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# Dengue encephalopathy in children in Northern India: Clinical features and comparison with non dengue

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**Abstract**

*Background:* Acute febrile encephalopathy (AFE) is a common cause of childhood hospital admissions in Lucknow. In recent years, many patients have been hospitalized with AFE and hemorrhagic manifestations, some of whom were proven to have dengue viral infection.
*Objectives:* To (i) define the role of dengue encephalopathy (DE) as a cause of AFE in children in Lucknow, (ii) document features of dengue hemorrhagic fever (DHF) in them and (iii)compare clinical features of definite dengue and non dengue AFE.
*Study design:* Prospective study at a teaching hospital in northern India. Children between 1–12 years of age hospitalized with fever and altered consciousness of 2 weeks or less duration were enrolled after excluding bacterial and tuberculous meningitis and frank hepatic encephalopathy. Clinical and laboratory details were charted. Haemagglutination inhibition (HI) test for dengue and Japanese encephalitis viruses in paired sera and IgM antibody capture ELISA for dengue were done. Real time PCR was done in those samples testing positive for dengue IgM. Those with either positive HI test or positive dengue PCR in CSF or serum were considered definite dengue infection and features of DHF were charted in them. Those negative for IgM antibodies after 5 days of illness or whenever done, HI test, were considered definite non dengue. Clinical and laboratory features were compared between definite dengue and non dengue groups.
*Results:* A total of 265 patients of AFE were enrolled over a 2 year period. HI test was positive in 15/49 (30.6%) and IgM in 52/238 (21.8%) patients thus tested. A total of 62 patients were positive for dengue antibodies by either test. Real time PCR assay for dengue virus genome was positive in 28/42 (69%) tested — 21/29 (72.4%) in CSF and 9/15 (60%) in serum. A total of 39 patients met the criteria for definite dengue infection of which only 2 fulfilled the WHO criteria for DHF. Comparing DE and non DE, rash, bleeding, swelling over body, and hepatomegaly were significantly more common and meningeal signs less frequent in DE. Mean platelet counts and serum albumen were lower and liver enzymes and INR were significantly higher in DE.
*Conclusions:* Dengue viral infection is a cause of AFE in children in this region. Majority of DE here appears to be due to viral invasion of brain as suggested by high PCR positivity in CSF and lack of WHO criteria for DHF. Differentiating features of DE include swelling and hepatic dysfunction.


*Keywords:* Acute febrile encephalopathy; Dengue encephalopathy; Dengue hemorrhagic fever; Viral encephalitis

## 1. Introduction

Dengue infections are a global public health problem, being the most important arbovirus infection of humans and the most important tropical infectious disease after malaria [1]. Over the last 50 years more complicated forms of the infection — dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) have been recognized [1]. DHF is known to have neurological manifestations including encephalopathy [2–9].

Acute febrile encephalopathy (AFE), defined as fever and altered sensorium of 14 days or less duration in a previously

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normal child [10], is a common problem presenting to us in Lucknow, India. Causes include cerebral malaria, bacterial meningitis, viral encephalitis etc. Over the last few years, many patients have been admitted in our wards with AFE along with hemorrhagic manifestations, some of which were proven to be dengue viral infections. The present study was therefore undertaken to define the role of dengue viral infection as a cause of AFE in children in Lucknow and to delineate clinical differentiating features of dengue encephalopathy (DE).

## 2. Subjects and methods

The study was conducted in the Childrens' wards of Chhatrapati Shahuji Maharaj (CSM) Medical University, Lucknow, which is the capital city of the state of Uttar Pradesh (or the northern state). This state is India's most populous (with a population of a 160 million, about 1/6th of that of the whole country) and also one of its poorest with lowest human development indices. It is divided into 70 districts or administrative units. This part of the country is the gangetic plain of northern India. The state's population density is 689 people/km$^2$ and children below 12 years account for roughly 40% of the population [11]. About 79% of the people live in rural areas. There are 3 seasons — summer (March to June), rainy or monsoon season (July to October) and winter (November to February). Temperatures may soar up to 45 °C in summer and fall as low as 3 °C in winter.

Lucknow, the state capital has a population of 4 million. The CSM Medical University Hospital is a tertiary care teaching hospital which caters mostly to the poor and severely ill. The catchment area of the hospital includes the Lucknow city and district and other eastern districts extending up to Nepal. The pediatric services of this hospital which cater to medical problems from birth to 12 years of age, have been admitting about 150–200 cases of 'encephalitis' annually — mostly in the monsoon and post monsoon season. Since facilities for viral diagnosis are not regularly available or are too expensive, most cases do not undergo virological investigations but are presumed to be Japanese encephalitis which is known to be endemic in the eastern regions of the state. Over the last decade, the region is recognized as a high dengue transmission area and a severe epidemic of DF/DHF occurred in late 2003.

Children between 1–12 years of age hospitalized with AFE were screened for enrollment in the study. Those in whom a firm diagnosis of bacterial or tubercular meningitis was made on the basis of CSF examination or imaging or those in whom consent was not obtained were excluded. Frank cases of hepatic encephalopathy with jaundice were also excluded and the remaining cases were enrolled in the study. The enrolled children were worked up according to a pre-designed protocol incorporating a detailed history and examination including general, systemic and neurological examination. Signs of raised intracranial tension were hypertension or bradycardia with hyperventilation or decerebration. Investigations done at admission were complete blood counts including packed cell volume (PCV) and platelet count, liver function tests including s. bilirubin, s AST and ALT, serum proteins, albumen and prothrombin time. Wherever possible and indicated, cerebrospinal fluid (CSF) was tapped at admission and examined for cells, protein and sugar. In addition, 5 ml blood was also collected on admission in Eppendorf tubes and transported to the Virology Laboratory of CSM Medical University, Lucknow for serological investigations. Convalescent sera were obtained whenever possible at least one week after acute serum or at the time of discharge. Daily follow up was done till discharge. Haemagglutination inhibition (HI) test was done in 2003 on acute and convalescent phase sera by standard methods [12] for dengue and Japanese encephalitis (JE) viruses. The test was considered positive for dengue if paired sera showed a 4 fold rise or fall in dengue HI antibodies without a similar response in JE virus antibodies. IgM estimation for dengue virus was also done in acute serum by IgM antibody capture ELISA using commercial kits marketed by Focus Technologies, USA.

### 2.1. Real time quantitative PCR

Serum and CSF samples from patients testing positive or borderline (equivocal) for dengue IgM were subjected to Real Time polymerase chain reaction (PCR) assays. Total RNA was extracted by column based extraction kits of Qiagen (Viral RNA Mini kit). Real time PCR was performed on Rotor Gene 3000, (Corbett Research Australia) using TaqMan probes, and primers designed to pick up all the four types of dengue virus synthesized according to Drosten et al. by Tibmolbiol, Berlin, Germany [13] (provided by Professional Biotech, New Delhi). Single tube RT-PCR was performed using Single tube RT/PCR premix obtained from Professional Biotech, Ltd. Standard curves were established with plasmids which were cloned PCR products obtained from clinical samples (Tibmolbiol) and contained complete cDNA sequence of the gene. Serial dilutions were prepared, starting at $10^5$ copies of the specific plasmid, to interpolate the unknown copy number of specific RNA in each sample.

A case was considered to have 'definite' dengue infection if either HI test in paired serum was positive or if Real Time PCR was positive in serum or CSF. A case was considered as 'non dengue' if IgM test done on blood sample collected after 5 days from onset of illness was negative and wherever done, HI test in paired sera showed no rise or fall in HI antibodies and PCR for dengue genome was negative. A case of dengue viral infection was considered as dengue encephalitis if CSF Real Time PCR was positive. A case testing IgM positive but equivocal or not tested by HI test and negative or not tested on PCR was considered 'probable dengue'.

WHO criteria for DHF [14] were looked for in those with proven dengue viral infection.

*Ethical Approval* was obtained for the study by the Institutional Review Board of Indian Clinical Epidemiology