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【 CASE REPORT 】

An Acute Case of Granulomatous Amoebic Encephalitis-*Balamuthia mandrillaris* Infection

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Abstract:

A 74-year-old woman who exhibited drowsiness was referred to our hospital. Enhanced head magnetic resonance imaging (MRI) revealed multiple ring-enhancing lesions and lesions showing partial mild hemorrhaging. The patient gradually progressed to a comatose condition with notable brain deterioration of unknown cause on follow-up MRI. On day nine, the patient inexplicably died, although brain herniation was suspected. Autopsy and histopathology revealed numerous amoebic trophozoites in the perivascular spaces and within the necrotic tissue. Brain immunostaining tested positive for *Balamuthia mandrillaris*. Infection due to free-living amoeba is rare in Japan; however, it may increase in the near future due to unknown reasons.

Key words: granulomatous amoebic encephalitis, *Balamuthia mandrillaris*, CNS infection

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Introduction

Similar to *Naegleria fowleri* and *Acanthamoeba*, *Balamuthia mandrillaris* is a free-living amoeba that causes fatal central nervous system (CNS) infections, with mortality rates of over 98% (1). More than 200 cases of *B. mandrillaris* infection have been reported worldwide since 1990. The highest prevalence of cases has been observed in temperate regions in the southern areas of North America and Latin America (2, 3). Nine cases of CNS infection due to *B. mandrillaris* have been reported in Japan (4, 5). Although only 3 cases were reported in the 23 years between 1986 and 2009 (the first case in 1986 was retrospectively diagnosed as a case of *B. mandrillaris* infection), infections have been reported almost every year since 2010.

We herein report a case of amoebic encephalitis caused by *B. mandrillaris*.

Case Report

In December 2015, a 74-year-old woman with a history of untreated diabetes mellitus (DM) and chronic hepatitis C virus infection (HCV) was referred to our hospital. Upon admission, the patient exhibited drowsiness that had persisted from the previous day. The patient had no dermatological disorders upon inspection. The test results on admission revealed mildly disturbed consciousness (Glasgow coma scale score of 13, E3V4M6); body temperature, 38.1℃; blood pressure, 108/67 mmHg; heart rate, 95 beats/min; white cell count, 10,060 cells/mm^3 (neutrophil, 85%; eosinophil, 0%; basophil, 0%; monocyte, 4%; lymphocyte, 8%); hemoglobin, 13.0 g/dL; platelets, 21.7×10^4 cells/mm^3; erythrocyte sedimentation rate, 30 mm/h (1 hour); ferritin, 380 ng/mL; aspartate aminotransferase, 36 U/L; alanine transaminase, 69 U/L; blood urea nitrogen, 28.9 mg/dL; creatinine, 0.39 mg/dL; lactate dehydrogenase, 363 U/L; glucose, 254 mg/dL; glycated hemoglobin (national glycohemoglobin standardization program), 12.1%; and soluble interleukin-2 receptor, 553 U/mL. The patient tested negative for the presence of C-reactive protein, procalcitonin, antinuclear antibody, anti-treponemal antibody, Toxoplasma IgM/IgG antibody, Cysticercus cellulose-IgG antibody, human immunodeficiency virus, hepatitis B virus, and a wide

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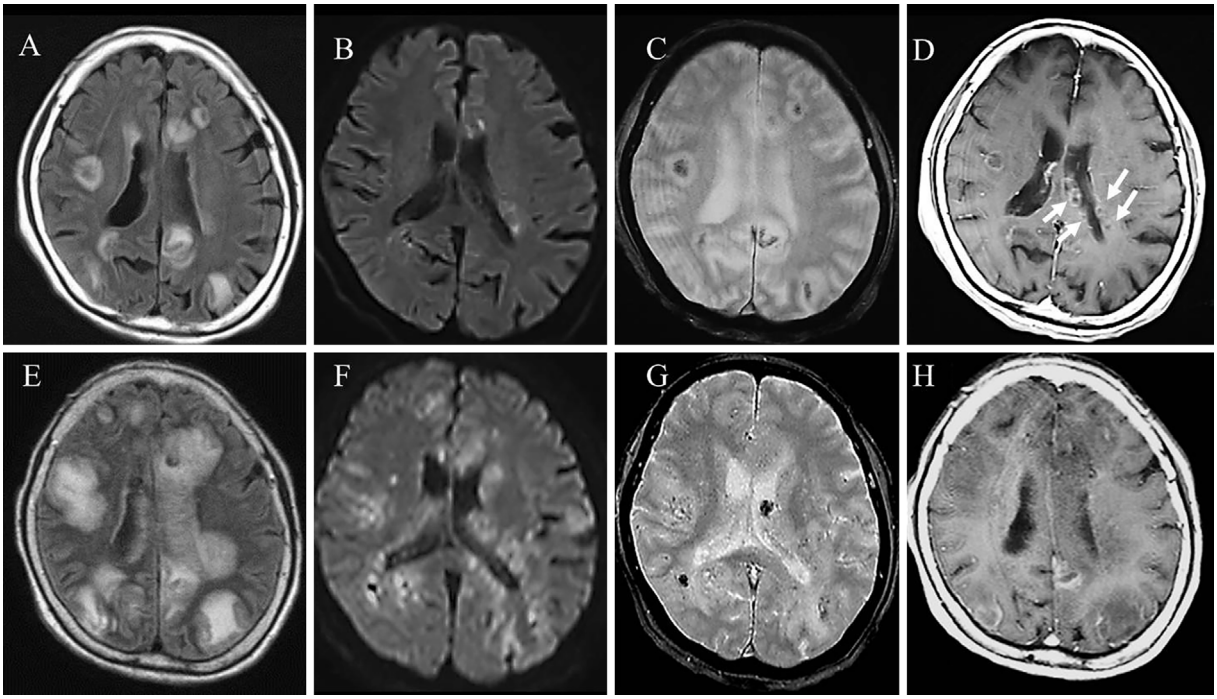

Figure 1. **(A) Magnetic resonance imaging (MRI) of fluid attenuated inversion recovery (FLAIR), (B) diffusion-weighted image (DWI), (C) T2*-weighted image, and (D) gadolinium-enhanced T1-weighted image (Gd-T1WI) performed on the first day of admission. Gd-T1WI shows multiple ring-enhancement regions in the brain, especially around the left lateral ventricle (arrow head). (E, F, G, H) Follow-up images on day 6 of admission showed deterioration. Locally, brain structures, especially around the lateral ventricle tissue, were destroyed, and there was a decrease rather than an increase in the gadolinium-enhanced oval lesions.**

range of tumor markers. Her interferon γ release assay results were also normal. A cerebrospinal fluid (CSF) analysis revealed moderate pleocytosis (cell count, 130/mm^3; monocytes, 85.4%); protein, 393 mg/dL; and glucose, 107 mg/dL; CSF tested negative for the presence of bacteria, Cryptococcus antigen, Aspergillus antigen, and β-D-glucan. The color of the CSF indicated xanthochromia. Enhanced head magnetic resonance imaging (MRI) revealed multiple ring-enhancing lesions and lesions showing partial mild hemorrhaging (Fig. 1A-D). Whole-body computed tomography (CT) and gallium-67 scintigraphy indicated the absence of abnormal lesions.

The patient's condition gradually progressed to a comatose state with notable brain deterioration on follow-up MRI performed six days after admission (Fig. 1E-H). On day 9 of admission, the patient died due to unexplained causes, although brain herniation was suspected. An autopsy on day 10 revealed liver cirrhosis and mild esophageal varices but no malignancy. The brain was macroscopically fragile and malacic. The lesions observed on MRI were fragile and crumbled upon handling. There was, however, no apparent brain herniation (Fig. 2A, B). A histological examination revealed multiple necrotic lesions with hemorrhaging in the cerebrum, mesencephalon, and brainstem. These brain regions demonstrated necrotizing angiitis. We observed large numbers of amoebic trophozoites in the perivascular spaces and within the necrotic tissue (Fig. 2C, D). Pathogen-specific polymerase chain reaction (PCR) using 18S rRNA gene DNA extracted from the specimen of CSF and brain tissues revealed a negative result for *B. mandrillaris*. Conversely, brain immunostaining tested positive for *B. mandrillaris*: the brain tissue section was incubated with rabbit antiserum against *B. mandrillaris*, followed by EnVision®+ Dual Link System-Horse radish peroxidase (HRP) (DAB+), which is based on an HRP-labelled polymer conjugated with secondary antibodies. Staining was performed with 3,3'-diaminobenzidine (Fig. 2E).

Discussion

The increasing number of observed cases of free-living amoebic infection may be due to the increasing availability of medical care, improvements in testing techniques, or other reasons. Immunocompromised hosts, including those with DM, are more susceptible than immunocompetent hosts to *B. mandrillaris* (1); indeed, our patient had untreated DM, HCV infection, and cirrhosis. In addition, the patient performed fieldwork and gardening throughout the year, which might have increased her risk of exposure. Typically, patients have ulcerated purple nodules at the site of skin lesions following percutaneous infection (6). Our patient, however, had no skin lesions.

Regarding the diagnosis of our patient, previous reports have described lymphocytic-predominant pleocytosis, ele-

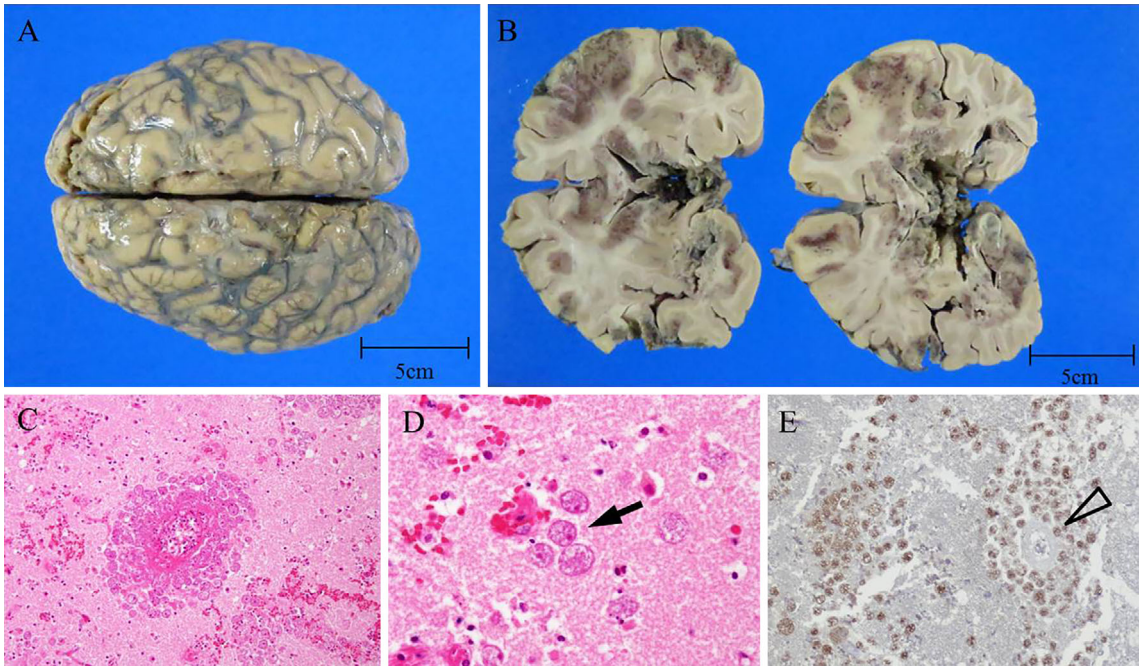

Figure 2. **(A, B) The brain was macroscopically fragile and malacic. The surface of the brain was clouded with apparent deformation and brain herniation. (C) The accumulation of numerous amoebic trophozoites was observed in the perivascular spaces and within the necrotic tissue that showed deficient granulomatous (Hematoxylin and Eosin (H&E) staining, ×200 magnification). (D) A high-power image of an amoebic organism (indicated by the arrow; H&E staining, ×600 magnification). At first glance, the amoebic organisms appeared to be cysts. However, the cysts of amoebic organisms typically have a double-walled structure, and no such double-walled structure was observed in the present case. Thus, the amoebic organism was considered to be in the trophozoite form rather than in the cyst form. (E) The arrowhead indicates the blood vessels and trophozoite accumulation in the perivascular spaces, which tested positive for *Balamuthia mandrillaris* (immunostaining, ×200 magnification).**

vated protein, and normal or low glucose levels in the CSF (2), as was true in our patient. The cytodiagnosis and PCR tests of CSF can fail to provide a diagnosis, as observed in our patient, so a biopsy is indispensable in many cases, a pathological diagnosis may take a long time to obtain. This is one of the reasons why a definitive diagnosis is difficult before patient death. Neuroimaging typically reveals multiple patchy lesions with hemorrhaging (4) exhibiting high intensity on diffusion-weighted imaging and ringed enhancement on T1-weighted imaging. Our case had multiple small lesions with ringed enhancement on T1-weighted imaging around the lateral ventricles (Fig. 1D). These findings are keys to an early diagnosis. In the present case, the differential diagnoses for neuroimaging with ringed enhancement included malignant lymphoma, metastatic brain tumor, primary brain tumor-like diffuse glioma, toxoplasmosis, neurocysticercosis, tuberculosis, brain abscess, and acute disseminated encephalomyelitis. Few of these diseases aside from bacterial infection present with day-by-day deterioration.

Generally, granulomatous amoebic encephalitis is reported to have a prolonged or chronic course (1). However, some patients have been reported to have an acute course (2). Indeed, our patient progressed from presenting with developing symptoms to death in only nine days. Pathologically, our patient demonstrated microhemorrhaging, diffuse invasion of inflammatory cells, trophozoite accumulation in the perivascular spaces that was more prominent than that in inflammatory giant cells, fibrinoid necrosis, epithelioid cells, and vascular cuffing. Such a case with a less granulomatous appearance than most might reflect an acutely or sub-acutely proceeding amoebic CNS infection (7). Acutely proceeding cases might be associated in some way with immunodeficiency; however, this link is still unclear.

Regrettably, our patient died before a diagnosis was confirmed; thus, antimicrobial therapy could not be initiated. Regarding the treatment, past reports have suggested antimicrobial therapy with flucytosine, pentamidine, fluconazole, sulfadiazine, and a macrolide antibiotic; recently, miltefosine has been suggested as a potential treatment for patients with *Balamuthia* infection (8, 9). However, there is currently no established treatment for this disease, and the survival of afflicted patients is extremely rare (10). Considering the acute progression of the disease in our patient, the outcome may have been the same even if an attempt at treatment had been initiated.

In conclusion, our patient presented with amoebic encephalitis by *B. mandrillaris*. Clinicians should be aware of

such infections because of the difficulty of the diagnosis, the high mortality rate, and the rapid increase in the number of cases observed worldwide.

The authors state that they have no Conflict of Interest (COI).

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