Knowledge Obtained from an Elderly Case of Japanese Encephalitis

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Abstract

The nationwide introduction of a Japanese encephalitis (JE) vaccine has contributed to a reduction in the annual infection rate of JE in Japan. However, the current neutralizing antibody prevalence ratio in Japan is approximately 20% in children 3-4 years of age and in people in their forties and fifties. We herein report a man with JE who was definitively diagnosed by multi-virus real-time polymerase chain reaction employing biopsied brain tissue and serological examinations. JE should be kept in mind when a patient has severe encephalitis of unknown etiology. In order to protect the susceptible population from JE, vaccination is recommended, especially for children and middle-aged people.

Key words: Japanese encephalitis, multi-virus real-time PCR, magnetic resonance imaging, pathology, vaccination, neutralizing antibody

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Introduction

Japanese encephalitis (JE) is caused by the Japanese encephalitis virus (JEV), a member of the genus Flavivirus in the family Flaviviridae, which is transmitted by C. tritaeniorhynchus, a species of mosquito (1-5). JE manifests as severe encephalitis with a fatality rate as high as 20-30%, and neurological sequelae are observed in approximately 50% of surviving patients (6).

In Japan, a nationwide vaccination program has successfully reduced the number of patients with JE. However, outbreaks of JE still occur annually in the summer, especially in the southwest part of Japan (7, 8). It has been reported that the JE vaccination rate decreased since adverse reactions, including post-vaccination events such as acute disseminated encephalomyelitis (ADEM), were recognized and reports were spread by the mass media (9). The nationwide JE surveillance program reported that approximately 10% of children 3-4 years of age were vaccinated with the JE vaccine in 2007 (10), whereas approximately 20% of children of identical ages possessed neutralizing antibodies against JE (11). Furthermore, the antibody prevalence ratio was less than 20% in individuals in their forties and fifties in Japan in 2013, and those populations face a high risk of infection when taking either short- or long-term trips to endemic regions worldwide (7). In Kyoto Prefecture, we experienced a case in which an elderly man with rapidly progressing encephalitis ultimately died. The diagnosis of JE was confirmed by multi-virus real-time polymerase chain reaction (PCR) employing biopsied brain tissue in combination with serological examinations. JEV should be kept in mind when a patient is encountered who is suffering from severe encephalitis of unknown etiology, and vaccination should be recommended for susceptible populations in Japan.

Case Report

A 72-year-old man complained of a high fever of 38.1°C and was medicated with antibiotics under a preliminary di-
agnosis of pneumonia in August. Three days later, he rapidly fell into a semicoma (Japan Coma Scale 30-100) with tonic and clonic convulsions. He had a past history of hypertension, chronic renal disease and bilateral cataracts without diabetes mellitus. He lived in the countryside and pig farms were in the vicinity of his home. He had no history of travelling abroad. At admission on the third day, his extremities were spastic and left hemiparesis was noted as being associated with abnormal reflexes, including Babinski and Chaddock reflexes and nuchal stiffness. The bilateral pupils showed miosis with a loss of light reflex.

Laboratory examinations showed leukocytosis (16,880/μL), a positive inflammatory reaction [C-reactive protein (CRP) 30.13 mg/dL], and renal dysfunction [blood urea nitrogen (BUN) 50.49 mg/dL, Creatinine 4.49 mg/dL]. A spirometry showed an increased intracranial pressure (27 cmH2O at start, 17 cmH2O at end), accompanied by an increased respiratory effort. Blood cultures were negative. A chest X-ray revealed bilateral pneumonia. A full blood cell count (556/3 μL: monocytes 86%, polymorphonuclear leukocytes 14%), mildly decreased glucose (79 mg/dL: 0.56 mmol/L), a positive inflammatory reaction [C-reactive protein (CRP) 30.13 mg/dL], and renal dysfunction [blood urea nitrogen (BUN) 50.49 mg/dL, Creatinine 4.49 mg/dL]. A sputum culture was negative. A transbronchial lung biopsy revealed a non-caseating granulomatous infiltrate with acid-fast bacilli. Analysis of pleural fluid showed no evidence of tuberculosis. The patient’s condition further deteriorated and he expired on the 108th day. No autopsy was performed.

**Neuropathology**

The biopsied brain tissue contained cortex and subcortical white matter of the right frontal lobe. Marked edema, perivascular cuffing of lymphocytes, microglial nodules and neuronophagia were observed (Fig. 2A and B). Intense glial fibrillary acidic protein (GFAP)-positive astrogliosis and microglial activation were noted (Fig. 2C and F). The perivascular lymphocytes were polyclonal, however, CD 8-positive lymphocytes were predominant, showing diffuse infiltration into the brain parenchyma (Fig. 2D and E). No inclusions were detected in the cytoplasm or nuclei of neurons and glia using immunohistochemical analyses for HSV type 1 and 2 or JEV.

**Multi-virus real-time PCR**

DNA and RNA were extracted from the formalin-fixed paraffin-embedded brain tissue sections. Multi-virus real-time PCR was performed as previously reported (12). Briefly, each probe-primer set was prepared in 96-well plates and the extracted DNA and RNA were mixed in each well, followed by a PCR reaction (QuantiTect Multiplex One-step real-time RT-PCR kit, Qiagen, Tokyo, Japan) and detection of the quantity of viral genome (Stratagene MX 3005P, Agilent Technologies, Santa Clara, USA). JEV genome was detected at 1.79×10^7 copies per 1 μg of RNA.

Figure 1. MRI findings of the patient. (A-C) On 1st day after admission; T2-weighted MRI-MRA (A) and fluid-attenuated inversion recovery (B) images showed a high intensity area in the right frontotemporal lobes, bilateral hemispheric white matter, corpus callosum, right basal ganglia, and bilateral midbrain, although those areas showed a low intensity on T1-weighted images (C). A nodular lesion measuring 14 mm in diameter showed a high intensity signal on T1-weighted imaging, whereas a low signal was noted on T2-weighted and FLAIR imaging (A-C). (D) On the 7th day after admission; the high intensity area was expanded to the left parietal white matter on T2-weighted imaging.
among the 163 viral species studied.

IgM capture ELISA

JEV-specific IgM was detected from the serum (P/N ratio: 19.5 on day 38, P/N ratio: 18.6 on day 79) and CSF (P/N ratio: 19.3 on day 38, P/N ratio: 20.5 on day 78) using an IgM capture ELISA for JEV.

Discussion

The annual number of JE patients has reduced from over 1,000 before 1960 to less than 10 after 1992 in Japan (13). This dramatic reduction in the prevalence of JE is the result of the introduction of a nationwide vaccination program in 1954 with a high-purity inactivated JE vaccine. In addition, this reduction in JE cases may be due to a decreased number of vector mosquitoes following the relocation of pig farms and rice fields to areas located away from residential zones, as well as a decrease in the transmission of JEV to humans through mosquitoes due to environmental improvements (3).

From a clinical aspect, the imaging findings of JE on CT and MRI reflect the pathologic changes. The temporal lobe involvement pattern in JE is fairly characteristic of primarily hippocampal involvement, with the rest of the temporal lobe spared. In addition, the concurrent involvement of the thalami, substantia nigra, and basal ganglia enables the differentiation of JE from herpes simplex encephalitis (14). Neuropathological findings of JE patients are characterized by glial nodules, perivascular lymphocytic infiltration and circumscribed necrotic foci, which are unspecific and common changes in viral encephalitis of uncertain etiology. Because the JEV genome cannot be detected 2 to 3 days after the occurrence of encephalitis, the multi-virus RT-PCR system is useful for detecting JEV in brain specimens from affected patients. This system has the potential to detect 163 human viruses (47 DNA viruses and 116 RNA viruses) with a high specificity and sensitivity, even when using formalin-fixed paraffin-embedded materials.

Pigs are the most important hosts for the maintenance and amplification of JEV with respect to human infections in Japan. The antibody to the JEV in pigs was surveyed in different regions in Japan by the National Institute of Infectious Diseases (NIID) in 2013 (7). Anti-JEV antibody detected by the hemagglutination-inhibition test (HI-Ab) was positive in pigs examined in 25 out of 33 prefectures, and IgM antibody to JEV was also detected in domestic pigs from 9 prefectures during the same analysis period. Furthermore, more than 50% of the pigs became positive for HI-antibody, as well as IgM, in 3 prefectures very close to Kyoto Prefecture. Konno et al. reported that a high positive rate of swine antibody may herald an outbreak of JE among humans in the same geographic locality (15). An interval of 18 days, as regularly observed between successive infections among swine and subsequent human infections, may be the summation of 4 days to the viremic stage in mammals plus 14 days of incubation in mosquitoes (7). Most JEV patients reported thus far in Japan ranged from 65 to 69 years of age, and 85% of the patients were older than 40 years of age. Considering that vaccination for JEV was initiated in 1954, Japanese people older than 61 years of age might not pos-
sess the antibody to JEV. In addition, NIID surveys conducted in 2004 and 2008 showed that less than 20% of the Japanese population, viz., 15.4% from 45 to 49 years of age and 18.9% from 50-54 years of age, had detectable levels of neutralizing JEV antibodies, despite the universal vaccination history (7).

In summary, the annual infection rate of JE has remained lower than 10% for the last 20 years (8). However, an active zoonotic cycle between mosquitoes and pigs has been reported in the southwestern parts of Japan, such as Kyushu, Okinawa, Shikoku, and Chugoku districts (7, 15). The vaccination rate has dropped in recent years, followed by a decrease in the number of people who have neutralizing antibodies against JEV. Taking into account the fact that JEV remains present in Japan, active vaccination should be recommended in order to protect susceptible populations from JE.

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

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References

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