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Original Research

Next-Generation Sequencing of Cerebrospinal Fluid for the Diagnosis of VZV-Associated Rhombencephalitis

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Abstract

Background: Rhombencephalitis (RE) is a general term for a group of inflammatory diseases of the rhombencephalon caused by different etiologies. Patients of RE caused by the varicella-zoster virus (VZV) are sporadic in medical practice. The VZV-RE is easily misdiagnosed and causes a poor prognosis for patients. **Methods**: In this study, we analyzed the clinical symptoms and imaging features of five patients with VZV-RE diagnosed by the next-generation sequencing (NGS) technique of cerebrospinal fluid. Magnetic resonance imaging (MRI) examination was used to characterize the imaging of the patients. The McNemar test was used to analyze the cerebrospinal fluid testing (CSF) values and MRI test of the 5 patients. **Results**: We finally used NGS technology to confirm the diagnosis in 5 patients with VZV-RE. MRI revealed T2/FLAIR high signal lesions in the patients' medulla oblongata, pons, and cerebellum. All patients had early signs of cranial nerve palsy; some had herpes or pain in the corresponding cranial nerve distribution areas. The patients develop headaches, fever, nausea, vomiting, and other signs and symptoms of brainstem cerebellar involvement. McNemar's test showed no statistical difference between multi-mode MRI and CSF values for diagnosing VZV-RE ($p = 0.513$). **Conclusions**: This study showed that patients with herpes in the skin and mucous membranes at the distribution area of the cranial nerves and with the underlying disease were prone to RE. We suggest that the NGS analysis should be considered and selected based on the level of parameters, such as MRI lesion characteristics.

Keywords: varicella-zoster virus; rhombencephalitis; cerebrospinal fluid; next-generation sequencing; magnetic resonance imaging

1. Introduction

Rhombencephalitis (RE) is a rare rhombencephalon inflammatory syndrome involving the brain stem and cerebellum, which is caused by numerous etiologies [1]. The rhombencephalon consists of metencephalon and myelencephalon. The metencephalon includes the pons, cerebellum, and pontine part of the fourth ventricle. The myelencephalon consists of the medulla oblongata and the medulla oblongata of the fourth ventricle. The causes of RE include infectious, autoimmune, and paraneoplastic syndromes. The most common infectious agents are Listeria, enterovirus 71, and herpes viruses, while RE caused by the varicella-zoster virus (VZV) is very rare [2].

VZV belongs to a double-stranded DNA herpes virus. When a primary infection occurs in a patient, VZV often lurks in the patient's cranial nerves, dorsal nerve roots, and autonomic ganglia. When cell-mediated immunity is reduced in the elderly and immunosuppressed patients, VZV can become activated and secondary to infectious disease in the central nervous system. Activation of VZV is an infectious disease secondary to the central nervous system. Virus isolation combined with polymerase chain reaction (PCR) detection is the "gold standard" for diagnosing VZV. However, both the isolation and identification of VZV are very time-consuming, and the detection rate of the virus is usually low. At the same time, PCR is limited by the nucleic acid fragments bound by its primers. Therefore, both of these assays are limited in clinical application.

Next-generation sequencing (NGS) technology is characterized by unrestricted targeting primers, fast detection speed, and a high detection rate, which can quickly and efficiently diagnose infectious diseases of the central nervous system. Since it was first reported in 2014, NGS has been progressively promoted and applied in detecting cerebrospinal fluid pathogens [3–5]. There are many causes of RE, while VZV-RE is relatively rare clinically. Clinicians are prone to delay the diagnosis and treatment of the disease, which can easily lead to a poor patient prognosis. Therefore, this study intends to analyze the clinical and imaging characteristics of five patients with VZV-RE diagnosed by cerebrospinal fluid NGS techniques to improve clinicians' knowledge of the disease; Provide readers with an understanding of rhombencephalitis and, in particular, to increase their awareness of the clinical and imaging features of VZV-associated rhombencephalitis; Enrich the imaging features of this infectious disease of the cen-

tral nervous system and to select the appropriate pathogen screening modality promptly for early diagnosis, treatment, and improved prognosis of the VZV-associated rhombencephalitis patients.

2. Materials and Methods

2.1 Patients

A total of 5 patients (4 males and one female) with VZV-RE were hospitalized and diagnosed in the Department of Neurology of our hospital from January 2019 to January 2021. The median age of the patients was 63.8 (41, 75) years old. The Ethics Committee approved this study at Hengshui People's Hospital, and the patient's and their families were informed of the study content and signed an informed consent form. All individual-level health and medical information, including the demographic, clinical, radiological and pathogenic findings, and treatment data, were recorded in the research project database.

Inclusion criteria of patients were: (1) Patients whose clinical features were consistent with the symptoms and signs of RE (cranial nerve palsy, cerebellar ataxia, long pyramidal signs, disturbance of consciousness, etc.); (2) Patients with lumbar puncture cerebrospinal fluid examination: white blood cell (WBC) count $>8 \times 10^6$/L; (3) Imaging examination was consistent with the imaging features of RE (T2/FLAIR hyperintense lesions were mainly located in the pons, medulla oblongata, cerebellum and other parts); (4) NGS of cerebrospinal fluid detected human VZV sequences; (5) Clinical and laboratory tests have ruled out evidence of other microbial infection; (6) Patients who signed informed consent.

Exclusion criteria of patients were: (1) Non-infectious encephalitis, such as autoimmune encephalitis (AE), paraneoplastic marginal encephalitis (PLE), neuromyelitis optica spectrum diseases (NMOSDs), immune rheumatic diseases involving the central nervous system; (2) Recent 4-week history of vaccination; (3) Patients who had contraindications to lumbar puncture or inability to obtain cerebrospinal fluid.

2.2 Methods

2.2.1 NGS of Cerebrospinal Fluid

NGS of Cerebrospinal fluid: (1) Specimen collection: 1–2 mL cerebrospinal fluid was obtained by lumbar puncture in patients, dripped into test tubes and stored in a refrigerator at –80 °C for 30 min and used for NGS assays. (2) Sample extraction and quality control: The DNA of cerebrospinal fluid samples was extracted using a micro-sample genomic DNA extraction kit (DP316, TIANGEN BIOTECH, Beijing, China), and fragmented into 200–300 bp fragments by DNA cutting ultrasonic disruptor(Bioruptor Pico, Diagenode, Belgium). After quality control of the pieces' size by a 2100 Bioanalyzer, the quality control DNA library's concentration was detected by quantitative PCR. (3) Library construction: DNA libraries were constructed through end-repair, poly(A)-tailing, adapter ligation, and PCR amplification. Roller amplification technology was used to increase single-stranded circular DNA by 2–3 sets to obtain DNA nanospheres. (4) Sequencing: The DNA nanospheres were loaded on the sequencing chip, and the BGISEQ-50 sequencing platform (Institute of Medical Laboratory, Tianjin BGI Technology Co., Ltd.Tianjin, China) was used to sequence the DNA nanospheres.

After the data analysis and quality control sequencing data were put on the machine, the reads with low quality, low complexity, and sequencing length <35 bp were eliminated. The high-quality sequencing data were obtained and compared with the BWA human genome database. After removing the interference of human genome information, we compare the remaining gene fragment information with the microbial gene database to identify pathogenic microorganisms such as bacteria, viruses, and fungi. The leading observation indicators are the type of virus and the number of copy sequences. The number of virus copy sequences detected in cerebrospinal fluid >1 is defined as positive.

2.2.2 Antibodies Detection

Indirect immunofluorescence assay (IIF) was used for autoimmune encephalitis antibodies. The Cell-based assay (CBA) has high diagnostic specificity and sensitivity for RE. The initial dilution titers of cerebrospinal fluid testing (CSF) and serum were 1:1 and 1:10, respectively (**Supplementary Fig. 1**).

2.3 Statistical Methods

Means $\pm$ standard deviations were used to represent continuous data that conformed to a normal distribution; medians (25%, 75%) were used for continuous data that did not conform to a normal distribution. The chi-square test was used for statistical analysis of the count data. The McNemar test was used to analyze the CSF values and MRI test results for the 5 patients. The patients were divided into three groups according to the number of leukocytes in the CSF: the mild group ($<50 \times 10^9$/L), the moderate group ($50–500 \times 10^9$/L), and the severe group ($>500 \times 10^9$/L). The imaging diagnosis was based on the MRI's number of brainstem lesions. It was divided into three groups: the mild group (No brainstem lesions), the moderate group (Brainstem lesions), and the severe group (Including lesions outside the brainstem).

3. Results

3.1 Clinical Findings

We used the NGS of CSF to identify the DNA sequence of VZV. The patients' clinical features with VZV-RE were summarized in Table 1 and Fig. 1. The patients' lumbar puncture pressure values, cerebrospinal fluid protein concentration, glucose, chloride, total blood cholesterol, triglyceride, the WBC and lymphocyte counts, and the number of sequences (NGS) are summarized in Ta-

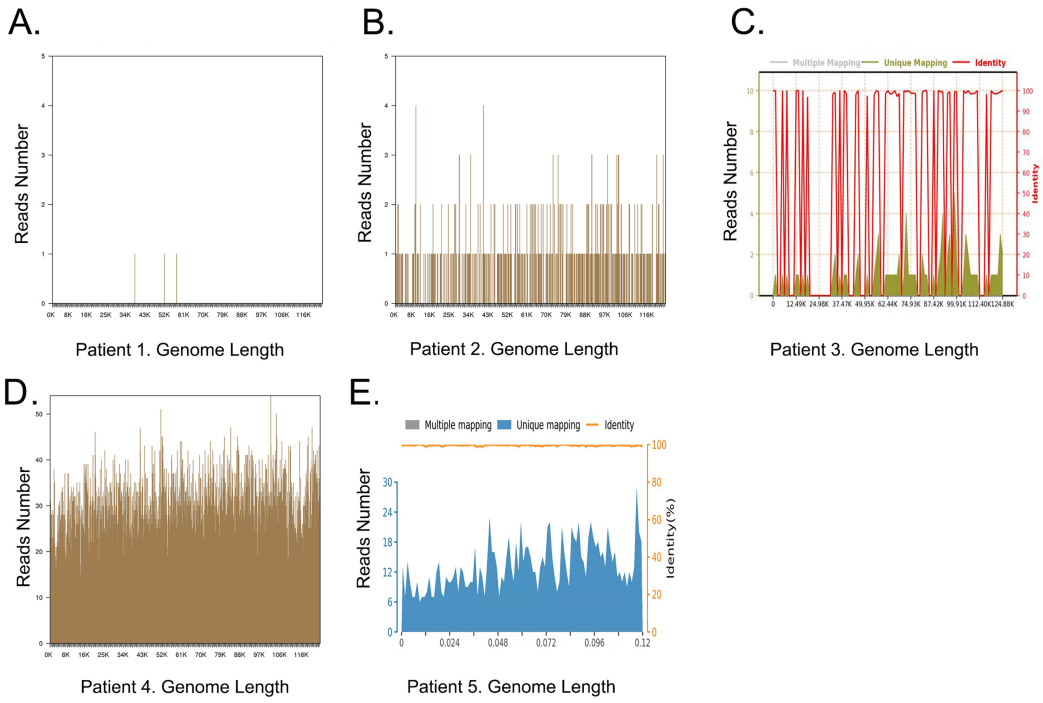

Fig. 1. Nucleotide Position along Human alphaherpesvirus 3 (Varicella-zoster virus) Genome. (A) Patient 1. (B) Patient 2. (C) Patient 3. (D) Patient 4. (E) Patient 5.

ble 2. The oligoclonal bands (OCB), aquaporin 4 (AQP4), myelin oligodendrocyte glycoprotein (MOG) antibodies, anti-GQ1b antibodies, and autoimmune encephalitis antibodies in all of the patients were negative. All five patients ruled out the diagnosis of HIV, syphilis, and hepatitis B infection. The patient's autoantibody spectrum and immune abnormalities were both normal.

3.2 Case 1

A 66-year-old man presented with cerchnus and dysphagia three days pre-hospitalization. This patient showed dysarthria and slowly pharyngeal reflex. The brain MRI showed low T1, high T2 and DWI signals in the medulla oblongata's left dorsal lateral position (Fig. 2A–C). Seven days later, the patient showed peripheral facial paralysis and herpes of the external auditory canal. Brain MRI reexamination revealed high DWI signals in the left cerebellar peduncles (Fig. 2D). This patient was finally diagnosed with VZV-RE and treated with acyclovir and immunoglobulins. After 12 weeks of follow-up, the patient still had left-sided peripheral facial palsy symptoms.

3.3 Case 2

A 41-year-old male patient developed facial distortion and dizziness 7 days before admission. The patient had many mottled blisters on the left auricle, and peripheral facial palsy occurred on the left side. MRI testing results revealed low T1 and high T2 signals in the left cerebellar medius of the patient's (Fig. 3A,B). The MRI showed the enhanced vestibulocochlear and facial nerve at the bottom

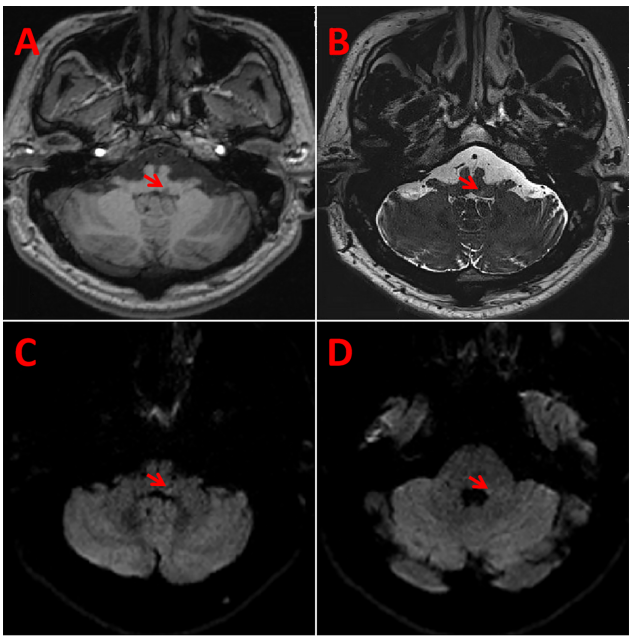

Fig. 2. MRI results of patient 1. The MRI showed low T1-MPRAGE, high T2 (3D-CISS), and DWI signals in the left medulla (A–C). Reexamination of brain MRI revealed high DWI signals in the left brachium pontis (D).

of the internal auditory canal (Fig. 3C,D). The patient was treated with acyclovir and immunoglobulins. Six months after discharge, the patient's hearing loss in the left ear had not improved.

Table 1. Clinical features of the five patients with VZV-RE.

Case NO.	Age	Cranial nerve	Underlying disease	Location of herpes	Fever	Headache	Epilepsy	Meningeal irritation	Antibody testing	Neuroimaging features	Treatment	Prognosis
1	66	VII, VIII, IX, X	Hypertension	Auricle	None	None	None	None	OCB, AQP4, MOG antibodies anti-GQ1b antibody, AE antibody are all negative	High T2 signals, FLAIR and DWI signals in the left medulla and left brachium pontis.	Acyclovir + immunoglobulins	Peripheral facioplegia
2	41	VII, VIII	NO	Auricle	None	None	None	None	idem	MRI of the brain after admission revealed high T2, FLAIR, and DWI signals in the left pedunculus cerebellaris medius. The gadolinium-enhanced MRI bottom showed that the facial nerve and vestibulocochlear nerve at the internal auditory canal was enhanced.	Acyclovir + immunoglobulins	Hearing loss
3	65	VII, VIII	Hypertension and diabetes	External auditory canal	Yes	Yes	None	Yes	idem	Head MRI of the brainstem showed no significant abnormalities. Contrast-enhanced MRI of the internal auditory canal revealed abnormal enhancement of the auditory nerve on the left internal auditory canal's inner surface.	Acyclovir	Peripheral facioplegia
4	72	VII	Hypertension	External auditory canal	Yes	Yes	Yes	Yes	idem	Head MRI revealed high T2 and FLAIR signals in the brainstem and cerebellum.	Acyclovir + immunoglobulins	Death
5	75	VII	Hypertension and diabetes	External auditory canal	Yes	Yes	None	Yes	idem	Head MRI showed T2/Flair hyperintensity in the back of the left pons.	Acyclovir + immunoglobulins	Peripheral facioplegia

VZV, Varicella-Zoster Virus; RE, Rhombencephalitis; DWI, Diffusion-Weighted Imaging; FLAIR, Fluid attenuated inversion recovery; T2, T2 weighted image; MRI, Magnetic Resonance Imaging; OCB, Oligoclonal Bands; AQP4, Aquaporin Protein 4; MOG, Myelin Oligodendrocyte Glycoprotein; AE, Autoimmune Encephalitis.

Table 2. Laboratory features of the five patients with VZV-RE.

NO.	CSF								
	Pressure (mmH_2O) (80–180)	WBC ($\times 10^6$ cells/L) (<5)	LYM (%) (60–70)	Protein (g/L) (0.15–0.45)	Glucose (mmol/L) (2.5–4.5)	Chloride (mmol/L) (120–130)	NGS (number of sequences)	Total cholesterol (mmol/L)	Triglycerides (mmol/L)
1	160	32	80%	0.68	Normal	Normal	14	4.90	1.61
2	150	36	80%	0.72	Normal	Normal	424	5.01	1.41
3	110	105	85%	0.702	Normal	Normal	84	5.25	2.10
4	220	1080	80%	5.3	10.03	108.5	20161	5.48	1.71
5	250	501	85%	18.575	5.62	104.1	1326	5.76	1.14

VZV, Varicella-Zoster Virus; RE, Rhombencephalitis; CSF, Cerebrospinal Fluid Testing; WBC, White Blood Cell; LYM, Lymphocyte Percentage; NGS, Next-Generation Sequencing Technology.

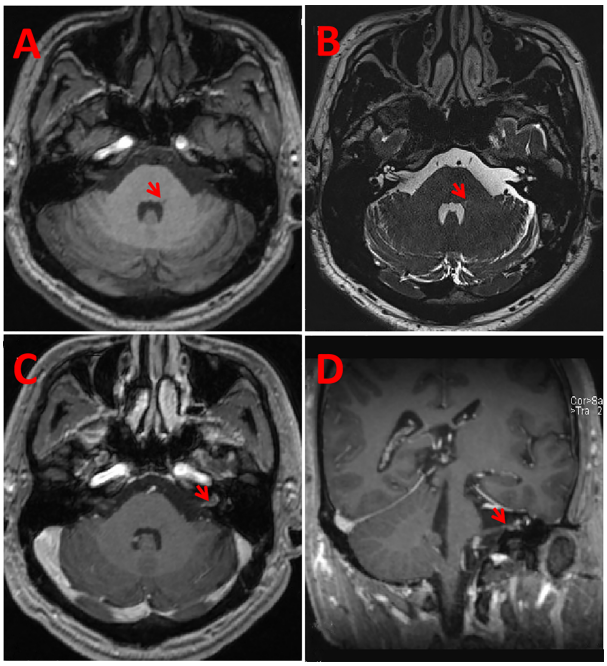

Fig. 3. MRI results of patient 2. MRI showed low T1-MPRAGE and high T2 (3D-CISS) in the left brachium pontis (A,B). The facial nerve and vestibular nerve in the central canal showed an enhanced sign in head MRI (red arrow), and the brain stem showed no abnormal enhanced signal (C,D).

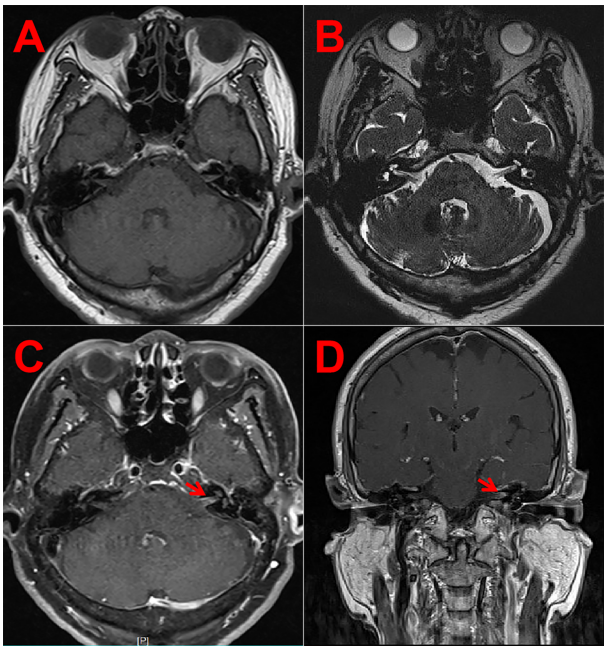

Fig. 4. MRI results of patient 3. MRI showed T1-MPRAGE and high T2 (3D-CISS) signals in the brainstem (A,B). Head MRI enhancement showed that the central canal's facial nerve and vestibular nerve had an enhanced signal (C,D, red arrow).

3.4 Case 3

A 65-year-old woman was admitted due to a deviation of the mouth for seven days and headache accompanied by dizziness with fever for one day. Fast-phase nystagmus toward the right level was seen in both eyes of the patient. The patient showed herpes in the left side of the external auditory canal, peripheral facial paralysis, and periodic limb movement disorder. Contrast-enhanced MRI of the internal auditory canal revealed abnormal enhancement of the auditory nerve on the left internal auditory canal (Fig. 4D). Although there was no abnormal signal on MRI (Fig. 4A–C), VZV-RE could still be diagnosed with brainstem involvement symptoms and signs. The patient was treated with acyclovir. Two months after discharge, the patient's dizziness improved, and he could walk independently but still had left peripheral facial paralysis.

3.5 Case 4

A 72-year-old man was admitted due to a headache for five days and convulsions accompanied by unconsciousness for one day. The patient developed a coma with a GCS of 4 (E1V1M2), a pupil diameter of 2.0 mm, and a slow light reflex was detected. He presented with a herpes scab in the left external auditory canal, left peripheral facial paralysis, and uncooperative muscle strength of the extremities. The patient had a positive response to the neck resistance test. MRI of the brain revealed high FLAIR signals in the brainstem and cerebellum (Fig. 5A–D). Cytologic analysis revealed abnormal cerebrospinal fluid cytology. The significant cellular abnormalities were manifested by lymphocyte reaction, activated monocytes, 2% plasma cells, 2% erythrophage, and the whole field of erythrocytes. The patient was diagnosed with VZV-RE and meningoencephalitis and received acyclovir and immunoglobulin combined with ventilator-assisted therapy.

3.6 Case 5

The patient was a 75-year-old man admitted due to deviated mouth for seven days and fever accompanied by psycho-behavioural abnormalities for three days. The patient presented drowsiness, irritability, suspected herpes in the left auricle, and peripheral facial palsy on the left side. The patient's neck resistance was positive. The patient's head MRI showed a high density of Flair in the left cerebral bridge (Fig. 6). The patient was diagnosed with VZV-RE. After combined treatment with acyclovir and immunoglobulin, the patient's consciousness became clear, psychiatric symptoms disappeared. Three-month follow-ups showed that the patient regained the ability to live normally but still had peripheral facial palsy symptoms.

3.7 McNemar Test for Diagnostic Methods

The McNemar's test was used to analyze the CSF values and MRI test of the 5 patients (Table 3). In the present study, there were no diagnostic differences in any of the three indices ($p = 0.400$).

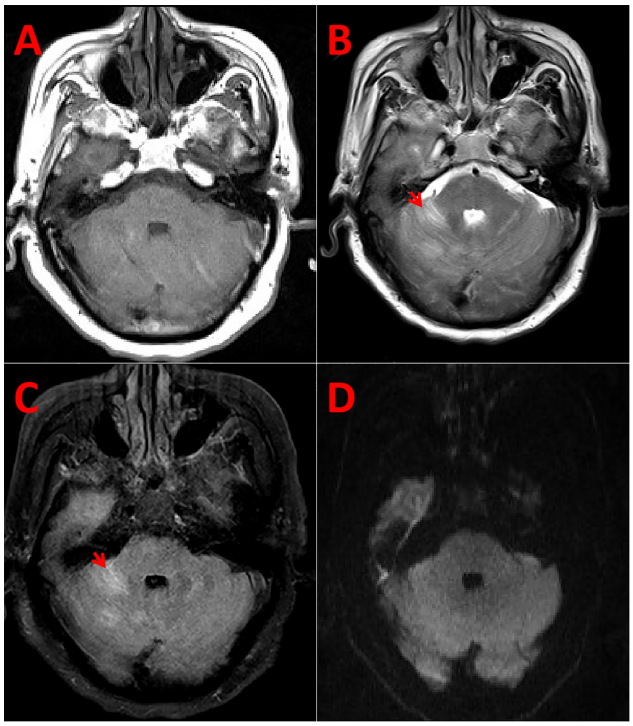

Fig. 5. MRI results of patient 4. The MRI of the head showed high T2/Flair signals (B and C, red arrow) and normal T1/DWI signals in brachium pontis (A and D). A, T1; B,T2; C, Flair; D DWI.

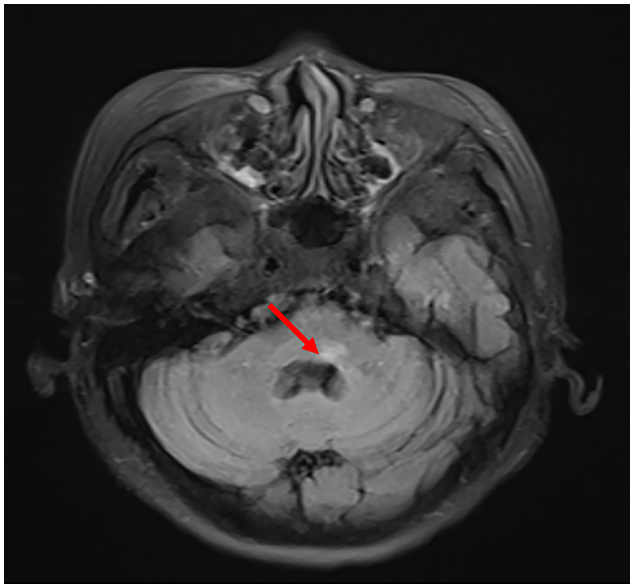

Fig. 6. MRI results of patient 5. MRI showed high Flair signals (red arrow) in the left medulla.

4. Discussion

RE is encephalitis involving the brainstem and/or cerebellum. Cranial nerve palsy is the disease's most common symptom, with a median incidence of about 75% (67%–86%), and the VII, VI, IX, X, and V cranial nerves

Table 3. McNemar's test was used to analyze the results of CSF values and MRI test.

		No-severe	Severe	Total	*p*
Group	No-severe	3	1	4	0.400
	Severe	0	1	1	
Total		3	2	5	

can be involved. Patients with this disease may present with clinical features such as long pyramidal tract signs (corticospinal tract, spinothalamic tract, posterior column), symptoms of cerebellar involvement (hemiataxia, vertigo, cerebellar dysarthria), disturbance of consciousness, and respiratory failure [1]. Some patients may also be accompanied by the symptoms such as headache, fever, and meningismus. MRI has a significant diagnostic value for RE patients. The main imaging manifestations are T2/FLAIR high signal lesions in the brain, medulla oblongata, upper cervical spine and cerebellum. Some patients may have only clinical signs and symptoms but lack typical MRI imaging [2]. In this study, all patients met the clinical manifestations of RE, and T2/FLAIR hyperintense lesions in the medulla oblongata, pons, cerebellum, and other parts were observed on head MRI. Although there was no clear abnormal signal in the brainstem, ataxia on the left side was observed, suggesting that the brainstem or cerebellum was also affected. These MRI changes in the patient were consistent with the diagnostic imaging features of RE.

There are numerous causes of RE, focusing on etiology and differential diagnosis [1,2]. A range of infectious and non-infectious diseases can cause the development of RE. In this study, the results of oligoclonal bands (OCB), AQP4, MOG antibodies, anti-GQ1b antibodies, autoimmune encephalitis antibodies and autoantibody tests in the patients' cerebrospinal fluid were not found to be abnormal. These test results largely exclude the possibility of RE triggered by immune-related diseases. In this study, all patients underwent lumbar puncture, and the results suggested an "inflammatory" response (elevated cerebrospinal fluid leukocyte count), and the diagnosis of infectious RE was largely confirmed. Due to the wide variety of infectious agents causing RE, conventional diagnostic methods are more challenging to reach a final diagnosis. In contrast, NGS technology can obtain information on pathogenic microorganisms directly from the patient's diseased brain tissue and cerebrospinal fluid, which will significantly shorten the diagnostic testing period for RE patients. In one study, Guan *et al*. [4] used NGS to test the cerebrospinal fluid of four patients suspected of having viral encephalitis. They detected two cases of herpes simplex virus type 1 (HSV1), one case of herpes simplex virus type 2 (HSV2), and one patient with VZV with genome-wide coverage of 12%–98%, respectively [4]. Their results indicate the significant value of NGS for diagnosing pathogenic CNS infections. In the present study, NGS was performed on the cerebrospinal

fluid in all patients. Our results showed that the patients developed VZV CNS infection. It further clarifies the diagnosis of VZV-RE and excludes RE caused by other pathogens. In addition, the differential diagnosis needs to consider the possibility of paraneoplastic syndrome-associated RE and brainstem lymphoma. In this study, no lymphoma-like enhancement was seen in the brainstem lesions, and the patient's follow-up and disease progression did not support the lymphoma diagnosis. Based on the patient's history and clinical auxiliary examination, the RE diagnosis can be ruled out as caused by progressive multifocal leukoencephalopathy and radiation encephalopathy. VZV is neurophilic; when it infects patients, it often lurks in cranial nerves, dorsal spinal nerve root, and autonomic ganglion. In the elderly or immunosuppressed, the virus can reactivate and extend the nerve retrogradely into the skull resulting in Encephalitis, meningitis, myelitis, and acute cerebrovascular diseases. In this study, the patients were all older, and most had underlying diseases such as hypertension and diabetes, activating factors for VZV.

This study showed that patients with early cranial nerve palsy or without herpes or pain in the corresponding distribution area, developed signs and symptoms such as headache, fever, nausea, vomiting, and involvement of the brainstem and cerebellum a few days later. These clinical features may be essential indicators for the latent activation of VZV, leading to the development of VZV-RE. Early damage to cranial nerves suggested the entry pathway of VZV. For example, patient 1 had retrograde entry through the glossopharyngeal vagus and facial nerves, and other patients had retrograde entry mainly through the facial and vestibular nerves. This study also predicted that in patients with cutaneous and mucosal herpes in the cranial nerves distribution area, VZV is prone to retrograde into the skull and cause the development of RE, which deserves the attention of clinicians. Limited case reports have shown that VZV-RHS can be accompanied by damage to the patient's brainstem, which is consistent with what was reported in this study [6–8]. In the present study, we also found that early VZV-RE brainstem injury sites were mainly related to cranial nerve connection brainstem sites and connecting nuclei, such as the suspected nucleus dorsolateral medulla oblongata and the facial nucleus at the base of the fourth ventricle. We suggest that the location of the injury may be related to the neurophilic properties of VZV, retrograde infection along the nerve, and the early nerve myelin's inhibitory effect on the virus's spread. Therefore, these features can serve as imaging manifestations that distinguish VZV infection from other infectious diseases causing RE. T2 imaging (3D-CISS) showed significant signal enhancement of the facial and vestibular nerves in the medial auditory canal. T2 and its enhancement allow early detection of the site and extent of injury in RE patients and allow assessment of the patient's prognosis. Therefore, this diagnostic method is also very effective in patients with cranial nerve palsy caused by VZV infection. It has been found that cytotoxic edema can be triggered in the early stages of nerve cell damage caused by VZV infection, with DWI showing a high signal [9]. However, the brightness of the DWI signal was significantly lower in patients with acute cerebral infarction, and the signal duration was more transient. In this study, although some patients exhibited high DWI signal intensities, these signals were significantly lower than those of patients with acute cerebral infarction. Instead, these high-intensity areas coincided with the vascular distribution areas but overlapped with the routes of cranial nerves into the cranial brain. Therefore, we believe it is more likely that the VZV virus directly invades the brainstem.

In this study, although all patients received active antiviral and immunosuppressive therapy, short-term follow-up showed poor prognosis, indicating that once damage occurred in the brain stem and parenchyma, the patient's recovery period would be significantly prolonged. Simultaneously, NGS technology found that the number of VZV copy sequences was closely related to patients' prognoses. A high copy number of genes tested for VZV in RE patients indicates a high number of viruses in the brainstem and severe brain tissue destruction, often indicating a poor prognosis.

We used the McNemar test to compare the CSF value and MRI of the five patients. The results showed no statistically significant difference in diagnostic efficacy between the two methods ($p > 0.05$). We considered that the results that were derived might be due to the lower clinical incidence of the VZV-RE's, which leads to a relatively small number of cases that could be included in this study. This deficiency has limited the conclusions of the study to some extent. Therefore, studies on VZV-RE still need to expand the sample size further, analyze clinical and imaging features, achieve early diagnosis and treatment, and improve patient prognosis. Moreover, clinicians must test patients with RE for NGS as soon as possible to avoid unnecessary treatment delays.

5. Conclusions

The clinical occurrence of VZV-RE is relatively rare. However, in patients with clinical and imaging features consistent with RE's diagnosis, the relationship between the distribution of the involved cranial nerve and the brainstem lesion should be focused on. We suggest that the NGS analysis should be considered and selected based on the parameters such as MRI lesion characteristics and the presence or absence of enhancement in the cranial nerve pool segment. The possibility of RE-VZV should be focused on if the site of early brainstem injury in RE patients is mainly related to the brainstem site of the involved cranial nerve connection and its associated nuclei. Cutaneous herpes in the cranial nerve distribution of RE patients can be highly suggestive of VZV-RE and should be promptly tested for VZV-DNA or cerebrospinal fluid mNGS. Although this study

showed no statistical difference between MRI and CSF in diagnosing VZV-RE, the smaller sample size may limit the present results. This study provides insight into the imaging features of VZV-associated rhombencephalitis and the pathogenic model of VZV retrograde cranial nerve entry causing rhombencephalitis. This study shows that looking for early VZV infection evidence can lead to early diagnosis and treatment of RE patients and improve their prognosis.

Abbreviations

RE, Rhombencephalitis; VZV, varicella-zoster virus; NGS, next-generation sequencing; MRI, magnetic resonance imaging; PCR,polymerase chain reaction; AE, autoimmune encephalitis; PLE, paraneoplastic marginal encephalitis; NMOSDs, neuromyelitis optica spectrum diseases; IIF, Indirect immunofluorescence assay; CBA, Cell-based assay; CSF, cerebrospinal fluid testing; OCB, oligoclonal bands; AQP4, aquaporin 4; MOG, myelin oligodendrocyte glycoprotein; DWI, diffusion-weighted imaging; FLAIR, fluid attenuated inversion recovery; HSV1, simplex virus type 1; HSV2, herpes simplex virus type 2; CNS, Central Nervous System; RHS, Ramsay-Hunt syndrome; 3D-CISS, 3D-Constructive interference in the steady state.

Author Contributions

JH, ZS and JZ conceived and designed the experiments; DC, YJ and ZK analyzed the data; YJ and ZK contributed reagents and materials; JH and NW wrote the paper.

Ethics Approval and Consent to Participate

This study was obtained with the informed consent of all participants. And this study was approved by the Ethics Committee of Hengshui People's Hospital, code 2019-1-024.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.jin2202036.

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