MEGA JOURNAL OF CASE REPORTS

Case Presentation

Compiled Date: April 18, 2023

Fatal Encephalitis Caused by Coinfection of Unusual Microorganisms: Case Report

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Abstract

Balamuthia mandrillaris amoebae and Epstein Barr virus (EBV) encephalitis are rare lesions that are difficult to confirm because of the lack of typical clinical symptoms, cerebrospinal fluid (CSF) analysis, and imaging features. We report a rare case that may enrich the recognition of the disease. A 68-year-old male patient who was admitted to the hospital initially presented with a two-week history of headache and dizziness. Because of rapidly changing conditions, intracranial imaging and routine laboratory findings in the short term, the patient was suspected of having an intracranial infection. Moreover, empirical anti-tuberculosis therapy was performed, even if CSF and peripheral blood cultures did not reveal the exact pathogen. Fortunately, high-throughput next-generation sequencing (NGS) detected the presence of high copy reads of Balamuthia mandrillaris amoebae and EBV in the patient's CSF. Although the treatment protocol was adjusted in time, the patient died due to quickly developing multiple organ failure.

Keywords: Encephalitis; Balamuthia mandrillaris amoebic; Epstein Barr virus; High-throughput next-generation sequencing

Introduction

Balamuthia mandrillaris infection in humans is rare and usually has a fatal result. In particular, when it invades the central nervous system, the mortality rate is as high as 98% [1]. Balamuthia mandrillaris can infect both immunocompromised and immunocompetent people [2]. To date, more than 200 cases caused by Balamuthia mandrillaris have been described worldwide, the majority of which are from the United States [3] and Peru [4]. Some cases have been reported in the Asia-Pacific region [5]. EBV is one of the common pathogens of central nervous system virus infection in children [6], but adult EBV encephalitis is rare and rarely reported. Here, we

report a rare case of encephalitis caused by Balamuthia mandrillaris and EBV confirmed by NGS.

Case Presentation

In January 2022, a previously healthy 68-year-old male farmer came to the outpatient clinic of our hospital. He initially presented with a two-week history of headache and dizziness in the absence of fever, nausea, or vomiting. Physical examination revealed no meningeal signs. He denied a history of trauma, drug abuse, or long-term use of immunosuppressant medication. The patient had no history of other major diseases, except for an appendicitis resection many years ago. Initial brain Computed Tomography (CT) on the same day showed multiple patchy hypodense lesions with irregular shapes and unclear boundaries in the bilateral frontal, parietal, and occipital lobes and the left cerebellum, and the enhancement was not obvious, which suggested the possibility of cerebral infarction **(Figure 1).** Further examination was recommended to rule out other diagnoses, but the patient went home for self-observation and refused to be admitted to the hospital. However, the patient returned to our hospital for hospitalization because his condition deteriorated rapidly after five days. He had been feverish for three days and unconscious and had limb weakness for one day. A CT scan of the head again revealed multiple patchy and nodular hypodense lesions in the bilateral frontal, parietal, and occipital lobes, right basal ganglia, right temporal lobe, and bilateral cerebellum, which were widely distributed and enlarged compared with the previous CT examination **(Figure 2).** In addition, brain MRI has more advantages in the display of lesions, which can detect more small lesions and show them more clearly. Brain Magnetic Resonance Imaging (MRI) showed the extensive distribution of nodular and patchy abnormal signal lesions with different sizes in the bilateral cerebrum and cerebellum. The largest lesion was located in the left cerebellum (4.7 cm×3.9 cm), and oedema was observed around the lesions. Lesions appeared hypointense on T1-weighted imaging (T1WI), hyperintense on T2-weighted imaging (T2WI), and isointense or hyperintense lesions on T2-Flair, with obviously high signal intensity on Diffusion-Weighted Imaging (DWI) and low signal intensity on the Apparent Diffusion Coefficient (ADC) map. The lesions exhibited annular enhancement after injection of contrast agent **(Figure 3).** Cerebral infection was suspected based on the imaging data. On the day of admission, lumbar puncture was performed, and CSF was extracted for genetic detection of pathogenic microorganisms by NGS. In addition, lumbar puncture revealed high intracranial pressure (240 mmH_2O), elevation in the white blood cell count (49×106/L) and protein (1780 mg/L), a slight decrease in chloride (115.7 mmol/L), and normal glucose (3.09 mmol/L). No acid-fast bacilli, fungi, or bacteria were found in the CSF or peripheral blood smears and cultures. Although the exact pathogen was not detected in the CSF and peripheral blood, the possibility of infectious lesions was still considered in combination with the clinical symptoms, rapidly developing condition, and neurological imaging; therefore, the patient was treated with empirical anti-tuberculosis therapy.

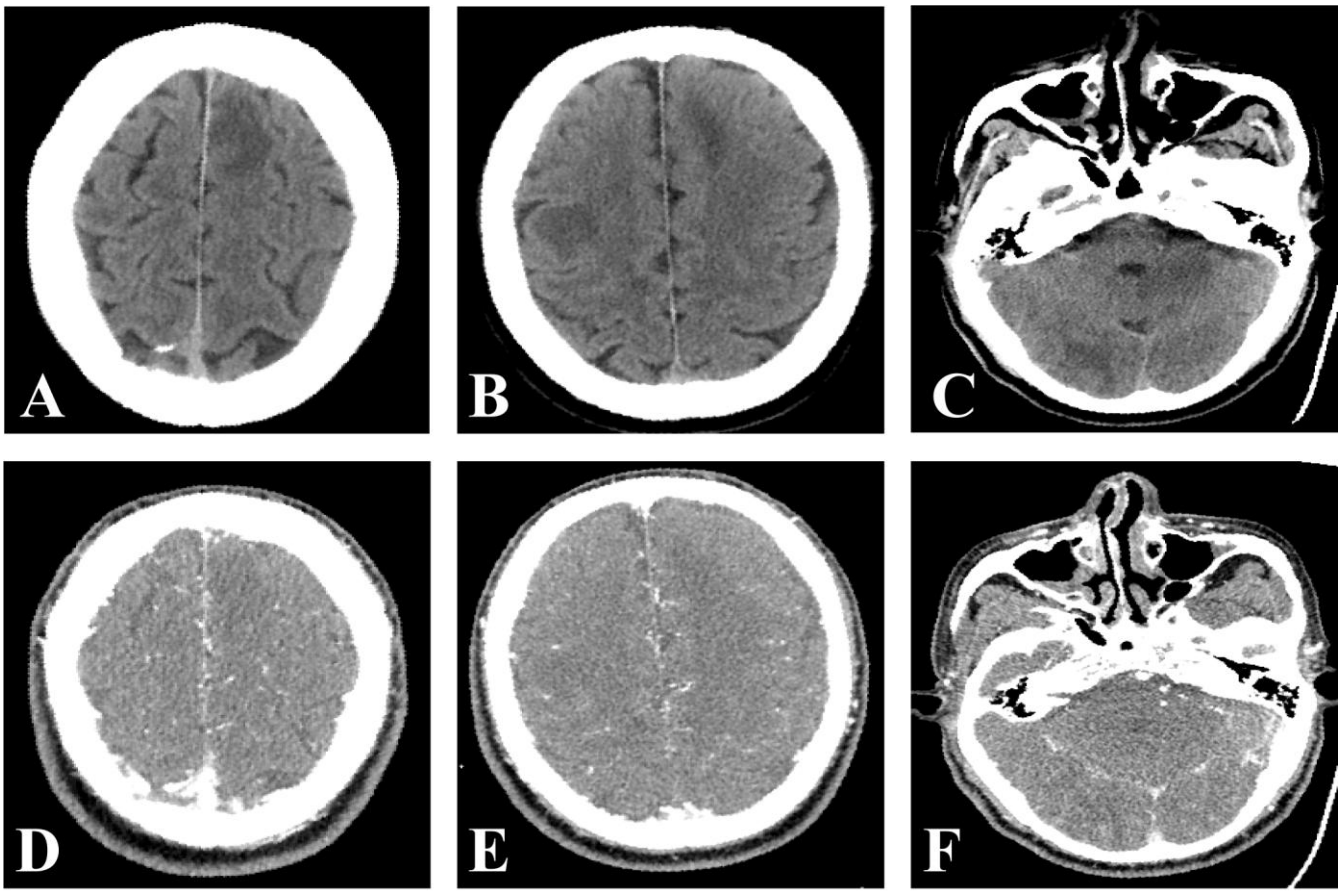

Figure 1: Initial brain CT scan. A-C Multiple patchy hypodense lesions with irregular shapes and unclear boundaries were found in the bilateral frontal, parietal and occipital lobes, and left cerebellum. D-F The enhancement of the lesions was not obvious.

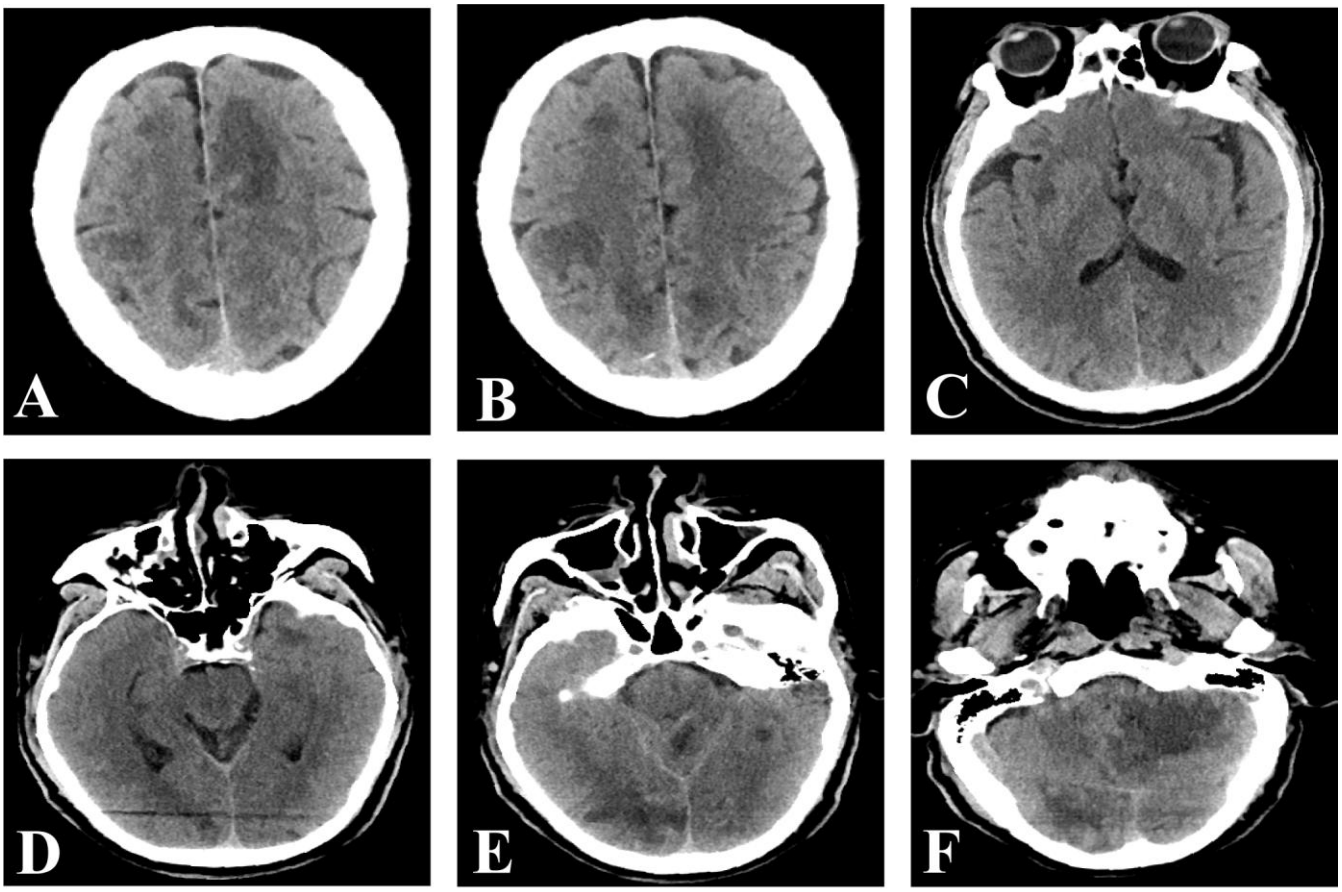

Figure 2: Re-examination CT scan after 5 days. A-F Multiple patchy and nodular hypodense lesions in the bilateral frontal, parietal, and occipital lobes, right basal ganglia, right temporal lobe, and bilateral cerebellum.

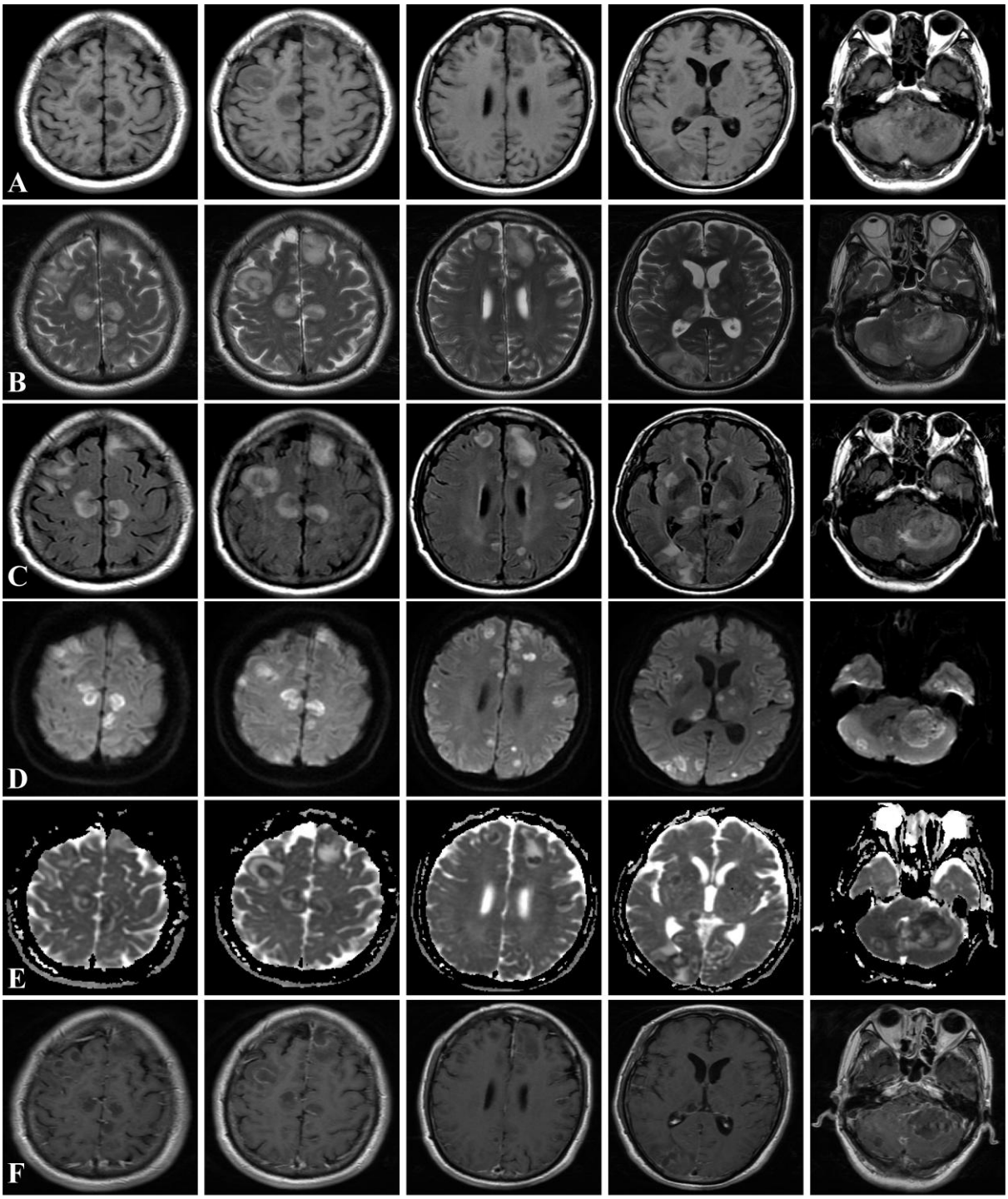

Figure 3: Brain MRI. Row A (T1WI), multiple nodular and patchy hypointense lesions were scattered in bilateral cerebrum and cerebellum. Row B (T2WI), the lesions presented hyperintense. Row C (T2-Flair), the lesions was isointense or hyperintense, and oedema was observed around the lesions. Row D (DWI) and Row E (ADC), the lesions showed obviously high signal intensity on DWI and low signal intensity on ADC map. Row F (T1-enhanced), the lesions showed annular enhancement.

The initial blood laboratory test revealed a C-Reactive Protein (CRP) level of 29.0 mg/ml (reference range, 0.0-8.0 mg/ml). The white blood cells, neutrophils, lymphocytes, eosinophils, and platelets were in the normal range, except for a slight increase in the percentage of neutrophils (84.0%, reference range, 50.0-70.0%). Other blood test indices, such as coagulation function, erythrocyte sedimentation rate, blood electrolytes, myocardial enzyme spectrum, renal function, and procalcitonin, were normal. Hepatitis B, HIV, tuberculosis, and Treponema pallidum antigens and antibodies were also negative. Although anti-tuberculosis therapy was continued, the patient had acute clinical deterioration. He was intubated, and mechanical ventilation was started due to deterioration of consciousness and decreased oxygenation. Fortunately, the NGS results were available on Day 4 of hospitalization. NGS detected the presence of Balamuthia mandrillaris with 1056 sequence copy reads and EBV with 68 sequence copy reads in the CSF. Thus, encephalitis caused by Balamuthia mandrillaris and EBV was diagnosed. At the same time, after consultation by the clinical pharmacy department, the treatment was adjusted to compound treatment with fluconazole (0.8 g iv drip Qd), flucytosine tablets (0.25 g po Qid), and sulfadiazine tablets (1 g po Bid). Furthermore, antiviral treatment with acyclovir (0.5 g iv drip Bid) was given, together with symptomatic and supportive treatment, such as dehydration, intracranial pressure reduction, and nutritional support.

Reexamined laboratory blood test indices of the patient after treatment revealed that the CRP value continued to increase (141.0 mg/ml, reference range, 0.0-8.0 mg/ml). Coagulation began to decline, plasma prothrombin time (PT) was 17.0 sec (normal reference value 9.4-12.5 sec), PT Percentage Activity (PTA) was 52.00%, International Normalized Ratio (INR) was 1.55 (normal reference value 0.8-1.2), and Fibrinogen (FBG) was 5.15 g/L (normal reference value 2-4 g/L). The Fibrin Degradation Product (FDP) was 62.27 µg/ml (normal reference value 0-2.01 µg/ml), and the Activated Partial Thrombin Time (APTT) and Thrombin Time (TT) were normal. Total protein (25.3 g/l, reference range, 65-85 g/l), albumin (26.4 g/l, reference range, 40-55 g/l), globulin (10.2 g/l, reference range, 20-40 g/l), and prealbumin (57 g/l, ng/mL (normal reference value 0-0.25 ng/mL). The percentage of neutrophils increased by 89.8% (reference range, 50-70%), and the percentage of lymphocytes decreased by 4.5% (reference range, 20-40%), whereas the total white blood cell count was normal. The creatinine value and glomerular filtration rate were normal. However, on Day 6 of hospitalization, the patient's condition deteriorated further, with bilateral pupil dilation, increased urine volume, and decreased renal function. The blood creatinine value was 114.5 µmol/L (reference range, 45-105 µmol/L), and the Glomerular Filtration Rate (EGFR) was 56 mL/min/L. Urine osmotic pressure began to decline (414 mosm/kg, reference range, 600-1000 mosm/kg). Coagulation disorders also worsened. Therefore, anti-infection treatment was continued, and appropriate fluid replacement and symptomatic treatment were carried out to balance the patient's internal environment. Even after a series of treatments, unfortunately, the patient’s condition deteriorated rapidly, and he quickly fell into a deep coma on Day 8 of hospitalization. Laboratory indicators showed that multiple organ failure (electrolyte disorder, renal failure, abnormal coagulation function) had occurred. After multidisciplinary consultation, it was planned to continue anti-infection treatment and symptomatic support treatment. However, due to the critical condition, the patient's family decided to give up treatment, and the patient died after being discharged from the hospital automatically on the same day.

Discussion

Infection of the central nervous system in adults, whether caused by amoeba or EBV, is rare and fatal. To the best of our knowledge, few cases of coinfection with Balamuthia mandrillaris and EBV have been reported. Here, we report a case of encephalitis caused by Balamuthia mandrillaris and EBV confirmed by NGS and present its diagnosis and treatment process, which may be helpful for the comprehensive recognition of the disease. Amoebas can be divided into two subgroups: parasitic and free-living amoebas. Parasitic amoeba live in the alimentary canal of a host animal, whereas free-living amoeba, which include Balamuthia mandrillaris, Acanthbamoeba species, and Naegleria fowleri, live in soil and fresh water **[7,8].** Although free-living amoebas rarely infect humans, they can cause fatal brain infections and are divided into two different clinical types of this disease. Balamuthia mandrillaris or Acanthbamoeba species mainly cause Granulomatous Amoebic Encephalitis (GAE), which usually shows subacute or chronic progression and eventually progresses to death, whereas Naegleria fowleri causes primary amoebic meningoencephalitis, which shows a highly acute progression and rapidly develops into a coma until death within a week or less **[9].** Unfortunately, early diagnosis is very difficult for clinicians, and the condition is associated with a high mortality rate of over 90% because none of these amoebae have characteristic clinical features **[3].** Balamuthia mandrillaris amoebae encephalitis is also a highly fatal disease with a known mortality rate of up to 98% **[1].** Unlike the other amoeba species, Balamuthia mandrillaris can infect not only immunocompromised but also immunocompetent individuals. At present, the pathogenesis of Balamuthia mandrillaris amoebae encephalitis is unclear.

The clinical symptoms of Balamuthia mandrillaris amoebae encephalitis are not specific and are similar to encephalitis caused by bacteria and viruses, which makes it difficult to make a correct diagnosis. The patient's common central nervous system symptoms include headache, fever, personality changes, focal seizures, nonspecific cranial nerve dysfunction, or localized motor deficit. Signs of increased intracranial pressure and alteration of consciousness may develop later as the disease progresses **[10].** The Peruvian case series reported that a majority of their patients initially presented with a skin lesion before invading the central nervous system **[11,12].** Typically, the skin lesion is a painless plaque or ulcer in the centre of the face or extremity **[12].** This is an important distinction, as the finding of a skin lesion presents an opportunity for an easier and earlier diagnosis and treatment of the disease. Nevertheless, most patients, including the present case, present with neurologic involvement from the start, without skin lesions **[3,10].** Although it seems to be a rare finding in patients from other countries, a careful skin examination should be conducted when Balamuthia is suspected, as detection of a lesion consistent with cutaneous balamuthias might allow for early diagnosis on easily accessible tissue. Balamuthia mandrillaris amoebae encephalitis is difficult to diagnose in a timely and accurate manner because of its rare incidence and lack of clinical specificity. Neuroimaging can be helpful in that almost all patients have abnormal brain imaging with parenchymal lesions. Lesions are usually multiple and widely distributed in the brain parenchyma. Both grey matter and white matter can accumulate **[3,5,13].** A few cases presented as solitary mass-like lesions **[14-18],** which mimic tumours of the central nervous system. Moreover, in more than 80% of cases, the lesion is enhanced by contrast medium. Small lesions tend to show solid and homogeneous enhancement, whereas large lesions tend to show nodular and/or ring enhancement **[3,5,7,13-18].** In our case, the size and the number of lesions changed as the disease progressed. Initially, the lesions were multiple and showed slightly hypodense

density on CT scan, but the enhancement of the lesions was not significant, and the oedema around the lesions was not obvious. As the condition deteriorated, the lesions increased rapidly in size and number, with annular enhancement and obvious perifocal oedema. Intracranial imaging features that change with the disease provide important information for the diagnosis of this disease. The CSF profile does not distinguish Balamuthia disease from other more common causes of encephalitis. Most Balamuthia patients have a mildly elevated white blood cell count with a lymphocytic predominance, elevated protein, and low-to-normal glucose **[3,5,7,9].** The nonspecific presenting clinical features and CSF profile of GAE are likely factors in the delayed diagnosis in most patients.

The diagnosis of amoebic encephalitis can be confirmed by microscopic identification of characteristic amoebic trophozoites or cysts in the skin or brain tissue or in CSF. However, Balamuthia trophozoites or cysts are rarely detected in the CSF of infected patients, either visually by microscopy or by PCR **[3,5].** Interestingly, in the present case, Balamuthia trophozoites or cysts were negative in both CSF and peripheral blood, and the CSF cultures remained negative. Thus, clinicians should be aware that a negative result on CSF does not rule out the diagnosis of Balamuthia disease because of unspecific symptoms and an absence of skin lesions. Many cases of GAE have been diagnosed via autopsy postmortem, sections of the skin, or brain tissues **[3].** Fortunately, with the development of NGS for pathogen detection, the diagnosis of GAE is determined in earlier stages of the disease than before. To the best of our knowledge, this is also one of the few cases of GAE reported within China confirmed by NGS [19-21]. As a rapid and accurate method of pathogen identification, NGS has major potential in the diagnosis of difficult and rare cases. It also has guiding significance for clinicians on deciding the treatment strategy early for patients. One of the important causes of high mortality from diseases is the lack of effective treatment. At present, there are no unified and effective therapeutic medication guidelines in the clinic due to the small number of surviving patients. Combination regimens are used in almost all surviving cases, and commonly used antimicrobial agents include acyclovir, ceftriaxone, isoniazid, and rifampin **[3,5].** Surgical resection of the lesion combined with drug therapy was performed in a small number of surviving cases **[5,21].** It may offer a new option for the treatment of patients, especially in isolated mass-like cases. However, due to the limited number of surgical survivors, prognostic factors and effective treatment options need to be further studied. EBV, also known as human herpesvirus type 4, belongs to the family of γ-herpesviruses **[22].** Epstein and Barr first identified the virus in Burkitt's lymphoma in 1964 **[23].** More than 95% of healthy adults worldwide have been infected with EBV, which is primarily an invisible infection **[24].** EBV is an important cause of encephalitis in children and adolescents **[6,25].** However, infection of the nervous system in adults caused by EBV is relatively rare. The pathogenesis of EBV encephalitis is currently unknown. The possible mechanisms are as follows. First, EBV infects neurons or other specific nerve cells, thus producing a direct viral effect on brain cells. Second, infected B cells secrete inflammatory cytokines when they infiltrate the infected tissue, which leads to local nerve cell dysfunction. Third, after viral infection, antibodies are produced that are latent in the body and reactivated when immune function is inhibited, producing an anti-body-mediated immune response **[26].** The diagnosis of EBV encephalitis is also difficult and challenging. The main symptoms of EBV encephalitis include fever, twitching, vomiting, headache, and disturbance of consciousness to different degrees, and a few patients have cranial nerve involvement and ataxia **[6,24-26].** Central nervous system infection caused by EBV mostly involves grey matter, basal ganglia, deep white matter, and others, and a few involve the cerebellum, with diverse imaging manifestations **[6,24,25].** CFS analysis

usually presents lymphocytic pleocytosis, mildly elevated protein, and normal glucose. Detection of cerebrospinal fluid EBV-DNA, serology, and virus culture are also important bases for assisting diagnosis **[27].** Metagenomics next-generation sequencing technology has been widely used in recent years as a new detection method to diagnose pathogens contained in samples, especially for atypical clinical symptoms, rare, ineffective treatment, and some unknown infections. EBV encephalitis is usually treated by symptomatic treatment. Some groups have reported the use of acyclovir and ganciclovir to treat EBV encephalitis, but there is no strong evidence in the literature to support this approach [6]. Patients with EBV infection experience varying degrees of severity. Mild cases can relieve themselves, whereas severe cases not only affect the nervous system of the patients but also lead to the involvement of the immune system of the patients. The prognosis of critically ill patients is poor and even life-threatening.

Conclusions

Encephalitis caused by either Balamuthia mandrillaris amoebae or EBV can infect immunocompetent and immunodeficient individuals, and severe cases are associated with a poor prognosis or even death. Neurological manifestations are complex and diverse, with overlapping symptoms and a lack of specificity. Diagnosis is challenging and requires an adequate combination of clinical course, neuroimaging, and laboratory tests. In particular, brain MRI can better display and find lesions. NGS offers a new opportunity for the earlier diagnosis of diseases caused by rare pathogen infections. Moreover, further research is required to develop new therapies for unmet medical needs.

Declaration of Competing Interests

The authors declare that they have no competing interests.

Funding

Data collection, analysis, and interpretation of the study were supported by Grant from the Talent Project of Chongqing Province (CQYC202103075) which Dong Zhang is the Principal Investigator.

Author's Contributions

CL, LW, YY, and DZ were all directly involved in the clinical discussions and data collection of this case. CL was a major contributor in writing the manuscript under the guidance of DZ. DZ reviewed and revised the whole manuscript. All authors read and approved the final manuscript.

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Citation of this Article

Liang C, Wen L, Yang Y and Zhang D. Fatal Encephalitis Caused by Coinfection of Unusual Microorganisms: Case Report. Mega J Case Rep. 2023; 6: 2001-2011.

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