Identification and Serotyping of Dengue Virus from CSF of Acute Encephalitis Syndrome Cases: Case Reports of Two Cases Eyeing Dengue Encephalitis

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ABSTRACT

Introduction: Dengue, the second most common mosquito-borne human disease, is caused by a Flavivirus, Dengue virus (DENV), by either of its 4 serotypes. Though majority of Dengue cases are limited to febrile illness, but in the past decade an increasing trend of CNS involvement by DENV has been seen. We report 2 cases with features of Acute Encephalitis Syndrome (AES) in which molecular analysis identifies DENV serotypes.

Case report: Two patients, a 61years male and a 51years female, from Kolkata, were admitted with fever for 6 days and 8 days respectively, associated with chills and rigor, body rash, severe headache and retro-orbital pain. The patients were disoriented and unconscious at the time of admission. No history of convulsions and yellowish discolouration of eyes or urine were given. Serum and CSF were collected aseptically and referred to the Virology Unit, CSTM. The sera were tested for IgM of JE, Scrub typhus, Chikungunya, Dengue, West Nile fever and Leptospirosis by respective ELISA kits. CSF samples were tested for JE IgM by ELISA.

Conclusion: DENV should be considered as a possible etiological agent of AES cases especially in Dengue endemic area. Molecular analysis of CSF is valuable for confirmation of Dengue Encephalitis and its causative DENV serotype.

Keywords: Dengue, Acute Encephalitis Syndrome, Serotyping

INTRODUCTION

Dengue, the second most common mosquito-borne human disease, is caused by a Flavivirus, Dengue virus (DENV), by either of its 4 serotypes. Clinical manifestation of Dengue ranges from asymptomatic infection to life threatening Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Though neurological manifestations are quite rare in dengue,[2] in the past decade an increasing trend of CNS involvement by different serotypes of DENV is being observed. Neurological manifestations of Dengue have been classified by Murthy into 3 categories[3] namely neuro-tropic effect of Dengue virus (encephalitis, meningitis, myositis, and myelitis), systemic complications of Dengue (encephalopathy, stroke, hypokalemic paralysis and papilledema) and post-infection neurological features (acute disseminated encephalomyelitis (ADEM), myelitis, neuromyelitis optica, optic neuritis, Guillain Barre syndrome, oculomotor palsy, maculopathy etc). Here we report two cases, a 51years female and a 61years male from Kolkata who were admitted with fever for 8 days and 6 days respectively.

CASE REPORT

Case 1: A 51years old normotensive, non-diabetic female from slum area of Kolkata was admitted in the first week of July with chief complaints of high grade fever for 8 days, associated with chills and myalgia. After admission, her fever subsided, and after 3 days in afebrile state, she developed severe headache and retro-orbital pain. At the time of admission, she was conscious but disoriented. Her GCS score was 11/15 [E-4, V-2, M-5]. There was no associated abdominal pain, postural dizziness, reduced urine output, or any bleeding tendency. Maculo-papular rash was noticed all over the body. Her vital signs were stable with a pulse rate of 100 beats per minute (bpm) and blood pressure of 107/70mm Hg. Rest of the examination was unremarkable except mild hepatomegaly.

Case 2: A 61years old normotensive, non-diabetic male from Kolkata was admitted in the month of June with chief complaints of high grade fever for 6 days, associated with chills. He was suffering from severe headache, retro-orbital pain, myalgia and there was no history of convulsion. He was admitted to the hospital in unconscious condition. There was no history of travel outside Kolkata in the last 6 months. At the time of admission, patient was febrile, unconscious and his GCS score was 7/15[E-2, V-2, M-3]. Maculo-papular rash was present over trunk and upper limbs. Clinical examination on admission revealed pulse 110/min, blood pressure 130/80 mm Hg and temperature 102.4 °F. Other systemic examinations were within normal limits. A mild non-tender hepatomegaly was noted only.

Serum samples were collected aseptically from both patients and were referred to Virology unit, department of

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Lab investigations	Case 1	Case 2
Haemoglobin%	13.5g/dl	12gm/dl
PCV	40.3%	42%
Leucocyte count	TLC-13*10^3/mm^3, 90% lymphocytes	17.5*10^3/mm^3, 90% lymphocytes
Platelet count	70*10^3/mm^3 (8^{th} day)	80*10^3/mm^3 (6^{th} day)
Total serum bilirubin	1.5mg/dl	1.2mg/dl
SGPT	100mg/dl	70mg/dl
SGOT	85mg/dl	45mg/dl
Malaria parasite	Not found	Not found
Widal test (8^{th} day)	Non reactive	Non reactive
Blood culture	No growth	No growth
Serum for JE IgM	Non- reactive	Non- reactive
Seum for Dengue IgM	Reactive	Non- reactive
Scrub Typhus IgM	Non- reactive	Non- reactive
Leptospira IgM	Non- reactive	Non- reactive
CT brain	Within normal limit	Diffuse cerebral edema

Table-1: Important blood/serum investigations

CSF study	Case 1	Case 2
Cell type and cell count	10 cells/mm^3, predominantly lymphocytes	15 cells /mm^3, lymphocytes
CSF protein	25mg/dl	40mg/dl
CSF culture	No growth	No growth
CSF for JE IgM	Non- reactive	Non- reactive
DENV RNA with serotype	Detected, Serotype--DENV 1	Detected, Serotype--DENV 4
DNA of Herpes Simplex Virus	Not done.	Not detected
RNA of DEN V, Chikungunya V, Zika V.	Not done	Not detected
India ink preparation	No encapsulated budding yeast cells	No encapsulated budding yeast cells

Table-2: CSF study

Microbiology, School of Tropical Medicine. It was then tested for the presence of DENV IgM by ELISA.

Case-1

On receiving a positive DENV serology and also due to persistence of the severe headache and altered sensorium, aseptically collected CSF sample was sent for detection of DENV RNA. Real time PCR (Viasure RT-PCR trioplex RNA detection kit and serotyping kit) was performed on CSF sample strictly following the manufacturer's instructions and DENV-1serotype was detected (As shown in table no 2).

Case-2

Ig M for *DENV, JE, Chikungunya, Orientia tsutsugamushi, Leptospira, West Nile virus* all were non-reactive in the 2^{nd} case. But as the patient presented with AES features, CSF sample was collected with aseptic precaution and tested by Real Time PCR for detection of nucleic acids of HSV DNA and DenV, ChikV, ZikaV RNA(Viasure RT-PCR trioplex RNA detection kit and serotyping kit). DENV-4 serotype was detected in this 2^{nd} case.

Apart from this, reports of routine laboratory investigations of blood and CSF, blood culture, Widal test, and reports from parasitology and mycology unit were collected (Described in detail in table 1 and table 2)

DISCUSSION

Dengue is most common arthopod-borne viral disease in India. It is caused by single stranded, enveloped, RNA viruses belonging to the family Flaviviridae. It has four serotypes (DEN-1to DEN-4). The fifth serotype (DEN-5) was discovered in 2013 from Bangkok.[4] Symptomatic illness can vary from undifferentiated fever (viral syndrome), Dengue fever, Dengue Haemorrhegic Fever (DHF), Dengue Shock Syndrome (DSS) and dengue with isolated organopathies. Encephalitis and meningo-encephalitis have been found in 4–21% of cases of dengue.[5] This wide variation in the prevalence of Dengue encephalitis is due to differences in the populations studied and the use of different criteria to define dengue encephalitis. Distinguishing dengue encephalitis from other types of central nervous system (CNS) involvement is also challenging.[5]

The criteria for dengue encephalitis are: i) fever; ii) acute signs of cerebral involvement; iii) presence of anti-dengue IgM antibodies or dengue genomic material in the serum and/or cerebrospinal fluid; iv) exclusion of other causes of viral encephalitis and encephalopathy.[6] The afore-said two patients had history of high grade fever associated with features of CNS involvement like headache, disorientation, unconsciousness etc. Though serum for Dengue IgM test was reactive only in case-1, but we were able to detect viral RNA from CSF sample by RT-PCR in both the cases confirming the diagnosis. The other probable causes of encephalitis and encephalopathy were excluded, namely *Japanese encephalitis*, *Orientia tsutsugamushi*, *Chikungunya*, *Zika*, *Herpes simplex* and *Leptospira.*

Typical symptoms of dengue fever like myalgias, skin rash and bleeding manifestations are seen in less than 50% of encephalitis cases.[7] Both of our patients had classical features of dengue like myalgia, macula-papular skin rash and retro-orbital pain.

Though the serotypes most frequently implicated in causing neurological manifestations are DEN2 and DEN3[8], in our study, DEN-1 and DEN-4 were the two serotypes detected in the two cases respectively.

Dengue encephalitis has a good prognostic profile but chances of mortality varying from 1.9% to 3.7% have been reported.[9] Both the patients in our study were treated conservatively and discharged uneventfully.

CONCLUSION

These two case reports emphasize the importance of considering dengue as a differential diagnosis in a patient with features of encephalitis especially in a background of dengue endemic country like India. Another important fact is that although we have national guidelines to manage DF, DHF, DSS, we must individualize the plan of management of Dengue encephalitis. Molecular analysis and serotyping of CSF can play an important role for confirmation of Dengue Encephalitis and its causative serotype. More numbers of study are required to explore the reasons of neurotropism in case of DENV infection.

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