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Analysis of the Clinical Features of HSV-2 Encephalitis Confirmed by the mNGS Technique: Insights Derived from Seven Patient Studies

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Background: Herpes simplex virus type 2 (HSV-2) encephalitis is rare in immunocompetent adults. Diagnosis typically depends on cerebrospinal fluid (CSF) polymerase chain reaction (PCR), which has limited sensitivity and potential for false negatives. Metagenomic next-generation sequencing (mNGS) provides unbiased pathogen detection, facilitating rapid HSV-2 identification in CSF and minimizing misdiagnosis risks, especially in atypical cases or immunocompetent individuals. This study examines the diagnostic value of mNGS in a cohort of patients with HSV-2 encephalitis presenting atypically.

Methods: A retrospective analysis was performed on patients diagnosed with HSV-2 encephalitis using mNGS at our institution between January 2022 and January 2025. Clinical characteristics, ancillary test results, and patient outcomes were analyzed to evaluate the diagnostic value of mNGS.

Results: Seven patients (2 males, 28.57%; 5 females, 71.43%) with a mean age of 33.57 years were included; one had pre-existing immunodeficiency (14.28%). Most presented atypical symptoms; six treated within three days fully recovered, while one with delayed treatment died. Mean follow-up was 14.71 ± 5.82 months. Higher viral sequence counts correlated with worse outcomes. Initial CSF analysis showed normal cell counts in one patient; all exhibited lymphocytic pleocytosis and elevated protein levels.

Conclusion: This study contributes to the limited clinical data on adult HSV-2 encephalitis by summarizing clinical manifestations and treatment outcomes, thereby informing improved diagnostic and management strategies. It also highlights the prognostic importance of early diagnosis and immune status assessment through the application of mNGS.

Keywords: HSV-2, encephalitis, mNGS, clinical features

Introduction

Central nervous system (CNS) infections are life-threatening conditions, with HSV being the most common causative agent of viral encephalitis. This disease is primarily attributed to HSV-1 and HSV-2.[1] HSV-1 is the predominant pathogen in adult populations, whereas HSV-2 infections are relatively uncommon in immunocompetent adults and typically affect neonates or immunocompromised individuals.[2] The clinical manifestations of HSV-2 encephalitis are nonspecific and may include altered mental status, fever, headache, seizures, and neurological deficits such as memory impairment or language dysfunction. These symptoms closely resemble those of HSV-1 encephalitis but may also be mistaken for systemic infections or metabolic encephalopathies due to their atypical presentation.[3] HSV-2 encephalitis may occur during primary infection or through reactivation of latent virus within the CNS. Although rare in immunocompetent adults, HSV-2 encephalitis can result in severe neurological sequelae and even mortality. Thus, early diagnosis and prompt treatment are crucial for improving patient outcomes.

The current diagnosis of HSE primarily relies on the detection of HSV-specific nucleic acids in CSF via PCR. While this method has high diagnostic value, some patients with meningitis or encephalitis remain undiagnosed despite PCR testing. Studies have shown that approximately 4% of patients with severe HSE initially test negative on CSF PCR.[4] Furthermore,

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in clinical practice, patients with atypical HSE may present with normal CSF white blood cell counts, increasing the likelihood of misdiagnosis or missed diagnosis. Although viral isolation and PCR detection are considered the "gold standard" for HSE diagnosis, these techniques have limitations, including prolonged viral isolation times, low sensitivity, and restricted capacity to detect nucleic acid fragments, thereby limiting their clinical utility.[5] Therefore, diagnosing atypical HSE remains a significant challenge. Research indicates that the mortality rate among untreated HSE patients can reach up to 70%, but this rate drops to 8% when treatment is initiated within 4 days of symptom onset.[6] Hence, timely diagnosis and intervention are critical, as misdiagnosis or delayed diagnosis can lead to severe consequences.

mNGS offers unbiased, broad-spectrum pathogen detection, particularly beneficial for patients presenting with atypical clinical features, immunocompromised status, or co-infections involving multiple pathogens. Given that misdiagnosis often results in costly and inappropriate treatment, mNGS provides a unique opportunity to overcome these challenges through unbiased laboratory and computational approaches. Additionally, some patients may present with recurrent encephalitis or autoimmune complications such as anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis, further complicating the diagnostic process.[7]

In the context of viral encephalitis diagnosis, mNGS technology is increasingly utilized and serves as a rapid and accurate diagnostic tool. However, there remains a paucity of systematic clinical studies focusing on HSV-2 encephalitis diagnosed via mNGS, particularly in cohorts of patients with normal immune function. This study aims to investigate the clinical characteristics of HSV-2 encephalitis in patients presenting with atypical symptoms and normal immune status, as well as to evaluate the diagnostic value of mNGS, thereby contributing valuable insights for clinical practice.

Materials and Methods

Patients

Clinical data of patients with type 2 HSE diagnosed in our hospital between January 2022 and January 2025 were retrospectively collected. The study was approved by the ethics committee of our hospital and conducted in accordance with the principles of the 1964 Declaration of Helsinki. All patients and their families were fully informed regarding the study's purpose and procedures and provided written informed consent. Inclusion criteria for the study were as follows: (1) acute HSE confirmed by detection of human HSV-2 sequences in CSF using mNGS; (2) completion of standardized antiviral treatment (intravenous acyclovir at a dose of 10 mg/kg every 8 hours)[8] and follow-up for more than 3 months after discharge; (3) availability of written informed consent from patients and their families. Exclusion criteria were as follows: (1) central nervous system tumors; (2) encephalitis caused by multiple concurrent pathogens; (3) autoimmune encephalitis; (4) incomplete clinical data or nonstandardized medical records; (5) suspected HSV-2 infection without confirmation by mNGS testing of CSF.

Clinical and Investigational Data

Baseline characteristics were recorded, including demographic data, admission date, immune status, and presenting symptoms upon admission. Clinical data also included time to initiation of antiviral therapy and the number of HSV-2 viral sequences detected by mNGS. Laboratory assessments included routine blood tests, initial and post-treatment CSF analysis, and brain magnetic resonance imaging (MRI). Follow-up was conducted in-person or by telephone at 3, 6, 12, and 24 months after disease onset and recorded in the center's infectious disease database.

mNGS Experimental Procedure

Cerebrospinal fluid samples were collected from patients and stored at −20 °C. Samples were sent to Hugobiotech (Beijing, China) for mNGS testing. DNA was extracted and purified using the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. DNA concentration and quality were assessed using the Qubit 4.0 Fluorometer (Thermo Fisher Scientific, Massachusetts, USA). Libraries were prepared using the QIAseq Ultralow Input Library Kit (QIAGEN, Hilden, Germany). Qualified libraries were sequenced on the Nextseq 550 platform (Illumina, San Diego, USA). Raw sequencing reads were processed to remove adapter sequences, low-quality reads, and reads shorter than 35 bases to obtain clean data. Clean reads were aligned to the human reference genome (hg38) using Bowtie2 to filter out human-derived sequences. The remaining reads were analyzed against

a microbial genome database downloaded from the National Center for Biotechnology Information (ftp://ftp.ncbi.nlm.nih.gov/genomes/) for pathogen identification. Genotyping analysis was performed based on metagenomic data. For herpes simplex virus type 2, mNGS results were considered positive if the number of HSV-2-specific sequences was ≥3 and not detected in the no-template control.

Result

Clinical Characteristics of the Patient

The clinical data of seven patients were recorded in detail (Table 1). There were 2 males (28.57%) and 5 females (71.43%), with a mean age of 33.57 ± 12.79 years. One immunocompromised patient (14.28%) had a history of immune myocarditis and had been receiving treatment with methylprednisolone and mycophenolate mofetil. The remaining six patients (85.72%) had no history of immunodeficiency. Four patients (57.14%) presented with fever, all patients (100%) experienced headache, three patients (42.86%) exhibited nausea and vomiting, one patient (14.28%) had motor or infectious dysfunction, one patient (14.28%) showed higher cortical dysfunction, and one patient (14.28%) experienced seizures. Four patients (57.14%) exhibited signs of meningeal irritation, while three patients (42.86%) showed no neurological signs. Most of these seven patients presented with atypical symptoms and were prone to misdiagnosis. Six patients (85.72%) were admitted within 3 days after symptom onset and were cured at discharge. Only one patient (14.28%) experienced treatment delay (Patient 3), who remained unimproved at discharge and died within 6 months during follow-up. All seven patients were followed up for a mean duration of 14.71 ± 5.82 months. Among the seven patients, the individual with the highest viral sequence count (Patient 3) had the poorest prognosis (Figure 1).

Analysis of initial peripheral blood tests in the seven patients (Table 2) revealed that only two patients (28.57%) had elevated white blood cell counts, four patients (57.14%) had increased neutrophil counts, three patients (42.86%) demonstrated elevated neutrophil percentages, and all patients had normal lymphocyte counts. During the first lumbar puncture, six patients (85.72%) were found to have significantly increased cerebrospinal fluid (CSF) cell counts, while only one patient (14.28%) had a normal cell count. CSF cytology in all patients indicated a lymphocytic reaction (Figure 2). All patients had elevated CSF protein levels, while glucose and chloride levels remained normal in all

Table 1 Clinical Data, Number of mNGS Viral Sequences, and Prognoses of Seven Patients

Patient	P 1	P 2	P 3	P 4	P 5	P 6	P 7
Sex	F	M	F	F	F	F	M
Age	36	44	21	21	34	24	55
Immune status	N	N	N	N	N	N	D
Clinical symptoms							
Fever	–	+	+	+	–	+	–
Headache	+	+	+	+	+	+	+
Nausea/vomiting	–	–	–	+	–	+	+
Motor/sensory disorders	–	–	–	–	+	–	–
Seizure	–	–	+	–	–	–	–
Higher cortical dysfunction	–	–	–	–	+	–	–
Meningeal irritation sign	+	–	+	+	+	–	–
Time from onset to admission (d)	1	3	14	1	2	1	3
Time from onset to diagnosis (d)	4	6	19	5	5	3	7
Time from onset to initiation of antiviral therapy (d)	1	4	17	2	2	2	5
Time from admission to initiation of antiviral therapy (d)	1	1	1	1	1	1	2
Length of Stay	14	20	14	18	12	11	16
Conditions at Discharge	R	R	NR	R	R	R	R
Viral sequence count	5363	15,057	25,930	231	169	382	8331
Follow-up time (months)	21	23	6	12	13	16	12

Notes: +: indicates the presence of the corresponding symptom; -: indicates the absence of the corresponding symptom.
Abbreviations: P, Patient; N, Normal; D, Defects; R, Recovery; NR, Not Recovered.

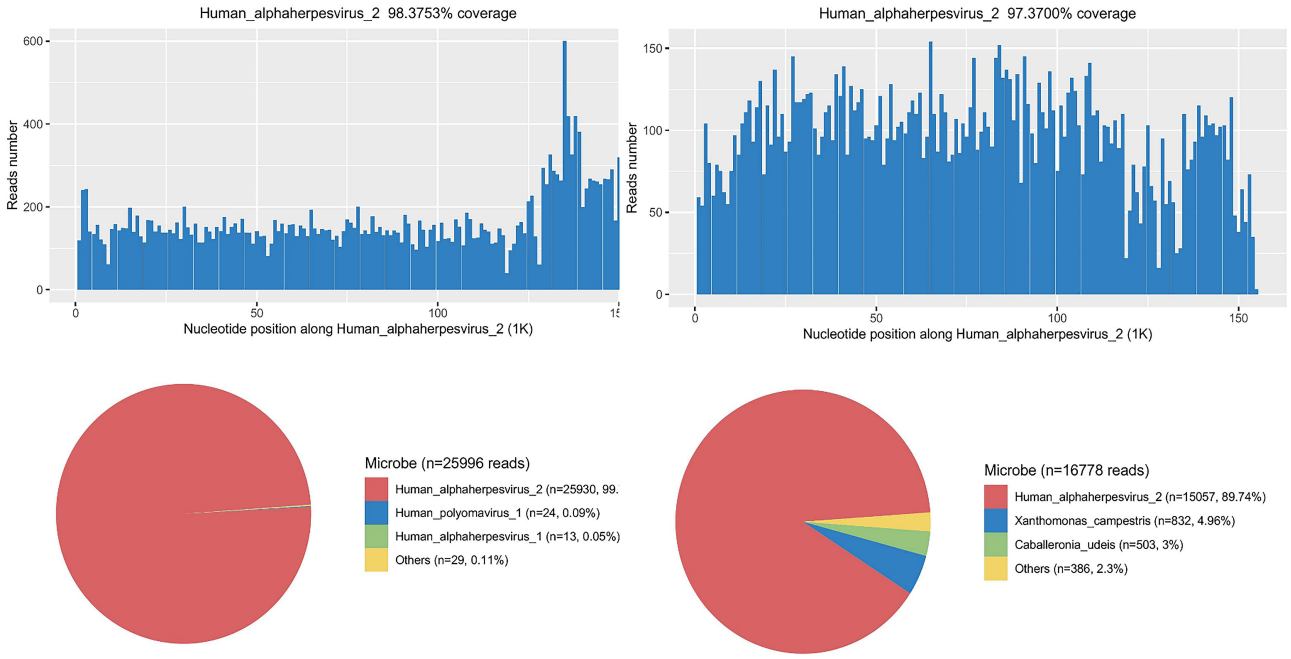

Figure 1 HSV-2 Species Coverage Map and Pie Chart Representation for Patients 2 and 3.
Notes: Left image (patient 3): A total of 25930 unique reads mapped to the Human_alphaherpesvirus_2 genome out of 25996 microbial reads with a genome coverage rate of 98.3753%. Right image (patient 2): A total of 15057 unique reads mapped to the Human_alphaherpesvirus_2 genome out of 16778 microbial reads with a genome coverage rate of 97.3700%.

Patients. Five patients (71.43%) had normal CSF pressure, and only two patients (28.57%) exhibited elevated CSF pressure. Six patients (85.72%) showed clinical improvement following antiviral and anti-inflammatory treatment. The family of the third patient refused to proceed with repeat lumbar puncture and opted to discontinue treatment due to the severity of the patient's condition.

Table 2 Results of Peripheral Blood and Cerebrospinal Fluid Analyses Before and After Treatment in Seven Patients

Patient		P 1	P 2	P 3	P4	P 5	P6	P 7
Peripheral blood testing	WBC count (×10^9/L,3.5–9.5)	8.13	11.58	6.34	5.76	8.98	6.92	10.53
	Neutrophil count (×10^9/L, 1.8–6.3)	6.53	8.58	4.45	3.15	7.12	5.3	7.89
	Proportion of neutrophils (%, 40–75)	80.4	74.1	70.3	54.7	79.4	76.6	74.9
	Lymphocyte count (×10^9/L, 1.1–3.2)	1.24	1.73	1.31	2.05	1.6	1.12	1.54
	Lymphocyte ratio (%, 20–50)	15.2	14.9	20.6	35.6	17.8	16.2	14.6
CSF before treatment	WBC count (cells/mm^3;0–8)	502	334	349	179	5	163	593
	Cytology	LR	LR	LR	LR	LR	LR	LR
	Protein (mg/dl, 15–45)	143.1	88.88	95.39	59.06	101.66	71.35	66.2
	Glucose (mmol/L, 2.5–4.5)	2.94	2.35	2.69	3.57	3.09	3.43	3.78
	Chloride (mmol/L, 120–132)	120.4	125.7	123.6	124.7	126.1	127.7	120.9
	IL-6 (pg/mL,<7)	1262	1424	431	664	995	335	192
	Peripheral blood Glucose (mmol/L;3.9–11.1)	6.7	7.4	13	8.5	6.4	5.4	8.9
	Pressure (mmH2O, 80–180)	180	120	320	160	180	130	400
CSF before discharge	WBC count (cells/mm^3;0–8)	8	67	/	84	89	59	6
	Cytology	LR	LR	/	LR	LR	LR	LR
	Protein (mg/dl, 15–45)	66.1	44.2	/	19.2	30.42	21.45	41.9
	Glucose (mmol/L, 2.5–4.5)	2.84	2.89	/	3.5	2.55	4.74	3.34
	Chloride (mmol/L, 120–132)	122.0	123.8	/	123.9	126.9	125.8	124.2
	IL-6 (pg/mL,<7)	7.75	3.93	/	2.83	5.93	3.05	6.22
	Peripheral blood glucose (mmol/L;3.9–11.1)	5.6	7.8	/	6.5	8	6.2	9
	Pressure (mmH2O, 80–180)	170	120	/	80	180	100	100

Abbreviations: WBC, White blood cell; CSF, Cerebrospinal fluid; LR, Lymphocyte response.

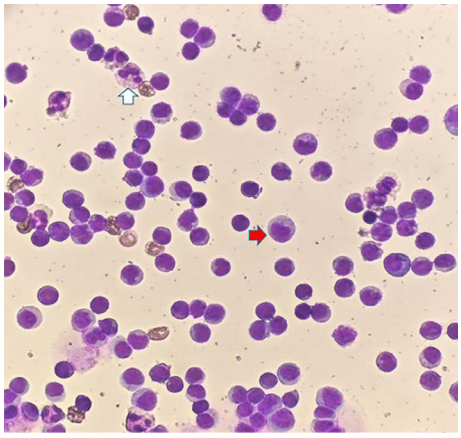

Figure 2 Light microscope images of Wright-Giemsa-stained cerebrospinal fluid (×400 magnification).
Notes: Microscopic examination revealed a predominance of lymphocytes, with numerous activated lymphocytes (Red arrow) present and a small number of neutrophils (White arrow).

Patient 1

The patient was a 36-year-old female government employee admitted to the hospital due to a one-day history of headache. The headache had an acute onset and was characterized as persistent, throbbing pain involving the entire head. Neurological examination revealed clear consciousness, normal responsiveness, intact orientation, comprehension, judgment, attention, calculation, and memory. Cranial nerve examination was unremarkable. Muscle strength in all extremities was grade 5 according to the Medical Research Council (MRC) scale, with normal muscle tone and symmetric tendon reflexes (++). Bilateral pathological signs were negative. Neck stiffness was present, and Kernig's and Brudzinski's signs were positive. Brain MRI, including plain and contrast-enhanced scans, as well as MR venography (MRV), revealed no abnormalities. Lumbar puncture showed CSF pressure at the upper limit of normal (180 mmH_2O; normal range: 80–180 mmH_2O). CSF analysis demonstrated pleocytosis 502 cells/mm^3 (normal range: 0–8 cells/mm^3), with lymphocytes accounting for 90%. CSF protein level was elevated at 143.10 mg/dL (normal range: 15–45 mg/dL), and CSF interleukin-6 (IL-6) was markedly elevated at 1262 pg/mL (normal range: <7 pg/mL). Other CSF parameters were within normal limits. Peripheral blood analysis revealed neutrophilia (80.4%; normal range: 40–75%) and relative lymphopenia (15.2%; normal range: 20–50%). Liver and kidney function tests, blood glucose, lipid profile, C-reactive protein (CRP), and procalcitonin (PCT) levels were all within normal ranges. Based on clinical presentation, initial diagnosis was viral meningitis. Empirical antiviral therapy with acyclovir (0.5 g every 8 hours) was initiated, and CSF samples were sent for mNGS. Two days later, mNGS results identified HSV-2 with 5363 viral sequence reads. After 14 days of treatment, the patient was discharged with complete resolution of headache. No recurrence was observed during 2 years of follow-up.

Patient 2

The patient was a 44-year-old man admitted with a 3-day history of headache and fever. Three days prior to admission, the patient developed acute-onset generalized headache characterized by a throbbing and tingling sensation, accompanied by fever peaking at 38.2°C, without additional neurological symptoms. She had been treated with cephalosporin antibiotics at a local hospital without improvement in headache, although fever persisted with temperature fluctuations between 37.3°C and 38.5°C. There was no history of immunodeficiency. On admission, her temperature was 38.5°C. Neurological examination revealed no focal deficits. Laboratory tests showed leukocytosis (11.58 × 10^9/L; normal range: 3.5–9.5 × 10^9/L). Liver and kidney function, CRP, erythrocyte sedimentation rate (ESR), IL-6, and PCT levels were all within normal limits. Lumbar puncture revealed normal CSF pressure (120 mmH_2O; normal range: 80–180 mmH_2O). CSF analysis showed mild pleocytosis 334 cells/mm^3 (normal range: 0–8 cells/mm^3), with lymphocytes comprising 93%. CSF protein was elevated at 88.88 mg/dL (normal range: 15–45 mg/dL), and CSF IL-6 was significantly elevated at 1424 pg/mL (normal range: <7 pg/mL). Empirical acyclovir therapy was promptly initiated. Headache symptoms gradually improved, and fever resolved on the

third day of treatment. mNGS of CSF confirmed HSV-2 infection with 15057 viral sequence reads. The patient achieved full clinical recovery after 20 days of treatment. No recurrence was observed during 2 years of follow-up.

Patient 3

The patient was a 21-year-old woman with no prior history of immunodeficiency, admitted to the hospital due to episodic unconsciousness accompanied by limb convulsions for two weeks. Twenty days prior to admission, the patient experienced symptoms of upper respiratory tract infection, which improved after treatment with ceftriaxone. Fourteen days prior to admission, she experienced a transient episode of unconsciousness without obvious precipitating factors, resulting in a fall with impact to the head. She regained consciousness approximately one minute later but was unable to recall the event. She was promptly transported to a local hospital, where an emergency brain CT scan revealed scalp hematoma and subcutaneous gas in the right temporal, parietal, and occipital regions. Following the examination, the patient developed limb convulsions, upward gaze deviation, and loss of consciousness. She was diagnosed with "cerebral contusion and laceration, suspected encephalitis". The patient presented with lethargy, was unable to provide a clear medical history, did not cooperate with physical examination, and was non-cooperative during assessment of orientation, memory, and calculation. Bilateral pupils were 2.5 mm in diameter, round and equal in size, with brisk light reflexes. Meningeal irritation signs and pathological reflexes were negative. In summary, the patient was a young female presenting with seizures and impaired consciousness, suggesting extensive cortical involvement. Therefore, lumbar puncture was promptly performed after admission. CSF pressure was elevated at 320 mmH2O (normal range: 80–180 mmH2O), with a CSF cell count of 349 cells/mm^3 (normal range: 0–8 cells/mm^3), lymphocyte percentage of 87.1%, and CSF biochemistry showing lactate dehydrogenase: 168.00 U/L (normal range: 0–40 U/L), CSF protein: 95.39 mg/dL (normal range: 0–45 mg/dL), and CSF IL-6: 431 pg/mL (normal range: <7 pg/mL). CSF smears for Cryptococcus neoformans, acid-fast bacilli, and tuberculosis were all negative. Peripheral blood tests showed normal complete blood count, liver and kidney function, CRP, PCT, and ESR. Due to the patient's agitation and inability to cooperate with examination, brain MRI (plain and contrast-enhanced scans) was performed under sedation (Figure 3), revealing extensive abnormal signals in the bilateral cerebral hemispheres (predominantly in the left temporal, parietal,

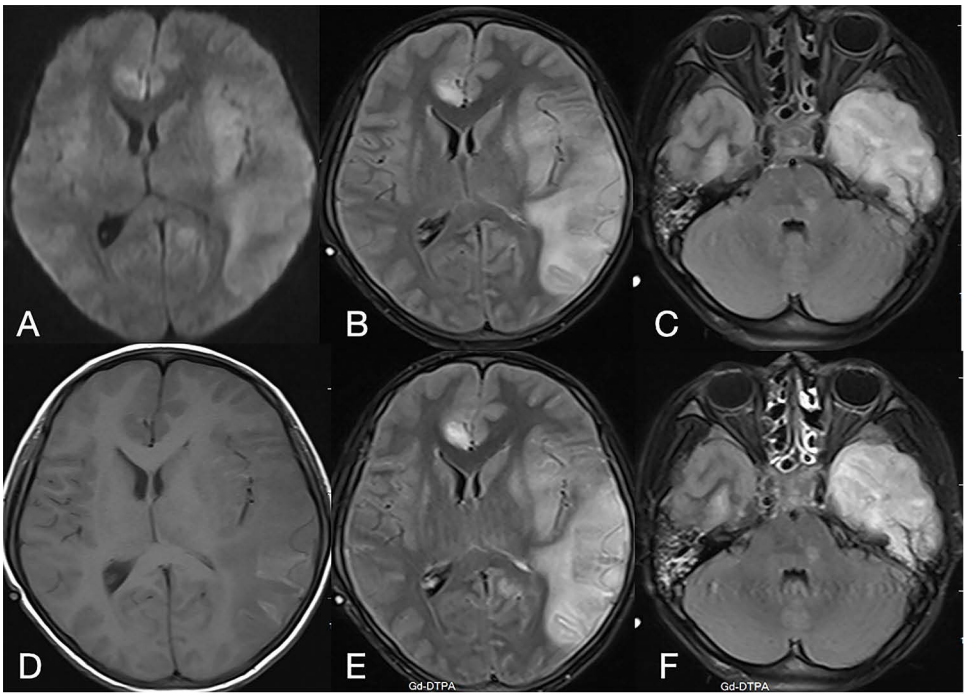

Figure 3 Plain and contrast-enhanced MRI images of the brain of Patient 3.
Notes: (**A**) DWI sequence; (**B** and **C**) T2 FLAIR; (**D**) T1-weighted image; (**E** and **F**) T2 FLAIR + C. Extensive hyperintense lesions were observed in the right frontal and temporal lobes, left frontal lobe, and left brachium pontis, with associated compression and displacement of the right lateral ventricle.

and occipital lobes) and pons, along with meningeal thickening and enhancement. Meningoencephalitis with laminar necrosis was suspected. Based on clinical manifestations including fever, seizures, impaired consciousness, and abnormal CSF findings, intracranial infection was initially diagnosed. On the day of admission, antiviral therapy with acyclovir, intracranial pressure reduction with mannitol, and anticonvulsant therapy were initiated. Simultaneously, CSF mNGS testing was performed. Body temperature normalized on day 3 of treatment, and mNGS results from CSF on day 3 confirmed HSV-2 infection (viral sequence count: 25930). Regrettably, the patient developed fever again on day 4 of treatment, complicated by aspiration pneumonia due to impaired consciousness and bulbar paralysis. Following endotracheal intubation, ventilatory support, anti-infective, and antiviral treatment, pulmonary infection was controlled, but the patient sustained severe neurological damage with persistent communication difficulties. After 14 days of hospitalization, the patient's family requested discontinuation of treatment due to financial constraints. We continued follow-up for six months post-discharge, and ultimately, the patient succumbed to pneumonia.

Patient 4

The patient was a 21-year-old woman who was admitted to the hospital with a 1-day history of headache, which worsened with fever 4 hours prior to admission. There was no previous history of immunodeficiency. The specific course of the disease was as follows: the patient developed headache without any obvious precipitating factors on the afternoon of the day before admission. The headache was characterized as pulsating pain involving the entire head, accompanied by nausea without vomiting, general fatigue, and loss of appetite. There was no dizziness, visual disturbance, fever, cough, sputum production, chest tightness, dyspnea, altered mental status, speech disturbance, limb weakness, or convulsions. Four hours before admission, the headache significantly worsened, becoming severe and unbearable, accompanied by a fever of 38.4 °C and poor general condition. Neurological examination upon admission revealed clear consciousness, normal responsiveness, fluent speech, and intact comprehension, orientation, and memory. Cranial nerve examination was unremarkable. Babinski's sign was positive on the left side and negative on the right side. Given the clinical presentation of fever, headache, and signs of meningeal irritation, central nervous system infection was initially suspected. Peripheral blood tests showed normal inflammatory markers, including complete blood count, CRP, PCT, and IL-6. Serological tests for HIV, hepatitis B, and COVID-19 were all negative. CSF analysis revealed an opening pressure of 160 mmH2O (normal range: 80–180 mmH2O), protein level of 59.06 mg/dL (normal range: 15–45 mg/dL), and a cell count of 179 cells/mm^3 (normal range: 0–8 cells/mm^3), with an interleukin-6 level of 664 pg/mL (normal range: <7 pg/mL). Brain MRI, including plain and contrast-enhanced sequences, showed no abnormalities. Empirical antiviral treatment with acyclovir was initiated immediately upon receipt of the CSF results on the first day of admission. On the second day of antiviral therapy, the headache began to resolve. CSF mNGS results confirmed HSV-2 infection with a viral sequence count of 231. After a 2-week hospitalization, the patient recovered and was discharged without recurrence during 1 year of follow-up.

Patient 5

The patient is a 34-year-old woman with previously good health and no history of immunodeficiency. She was admitted due to a 2-day history of headache. Two days prior to admission, the patient developed persistent bilateral frontotemporal headache, without accompanying symptoms such as fever, chills, blurred vision, limb weakness, nausea, vomiting, or seizures. The headache could not be relieved with self-administered analgesics. Emergency head CT performed one day prior to admission at our hospital showed no abnormalities, and she was treated in the emergency department with non-steroidal anti-inflammatory drugs. The headache recurred persistently on the day of admission. Neurological examination revealed clear consciousness, but with decreased responsiveness, orientation, memory, calculation, and comprehension. Cranial nerve examination was unremarkable. Muscle tone and strength (grade 5) in all limbs were normal. Superficial and deep sensory functions were intact. Tendon reflexes were normal, and no pathological signs were observed bilaterally. Neck stiffness was present, with a chin-to-chest distance of three transverse fingers, and Kernig's sign was positive. Given the clinical presentation of headache and positive meningeal irritation signs, central nervous system infection was initially suspected. Peripheral blood tests showed a neutrophil percentage of 79.4% (normal range: 40–75%), lymphocyte percentage of 17.8% (normal range: 20–50%), and monocyte percentage of 2.4% (normal

range: 3–10%). CRP, PCT, and IL-6 levels were within normal limits. Serological tests for HIV, hepatitis B, and COVID-19 were negative. Lumbar puncture revealed an opening pressure of 180 mmH2O (normal range: 80–180 mmH2O). CSF analysis showed a white blood cell count of 5 cells/mm^3 (normal range: 0–8 cells/mm^3), protein level of 101.66 mg/dL (normal range: 15–45 mg/dL), and IL-6 level of 990 pg/mL (normal range not specified). Based on clinical findings and CSF evidence, empirical antiviral therapy with acyclovir was initiated on the day of admission, and CSF samples were sent for mNGS. Headache symptoms improved on the second day of antiviral treatment. Subsequently, the mNGS report confirmed HSV-2 infection with a viral sequence count of 169. The patient's headache completely resolved after 12 days of hospitalization. Follow-up for 13 months post-discharge revealed no recurrence.

Patient 6

The patient was a 24-year-old woman with no prior history of immunodeficiency. She was admitted to the hospital due to a one-day history of headache. One day prior to admission, the patient developed headache without any apparent trigger, characterized by diffuse, pulsating pain persisting without remission, accompanied by dizziness and nausea. There was no associated fever, seizure activity, or vomiting. The headache was unresponsive to non-steroidal analgesics. Neurological examination revealed no focal neurological deficits. Given the isolated presentation of headache and normal neurological examination on admission, peripheral blood monitoring was conducted on the day of admission. Complete blood count showed a neutrophil percentage of 79.2% (normal range: 40–75%) and a lymphocyte percentage of 15.6% (normal range: 20–50%). Hemoglobin was 94 g/L (normal range: 115–150 g/L). CRP, PCT, and IL-6 levels were within normal limits. Serological tests for HIV, hepatitis B virus, and COVID-19 were negative. Considering the patient's presentation of headache with slightly elevated inflammatory markers, empirical antibiotic therapy with amoxicillin was initiated on the day of admission. However, on the second hospital day, the patient developed a fever of 38.8 °C and experienced worsening headache. A lumbar puncture was performed for differential diagnosis. CSF opening pressure was 130 mmH2O (normal range: 80–180 mmH2O), protein level was 71.35 mg/dL (normal range: 15–45 mg/dL), and total nucleated cell count was 163 cells/mm^3 (normal range: 0–8 cells/mm^3); IL-6 level was 33 pg/mL (normal range: <7 pg/mL). Microscopic examination of CSF revealed a predominance of lymphocytes. Empirical antiviral therapy with acyclovir was promptly initiated, and CSF samples were sent for mNGS. After 48 hours of antiviral treatment, the patient's fever resolved and headache began to improve. The patient was followed up for 16 months post-discharge, with no evidence of recurrence.

Patient 7

The patient was a 55-year-old man admitted with a three-day history of headache. He had been diagnosed with autoimmune myocarditis two months earlier and was receiving treatment with methylprednisolone and mycophenolate mofetil. The headache began three days prior to admission without any identifiable trigger, primarily involving the occipital region, and was characterized by persistent distending pain. It was accompanied by a single episode of emesis of gastric contents. There was no associated fever, tinnitus, hearing loss, visual disturbance, diplopia, seizure, or limb weakness. The headache did not improve with over-the-counter cold medications or analgesics administered at a community clinic. Neurological examination was unremarkable. On admission, routine blood biochemistry was normal except for a mildly elevated white blood cell count of 10.53×10^9/L (normal range: $3.5–9.5 \times 10^9$/L). Brain MRI, including T1-weighted, T2-weighted, and contrast-enhanced sequences, showed no abnormalities. Initially, a diagnosis of neuropathic headache was considered. However, given the patient's ongoing immunosuppressive therapy, an infectious etiology was also suspected, prompting lumbar puncture. CSF opening pressure was elevated at 400 mmH2O (normal range: 80–180 mmH2O), CSF protein was 66.20 mg/dL (normal range: 15–45 mg/dL), and IL-6 level was significantly elevated at 192 pg/mL (normal range: <7 pg/mL). Total nucleated cell count was 593 cells/mm^3 (normal range: 0–8 cells/mm^3), with lymphocytes accounting for 97.4%. Intravenous acyclovir was initiated immediately. Two days later, CSF mNGS results confirmed HSV-2 infection, with 8331 viral sequence reads detected. The patient showed clinical improvement after three weeks of antiviral therapy and was discharged without neurological sequelae. Follow-up for 12 months post-discharge revealed no recurrence.

Discussion

Most previous clinical studies on HSE have primarily focused on HSV-1 as the causative agent, with HSV-2 being relatively less reported. This study investigated the clinical characteristics, treatment approaches, and outcomes in seven patients with HSV-2 encephalitis, emphasizing the influence of atypical symptom presentation, immune status, and early intervention on prognosis. The mean age of the patients was 33.57 years, with a male-to-female ratio of 2:5, indicating a predominance of young female patients. This aligns with the commonly reported bimodal age distribution of HSV encephalitis (infants and adults);[9] however, due to the limited number of Patients, an infantile peak was not observed. All patients presented with headache, four had fever, three experienced nausea and vomiting, and only one patient (Patient 3) exhibited motor dysfunction, higher cortical dysfunction, and seizures. In contrast to the classical triad of HSV encephalitis (fever, headache, and altered consciousness) typically described in the literature,[3] patients in this study frequently presented with atypical symptoms, such as mild neurological signs (meningeal irritation signs in four patients and no neurological signs in three patients), which increases the likelihood of misdiagnosis.[3] This highlights the variable and nonspecific clinical manifestations of HSV encephalitis, corroborating previous findings that HSV-2 encephalitis often presents with atypical features that may delay diagnosis.[10]

In this cohort, six patients (85.72%) exhibited significantly elevated CSF cell counts, while only one patient (14.28%) had normal cell counts. All patients (100%) showed elevated CSF protein levels. A marked increase in CSF IL-6 was observed in all patients, with a notable decline in inflammatory markers following treatment. IL-6 serves as a key mediator of neuroinflammation in HSV-2 encephalitis, and its elevated levels are associated with disease severity, immunopathological damage, and potential autoimmune complications.[11] Although clinical data specifically targeting HSV-2 remain limited, IL-6 may represent a potential biomarker for evaluating disease activity and therapeutic response, based on shared immune mechanisms in HSV infection, such as hyperinflammatory responses, and evidence from animal models.[12] Further research is warranted to clarify the specific role of IL-6 in HSV-2 encephalitis. Six patients (85.7%) were hospitalized within three days of symptom onset and demonstrated favorable recovery following prompt antiviral therapy. However, Patient 3 experienced a poor outcome and died within six months of follow-up. This finding is consistent with previous reports indicating that early acyclovir administration significantly improves prognosis in HSV encephalitis,[13] whereas delayed or absent treatment correlates with high mortality.[10] Additionally, Patient 3 had the highest viral sequence count (25,930) and the most severe outcome (death), which aligns with prior studies underscoring the impact of immune response and viral clearance efficiency on clinical outcomes. The prognosis of HSV encephalitis is influenced by the host's immune status and inflammatory response, with persistent viral presence potentially leading to adverse outcomes.[14]

The patient's immune status also has a significant impact on prognosis. In this cohort, one immunocompromised patient (14.28%) received timely treatment and subsequently recovered; however, immunosuppression is recognized as a high-risk factor for HSV encephalitis. Immunocompromised individuals, including those undergoing immunosuppressive therapy,[15] are more susceptible to severe HSV encephalitis and exhibit higher mortality rates,[1] primarily due to the diminished capacity of the immune system to control viral replication.[16] Although a poor prognosis was not observed in this particular Patient (Patient 7), previous studies emphasize that immune status remains a critical determinant of both susceptibility and clinical outcome in HSV encephalitis. Larger studies have demonstrated that autoimmune disorders or the use of immunosuppressive therapy increase the risk of HSV encephalitis and are associated with worse clinical outcomes.[1] Furthermore, autoimmune encephalitis may develop following infection, potentially leading to neurological sequelae.[17] Although such sequelae were not observed during long-term follow-up in this cohort, vigilance regarding this potential risk in immunocompromised patients is warranted.

This cohort highlights the atypical presentation of HSV encephalitis: absence of meningeal irritation signs (3 Patients, 42.86%), mild neurological manifestations, and normal CSF cell count (Patient 5), all of which contribute to the risk of misdiagnosis. Clinical presentations of HSV-2 encephalitis are frequently atypical and may manifest only as mild headache or lack specific neurological signs. These atypical features pose significant diagnostic challenges and may lead to misdiagnosis as other conditions.[3] Although previous diagnostic approaches have heavily relied on CSF PCR testing for HSV,[3] false-negative results may occur (approximately 4% false-negative rate), which itself is an independent

risk factor for poor neurological outcomes.[3] In this cohort, none of the patients underwent PCR testing; all diagnoses were made using mNGS technology. All patients were diagnosed within five days of hospital admission, indicating a timely and efficient diagnostic process.

In our study, all six patients who received prompt treatment (within 3 days of symptom onset) achieved full recovery, whereas Patient 3, who experienced treatment delay, succumbed, underscoring the critical importance of early intervention. Evidence suggests that delayed initiation of acyclovir therapy is an independent risk factor for mortality and neurological disability in HSV encephalitis. Prompt treatment, particularly within 24 hours of symptom onset, can significantly improve functional outcomes.[13] Standard acyclovir therapy is generally effective in improving prognosis;[3] however, treatment delays can lead to continued viral replication and progressive neural damage.[13] In contrast, the death of Patient 3 was attributed to delayed presentation, as standardized antiviral therapy with acyclovir was initiated on the third hospital day, and the diagnosis was confirmed on the fifth hospital day. Additionally, relapse or autoimmune complications may occur in some patients, particularly following premature discontinuation of therapy,[18] although such events were not observed in this cohort. During a mean follow-up period of 14.71 months, one patient (Patient 3) died. Prognosis was associated with both the quantity of viral sequences detected and the timing of treatment initiation.

Therefore, our findings suggest that high-risk populations should be proactively screened. For patients presenting with atypical symptoms (eg, headache without fever) or a history of immunocompromise, early CSF testing via mNGS should be considered for the timely diagnosis of HSV encephalitis. Once viral encephalitis is suspected, empirical antiviral therapy should be initiated immediately to minimize the risk of treatment delay. Moreover, long-term follow-up should include monitoring for potential autoimmune sequelae, particularly in patients with high viral sequence count.

Conclusion

This cohort highlights the atypical clinical manifestations and diagnostic challenges associated with HSV-2 encephalitis, thereby expanding the current understanding of this rare form of encephalitis. While previous studies have identified HSV-2 as a significant causative agent of encephalitis with the potential to induce severe neurological complications, comprehensive clinical data regarding HSV-2 encephalitis in adult populations remain limited. By summarizing key clinical features, including a female predominance (71.43%), a mean age of 33.57 years, and favorable treatment outcomes in most Patients, this study provides valuable insights that may inform future clinical practice. Furthermore, the findings emphasize the prognostic implications of delayed diagnosis and immune status, underscoring the need for prompt recognition and tailored therapeutic strategies. Nevertheless, the limited sample size in this study constrains the generalizability of the findings. Future research involving a larger cohort is warranted to further elucidate the molecular mechanisms and immune responses associated with HSV-2 encephalitis, ultimately facilitating the development of more targeted interventions.

Institutional Review Board Statement

This study was conducted in accordance with the Helsinki Declaration and approved by the Clinical Research Ethics Subcommittee of Ganzhou People's Hospital (protocol code TY-ZKY2024-084-01 and approval date: July 30, 2024).

Informed Consent Statement

In the case of Patient 3, written informed consent was obtained from a legally authorized family member (spouse, verified by official identification), and the specific reason for the patient's inability to provide consent in person was that irreversible cognitive impairment prevented understanding the research/publication; this information was recorded in the patient's medical record and was reviewed and approved by the ethics committee. The other six patients who participated in this study were all able to understand the purpose of the study and the scope of information to be published. They each directly provided written informed consent to participate in the study and authorized the use of their clinical data. This procedure was documented in the patient's medical records and in our ethical application documents.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that there are no financial or commercial relationships that could be viewed as potential conflicts of interest with this research.

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