Liu *et al. Virology Journal* (2025) 22:141
https://doi.org/10.1186/s12985-025-02768-w

Virology Journal

RESEARCH Open Access

Clinical features and risk factors for Epstein-Barr virus-associated encephalitis: a retrospective cohort study

Zhu Liu[1], Anjiao Peng[1], Luyan Huang[1], Leihao Sha[1], Yusha Tang[1], Yongzhao Zhou[2*] and Lei Chen[1*]

Abstract

Background Epstein-Barr virus (EBV) has been gradually recognized as an important pathogen of encephalitis, but our knowledge of EBV-associated encephalitis is still limited. The aim of this study was to describe the clinical features and explore the risk factors for EBV-associated encephalitis in a large cohort of Chinese patients.

Methods All patients with confirmed encephalitis in our center from June 2020 to April 2021 were enrolled. Data were extracted from the electronic medical record system and analyzed by Student's t test, Mann-Whitney U test, chi-square test, and logistic regression analyses.

Results This study included a total of 364 patients diagnosed with encephalitis, among which 86 cases (23.6%) had EBV detected in their cerebrospinal fluid (CSF), and 39 cases were diagnosed with EBV-associated encephalitis. The clinical characteristics of EBV-associated encephalitis differ from those caused by other herpes viruses. Compared to other herpes virus-related encephalitis, patients with EBV encephalitis exhibited significantly higher protein levels (1.310 [0.695, 1.840] vs. 0.710 [0.490, 1.700], $p < 0.001$), lower glucose levels (3.030 [2.585, 3.640] vs. 3.380 [2.990, 4.030], $p < 0.001$), and a higher incidence of meningeal involvement on MRI (11 (30.6%) vs. 3 (6.5%), $p = 0.010$). Additionally, univariate logistic regression analysis revealed that age (OR = 1.015, 95% CI: 1.002–1.029), HIV infection (OR = 4.285, 95% CI: 1.582–11.816), non-tumor immunosuppression (OR = 5.713, 95% CI: 1.588–22.883), and peripheral blood EBV infection (OR = 10.204, 95% CI: 4.231–26.346) were independent risk factors for CNS EBV infection.

Conclusion Compared to other herpes virus-associated encephalitis, EBV encephalitis is characterized by a higher degree of meningeal involvement, elevated CSF protein levels, lower glucose levels, and a reduced T-lymphocyte count in peripheral blood. These characteristics suggest that EBV encephalitis may have a distinct pathophysiology.

Zhu Liu, Anjiao Peng and Luyan Huang equally contributed to this work.

*Correspondence:
Yongzhao Zhou
yongzhaozhou001@wchscu.cn
Lei Chen
leilei_25@126.com
[1]Department of Neurology, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang, Chengdu, Sichuan 610041, China
[2]Department of Respiratory and Critical Care Medicine, Frontier Science Center of Disease Molecular Network, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China

Introduction

Encephalitis is a severe central nervous system infectious disease with an estimated incidence of 1.7–7.4 cases per 100,000 person-years worldwide [1]. The mortality of encephalitis in the acute phase has been reported to be as high as 10%, and many survivors develop long-term neurological deficits such as cognitive decline, disability, and mental illness, causing great burden to their family and the society [2, 3]. Pathogens could only be determined in nearly half of patients with encephalitis and 20–50% of them are attributed to viruses [4]. Among them, herpes simplex virus, varicella-zoster virus, enteroviruses, and arboviruses-associated encephalitis have been well recognized.

Epstein Barr virus (EBV), also known as human herpes virus γ, is a typical human lymphotropic herpesvirus with latent infection in B lymphocytes and productive infection in oral mucosal epithelium [5, 6]. EBV has been widely recognized to cause Burkitt lymphoma, lymphoid hyperplasia disease, and nasopharyngeal carcinoma [6]. In recent years, EBV has also been found to be closely associated with autoimmune diseases such as systemic lupus erythematosus [7] and multiple sclerosis [8]. Moreover, compelling evidence has shown that EBV is an important pathogen for encephalitis, [9–13] and EBV-associated encephalitis has receiving great attention. Compared with other viruses, EBV is more likely to induce early autoimmune responses against nerve cells and causes a worse prognosis in patients with encephalitis [14]. However, our knowledge of EBV-associated encephalitis is still limited.

To deepen our understanding of EBV-associated encephalitis, we conducted this retrospective cohort study and reviewed the detailed clinical features of EBV-associated encephalitis in our center. We also explored the potential risk factors for EBV-associated encephalitis.

Methods

Participants

With approval from the ethics committee of West China Hospital, Sichuan University, we enrolled all patients with confirmed encephalitis in our center from June 2020 to April 2021. All electronic medical records were reviewed, and the diagnoses of all patients were further confirmed by two authors (Peng and Liu) according to previous criteria [15]. Patients with symptoms consistent with meningitis and a cerebrospinal fluid (CSF) leucocyte count $>4\times10^6$ cells per L were diagnosed with meningitis. The criteria of encephalitis were altered consciousness for >24 h (including lethargy, irritability, or a change in personality) with no other cause found and two or more of the following signs: fever or history of fever (≥38 °C) during the current illness; seizures or focal neurological signs (with evidence of brain parenchyma involvement); CSF pleocytosis ($>4\times10^6$ cells per L); electroencephalogram suggesting encephalitis; and neuroimaging suggestive of encephalitis [15]. Cases without key clinical information were excluded.

Data extraction

A standardized form was used to extract the following information from all patients from the electronic medical record system: age, gender, consciousness, behavioral and psychological symptoms, seizure, ventilator-assisted ventilation, immunosuppression (i.e., human immunodeficiency virus infection, immunosuppressive therapy for malignant tumor (chemotherapy, radiotherapy, organ transplantation, or bone marrow transplantation), and immunosuppressive therapy for non-neoplastic condition (systemic lupus erythematosus, kidney disease, rheumatoid arthritis, etc.), cell count, protein, glucose, chlorine and intrathecal IgG synthesis rate in CSF, white blood cell, lymphocyte, monocyte, CD3 + lymphocyte, CD4 + lymphocyte, and CD8 + lymphocyte, EBV copies in peripheral blood (categorized as negative; latent: positive but less than 50 copies/mL; active: more than 50 copies/mL), and findings of brain magnetic resonance imaging (MRI). Statuses at discharge were also reviewed, and those with death, persistent disturbance of consciousness or mechanical ventilation were regarded as having a poor prognosis.

Data of pathogen detection

EBV and other pathogens in CSF were detected by metagenomic next-generation sequencing (mNGS). We also reviewed the information of conventional testing for pathogens in CSF, brain tissue, and peripheral blood for all patients. Cryptococcal antigen assay in CSF and peripheral blood, CSF ink test examination, and CSF cryptococci culture were reviewed for cryptococcus [16, 17]. CSF tuberculous smear and culture, and Mycobacterium tuberculosis and rifampicin resistance test (Xpert MTB/RIF) were reviewed for tuberculous [18, 19]. CSF smear and culture were reviewed for bacteria, PCR in CSF and peripheral blood and IgM in peripheral blood were reviewed for viruses [4]. Information of other pathogens, such as John Cunningham virus, Toxoplasma gondii, and Treponema pallidum were also extracted.

Statistical analysis

Continuous variables were presented as median [interquartile range (IQR)] due to non-normality identified via Shapiro-Wilk tests, while categorical variables were described using frequencies (%). Group comparisons were performed using Mann-Whitney U tests for continuous variables and Chi-square tests (or Fisher's exact tests when expected cell counts < 5) for categorical variables between two groups.

For subgroup analyses, EBV-positive cases were further stratified into EBV-caused and non-EBV-caused subgroups, with pairwise comparisons between EBV-negative, EBV-caused, and non-EBV-caused groups using Mann-Whitney U tests for continuous variables and Chi-square/Fisher's exact tests for categorical variables. A similar analytical approach was applied when comparing EBV-associated and other herpes virus-associated subgroups.

Univariate logistic regression models were constructed to evaluate associations between candidate variables and EBV positivity, yielding odds ratios (ORs) with 95% confidence intervals (CIs). Statistical significance was defined as two-tailed $p < 0.05$, and data were analyzed using SPSS Statistics version 25.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic composition

Initially, a total of 379 patients with acute encephalitis were included in the study. However, 15 patients who underwent lumbar puncture and CSF mNGS testing during emergency admission were excluded from the final analysis due to incomplete medical records caused by their spontaneous discharge and failure to re-admit. Ultimately, 364 patients were included in the study, among which 86 (23.6%) patients had EBV detected in their CSF (Tables 1) (Fig. 1). Additionally, 39 patients were diagnosed with EBV-associated encephalitis, while 49 patients were diagnosed with herpes virus associated encephalitis (Table 3).

Clinical features between patients with or without EBV infection

Generally, there was no gender difference between the two groups, but patients in EBV-positive (EBV+) group were slightly older than EBV-negative (EBV-) group (Table 1).

There were several differences in clinical characteristics between the two groups. Firstly, immunosuppression was more common in the EBV + group compared to the EBV - group, particularly in cases of HIV infection and immunosuppression for non-neoplastic diseases. Secondly, significant differences were observed in CSF characteristics: the EBV + group had relatively higher cell counts and protein levels, while glucose and chloride levels were lower.

Significant changes were also observed in peripheral blood. Compared to the EBV - group, the EBV + group exhibited significantly higher white blood cell counts and monocyte counts, while lymphocyte counts, as well as CD3+, CD4+, and CD8 + T cell counts, showed an opposite trend. EBV in peripheral blood was tested in 213 patients (58 in the EBV + group and 155 in the EBV - group). The results showed that patients with CNS EBV infection were more likely to have active peripheral EBV infection. A total of 326 patients (89.6%) completed brain MRI scans. Although the EBV + group showed more meningeal involvement on MRI, there was no statistically significant difference observed (35.6% vs. 24.1%, $p>0.05$) (Table 1).

To further explore the risk factors for CNS EBV infection, logistic regression was used to calculate ORs and 95% CIs. Immunosuppression, active peripheral EBV infection, and blood cells were analyzed after age and gender adjustment. The results showed that age, immunosuppression and active infection of peripheral EBV were independent risk factors for CNS EBV infection.

Pathogens were determined in a total of 181 (49.8%) patients. The results showed that compared with the EBV - group, the EBV + group had relatively higher rates of cryptococcus and tuberculosis encephalitis, but viral and bacterial infections were similar between the two groups (Fig. 2).

Comparison of clinical features in subgroups

A comparison of clinical features among three groups: EBV-positive encephalitis, EBV-positive encephalitis of other etiology, and EBV-negative encephalitis, revealed significant differences. Specifically, EBV-negative encephalitis was characterized by significantly lower values in age, CSF white blood cell count, protein level, and peripheral blood monocyte count compared to both the EBV-positive encephalitis and EBV-positive encephalitis of other etiology groups. Concurrently, the EBV-positive encephalitis group exhibited notably higher levels of CSF glucose and chloride, as well as elevated counts of peripheral blood CD3+, CD4+, and CD8 + T cells compared to the other two groups.

When comparing EBV-positive encephalitis to EBV-positive encephalitis of other etiology, the former group demonstrated significantly higher values in age, CSF glucose level, peripheral blood lymphocyte and monocyte counts, as well as CD3+, CD4+, and CD8 + T cell levels. Conversely, they had lower values in CSF white blood cell count, protein level, chloride level, and peripheral blood white blood cell count.

Regarding the comparison between EBV-positive encephalitis of other etiology and EBV-negative encephalitis, additional differences were observed. Notably, the presence of immunosuppression, intrathecal IgG synthesis rate in CSF, and active infection of peripheral EBV were found to be higher in the EBV-positive encephalitis of other etiology group, alongside the previously mentioned disparities in clinical features (Table 2).

Clinical features between EBV and other herpes viruses associated encephalitis

A total of 39 patients were diagnosed with EBV-associated encephalitis among whom only EBV was detected

Table 1 Clinical features between patients with or without EBV infection

Characteristic	EBV positive ($n=86$)	EBV negative ($n=278$)	*p* value	miss(%)	OR	95%CI
Age (years, median and IQR)	53.000 [34.000, 62.000]	47.000 [28.250, 57.000]	**< 0.001**	-	1.015*	1.002–1.029
Gender (male, %)	63 (73.256)	180 (64.748)	0.183	-	1.491	0.882–2.592
Clinical manifestation (%)						
Consciousness disturbance	38 (44.186)	108 (38.849)	0.449	-	1.246	0.762–2.030
Behavioral and psychological symptoms	22 (25.581)	85 (30.576)	0.451	-	0.781	0.444–1.333
Seizure	14 (16.279)	62 (22.302)	0.294	-	0.677	0.346–1.252
Mechanical ventilation	23 (26.744)	56 (20.144)	0.251	-	1.447	0.816–2.513
Immunocompromised (%)			**0.001**			1.258–2.561
HIV	9 (10.465)	8 (2.878)		-	4.285*	1.582–11.816
Malignancy	3 (3.488)	7 (2.518)		-	1.632	0.345–6.041
Immunosuppression for non-neoplastic condition	6 (6.977)	4 (1.439)		-	5.713*	1.588–22.883
CSF features						
Cell counts (median and IQR)	105.000 [20.000, 180.000]	37.500 [3.000, 157.500]	**< 0.001**	-	1.000	1.000–1.000
Proteins (g/L, median and IQR)	1.310 [0.702, 2.077]	0.850 [0.450, 1.630]	**< 0.001**	-	1.032	0.943–1.128
Glucose (mmol/L, median and IQR)	2.900 [2.183, 3.607]	3.290 [2.652, 4.008]	**< 0.001**	-	0.783*	0.650–0.932
Chlorine (mmol/L, median and IQR)	121.000 [115.000, 126.000]	123.000 [116.000, 127.750]	**< 0.001**	-	0.990	0.966–1.015
Leukocytosis (%)	72 (83.721)	200 (71.942)	**0.040**	-	2.006*	1.097–3.895
Proteins increasing (%)	76 (88.372)	207 (74.460)	**0.010**	-	2.607*	1.331–5.612
Glucose reduction (%)	27 (31.395)	59 (21.223)	**0.073**	-	1.699	0.983–2.895
Chlorine reduction (%)	37 (43.023)	100 (35.971)	**0.293**	-	1.344	0.819–2.196
Increased IgG synthesis rate (%)	32 (76.190)	77 (47.531)	**0.002**	44.0	3.532*	1.680–8.012
MRI features (%)						
Positive findings of parenchyma	40 (54.795)	118 (46.640)	0.273	10.4	1.387	0.823–2.349
Positive meningeal findings	26 (35.616)	61 (24.111)	0.071	10.4	1.741	0.988–3.032
Peripheral blood (median and IQR)						
White blood cells (10^9/L)	8.325 [6.192, 10.545]	7.895 [5.920, 10.510]	**< 0.001**	-	1.006	0.949–1.064
Lymphocytes (10^9/L)	1.080 [0.715, 1.447]	1.165 [0.832, 1.647]	**< 0.001**	-	0.793	0.530–1.163
Monocyte (10^9/L)	0.660 [0.450, 0.947]	0.570 [0.422, 0.780]	**< 0.001**	-	1.556	0.779–3.063
CD3 + T lymphocytes (cell/μl)	488.500 [318.750, 782.250]	636.000 [408.500, 935.000]	**< 0.001**	56.9	0.999*	0.998-1.000
CD4 + T lymphocytes (cell/μl)	237.000 [129.500, 450.000]	334.000 [189.500, 518.500]	**< 0.001**	56.9	0.998*	0.996-1.000
CD8 + T lymphocytes (cell/μl)	214.000 [96.500, 330.750]	223.000 [163.500, 377.500]	**< 0.001**	56.9	0.998	0.996-1.000
EBV in peripheral blood (%)			**< 0.001**			
Negative	24 (41.379)	116 (74.839)				
Latent infection	15 (25.862)	30 (19.355)		41.5	2.417*	1.117–5.151
Active infection	19 (32.759)	9 (5.806)		41.5	10.204*	4.231–26.346
Poor prognoses (%)			0.600			
Consciousness disturbance	10 (11.628)	20 (7.194)		-	1.724	0.742–3.785
Mechanical ventilation	7 (8.140)	20 (7.194)		-	1.207	0.457–2.855
Death	2 (2.326)	7 (2.518)		-	0.985	0.144–4.190

HIV: human immunodeficiency virus; IQR: interquartile range

Miss (%) If there is no missing data for the corresponding indicator, it is identified with "-", otherwise, the specific missing proportion value is displayed after it

* $P<0.05$ Single factor analysis was statistically significant

and deemed as the pathogen. Another 49 cases were diagnosed with other herpesviruses-associated encephalitis (including 23 herpes simplex virus, 12 varicella-zoster virus, 2 cytomegalovirus, 1 human herpesvirus-6 and 11 human herpesvirus-7). Patients with EBV encephalitis tend to be older compared to those with encephalitis caused by other herpes viruses. In terms of CSF characteristics, patients with EBV encephalitis exhibit higher

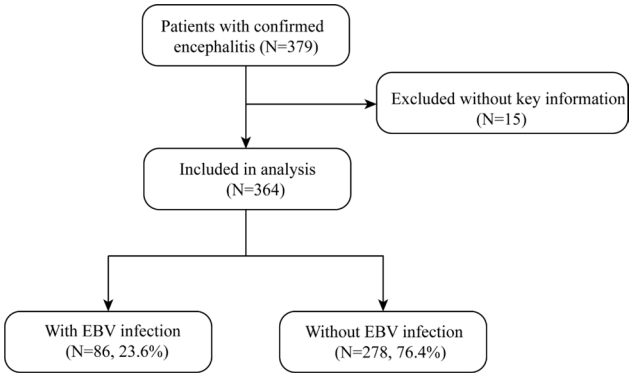

Fig. 1 The inclusion flow chart

white blood cell counts and protein levels, while glucose and chloride levels are relatively lower. Compared to other herpes virus infections, individuals infected with EBV demonstrate higher peripheral blood white blood cell counts and monocyte counts, but lower lymphocyte, CD3+, CD4+, and CD8 + T-cell counts. This observation is similar when comparing EBV + and EBV - groups. However, a notable difference is the higher incidence of meningeal involvement among patients with EBV encephalitis. Although the prognosis of EBV encephalitis is generally poor, the results of this study did not reveal a statistically significant difference (20.5% vs. 10.2%, $p>0.05$) (Table 3).

Discussion

The main findings of the study were as follows: (1) EBV could be found in CSF in approximately 24% of patients with acute encephalitis; (2) clinical characteristics of EBV-associated encephalitis differ from those of other herpes virus-associated encephalitis, specifically featuring leukocytosis and higher protein content, lower glucose and chloride levels in CSF, and more frequent meningeal involvement on MRI; and (3) age, immunosuppression and active peripheral EBV infection were independent risk factors for CNS EBV infection.

In this retrospective cohort study, we found that EBV could be detected in CSF in nearly 24% of patients with confirmed acute encephalitis (including viral, tuberculous, bacterial, cryptococcal, and unknown cause). In line with our results, a previous study found that EBV exited the CSF of 26% of patients with bacterial meningitis using PCR [20]. Similar results from two studies with different populations and different techniques confirmed the commonness of EBV infection in acute encephalitis. Furthermore, we found that the rates of tuberculous and cryptococcal encephalitis were relatively higher in patients with CNS EBV infection, suggesting that CNS EBV may be associated with tuberculosis and cryptococcal infections, but the causal relationship deserves further research.

In previous studies, glucose and chloride levels in CSF were often regarded as typical biomarkers for viral encephalitis. However, this study found that both EBV positive and negative encephalitis patients experienced varying degrees of reduction in CSF glucose and chloride levels. To eliminate interference from other pathogens such as cryptococcus and tuberculosis, we further excluded cases of related pathogen infections from the analysis, and the results still showed the presence of the aforementioned characteristics. To further investigate whether this phenomenon was limited to EBV-associated encephalitis, we selected 39 cases diagnosed with

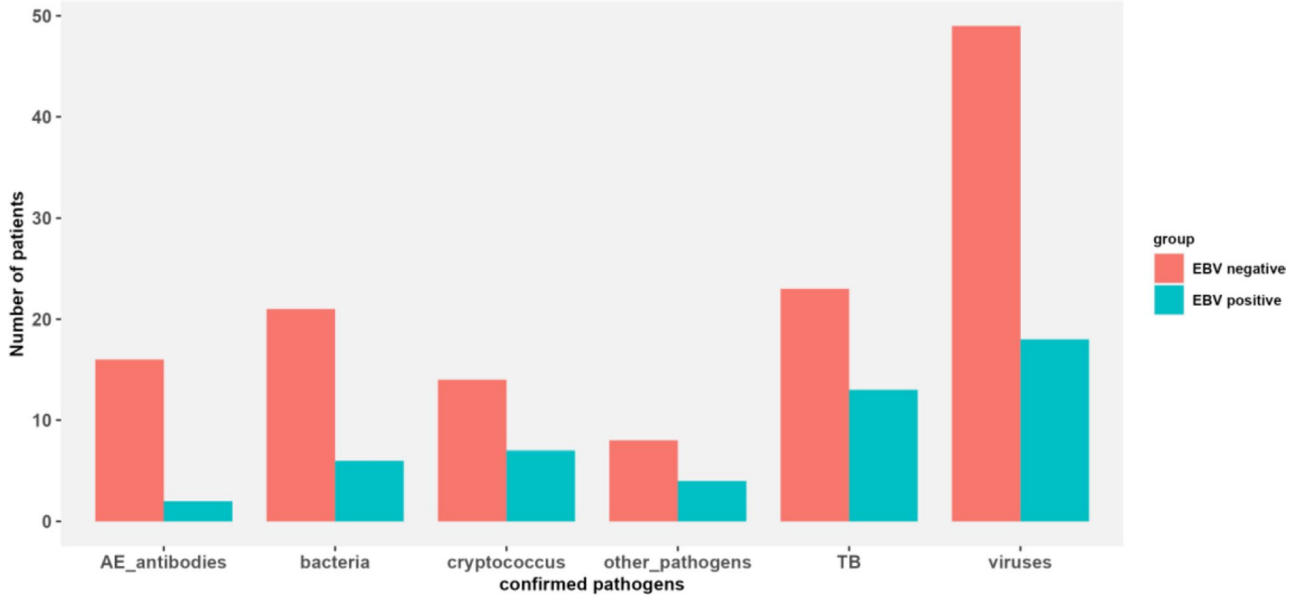

Fig. 2 The prevalence of Epstein Barr virus (EBV) in encephalitis with confirmed pathogens

Table 2 Clinical features among patients with encephalitis caused by EBV, with encephalitis not caused by EBV, and with encephalitis negative EBV

Characteristic	EBV caused (*n* = 39)	Non-EBV caused (*n* = 47)	EBV negative (*n* = 278)	*p* value (EBV caused VS Non - EBV caused)	*p* value (EBV caused VS EBV negative)	*p* value (non-EBV caused VS EBV negative)
Age (years, median and IQR)	55.000 [43.500, 62.500]	52.000 [32.500, 61.500]	47.000 [28.250, 57.000]	**<0.001**	**<0.001**	**<0.001**
Gender (male, %)	28 (71.795)	35 (74.468)	180 (64.748)	0.973	0.492	0.256
Clinical manifestation (%)						
Consciousness disturbance	19 (48.718)	19 (40.426)	108 (38.849)	0.580	0.316	0.965
Behavioral and psychological symptoms	6 (15.385)	16 (34.043)	85 (30.576)	0.084	0.076	0.761
Seizure	5 (12.821)	9 (19.149)	62 (22.302)	0.618	0.251	0.770
Mechanical ventilation	14 (35.897)	9 (19.149)	56 (20.144)	0.133	**0.044**	1.000
Immunocompromised (%)				0.725	**0.001**	**0.031**
HIV	4 (10.256)	5 (10.638)	8 (2.878)			
Malignancy	1 (2.564)	2 (4.255)	7 (2.518)			
Immunosuppression for non-neoplastic condition	4 (10.256)	2 (4.255)	4 (1.439)			
CSF features						
Cell counts (median and IQR)	70.000 [6.000, 140.000]	120.000 [41.000, 287.000]	37.500 [3.000, 157.500]	**<0.001**	**<0.001**	**<0.001**
Proteins (g/L, median and IQR)	1.310 [0.695, 1.840]	1.310 [0.795, 2.375]	0.850 [0.450, 1.630]	**<0.001**	**<0.001**	**<0.001**
Glucose (mmol/L, median and IQR)	3.030 [2.585, 3.640]	2.670 [1.425, 3.605]	3.290 [2.652, 4.008]	**<0.001**	**<0.001**	**<0.001**
Chlorine (mmol/L, median and IQR)	119.000 [115.000, 125.000]	121.000 [115.000, 126.000]	123.000 [116.000, 127.750]	**<0.001**	**<0.001**	**<0.001**
Leukocytosis (%)	32 (82.051)	40 (85.106)	200 (71.942)	0.929	0.254	0.085
Proteins increasing (%)	34 (87.179)	42 (89.362)	207 (74.460)	1.000	0.123	**0.041**
Glucose reduction (%)	8 (20.513)	19 (40.426)	59 (21.223)	0.081	1.000	**0.008**
Chlorine reduction (%)	20 (51.282)	17 (36.170)	100 (35.971)	0.234	0.095	1.000
Increased IgG synthesis rate (%)	14 (77.778)	18 (75.000)	77 (47.531)	1.000	**0.029**	**0.022**
MRI features (%)						
Positive findings of parenchyma	19 (52.778)	21 (56.757)	118 (46.640)	0.915	0.609	0.330
Positive meningeal findings	11 (30.556)	15 (40.541)	61 (24.111)	0.518	0.528	0.055
Peripheral blood (median and IQR)						
White blood cells (10^9/L)	7.790 [6.035, 10.785]	8.340 [6.485, 10.165]	7.895 [5.920, 10.510]	**<0.001**	**<0.001**	**<0.001**
Lymphocytes (10^9/L)	1.120 [0.665, 1.305]	1.020 [0.750, 1.525]	1.165 [0.832, 1.647]	**<0.001**	**<0.001**	**<0.001**
Monocyte (10^9/L)	0.660 [0.455, 0.945]	0.660 [0.425, 0.910]	0.570 [0.422, 0.780]	**0.004**	**<0.001**	**<0.001**
CD3 + T lymphocytes (cell/μl)	504.000 [414.000, 785.500]	453.000 [248.500, 751.000]	636.000 [408.500, 935.000]	**<0.001**	**<0.001**	**<0.001**
CD4 + T lymphocytes (cell/μl)	254.000 [222.500, 454.500]	193.000 [119.000, 378.000]	334.000 [189.500, 518.500]	**<0.001**	**<0.001**	**<0.001**
CD8 + T lymphocytes (cell/μl)	220.000 [101.000, 305.500]	213.000 [90.500, 327.500]	223.000 [163.500, 377.500]	**<0.001**	**<0.001**	**<0.001**
EBV in peripheral blood (%)				0.769	**< 0.001**	**< 0.001**
Negative	15 (45.455)	9 (36.000)	116 (74.839)			
Latent infection	8 (24.242)	7 (28.000)	30 (19.355)			
Active infection	10 (30.303)	9 (36.000)	9 (5.806)			
Poor prognoses (%)				0.982	0.921	0.596
Consciousness disturbance	4 (10.256)	6 (12.766)	20 (7.194)			

Table 2 (continued)

Characteristic	EBV caused (*n* = 39)	Non-EBV caused (*n* = 47)	EBV negative (*n* = 278)	*p* value (EBV caused VS Non - EBV caused)	*p* value (EBV caused VS EBV negative)	*p* value (non-EBV caused VS EBV negative)
Mechanical ventilation	3 (7.692)	4 (8.511)	20 (7.194)			
Death	1 (2.564)	1 (2.128)	7 (2.518)			

EBV caused: EBV associated encephalitis

Non-EBV caused: EBV-positive patients with other diagnoses of encephalitis

Table 3 Clinical features between EBV and other herpes viruses associated encephalitis

Characteristic	EBV associated (*n* = 39)	Other herpes viruses associated (*n* = 49)	*p* value
Age (years, median and IQR)	55.000 [43.500, 62.500]	37.000 [27.000, 57.000]	**<0.001**
Gender (male, %)	28 (71.795)	27 (55.102)	0.166
Clinical manifestation (%)			
Consciousness disturbance	19 (48.718)	19 (38.776)	0.472
Behavioral and psychological symptoms	6 (15.385)	12 (24.490)	0.432
Seizure	5 (12.821)	14 (28.571)	0.128
Mechanical ventilation	14 (35.897)	10 (20.408)	0.168
Immunocompromised (%)			0.123
HIV	4 (10.256)	2 (4.082)	
Malignancy	1 (2.564)	0 (0.000)	
Immunosuppression for non-neoplastic condition	4 (10.256)	1 (2.041)	
CSF features			
Cell counts (median and IQR)	70.000 [6.000, 140.000]	50.000 [10.000, 190.000]	**<0.001**
Proteins (g/L, median and IQR)	1.310 [0.695, 1.840]	0.710 [0.490, 1.700]	**<0.001**
Glucose (mmol/L, median and IQR)	3.030 [2.585, 3.640]	3.380 [2.990, 4.030]	**<0.001**
Chlorine (mmol/L, median and IQR)	119.000 [115.000, 125.000]	125.000 [120.000, 128.000]	**<0.001**
Leukocytosis (%)	32 (82.051)	39 (79.592)	0.985
Proteins increasing (%)	34 (87.179)	38 (77.551)	0.376
Glucose reduction (%)	8 (20.513)	2 (4.082)	**0.038**
Chlorine reduction (%)	20 (51.282)	12 (24.490)	**0.018**
Increased IgG synthesis rate (%)	14 (77.778)	21 (61.765)	0.390
MRI features (%)			
Positive findings of parenchyma	19 (52.778)	20 (43.478)	0.593
Positive meningeal findings	11 (30.556)	3 (6.522)	**0.010**
Peripheral blood (median and IQR)			
White blood cells (10^9/L)	7.790 [6.035, 10.785]	7.150 [6.170, 9.900]	**<0.001**
Lymphocytes (10^9/L)	1.120 [0.665, 1.305]	1.180 [0.700, 1.510]	**<0.001**
Monocyte (10^9/L)	0.660 [0.455, 0.945]	0.550 [0.440, 0.630]	**0.004**
CD3 + T lymphocytes (cell/ul)	504.000 [414.000, 785.500]	589.000 [458.000, 806.000]	**<0.001**
CD4 + T lymphocytes (cell/ul)	254.000 [222.500, 454.500]	309.000 [152.000, 371.000]	**<0.001**
CD8 + T lymphocytes (cell/ul)	220.000 [101.000, 305.500]	240.000 [176.000, 395.000]	**<0.001**
EBV in peripheral blood (%)			**0.005**
Negative	15 (45.455)	27 (75.000)	
Latent infection	8 (24.242)	8 (22.222)	
Active infection	10 (30.303)	1 (2.778)	
Poor prognoses (%)			0.250
Consciousness disturbance	4 (10.256)	1 (2.041)	
Mechanical ventilation	3 (7.692)	4 (8.163)	
Death	1 (2.564)	0 (0.000)	

The EBV negative group showed relatively higher level of central nervous cryptococcus and TB (Mycobacterium tuberculosis) infections, but the prevalence of bacteria, viruses, autoimmune encephalitis antibodies (AE), and others pathogens were comparable

EBV-associated encephalitis from all 86 patients with EBV detected in their CSF and conducted a comparative analysis with another 49 cases of encephalitis caused by other herpes viruses. The results indicated that the decrease in glucose and chloride levels in the CSF of patients with EBV-associated encephalitis was more significant. This finding is consistent with reports in some case studies that showed reduced CSF glucose concentrations in patients with viral encephalitis [21–23]. Currently, the mechanism underlying this phenomenon is not fully understood and may be related to multiple factors, including changes in blood-brain barrier function that limit glucose entry into the subarachnoid space, the rate of glucose transport through arachnoid villi, increased glycolysis in leukocytes and bacteria, and elevated metabolic rates in the central nervous system. This phenomenon suggests that subsequent changes in CSF glucose levels in viral encephalitis may be associated with the severity of the disease [24].

Furthermore, this study found characteristics of leukocytosis and elevated protein levels in the CSF of patients with EBV-associated encephalitis, which is consistent with the findings of a previous retrospective analysis of 97 cases of EBV encephalitis [25]. It is worth noting that we also discovered that meningitis caused by the EB virus more commonly shows meningeal involvement on MRI. These manifestations further indicate that EB virus-associated encephalitis is a special type of encephalitis with characteristic presentations.

Currently, the mechanism by which viruses cause encephalitis is not clear, but it is commonly believed that retrograde infection through the cranial nerve is the main pathway. However, the results of this study showed meningeal involvement on MRI in nearly 30% of patients with EBV-associated encephalitis, far higher than 6% in patients with other herpesviruses-associated encephalitis. These results suggest that destruction of the blood-brain barrier may be an important mechanism for CNS EBV infection. A major discovery in recent years is the lymphatic system in the brain, which connects the subarachnoid space, venous system, lymphatic vessels and deep cervical lymph nodes [26–28]. We infer that EBV (a typical lymphotropic herpesviruses) may enter the CNS through these channels from the peripheral lymphatic and blood system.

Significantly, EBV-associated encephalitis showed more common of immunosuppression and peripheral EBV infection. The results of logistic analyses also confirmed that immunosuppression and peripheral EBV infection were independent risk factors for CNS EBV infection, implying that EBV is an opportunistic infection in the CNS. Generally, EBV lurks in B lymphocytes in adults, [5, 20] and is controlled by a sustained immune response [29]. When the immune system is disrupted, EBV may proliferate extensively and invade the CNS. This hypothesis was supported by the results that patients with CNS EBV infection showed a relatively lower average value of CD3 + and CD4 + T lymphocytes than those without CNS EBV infection.

It is noteworthy that this study found a general decrease in peripheral blood CD3+, CD4+, and CD8 + T lymphocytes in patients with EBV encephalitis, suggesting a significant impact of EBV encephalitis on the immune system. Given the increasing understanding in recent years of the possibility of autoimmune encephalitis subsequent to herpes simplex encephalitis, and research indicating poor immune outcomes subsequent to EBV viral encephalitis [14]. It is essential to conduct relevant tests such as NMDA receptor antibody testing for patients with EBV encephalitis, if necessary, to provide a theoretical basis for combined immunotherapy [30].

Limitations

First, due to the limitations of mNGS itself, we could not rule out the possibility of false positives or false negatives, but similar results from different analyses confirmed the robustness of the results. Second, some patients in our center were transferred from other regional hospitals, and many of them received antiviral treatment before arriving our center. Thus, the positive rate of EBV in untreated encephalitis patients may be higher than that reported here. Third, EBV can be detected in many patients with tuberculous, cryptococcal, or bacterial encephalitis, but the causal relationship between EBV and these pathogens needed to be explored in further studies. Finally, it is important to note that the data were collected during the COVID-19 pandemic, a period when lower circulating infections in society may have affected the prevalence of infections observed in our study. This potential limitation should be considered when interpreting the results.

Conclusions

Our results indicate that EBV infection is common and may be an opportunistic infection in patients with encephalitis, which may enter CNS mainly through the destruction of the blood-brain barrier. EBV-associated encephalitis is characterized by relatively reduced glucose and chloride levels, and more meningeal involvement on MRI than other herpesviruses-associated encephalitis.

Acknowledgements

Not applicable.

Author contributions

Concept and design, Yongzhao Zhou. and Anjiao Peng; drafting of the manuscript and interpretation of data, Zhu Liu. and Luyan Huang; data acquisition, analysis, Yusha Tang; data analysis, Leihao Sha; critical revision of the manuscript for intellectual content; study supervision, Lei Chen. All authors have read and agreed to the published version of the manuscript.

Funding
This study was supported fund of West China Hospital of Sichuan University and University of Electronic Science and technology (No. HXDZ21006), and the National Natural Science Foundation of China (82201752).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study has been approved by the Ethics Committee of West China Hospital, Sichuan University (No. 2022 – 857). Our data are extracted from the electronic medical record system and do not involve patient privacy, so a patient consent statement is not needed.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

EBV caused
EBV associated encephalitis.

Non-EBV caused
EBV-positive patients with other diagnoses of encephalitis.

Received: 2 October 2024 / Accepted: 28 April 2025
Published online: 13 May 2025

References

1. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and National age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the global burden of disease study 2013. Lancet. 2015;385(9963):117–71.
2. Mailles A, De Broucker T, Costanzo P, et al. Long-term outcome of patients presenting with acute infectious encephalitis of various causes in France. Clin Infect Dis. 2012;54(10):1455–64.
3. Venkatesan A. Epidemiology and outcomes of acute encephalitis. Curr Opin Neurol. 2015;28(3):277–82.
4. Tyler KL. Acute viral encephalitis. N Engl J Med. 2018;379(6):557–66.
5. Thorley-Lawson DA. EBV Persistence–Introducing the virus. Curr Top Microbiol Immunol. 2015;390(Pt 1):151–209.
6. Shannon-Lowe C, Rickinson A. The global landscape of EBV-Associated tumors. Front Oncol. 2019;9:713.
7. Zhang F, Zhang B, Ding H, et al. The oxysterol receptor EBI2 links innate and adaptive immunity to limit IFN response and systemic lupus erythematosus. Adv Sci (Weinh). 2023;10(27):e2207108.
8. Debuysschere C, Nekoua MP, Hober D. Markers of Epstein-Barr virus infection in patients with multiple sclerosis. Microorganisms. 2023;11(5):1262.
9. Tsuruyama Y, Mori N, Yoshida S, et al. Epstein-Barr virus-related encephalitis in a young woman: A case report. J Infect Chemother. 2020;26(7):741–4.
10. Nakamura J, Yanagida M, Saito K, et al. Epstein-Barr virus encephalitis in a patient with rheumatoid arthritis. Mod Rheumatol Case Rep. 2022;6(2):160–2.
11. Patnaik S, Samal P, Sahoo A, et al. A fulminant case of Epstein-Barr virus encephalitis with multiorgan dysfunction. J Neurovirol. 2022;28(3):464–6.
12. Zhao H, Zhang X, Yang H, Gu J. Epstein-Barr virus encephalitis with excessive daytime sleepiness as the main manifestation: two case reports. Med (Baltim). 2022;101(34):e30327.
13. Schwenkenbecher P, Skripuletz T, Lange P, et al. Intrathecal antibody production against Epstein-Barr, herpes simplex, and other neurotropic viruses in autoimmune encephalitis. Neurol Neuroimmunol Neuroinflamm. 2021;8(6):e1062.
14. Liu D, Lin PH, Li HL, et al. Early autoimmunity and outcome in virus encephalitis: a retrospective study based on tissue-based assay. J Neurol Neurosurg Psychiatry. 2023;94(8):605–13.
15. McGill F, Griffiths MJ, Bonnett LJ, et al. UK meningitis study investigators. Incidence, aetiology, and sequelae of viral meningitis in UK adults: a multicentre prospective observational cohort study. Lancet Infect Dis. 2018;18(9):992–1003.
16. Guidelines for The Diagnosis. Prevention and management of Cryptococcal disease in HIV-Infected adults, adolescents and children. Supplement to the 2016 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: World Health Organization. 2018.
17. Stott KE, Loyse A, Jarvis JN, et al. Cryptococcal meningoencephalitis: time for action. Lancet Infect Dis. 2021;21(9):e259–71.
18. Schaller MA, Wicke F, Foerch C, Weidauer S. Central nervous system tuberculosis: etiology, clinical manifestations and neuroradiological features. Clin Neuroradiol. 2019;29(1):3–18.
19. Ssebambulidde K, Bangdiwala AS, Kwizera R, et al. Symptomatic Cryptococcal antigenemia presenting as early Cryptococcal meningitis with negative cerebral spinal fluid analysis. Clin Infect Dis. 2019;68(12):2094–8.
20. Kelly MJ, Benjamin LA, Cartwright K, et al. Epstein-barr virus coinfection in cerebrospinal fluid is associated with increased mortality in Malawian adults with bacterial meningitis. J Infect Dis. 2012;205(1):106–10.
21. Cao LJ, Zheng YM, Li F, et al. Varicella-zoster virus meningitis with hypoglycorrhachia: A case report. World J Clin Cases. 2023;11(29):7101–6.
22. Fadhel M, Campbell N, Patel S, et al. Varicella Zoster meningitis with hypoglycorrhachia on cerebrospinal fluid (CSF) analysis in a young immunocompetent host without a rash. Am J Case Rep. 2019;20:701–4.
23. Spernovasilis N, Milioni A, Gialamas I, et al. Varicella-zoster virus meningitis with hypoglycorrhachia in a young immunocompetent adult without rash: A case report and literature review. IDCases. 2018;12:104–6.
24. Shrikanth V, Salazar L, Khoury N, et al. Hypoglycorrhachia in adults with community-acquired meningitis: etiologies and prognostic significance. Int J Infect Dis. 2015;39:39–43.
25. Peuchmaur M, Voisin J, Vaillant M, et al. Epstein-Barr virus encephalitis: A review of case reports from the last 25 years. Microorganisms. 2023;11(12):2825.
26. Wei CJ, Yassine HM, McTamney PM, et al. Elicitation of broadly neutralizing influenza antibodies in animals with previous influenza exposure. Sci Transl Med. 2012;4(147):147ra114.
27. Aspelund A, Antila S, Proulx ST, et al. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med. 2015;212(7):991–9.
28. Louveau A, Smirnov I, Keyes TJ, et al. Structural and functional features of central nervous system lymphatic vessels. Nature. 2015;523(7560):337–41.
29. Afrasiabi A, Parnell GP, Fewings N, et al. Evidence from genome wide association studies implicates reduced control of Epstein-Barr virus infection in multiple sclerosis susceptibility. Genome Med. 2019;11(1):26.
30. Venkatesan A, Michael BD, Probasco JC, et al. Acute encephalitis in immunocompetent adults. Lancet. 2019;393(10172):702–16.

Publisher's note