Original Research Paper

Volume - 11 | Issue - 10 | October - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Paediatrics

A STUDY ON AETIOLOGY OF ACUTE ENCEPHALITIS SYNDROME IN A TERTIARY CARE CENTRE IN EASTERN BIHAR.

Dr. Kishore Kumar Sinha Assosciate Professor & HOD, Department of Pediatrics, JLNMCH, Bhagalpur, Bihar.

Dr. Brajesh Kumar* Assistant Professor, Department of Pediatrics, JLNMCH, Bhagalpur, Bihar.
*Corresponding Author.

ABSTRACT **Objectives:** The objective is to study the clinico-epidemiological features of viral acute encephalitis syndrome (AES) cases in children for patient management and policy making. The present study was carried out to determine the prevalence of common aetiological agents of AES in Eastern Bihar.

Methods: This study was conducted at a tertiary care centre at Jawahar Lal Nehru Medical College and Hospital in Eastern Bihar. Serum and/or CSF samples were collected from admitted AES patients during September 2019 to August 2021. Cerebrospinal fluid (CSF) and serum samples from cases were tested for IgM antibodies against Japanese encephalitis virus (anti-JEV), and dengue virus (anti-DENV) by ELISA; and for enterovirus, herpes simplex virus (HSV) and varicella zoster virus (VZV) by real-time PCR. Serum samples of cases having sufficient CSF volume, were also tested for anti-scrub typhus IgM antibodies and for *Neisseria meningitides, Haemophilus influenza* and *Streptoccus pneumonia.*

Results: In the present study, JEV and DENV (8% each) were the most common identified aetiology from the 2046 enrolled patients. Enterovirus, HSV and VZV, each were detected in <1% AES cases. Co-positivity occurred in 24 cases. Scrub typhus (31.8%) was the most common aetiology detected. *Haemophilus influenzae* and *S. pneumoniae* were detected in 0.97 and 0.94% cases, respectively, however, *N. meningitides* was not detected in any of the cases. The gap between the total number of AES cases and those with JEV/ DENV infection increased during monsoon and post-monsoon seasons.

Conclusion: Scrub typhus, JEV and DENV are the main aetiological agents of AES in Eastern Bihar. DENV and JEV can no longer be considered paediatric diseases. The prevalence of non-JEV/DENV aetiology of AES increases in the monsoon and post-monsoon seasons.

KEYWORDS : Acute encephalitis syndrome; Japanese encephalitis; aetiology; dengue virus; scrub typhus; Children.

INTRODUCTION:

Acute encephalitis syndrome (AES) is characterized by an acute onset of fever and clinical neurological manifestation that includes mental confusion, disorientation, delirium, or coma [1]. Viruses have been mainly attributed to be the cause of AES in India although other sources such as bacteria, fungus, parasites, spirochetes, chemical, and toxins have been reported over the past few decades [1].

Acute encephalitis is the clinical diagnosis of children with acute onset of symptoms and signs of inflammatory lesions in the brain. Changes in sensorium, seizures and upper motor neuron type of altered muscle tone point to cerebral dysfunction. Brain tissue would show the pathology, but at the bedside, inflammation is surmised from pleocytosis of the cerebrospinal fluid (CSF) to predominantly lymphocytes, since the aetiology is mostly non-pyogenic infection[2].
Worldwide, acute encephalitis syndrome (AES) has been a major health problem because of associated high morbidity and mortality. AES is defined as a person of any age at any time of year with the acute onset of fever and a change in mental statuses such as confusion, disorientation, coma, or inability to talk and/or new onset of seizures (except febrile seizure) [3].

Acute encephalitis syndrome (AES) is an important public health problem worldwide. Population-based studies have estimated, a global incidence of 3.5 to 7.4 for AES per 100,000 patients in an year [4]. The disease has been reported to be associated with several complications including limb paralysis, seizures, impaired consciousness or even death[5] In non-fatal cases, AES may often lead to severe permanent physical, cognitive, emotional, behavioural and social difficulties in affected individuals[6].

Japanese encephalitis virus (JEV) is the major identified cause of AES in India, affecting 50,000 people annually and causing 8–30% mortality and 50-60% disability[7]. The state of Eastern Bihar is one of the worst hit states in India [8], where JE outbreaks occur annually in the rainy season. In 2005, JE caused a massive outbreak in Bihar affecting 5737 persons including 1344 deaths. Consequently, in 2006, the Government of India launched JE vaccination drive in Bihar, which resulted in overall decrease in the incidence of JE (7.1% of AES) [9] But the number of AES cases has not decreased over the years resulting in an increased gap between the total AES and JE positive cases [10]. This implies that a majority of AES cases in Bihar are caused by non-JE aetiology which may include other viruses, bacteria, parasites, protozoa or fungi; viral aetiology, though is considered to be the most common cause of AES.

Identifying the aetiology of AES in patients is important, not only for patient management but also to understand the epidemiology, identify appropriate targets for immunization, and for formulating public health policies. Therefore, the present study was carried out to determine the prevalence and epidemiology of the common aetiological agents of AES in Eastern Bihar.

MATERIAL AND METHODS:

This was an observational cross-sectional study done over a period of two years. Patients with a clinical diagnosis of AES admitted at a tertiary care centre at Jawahar Lal Nehru Medical College and Hospital in Eastern Bihar, India and referred to the Virology Laboratory for testing, were enrolled consecutively in the study from September 2019 to August 2021. A case of AES was clinically defined as a constellation of symptoms comprising of acute onset fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures) in a person of any age at any time of the year [11]. Cases with known non-infectious aetiology like trauma, metabolic causes, hypoxia, *etc*. were excluded from the study. CSF and/or serum samples were collected from the enrolled patients.The study was approved by the Institutional Ethics Committee (No. 7139/Ethics/R.cell-15).CSF and/ or serum samples were collected after obtaining written informed consent from the patients/guardians.

The CSF sample was tested for anti-JEV IgM antibodies (MAC ELISA kit developed by the National Institute of Virology, Pune, India) as per the manufacturer's instructions and for nucleic acids of JEV (JEV-RNA), enterovirus (enterovirus RNA), herpes simplex virus (HSVDNA) and varicella zoster virus (VZV-DNA) by real-time PCR. The serum sample was tested for anti-dengue virus IgM (anti-DENV) antibodies (MAC ELISA kit—National Institute of Virology, Pune). In case CSF sample was not available, anti-JEV IgM antibodies were tested in serum sample.

Cases, for whom both CSF and serum samples were available in sufficient quantity for further testing, the serum samples were also tested for anti-scrub typhus IgM antibodies by ELISA (Inbios International, USA) following the manufacturer's instructions. Samples with an optical density (OD) >0.5 were considered positive

for anti-scrub typhus IgM antibodies. CSF samples from these cases were also tested for *Neisseria meningitides, Streptococcus pneumoniae* and *Haemophilus influenzae* by real-time PCR.

Nucleic acids were extracted from 140 µl CSF samples following the manufacturer's protocol (Qiagen, Hilden, Germany). The real-time PCRs were standardised. For RNA detection, the reaction mixture contained 12.5 µl of 2× RT-Buffer and 1µ of 25×RT enzyme (AgPath, Life Technologies, CA, USA) and for DNA detection, the reaction mixture contained 12.5 µl TaqMan universal PCR master mix (Life Technologies, CA, USA). For each real-time PCR reaction mixture, the final volume was 25 µl containing 7.5 µl nucleic acid, 0.5 µl each primer (10 pmol), and 0.5 µl probe (5 pmol). The cycling conditions consisted of an initial denaturation of 95°C for 10 min followed by 45 cycles of denaturation at 95°C for 15 sec and annealing and extension for 60°C for 1 min for DNA pathogens and included an extra step of pre-amplification at 45°C for 10 min for RNA pathogens. The threshold cycle (Ct value) of 35 was taken as the cut-off and only the curves showing the value of <35 were considered positive.

The statistical analyses were done using Graph-Pad Prism software version 5. Intergroup comparisons of categorical and continuous variables were done using Fischer's exact test and Chi-square test, respectively in the present study.

RESULTS:

In total 2046 cases of AES were enrolled during the study period, from which 1930 CSF and 1414 serum samples were available. JEV (8.3%) and DENV (7.8%) were the most common aetiological identified among the enrolled cases. Enterovirus, HSV and VZV, each were detected in < 1% AES cases. RT PCR for JEV (JEV PCR) detected three, eight and four extra cases in 2019, 2020 and 2021, respectively in anti- JEV IgM negative cases.

Of the anti-DENV IgM or anti-JEV IgM positives, 8, 7 and 5 cases tested positive for both the antibodies in the years 2019, 2020 and 2021, respectively. Three patients tested positive for both anti-DENV IgM and JEV PCR and one patient tested positive for anti-DENV IgM antibodies and VZV PCR.

It was found that 31.8% tested AES cases were positive for anti-scrub typhus IgM antibodies. *Haemophilus influenzae* and *S. pneumoniae* were detected in 0.97 and 0.94% cases, respectively while *N. meningitides* was not detected in any case.

When the age and sex distribution of the two most commonly detected aetiologies (JEV and DENV) were studied, it was observed that they mostly affected children between 1 and 15 year of age. It was important to note that approximately 40% of the JEV/DENV positive AES cases were adults. Also 0.6% JE and 2.4% DENV positive cases were infants. Males were more commonly affected than females in both the cases.

The seasonal distribution of AES cases showed that AES occurred throughout the year, though the number of cases increased steeply between August and October. DENV showed a similar pattern, with cases occurring throughout the year mostly during August to October. JEV had a distinct seasonality with no JE positive cases during February to June followed by a sudden rise in the number of cases from August to October and occasional cases in July, November and December. The gap between the total number of AES cases and those with JEV/DENV infection also increased during August to October.

DISCUSSION:

Acute encephalitis syndrome is a major health problem in the Eastern Bihar, India, because of the multiple aetiologies involved, lack of standardized case definitions and availability of limited resources. The present study was an attempt to study the prevalence of common aetiologies of AES cases from Bihar.

JEV was examined in the AES cases because the World Health Organization has paralleled JEV surveillance with AES surveillance in the Southeast Asian countries [11] and the virus is also known to cause constant outbreaks in Bihar since 1978 [12]. DENV was examined in the AES cases, since it has increasingly been reported as a cause of AES and other neurological manifestations [13,14]. Enteroviruses were selected, since they were implicated in 2006 and 2008 AES outbreaks in eastern Bihar [15]. HSV and VZV were tested, since well-established antiviral treatment is available only against these viral etiologic agents of AES. Other known viral aetiologies like mumps, measles and rubella viruses were not tested since these viruses contribute to only few sporadic cases, due to availability of effective vaccination [16]. Moreover, they usually have a preceding suggestive history.

Majority of the tests were carried out from CSF samples, as detection of an etiologic agent (antibodies or nucleic acid) in CSF represents direct neuro-invasion, and hence, CSF remains the sample of choice for diagnosing AES aetiology. Anti-DENV IgM and anti-scrub typhus IgM antibodies were detected in serum as these kits are standardized only for the serum samples. The limitation of detecting these antibodies in serum is that these might be pre-existing antibodies and not the cause of present illness, since IgM antibodies may persist for up to six months after infection [17].

Infections by JEV, DENV and scrub typhus were diagnosed by detecting IgM antibodies because by the time patients develop encephalitis and present to the hospital, viremia usually disappears (best detected within first week of illness) and neutralizing antibodies appears [11,18]. Hence, IgM capture assays are the most sensitive tests for diagnosing recent viral [11] / rickettsial [19,20] infections, though few extra JE cases were detected by real-time PCR, which could have been missed if only IgM testing was done. Infections by the remaining pathogens were diagnosed by detecting nucleic acid by real-time PCR, since viruses like HSV, VZV are known to establish latent infections with intermittent reactivations and, therefore, PCR remains the gold standard test for their detection [21]. For bacterial infections *(H. influenzae, S. pneumoniae, N. meningitides)* the Center for Disease Control, USA, has recommended the use of real-time PCR in CSF samples [22], so that targeted therapy may be initiated as soon as possible in a patient.

In the study, JEV and DENV were identified to be the most common aetiological agents of AES each contributing to approximately 8% cases. In the years 2011–12, JEV and DENV contributed to 16 and 11% AES cases, respectively [23]. The incidence of JEV has decreased, probably as a result of mass vaccination campaigns. This decrease has also been observed from other states like West Bengal [23]and Assam [25] where vaccination campaigns are going on. It is interesting to note that the proportion of DENV causing AES is constantly decreasing; from 23.4% in 2008 to 11% in 2012 [24] and to 8% in 2016 [24,25]. Change in the predominantly circulating DENV serotype might be accountable for this observation.

Few cases were positive for more than one test. In a hyperendemic region, for cases that were positive for both anti-DENV IgM and anti-JEV IgM antibodies, three possibilities exist: (i) sequential arbovirus infection; (ii) cross-reacting antibodies due to the common antigenic epitopes in the major envelope (E) protein; and (iii) coinfection [26]. Further, testing is required to resolve the issue. Simultaneous detection of anti-DENV IgM antibodies and JEV PCR favour co-infection.

The age preference of both JEV and DENV has changed over the years. Both the viruses were earlier considered to be predominantly affecting children <15 year of age. But probably as a result of vaccination (JEV) or long-standing endemicity (DENV), adults were observed to be majorly affected (40% of the cases were adults) in the study. Similar age shifts have been observed earlier in areas with good quality, long-standing childhood JE vaccination programmes[27]; and from other Asian countries including Bangladesh, Indonesia, Thailand and Singapore with long-standing dengue epidemic activities[28].

The cases of AES occurred throughout the year, though maximum cases were observed in the monsoon and post-monsoon seasons. A gap between the number of AES cases and those positive for JEV/DENV was also observed throughout the year, which widened in the monsoon and post-monsoon seasons despite the increase in JEV/DENV cases. This indicates the presence of other aetiologies probably with a definite seasonality.

In this study scrub typhus was observed as an important cause of AES in Eastern Bihar contributing to about 32% of the tested cases. Scrub typhus has also been incriminated in about 30% AES cases in Tamil Nadu [29] and was also proved to be a cause of AES in a case control study from Gorakhpur, UP [30]. Neurological complications (cerebellar ataxia and encephalitis) by VZV have been observed previously [31,32]. Minor difference could be due to underlying virus, timing of presentation, and demography of the study population.

This is a treatable infection if detected on time. Therefore, it is suggested to test all AES cases for scrub typhus in Eastern Bihar and initiate early and appropriate treatment on an empirical basis.

CONCLUSION:

From the present study it was concluded that JEV, DENV and scrub typhus remain the main aetiologies of AES in Bihar. Enteroviruses, herpes viruses and bacteria cause only rare sporadic infections. The age preference of DENV and JEV is shifting and adults are now equally affected. The prevalence of non-JEV/DENV aetiology of AES increases in the monsoon and post monsoon seasons. Aetiology could not be determined in a large number of cases, which warrants further studies for detection of other agents of encephalitis. The landscape of AES in India has changed in the previous decade, and both outbreak investigations and surveillance studies needs to be analysed properly. Because of these findings, there is a need to explore additional strategies to prevent AES beyond vector control and JEV vaccination.

REFERENCES:

1. Joshi R, Kalantri SP, Reingold A, Colford JM., Jr Changing landscape of acute encephalitis syndrome in India: a systematic review. *Natl Med J India.* 2012;25:212–220. [PubMed] [Google Scholar]
2. Prober CG, Srinivas NS, Mathew R. Central nervous system infections. In: Kliegman RM, Stanton BF, St. Geme JW, Schor NF, editors. *Nelson textbook of pediatrics*. 20th ed. New Delhi: Reed Elsevier India Pvt. Ltd.; 2016. p. 2936-48.
3. Sharma S, Mishra D, Aneja S, Kumar R, Jain A, Vashishtha VM, *et al.* Consensus guidelines on evaluation and management of suspected acute viral encephalitis in children in India. Indian Pediatr 2012;49:897-910.
4. Granerod J, Crowcroft NS. The epidemiology of acute encephalitis. *Neuropsychol Rehabil* 2007; *17:* 406-28. [PUBMED].
5. Rayamajhi A, Singh R, Prasad R, Khanal B, Singhi S. Study of Japanese encephalitis and other viral encephalitis in Nepali children. *Pediatr Int*2007; *49(6):* 978-84.
6. Clarke M, Newton RW, Klapper PE, Sutcliff EH, Laing I, Wallace G. Childhood encephalopathy: Viruses, immune response, and outcome. *Dev Med Child Neurol* 2006; *48:* 294-300.
7. *Details of AES/JE cases and deaths from 2008–2014*. Delhi: National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health & Family Welfare. Available from: *http://www.nvbdcp.gov.in/Doc/je-aes-cdfeb14.pdf* (Accessed on July 1, 2017).
8. Parida M, Dash PK, Tripathi NK, Ambuj, Sannarangaiah S, Saxena P, *et al*. Japanese encephalitis outbreak, India, 2005. *Emerg Infect Dis* 2006; 12(9): 1427-30.
9. Gore MM. Acute encephalitis syndrome in India: Complexity of the problem. *J Commun Dis* 2014; 46(1): 35-49.
10. Sen PK, Dhariwal AC, Jaiswal RK, Lal S, Raina VK, Rastogi A. Epidemiology of acute encephalitis syndrome in India: Changing paradigm and implication for control. *J Commun Dis* 2014; 46(1): 4-11.
11. *Japanese encephalitis surveillance standards. From WHO-recommended standards for surveillance of selected vaccine-preventable diseases (updated January 2006)*. Geneva, Switzerland: World Health Organization. Available from: *http://www. path.org/files/ WHO_surveillance_standards_ JE.pdf* (Accessed on May 5, 2017).
12. Kumari R, Joshi PL. A review of Japanese encephalitis in Uttar Pradesh, India. *WHO South East Asia J Public Health* 2012; 1(4): 374-95.
13. Francisco Javier Carod-Arta. Neurological manifestations of dengue viral infection. *Res Rep Trop Med* 2014; *5:* 95-104.
14. Kumar R, Tripathi S, Tambe JJ. Dengue encephalopathy in children in northern India: Clinical features and comparison with non-dengue. *J Neurol Sci* 2008; *269:* 41-8.
15. Sapkal GN, Bondre VP, Fulmali PV, Patil P, Gopalkrishna V, Dadhania V, *et al*. Enteroviruses in patients with acute encephalitis, Uttar Pradesh, India. *Emerg Infect Dis* 2009; 15(2): 295-8.
16. Koskiniemi M, Vaheri A. Effect of measles, mumps, rubella vaccination on pattern of encephalitis in children. Lancet 1989; 333: 31-4.
17. Roehrig JT, Nash D, Maldin B, Labowitz A, Martin DA, Lanciotti RS, *et al*. Persistence of virus-reactive serum immunoglobulin M antibody in confirmed West Nile virus encephalitis cases. *Emerg Infect Dis* 2003; 9(3): 376-9. doi: 10.3201/ eid0903.020531.
18. Swami R, Ratho RK, Mishra B, Singh MP. Usefulness of RT-PCR for the diagnosis of Japanese encephalitis in clinical samples. *Scand J Infect Dis* 2008; 40(10): 815-20.
19. Blacksell SD, Tanganuchitcharnchai A, Nawtaisong P, Kantipong P, Laongnualpanich A, Day NPJ, *et al*. Diagnostic accuracy of the InBios scrub typhus detect enzyme-linked immunoassay for the detection of IgM antibodies in northern Thailand. In: Rosenberg HF, editor. *Clin Vacc Immunol* 2016; 23(2): 148-54.
20. Rahi M, Gupte MD, Bhargava A, Varghese GM, Arora R. DHR-ICMR guidelines for diagnosis and management of rickettsial diseases in India. *Indian J Med Res* 2015; *141:* 417-22. [PUBMED]
21. DeBiasi RL, Tyler KL. Molecular methods for diagnosis of viral encephalitis. *Clin Microbiol Rev* 2004; *17(4):* 903-25.
22. *PCR for detection and characterization of bacterial meningitis pathogens: Neisseria meningitidis, Haemophilus influenzae*, and *Streptococcus pneumoniae*. Chapter 10. Available from: *https://www.cdc.gov/meningitis/lab-manual/chpt10-pcr.html*.
23. Bandopadhyay B, Bhattacharyya I, Adhikary S, Mondal S, Konar J, Dawar N, *et al*. Incidence of Japanese encephalitis among acute encephalitis syndrome cases in West Bengal, India. *Bio Med Res Int* 2013; *2013* (Article ID 896749). Avaiable from: *http://dx. doi. org/10.1155/2013/896749*.
24. Jain P, Jain A, Kumar A, Prakash S, Khan DN, Singh KP. Epidemiology and etiology of acute encephalitis syndrome in north India. *Jpn J Infect Dis* 2014; *67:* 197-203.
25. Borkotoki U, Borkotoki S, Barua P, Das A, Rajkhowa A. Japanese encephalitis (JE) among acute encephalitis syndrome (AES) cases— A hospital based study from Upper Assam, India. *Int J Health Sci Res* 2016; 6(5): 72-7
26. Singh KP, Mishra G, Jain P, Pandey N, Nagar R, Gupta S. Co-positivity of anti-dengue virus and anti-Japanese encephalitis virus IgM in endemic area: Co-infection or cross reactivity? *Asian Pac J Trop Med* 2014; 7(2): 124-9.
27. Arai S, Matsunaga Y, Takasaki T, Tanaka-Taya K, Taniguchi K, Okabe N, *et al*. Vaccine preventable diseases surveillance program of Japan, Japanese encephalitis: Surveillance and elimination effort in Japan from 1982 to 2004. *Jpn J Infect Dis* 2008; *61:* 333-8.
28. Bhatia R, Dash AP, Sunyoto T. Changing epidemiology of dengue in South-East Asia. *WHO South-East Asia J Public Health* 2013; *2:* 23-7.
29. Kar A, Dhanaraj M, Dedeepiya D, Harikrishna K. Acute encephalitis syndrome following scrub typhus infection. *Int J Crit Care Med* 2014; 18(7): 453-5.
30. Mittal M, Thangaraj J, Rose W, Verghese V, Kumar C, Mittal M, *et al*. Scrub typhus as a cause of acute encephalitis syndrome, Gorakhpur, Uttar Pradesh, India. *Emerg Infect Dis* 2017; 23(8): 1414-6.
31. Bozzola E, Tozzi AE, Bozzola M, Krzysztofiak A, Valentini D, Grandin A, *et al.* Neurological complications of varicella in childhood: Case series and a systematic review of the literature. Vaccine 2012;30:5785-90.
32. Gowin E, Wysocki J, Michalak M. Don't forget how severe varicella can be – Complications of varicella in children in a defined Polish population. Int J Infect Dis 2013;17:e485-9.