
**Association of adenovirus type 2 with encephalitis: A new case report
from Kerala, India.**

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ABSTRACT

Adenovirus is a common respiratory pathogen in children that sometime produces encephalitis symptoms in affected children. Very few reports are available on association of adenovirus type 2 with encephalitis cases. However, none of the cases have been reported from India. We have detected and characterized the adenovirus from the clinical specimens collected from a six year old boy who had the symptoms of encephalitis.

The adenovirus was isolated from all the three specimens; CSF, serum and throat swab in HEK-293 cells. The presence of adenovirus was confirmed by PCR. Sequencing and BLAST search analysis confirmed that the isolated adenovirus belonged to adenovirus type 2. As per our knowledge this is the first report of adenovirus type 2 associated with encephalitis case from India.

Keywords: encephalitis, Human adenovirus type 2

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INTRODUCTION

Encephalitis refers to an acute, usually diffuse, an inflammatory process in the brain parenchyma associated with clinical evidence of the brain dysfunction^[1, 2]. The characteristic presentation of viral encephalitis usually consists of fever,

headache, drowsiness, fatigue and clouding of consciousness together with seizures^[1].

Central nervous system (CNS) manifestations in association with adenovirus infections have rarely been documented. Association of adenovirus infection and encephalitis were first been

reported in the year 1958 after isolating adenovirus type 7 from cerebrospinal fluid (CSF) and brain tissue^[3]. Since then, many reports of adenovirus associated with CNS infection had been published. So far, adenovirus types 1, 2, 3, 5, 6, 7, 11, 12, 26, 31, 32, and 49 had been reported in association with CNS infections^[4, 5, 6, 8, 9, 10, 12]. Among the encephalitis causing adenovirus types, type 7 is the most dominant type followed by type 3, 1, and 2^[4, 7]. However, none of the cases of adenovirus type 2 associated encephalitis have been reported from India. We report the first case of adenovirus type 2 associated with encephalitis-case from India.

CASE REPORT

Clinical samples (blood, CSF, urine and throat swab) of six year old boy were referred to National Institute of Virology (NIV) Kerala Unit from Institute of Child Health (ICH) Kottayam in April, 2014 for virological investigation. The boy had a history of fever (102°F) for two days, running nose and altered sensorium. His routine blood analysis revealed that the white blood cell (WBC) count was slightly elevated from normal (13200 cells/mm³). Peripheral blood smear examination

indicated that 51% of the cells were polymorphonuclear cells and 49% were lymphocytes.

The platelet count and the haemoglobin (Hb) levels were normal. CSF contained abnormal level of WBC (28cells/mm³), predominantly lymphocytes (85%). Other biochemical parameters like sugar (71 mg %) and protein (65 mg %) were elevated in CSF. Renal and liver biochemical profiles were normal, however, the alkaline phosphatase (ALP) level was elevated significantly (424 IU/L).

Based on his symptoms and the clinical data, the investigations were therefore restricted to the common viruses involved in encephalitis and respiratory illness. Japanese encephalitis virus (JEV), West Nile virus (WNV), herpes simplex viruses (HSV), enterovirus, influenza type A and adenovirus were looked in the samples.

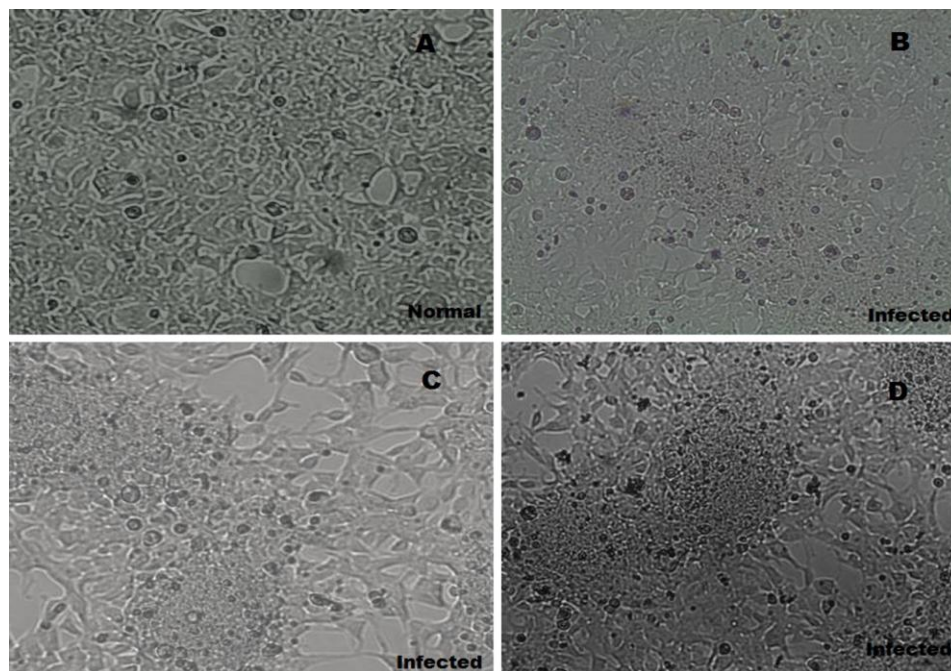
The presence of JEV and HSV type 1 and 2 specific IgM in the serum specimen were tested by using in house JE Mac-ELISA kit and HSV 1 and 2 IgM ELISA kit (Calbiotech Inc, USA) and the results were found to be negative. In addition to the ELISA tests, real time PCR was performed for the detection of other suspected viruses.

The total RNA and DNA were extracted from the specimens using MagMAX viral RNA isolation kit (Applied Biosystem, Foster City, CA, USA) and QIAmp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Real time PCR for WNV, JEV, enterovirus, influenza virus and adenovirus were carried out using different sets of diagnostic primers published elsewhere^[13]. Out of all the

viruses tested, adenovirus was found to be positive.

In addition to the serological and molecular investigations, all the specimens (serum, CSF and throat swab) were simultaneously processed for virus isolation in HEK-293 cells. Cytopathic effect (CPE) was observed in HEK-293 cells inoculated with all the three specimens (Figure 1). The isolates were confirmed by adenovirus hexon gene specific PCR (Figure 2a).

Figure 1: Cytopathic effect (CPE) in HEK-293 cell line showing cell lysis and aggregation on day 2 of second passage (magnification 200x). (A) Normal morphology of HEK-293 cell line. (B) CPE in HEK-293 cells inoculated with serum (C) CPE in HEK-293 cells inoculated with CSF (D) CPE in HEK-293 cells inoculated with throat swab.



The conventional multiplex fiber gene specific PCR was further performed using the published primers and EmeraldAmp GT PCR Master Mix (TaKaRa, Japan) for the identification of adenovirus subgroups^[14]. The PCR reaction was carried out in 25 μ L volume with 0.2 μ M of each primers and 2.5 μ L DNA. Amplification reaction was carried out at 94 $^{\circ}$ C for 5 min followed by 30 cycles 94 $^{\circ}$ C for 1 min, 54 $^{\circ}$ C for 45 sec and 72 $^{\circ}$ C for 2 min with a final extension at 72 $^{\circ}$ C for 5 min in S1000 thermal cycler (Bio-Rad, CA, USA). The amplified PCR products were resolved in 1% agarose gel stained with SYBR green, a DNA binding dye. The adenovirus was identified as subgroup C (Fig 2b).

Figure 2: SYBR green-stained agarose gels showing PCR products of the adenovirus isolated from the patient's specimens (serum, CSF and throat swab).

Figure 2a: Adenovirus hexon gene (conserved region) specific PCR products with the 1 kb plus molecular weight marker (GeneRuler, Thermo scientific, USA) and no template control (NTC).

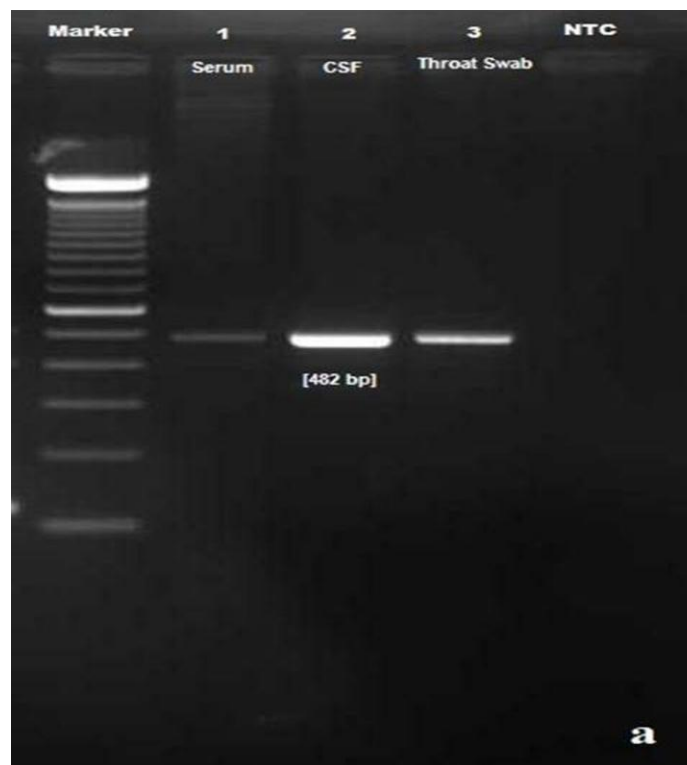
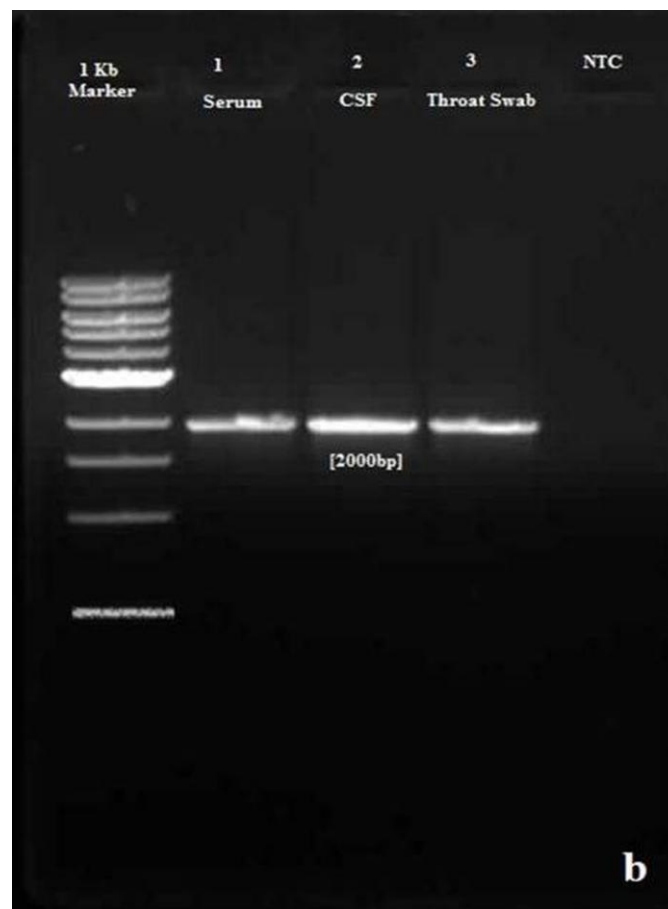


Figure 2b: Adenovirus fiber gene specific PCR with 1 kb molecular weight marker (NEB, Biolabs, New England) and NTC.



Hexon and fiber gene amplicons were purified using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) as per the manufacturer's instructions and outsourced to SciGenomic Technology Pvt. Ltd, India for sequencing. The fiber and hexon gene sequences were subjected to Basic Local Alignment Search Tool (BLAST) search. The search result revealed that the sequences belonged to adenovirus type 2. The sequences have been deposited in

the National Center for Biotechnology Information (NCBI) GenBank (Acc.Nos. KT005573 and KT005574).

DISCUSSION

Adenovirus in human beings is usually associated with respiratory illness. Although CNS involvement is a rare phenomina in adenovirus-infections, sometime it establishes CNS infection. Adenovirus types associated with CNS infections include subgroup A (31 and 12), B (3, 7 and 11), C (1, 2, 5 and 6) and D (26, 32 and 49). The most common adenovirus type associated with CNS infection is adenovirus type 7^[7, 11]. Furthermore, some reports are available for fatal adenoviral encephalitis caused by adenovirus types 6 and 11^[5, 8].

In the present case, neurological manifestation with respiratory symptoms suggested the association of adenovirus. Presence of cells in CSF (28 cells/mm³) and altered sensorium suggested the patient had encephalitis. Magnetic resonance imaging (MRI) results were not available to confirm the involvement of CNS. However; the isolation of adenovirus from the CSF specimen and the neurological manifestation

confirmed the patient had been suffered from the adenoviral encephalitis. This circumstance was, perhaps, surprising because adenoviral encephalitis has not yet been reported in this region.

Although cases had been reported for adenovirus in association with CNS infection in other countries, this could be the first report of adenovirus associated with encephalitis in India. There are few reports of this kind in Indian sub-continent where mostly JEV plays a major role in viral encephalitis^[15]. Adenovirus type 2 has been isolated previously in our institute from a fatal respiratory case^[16]. This finding along with previous finding confirmed the prevalence of adenovirus type 2 with different manifestations in the region.

CONCLUSION

Although adenovirus type 2 is a common respiratory pathogen of children, there have been few reports on encephalitis caused by this adenovirus type. To the best of our knowledge, this is the first Indian case of encephalitis associated with adenovirus type 2 infections in a young child. Our finding and the previously reported cases exhibit a

wide clinico-pathological spectrum by adenovirus type 2.

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