Detection of Chikungunya virus from a case of encephalitis, Bangalore, Karnataka State

Dear Editor,

Chikungunya (CHIK) is a self-limiting viral infection usually characterized by sudden onset of high fever, skin rash and joint pain with or without swelling caused by an Arbo virus and is transmitted by Aedes mosquitoes (Aedes aegypti).[1] The incubation period of CHIK is usually between 2–10 days and mostly affects adult population in comparison to young population. Till date, no specific treatment is available for this infection. Vector control through proper intervention is the only mode of preventing this epidemic. It is evidenced from prior literature that the most effective strategy for reducing density of Aedes mosquitoes is community-based intervention and personal protective measures. As CHIK is a self-limiting disease and treatment is mainly supportive, the best strategy for control of such outbreak is raising awareness of the community through mass education by public health officials.

A boy aged 12 years was admitted in Bangalore, Karnataka State, with fever for 2 days, convulsion (tonic posturing) two episodes, vomiting, altered sensorium, pulse rate 108/min, respiration rate 20/min, shallow breathing, saturation 94%, temperature afebrile. Examination showed that the child had slowness in activities mainly with right upper limb, stiffness of right limbs, occasional tremors in right upper limb, biceps reflex, brachioradialis reflex, triceps reflex, quadriceps reflex and planter, clonus were found to be absent. Central nervous system (CNS) findings showed B/L pupils unequal, dilated and sluggishly reactive. MotorTone increased in all four limbs; power: 2/5 in all four limbs. Computed tomography (CT) scan shows bulky hypodense areas involving bilateral thalami, brainstem, left temporal lobe and also the right external capsule showed irregular hyperdense areas in the pontine hypodensities. The features are likely representing acute viral encephalitis changes. The hyperdense areas in pons may represent haemorrhagic areas. The child expired on the sixth day of admission.

Suspecting as encephalitis, the case was referred to National Institute of Virology, Bangalore for virological investigations. Blood and cerebrospinal fluid (CSF) were collected on 3PID. Second CSF was collected on 5PID. Samples were processed for virological investigation.

Serum and CSF samples were subjected to enzyme-linked immunosorbent assay (ELISA) to detect the presence of immunoglobulin M (IgM) antibodies against Japanese encephalitis (JE), CHIK and Dengue (Den) viruses. Interestingly, only CHIK antibodies could be detected from serum as well as CSF samples. The samples were inoculated on to C6/36 mosquito cell line (*Aedes albopictus)* for virus isolation. Fourth day of inoculation showed typical cytopathic effect (CPE) of CHIKV.

Total ribonucleic acid (RNA) was extracted from CSF, serum and tissue culture fluid (TCF) using a QIAmp viral RNA kit (Qiagen, Germany).

RT-PCR was used to rule out JE, CHIK and Den viruses based on clinical manifestation. All the precautions to prevent cross-contamination were observed as per the World Health Organisation (WHO) protocol. Standard primer pairs were used. Extracted RNAs were negative for JE and Den, but positive for CHIK in reverse transcription polymerase chain reaction (RT-PCR) and confirmed by sequencing. CHIK virus (CHIKV) sequence contained 600nt and comparison of the nucleotide sequence was performed using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) program with the GenBank database. Sequence information revealed that the sequence from infected CHIKV shared 99% homology with CHIKV isolate SVUKDP-09 structural polyprotein gene having GenBank accession no. JN558836 from Andhra Pradesh, India and genotype matching with Central/East African strain of CHIKV as earlier reported.[1]

CHIKV is a mosquito-borne alphavirus which causes fever, rash and arthralgia. It affects all age groups of humans. In the past, life-threatening complications were very rarely reported. However, during the recent worldwide outbreaks, there have been several reports of unusually severe complications and deaths.[2] Recently, neurologic involvement and deaths have been reported in Malaysia and Thailand.[3,4] Apart from typical clinical triad of high-grade fever, arthralgia and vomiting in CHIK infection, we have observed a spectrum of neurological abnormalities in terms of altered mental functions, seizures, focal neurological deficit with abnormal CT scan of head and altered CSF biochemistry. The widely available and easily used tests are, however, serological tests in the initial few days of the illness. RT-PCR can also be used for viral RNA detection and rapid diagnosis during viraemic phase.[5] Now it has become an important issue in domain of public health due to its rapid onset, its potential for epidemic and high morbidity.

Typical history of CHIK infection, neurological complications associated with CNS abnormalities, supportive laboratory evidence C6/36 cell line showing typical cytopathic effect after inoculation with serum and CSF as well as findings of IgM antibodies against CHIK and positive serum, CSF and TCF with standard CHIK primers for RT-PCR-positive and sequencing results allow us to conclude this as the case of CHIK encephalitis. The present study indicates the fact that CHIKV infection is an important but unrecognized cause of febrile illness and emphasizes the need of surveillance for CHIKV disease using multiple diagnostic tests.

Acknowledgement

We thank Director, National Institute of Virology, Pune for providing all the facilities to carry out the research work.

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Received: 28-10-2014
Accepted: 20-01-2015

Access this article online	
Quick Response Code:	Website: www.ijmm.org
	DOI: 10.4103/0255-0857.158600