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A report on Japanese encephalitis in Mizoram: clinical investigation and diagnosis

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Abstract

Japanese encephalitis (JE) is a leading cause of viral encephalitis in Asia. Mizoram does not have record of JE till now, however, mortality due to 'viral encephalitis' among 7 to 20 aged groups during pre-monsoon and post- monsoon season has been recorded. Pathologic studies of fatal human encephalitis caused many common findings. The clinical syndrome may include many neurological effects and many other similarities in the clinical presentations. Such neurologic disease may manifest as subtle changes resulting in an initial misdiagnosis. Several serological methods have been developed for investigation and diagnosis of the disease. However, reverse transcriptase polymerase chain reaction (RT-PCR) based methodologies have resulted in increased sensitivity for the detection of JEV viruses in clinical samples, and offers rapid and sensitive method.

Key words: Japanese encephalitis; diagnosis; detection; diagnosis; RT-PCR; Mizoram.

Introduction

Japanese encephalitis (JE) is a leading cause of viral encephalitis in Asia with 68,000 clinical cases reported annually.[1] The virus is one of Arboviruses belonging to the genus *Flavivirus*.[2] Epidemics of JE were recognized as early as 1871 in Japan and were common in Japan, Korea and China in the first half of 1900s.[3] JE was not recognized as a threat in Southeast Asia until 1969 when its case was reported from Chiangmai valley in Thailand.[4] The disease subsequently continued to spread in two distinct epidemiologic patterns. In the summer months of temperate zones, such as the Korean peninsula, Japan, China, Nepal, and northern India; in rainy season of the tropical areas of southern Vietnam, southern Thailand, Indonesia, Malaysia, the Philippines, and Sri Lanka.[5-6]

In India, the disease was first observed in Vellore district in Tamil Nadu in 1955.[7] Since

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then, the virus is active in many parts of India and outbreaks have been reported from the states of Bihar, Uttar Pradesh, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa and union territories of Goa and Pondicherry and in the northeast India, the disease has been appearing in endemic forms or sporadic outbreaks, in Assam and Arunachal Pradesh.[8-10]

Mizoram (92.15-93.29°E and 21.58-24.35°N) belongs to the Indo-Burma region. The moist climate during monsoon season, deep forests and dense network of water streams, water-logged rice fields are conducive for breeding of mosquito including 20 species of *Anopheles* species, vector of malaria, and 3 species of *Culex*, vector species of JE.[11-13] So far the region have no record of JE, however, mortality due to 'viral encephalitis' among 7 to 20 aged groups (67 cases from 2008 to 2014) during pre-monsoon (March-June) and post-monsoon season (August -October) has been recorded.[14] It is, therefore, pertinent to study the status of the disease in the region. The epidemiological data is given in Figure 1.

Materials and Methods

Collection of sample

Whole blood (5 ml) was drawn from a patient admitted in Aizawl Hospital and Research Centre, Aizawl, Mizoram which was used for different medical examinations. 1 ml of collected blood was used for JEV analysis with the permission of the patient's parent and the attending medical officer.

Virus isolation and cDNA synthesis

Total RNA was extracted from blood using Trizol (Fermentas), phenol-chloroform and finally precipitated in ice-cold ethanol with slight modification. The quantity and quality of ex-

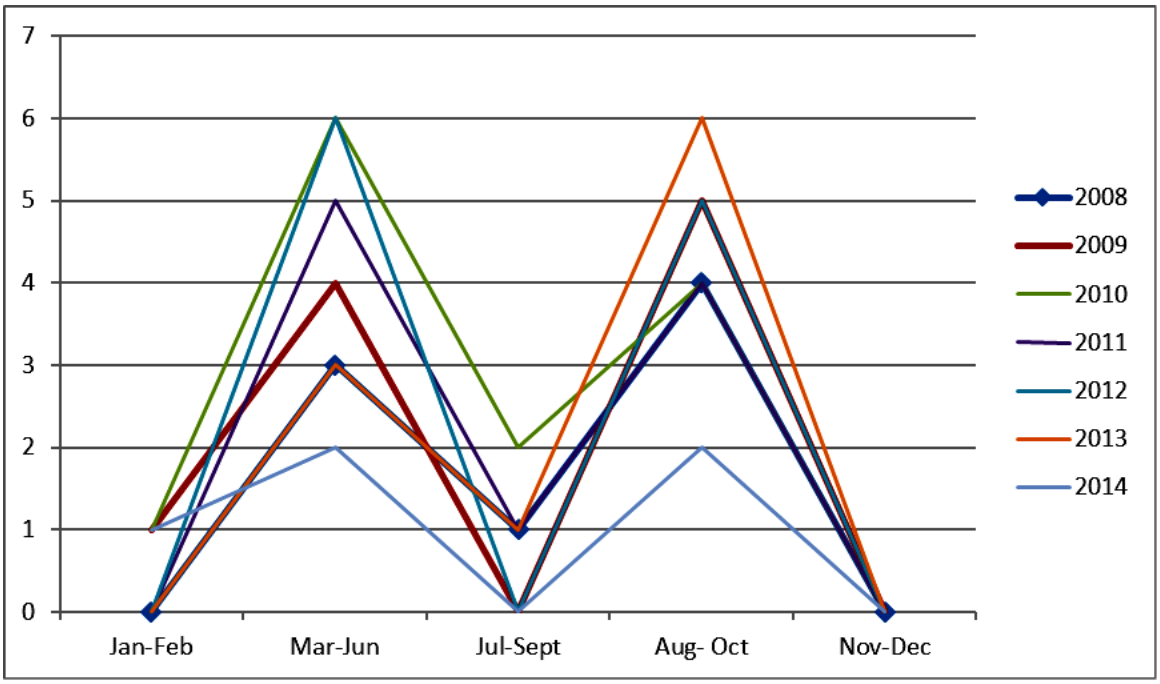

Figure 1. Epidemiological data of mortality due to 'Viral Encephalitis' in Aizawl Civil Hospital, Government of Mizoram during 2008-2014.

tracted RNA was visualised using 2% Agarose gel with 1 µl 10 mg/ml ethydium bromide. The RNA samples were treated with 1 µl of DNAase (Merck, Biosciences); and incubated at 37°C for and at 75°C for 15 mins.[15-16] RNA was reverse transcribed using RevertAid First strand cDNA synthesis kit (Fermentas) following the manufacturer's protocol.

Detection of JE through reverse transcription-PCR (RT-PCR)

The resultant cDNA (2µl) was used to amplify targeting the C-prM region of JE virus using the forward and reverse primers 5'-GCAGAAAGCAAAACAAAAGAG-3' and 5'-ACGGATCTCCTGCTTCGCTTG-3' respectively.[17] PCR amplification was carried out by denaturing the DNA at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 54°C for 60 seconds, and 72°C for 60 seconds, and a final extension at 72°C for 7 minutes using Taq DNA polymerase (Merck, Biosciences).

Result and Discussion

As stated in the introduction, mortality due to 'viral encephalitis' has been recorded in Aizawl civil hospital. Pathologic studies of fatal human encephalitis caused by arboviruses show many common findings.[18] For most arboviral infections, viremias are so low and brief that they are undetectable by the time a patients comes to the hospital.[19] The clinical syndrome following neurological infection with arboviruses (JEV, WNV, dengue, etc.) may include attack on meningis (meningitis), inflamation of the brain parenchyma (encephalitis) and damage of the spinal cord (myelitis) – *meningoencephalomyelitis*,[19] whose mortality cases has also been observed in hospitals.[14] Many other similarities in the clinical presentations such as - seizures, fever, headache, nausea, vomiting, cough, sore throat, abdominal pain and diarrhoea etc and also been observed with infections caused by these virus. Such neurologic disease may manifest as subtle changes resulting in an initial misdiagnosis.[19]

Several methods have been developed for investigation and diagnosis of these viruses. Serological methods such as hemagglutination inhibition, indirect immunofluorescence, neutralization tests,[20] etc. have practical limitations and do not give an accurate diagnosis. The detection of JEV-specific IgM from cerebrospinal fluid (CSF) and/or serum and have been found successful.[21-23] However, even with the best laboratory facilities, JEV cannot usually be isolated from clinical specimens. This is probably because of low circulating viral numbers, rapid development of neutralizing antibodies[24] and quick clearance of transient viremia after onset of illness.[25] Further, there is also cross-reactivity of antibody with other flaviviruses, particularly dengue, which means that dengue infection can be misdiagnosed as JE unless antibodies for both are tested for in parallel.[26]

PCR-based methodologies offer increased sensitivity for the detection of most JEV viruses in clinical samples. They offer better sensitivity compared to virus isolation with a much more rapid turn-around time.[27] The introductions of reverse transcriptase polymerase chain reaction (RT-PCR)[28] and TaqMan RT-PCR[29] based molecular techniques have proved to be rapid, sensitive, specific, rapid and quantitative for the detection of JEV from laboratory and field samples. Although TaqMan RT-PCR has proved to be more sensitive than traditional RT-PCR,[30] it was not found to be cost-effective and needs a special thermocycler.

Unfortunately, till date, no form of clinical diagnosis tool for JEV infection is available within the state of Mizoram. Therefore the present study targeted the specific C-prM region of JEV. During September 2014, a JEV-suspected sample was collected from Aizawl Hospital and Research Centre, Aizawl and was processed immediately for total RNA extraction and later processed using RT-PCR (in duplicate). The result of the RT-PCR gave amplification of approximately 350 bp as shown in Figure 2.

In conclusion, though the use of RT-PCR

Figure 2. Agarose gel showing RT-PCR amplified products. Lane 1: Low range DNA ruler; Lane 2: 356 bp product of RT-PCR; Lane 3: Negative control.

may be more expensive than any of the serological diagnosis described above, its use is pertinent as an emergency clinical diagnostic tool for the state because of its sensitivity, rapid detection and reliability and hence prevention of misdiagnosis with other flaviviruses.

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