17].

3. Results

3.1. Demographic and Clinical Characteristics of Included Patients

This study included 388 participants in total. The majority (76.03%, n = 295) of

the participants were from Treichville Teaching Hospital, followed by 15.46% (n = 60) from Angré teaching hospital, 7.22% (n = 28) from Bouaké teaching hospital, and 1.29% (n = 5) from Cocody teaching hospital. The participants comprised 222 (57.22%) women and 166 men (42.78%), with a sex ratio (M/F) of 1.34. The age of the participants ranged from 15 to 79 years, with a mean age of 45.36 ± 13.56 years. With regard to symptoms, the principal clinical manifestations observed in patients suspected of suffering from herpesvirus encephalitis were altered consciousness (confusion), fever, and convulsions. Fever was the most common symptom, observed independently in 13 patients (3.35%), or in association with altered consciousness (AC) in 185 patients (47.68%), with convulsions in 23 patients (5.93%), or with both manifestations in 26 patients (6.70%). Other symptoms were observed in 141 (36.34 %) patients. In addition, 282 patients (72.68%) included in this study were HIV-infected. The demographic and clinical characteristics of the patients are shown in Table 2.

Table 2. Summary of demographic and clinical characteristics of the patients.

Variable	Category	N (%)
Age	[15 - 29[	53 (13.66%)
	[30 - 44[	150 (38.66%)
	[45 - 59[	111 (28.61%)
	≥60	74 (19.07%)
Gender	Men	166 (42.78%)
	Women	222 (57.22%)
Provenance	Treichville	295 (76.03%)
	Angré	60 (15.46%)
	Cocody	5 (1.29%)
	Bouaké	28 (7.22%)
Symptoms	Fever	23 (5.93%)
	Fever + AC*	185 (47.68%)
	Fever + AC* + Seizures	13 (3.35%)
	Fever + Seizures	26 (6.70%)
	Other symptoms	141 (36.34%)
HIV Status	HIV+	282 (72.68%)
	HIV−	11 (2.84%)
	Not available	95 (24.48%)

*AC = altered consciousness.

3.2. Viral Etiologies

PCR test results revealed the presence of viruses in sixty (60) patients, corresponding to a prevalence of 15.46%. The *Epstein-Barr virus* (EBV) was the most frequently detected virus. Among the patients with a positive PCR for *Herpesviridae*,

10 (16.67%) were co-infected with at least two *Herpesviridae* viruses. The co-infection associations were varied and mostly included CMV, EBV and VZV. In 70% of cases, the most frequent associations were CMV + EBV, EBV + HHV6, VZV + HHV6 and CMV + VZV. Only 30% of cases showed triple associations of CMV + HSV + VZV, CMV + EBV + HHV6 and CMV + VZV + HHV6. Table 3 shows the detection frequency of each targeted herpesvirus in the study population.

Table 3. Prevalence of viruses detected.

		PCR+	Prevalence of positivity (n = 60)	Overall prevalence (n = 388)
Virus	EBV	33	55.00%	8.50%
	CMV	20	33.33%	5.15%
	VZV	12	20.00%	3.09%
	HHV6	7	11.67%	1.80%
	HSV1/2	1	1.67%	0.26%

3.3. Factors Associated with Viral Infection

Analysis of demographic variables revealed no statistically significant association between age (P = 0.09) or sex (P = 0.54) and PCR positivity for Herpesviridae. Comparison between hospital centers revealed variable detection rates: 25% at Bouaké teaching hospital (7/28), 16.61% at Treichville teaching hospital (49/295), 6.7% at Angré teaching hospital (4/60), and 0% at Cocody teaching hospital. However, these differences were not statistically significant (P = 0.08).

From a clinical aspect, a significant association was observed between the detection of Herpesviridae and the concomitant presentation of fever and disorders of consciousness (P = 0.005). In addition, HIV infection appeared to be a risk factor strongly associated with PCR positivity in this study. HIV-positive patients represented 91.67% (55/60) of herpesvirus PCR-positive patients, compared with 1.67% (1/60) of HIV-negative patients and 6.67% (4/60) of patients with unknown status. This correlation was statistically significant (OR = 3.9; 95% CI: [1.4 - 9.3]; P < 0.001). All associations between clinical and epidemiological characteristics and PCR results are presented in Table 4.

4. Discussion

The introduction of virological diagnostic techniques in the laboratory reduces the burden on both patients and health services. Indeed, some encephalitis etiologies have specific treatments, such as herpesviruses, for which there are well-established antiviral treatments [18]-[20]. Rapid identification of the etiology is essential, as these neurological infections are fatal and therefore require rapid and effective laboratory tests to diagnose the etiological agents. Once the etiology is established, therapeutic interventions are less difficult.

Table 4. Summary of factors associated with PCR status.

Variable	Category	PCR+ (n = 60)	PCR− (n = 328)	P-value
Age	[15 - 29[	8 (15.09%)	45 (84.91%)	0.09
	[30 - 44[	17 (11.33%)	133 (88.67%)	
	[45 - 59[	25 (22.52%)	86 (77.48%)	
	≥60	10 (13.51%)	64 (86.51%)	
Gender	Men	23 (13.86%)	143 (86.14%)	0.54
	Women	37 (16.67)	185 (83.33%)	
Provenance	Treichville	49 (16.61%)	246 (83.39%)	0.08
	Angré	4 (6.67%)	56 (93.33%)	
	Cocody	0 (0.0%)	5 (100.0%)	
	Bouaké	7 (25.0%)	21 (75.0%)	
Symptoms	Fever	2 (8.70%)	21 (91.30%)	0.005
	Fever + AC	42 (22.70%)	143 (77.30%)	
	Fever + AC + Seizures	0 (0.00%)	13 (100.00%)	
	Fever + Seizures	7 (26.92%)	19 (73.08%)	
	Other symptoms	9 (6.38%)	132 (93.62%)	
HIV Status	HIV+	55 (19.50%)	227 (80.50%)	< 0.0015
	HIV−	1 (9.1%)	10 (90.90%)	
	Not available	4 (4.2%)	91 (95.79%)	

In our study, encephalitis predominantly occurred in adult patients. This observation differs from those of studies conducted in other countries, such as Brazil [21], Australia [22] or Sweden [23]. In these studies, encephalitis was more frequent in the juvenile population. The mean age of the patients in the present study was 45.36 ± 13.57 years, and the predominance of females (57.22%) suggests a trend that diverges slightly from the global data. Indeed, viral encephalitis most often affects children and the elderly, in contrast to the results of the present study, which predominantly involved young adults. This difference could be explained by the medical history of the patients included in this study. Indeed, 91.67% of those infected with Herpesvirus were also HIV-positive, highlighting an interaction between these pathogens. By weakening the immune system, HIV promotes the reactivation of latent herpesviruses, which can lead to serious complications, such as encephalitis [24]. This observation is consistent with the literature, where it is well documented that HIV patients have an increased risk of herpesvirus encephalitis, particularly EBV, CMV, and HHV6 [25]. Young adults are more affected by HIV and are more vulnerable to opportunistic infections, including viral encephalitis. The hypothesis of co-infection favoring viral reactivation could explain this difference [26].

In terms of clinical signs, fever, disturbances of consciousness, and convulsions are characteristic of herpesvirus encephalitis, as described in other studies [27].

The frequencies observed in the present study were consistent with the results of other studies on viral encephalitis [28] [29]. PCR revealed that 15.46% of patients were positive for at least one herpesvirus. This rate is consistent with the expected figures for opportunistic infections in immunocompromised patients [30] [31]. However, the viral etiologies observed differed from those in other parts of the world. In this study, *Epstein-Barr virus* (EBV) was the most frequent pathogen, accounting for 55.00% of positive cases, followed by *Cytomegalovirus* (CMV), *Varicella zoster virus* and *Human Herpesvirus* type 6 (HHV6), and *Herpes simplex virus* 1 and 2, with 20.00%, 11.67%, and 1.67%, respectively.

This distribution is unusual in the literature. Indeed, Herpes Simplex virus (HSV) has been described in numerous studies as the most frequent cause of herpetic encephalitis, particularly in high-income countries [32]. However, this virus had the lowest rate in the present study. This low rate of HSV could be explained by local factors (vaccination, local viral prevalence, and influence of co-infections) or epidemiological factors specific to Côte d'Ivoire. Indeed, the high frequency of EBV in the present study could also be linked to the prevalence of HIV in the patients tested, since this virus is strongly associated with complications such as lymphoma in immunocompromised individuals [33].

The results of this study also highlight several significant associations between clinical and demographic factors, and the detection of Herpesviridae by PCR, with important clinical and epidemiological implications. The significant association between HIV infection and *Herpesviridae* detection (OR = 3.9; IC 95%: [1.4 - 9.3]; $P < 0.0015$) confirms the key role of immune status in *Herpesviridae* infections. This result corroborates global data highlighting the role of immunosuppression in the reactivation of latent herpesviruses, notably CMV and EBV [34] [35]. Previous studies have reported a higher prevalence of *Herpesviridae* infections in immunocompromised patients, sometimes reaching up to 20% - 30% in patients hospitalized for encephalitis [36]. The prevalence observed in this 55/282 study (19.50%) is therefore consistent with these data, underlining the importance of systematic screening for Herpesviridae in HIV + patients with neurological signs. In the sub-Saharan context, where HIV remains endemic, this association reinforces the need for systematic screening for *Herpesviridae* in HIV-positive patients, in line with WHO recommendations [3].

In this study, the rate of PCR positivity varied significantly between university hospitals. In Abidjan, the highest rate was observed at Treichville University Hospital (16.61%), compared to Angré (6.67%) and Cocody (0%). These disparities could reflect differences in the influx of patients or the severity of cases admitted. Treichville University Hospital, being a national referral center, probably receives more severe patients or cases referred from other establishments, which would explain the high rate of positive PCRs results [37]. However, the absence of PCR-positive cases at the CHU de Cocody could be due to low recruitment rates. Bouaké University Hospital (7 PCR + /28 patients) had a small number of patients (n = 28), which could limit statistical analysis, and is a frequent challenge in mul-

ticenter African studies [38]. The high rate of positive cases observed at Bouaké University Hospital (25%, 7/28) calls for particular attention. Bouaké, being located in a central area of Côte d'Ivoire, could reflect a higher prevalence in rural populations where access to early care is limited, or a higher concentration of untreated HIV + patients.

No statistically significant association was found between patient age (P = 0.09), sex (P = 0.54), and Herpesviridae detection. These results are consistent with previous studies, which indicate that *Herpesviridae* infections are not strictly linked to age groups or sex differences, although some studies report a slight male predominance for certain viral encephalitides, probably due to behavioral or exposure factors [39]. Clinically, the complete absence of PCR + in patients presenting with fever, altered consciousness, and convulsions is an intriguing finding. This result contrasts with Asian studies, where HSV-1 is often associated with convulsions [40]. This triad could be linked to bacterial, parasitic, or autoimmune causes, which are dominant in this subgroup [41]. The classic triad usually associated with herpetic encephalitis was absent in all PCR-positive patients. This suggests that *Herpesviridae* infections may present with varied or atypical clinical manifestations, particularly in immunocompromised patients. Studies have shown that fever alone, or associated with disturbances of consciousness without convulsions, can represent a significant proportion of clinical presentations [6] [42].

Bivariate analysis confirmed that HIV infection was an independent risk factor for PCR positivity (OR 3.9; IC 95%: [1.4 - 9.3]; P = 0.0015). This highlights the importance of integrating *Herpesviridae* screening into the management of HIV + patients, particularly those with neurological symptoms.

These results highlight the importance of tailored care for patients with neurological disorders, depending on their HIV status and their place of origin. Disparities between university hospital centers highlight the need to strengthen diagnostic capabilities and access to care in the country's underserved areas. A longitudinal evaluation of *Herpesviridae* encephalitis cases and their associated factors is essential for improving prevention and management strategies.

5. Conclusion

This study highlights the high burden of herpesvirus encephalitis in immunocompromised patients in Ivorian hospitals. The high proportion of EBV and CMV viruses and the low rate of HSV suggest local epidemiological specificities or are probably linked to HIV infection. These results underline the importance of rapid detection, notably by PCR, for the appropriate management of herpesvirus encephalitis to reduce the morbidity and mortality associated with these infections.

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Authors' Contributions

Aboubacar Bamba, designed the study, drafted the protocol and the manuscript.

Aboubacar Bamba and **Kobina Amandze Adams Kofi** carried out the laboratory analyses.

Flora Ahonzo, Arouna Coulibaly, Sodji Emilie K N'Goran, Pacôme Monemo, Mbodje Ophélia Gnamon for collecting the samples.

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Kouadio Stephane Koffi validated the protocol and revised the document.

All authors have read and approved the manuscript.

Conflicts of Interest

All authors declare no conflicts of interest.

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