

Original Article

Epidemiology and Etiology of Acute Encephalitis Syndrome in North India

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SUMMARY: Acute encephalitis syndrome (AES) is a major public health problem in eastern Uttar Pradesh, claiming thousands of lives every year. Here we report the common viral etiologic agents of AES and its epidemiology in the vicinity of Lucknow in Uttar Pradesh, North India. Cerebrospinal fluid (CSF) samples collected from patients with AES, who were referred to a viral diagnostic laboratory from January 2011 to December 2012, were tested for IgM antibodies against Japanese encephalitis virus (JEV), dengue virus (DV), herpes simplex virus (HSV), measles virus, mumps virus, varicella zoster virus (VZV), and enterovirus using commercial enzyme immuno-assays. Of the 1,578 enrolled patients, JEV was the most commonly detected (16.2%), followed by DV (10.8%), HSV (9.3%), measles virus (8.9%), mumps virus (8.7%), VZV (4.4%), and enterovirus (0%). Co-positivity with more than 1 virus was observed in 12 patients. The demographic distribution of patients pertaining to age, sex, and geographic and seasonal variation is discussed. Maximum mortality was caused by JEV infection, while patients with HSV infection had maximum residual neuro-psychiatric disability. JEV and DV are the chief causative agents of AES in North India, although other viruses should also be considered in a differential diagnosis.

INTRODUCTION

The incidence of acute encephalitis syndrome (AES) has been reported worldwide. Viruses are the most common causative agents of AES, though bacteria, fungi, parasites, and toxins have also been implicated in its etiology. The incidence of AES varies from 0.9 per 100,000 adults in Nigeria, to 185 per 100,000 adults for a rural population in Nepal during an outbreak of Japanese encephalitis (JE) (1). In India, it has been estimated that a population of 375 million people residing in 171 endemic districts of 17 states are at a risk of acquiring AES (2). Approximately 70% of the disease burden is from the northern state of Uttar Pradesh (UP), which has become an epicenter for this killer disease. In the year 2012 alone, 3,494 patients suffering from AES were admitted to different government hospitals of Gorakhpur and Basti divisions, 588 of whom died (3). JE virus (JEV) has been the major and consistent causative agent of AES in UP, annually accounting for approximately 10%–15% of the patients (4). The growth of vector mosquito population is favored by the accumulation of water and extensive rice cultivation in the Terai region of eastern UP and other adjoining regions that run parallel to the lower ranges of the Himalayas. Besides JEV, other viruses that have contributed to the

high incidence of AES in India include the dengue virus (DV), enterovirus, herpes simplex virus (HSV), measles virus, and Chandipura virus (5); however, the etiology of AES remains unknown in 68%–75% of the patients (6). An accurate identification of the organism causing AES is essential for surveillance and patient management because some of these infections are preventable or treatable. Hence, the common viral etiologic agents of AES and their epidemiology were studied in the vicinity of Lucknow in UP, North India.

MATERIALS AND METHODS

Region of study: UP is the most populous state in India and the fifth largest state in the country by area; it is divided into 75 districts. Data obtained from samples collected from only 34 of these 75 districts is presented in this paper (Fig. 1).

Patient population and clinical information: The study was approved by the Institutional Ethics Committee. Consecutive patients referred to a viral diagnostic laboratory with a clinical diagnosis of AES were enrolled in the study over a 2-year period from January 2011 to December 2012. A case of AES was defined as a patient of any age with an acute onset (≤ 14 days) of fever at any time of the year, and at least 1 of the following: altered sensorium (confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures) (7). Patients with a history of head injury, poisoning, or hypertension were excluded. Written informed consent was obtained from the patients. In the event that the patient was unable to

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Fig. 1. Map of India with magnified map of Uttar Pradesh. Alphabets refer to the districts covered, number in parentheses refer to the percent cases with confirmed viral etiology from that district.

give consent, it was obtained from a close relative/family member/guardian. Detailed history that included the address and specific signs and symptoms, including seizures, headache, rashes, trauma, vomiting, arthralgia, and breathlessness, were obtained. Follow-up was done for all patients for 7 days till discharge.

Laboratory testing: Cerebrospinal fluid (CSF) samples were tested for anti-JEV IgM and anti-DV IgM antibodies using ELISA kits (Panbio Inverness Medical Innovations, Brisbane, Australia). The presence of IgM antibodies against HSV 1 and 2, measles virus, mumps virus, and VZV was tested using ELISA kits (NovaTec Immundiagnostica, Dietzenbach, Germany). The cut-off values and criteria for labeling a sample positive or negative are mentioned in Table 1. Real time PCR was performed for enteroviruses (Fast Track Diagnostics Research Ltd., London, UK). The probe and primer sequences were not disclosed by the manufacturing company. Reaction mixture was prepared as per the manufacturer's instructions, and included 12.5 μ l of AgPath buffer, 1.5 μ l of primer-probe mix, 0.5 U of Taq DNA polymerase, and 10 μ l of extracted DNA from the samples. The reaction mix was pre-incubated

at 45°C for 10 min followed by initial denaturation at 95°C for 10 min. This was followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 20 s, and extension at 72°C for 15 s.

Laboratory diagnostic criteria: As per national guidelines, a virus was considered to be the etiologic agent of AES if IgM antibodies specific to that particular virus/viral genome was detected in the CSF sample (8).

Statistical analysis: Data was analyzed using GraphPad Prism software, version 5. Intergroup comparison of categorical variables was done using Fischer's exact test.

RESULTS

A total of 1,578 patients were enrolled in the study over a 2-year period (January 2011–December 2012). Of these, a definite diagnosis of AES was established in 921 (58.4%) patients on the basis of clinical and virological investigations. The most common causative agent of AES was JEV (16.2%), followed by DV (10.8%), HSV (9.3%), measles virus (8.9%), mumps virus (8.7%), and VZV (4.4%); no enteroviruses were detected (Table 2).

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Table 1. Criteria for cut-off and interpretation of samples

Test done	Kit used	Manufacturer's criteria				
		Cut-off calculation	Sample calculation	Positive	Negative	Equivocal
Anti-Japanese encephalitis virus IgM	Panbio Inverness Medical Innovations, Brisbane, Australia	Average of two calibrators provided in the kit X calibration factor (mentioned on the kit)	(Sample OD/Cut-off) X 10 = no. of panbio units	> 11 panbio units	< 9 panbio units	9–11 panbio units
Anti-dengue virus IgM						
Anti-herpes simplex virus 1 and 2 IgM						
Anti-measles virus IgM	Novatec Immunodiagnostica Dietzenbach, Germany	Average of two calibrators provided in the kit	(Sample OD/Cut-off) X 10 = no. of novatec units	> 11 novatec units	< 9 novatec units	9–11 novatec units
Anti-mumps virus IgM						
Anti-varicella zoster virus IgM						

Table 2. Etiology and adverse outcome in patients with AES ($n = 1,578$)

Virus	No. of positive cases (%)	Adverse outcome	
		Mortality No. of deaths/no. of positive patients (%)	Residual neuro-psychiatric disability at discharge from hospital No. of patients with residual illness/no. of positive patients (%)
JEV	256 (16.2)	39/256 (15.2)	12/256 (4.7)
DV	170 (10.8)	20/170 (11.7)	0/170 (0)
HSV	148 (9.3)	10/148 (6.8)	39/148 (26.4)
Mumps virus	138 (8.7)	13/138 (9.4)	7/138 (5.1)
Measles virus	140 (8.9)	9/140 (6.4)	6/140 (4.3)
VZV	69 (4.4)	0/69 (0)	0/69 (0)
Cases with confirmed viral etiology	921 (58.4)	91/921 (9.9)	64/921 (6.9)
Cases with unknown etiology	657 (41.6)	39/657 (5.9)	21/657 (3.2)
<i>P</i> value		0.0052	0.001

Table 3. Age and sex-wise distribution of AES cases with established viral etiology

Virus	Age-wise distribution, Positive (%)						Sex-wise distribution, Positive (%)		
	6 m–1 y ($n = 75$)	> 1–5 y ($n = 430$)	> 5–15 y ($n = 540$)	> 15–50 y ($n = 435$)	> 50 y ($n = 98$)	<i>P</i> value	Males ($n = 1034$)	Females ($n = 544$)	<i>P</i> value
JEV ($n = 256$)	8 (10.7)	67 (15.6)	110 (20.4)	58 (13.3)	13 (13.3)	0.617	167 (16.2)	89 (15.8)	1.00
DV ($n = 170$)	9 (12)	59 (13.7)	56 (10.4)	44 (10.1)	2 (2.1)	0.005	117 (11.4)	53 (9.7)	0.348
HSV ($n = 148$)	0 (0)	45 (10.5)	58 (10.7)	41 (9.4)	4 (4.1)	0.946	119 (11.6)	29 (5.3)	<0.0001
Mumps virus ($n = 138$)	0 (0)	31 (7.2)	42 (7.8)	58 (13.3)	7 (7.1)	0.001	109 (10.6)	29 (5.3)	0.0003
Measles virus ($n = 140$)	9 (12)	59 (13.7)	34 (6.3)	33 (7.6)	5 (5.1)	0.0006	89 (8.7)	51 (9.4)	0.643
VZV ($n = 69$)	0 (0)	26 (6.1)	19 (3.5)	24 (5.5)	0 (0)	0.599	23 (2.2)	46 (8.4)	<0.0001
Total ($n = 1,578$)	26 (34.7)	287 (66.7)	319 (59.1)	258 (59.3)	31 (31.6)	0.012	624 (60.9)	297 (54.6)	<0.0001

IgM positivity for more than 1 virus was observed in 12 patients, 6 of whom were co-positive for JEV and DV, 3 for HSV and mumps virus, and 3 for measles and mumps viruses.

Mortality and residual neuro-psychiatric disability at the time of discharge from the hospital were significantly higher among patients with established viral etiology (9.9% and 6.9%, respectively) than in patients with unknown etiology (5.9% and 3.2%, respectively), with corresponding *P* values of 0.0052 and 0.001, respec-

tively (Table 2). Maximum mortality was caused by JEV, while HSV was the leading cause of residual neurological disability. Of the 39 HSV-positive patients with residual illness, 21 (53.8%) patients had intermittent seizures, while 18 (46.2%) patients displayed behavioral disturbances. Similarly, 3 of the 7 mumps virus-positive patients with residual illness, 3 were ataxic, 3 developed transverse myelitis, while 1 displayed behavioral disturbances.

The demographic distribution of AES patients is

Table 4. District-wise distribution of AES cases in Uttar Pradesh, India

No. (as shown in Fig. 1)	District (No. of cases referred)	Percent cases with confirmed viral etiology	Percent JEV positive cases	Percent DV positive cases	Percent HSV positive cases	Percent measles virus positive cases	Percent mumps virus positive cases
1	Lucknow (257)	46.2	10.4	8.8	6.8	3.9	9.9
2	Sitapur (140)	84.7	18.1	13.2	12.5	10.4	11.1
3	Barabanki (125)	67.2	23.5	16.8	16.8	3.4	6.7
4	Raebareilly (58)	63.5	21.2	15.4	11.5	5.8	9.6
5	Unnao (71)	55.4	12.3	16.9	3.1	3.1	9.2
6	Hardoi (132)	73	15.9	19	7.1	22.2	8.7
7	Lakeempur (57)	68.6	23.5	9.8	5.9	19.6	9.8
8	Behraich (78)	61.1	23.6	8.3	6.9	12.5	9.7
9	Gonda (81)	76.1	20	18.7	6.7	20	10.7
10	Faizabad (58)	42.3	15.4	7.7	3.8	5.8	9.6
11	Sultanpur (118)	75	17.9	16.9	17.9	12.5	9.8
12	Ambedkar Nagar (46)	45	10	17.5	7.5	0	10
13	Basti (61)	76.4	14.5	10.9	12.7	29.1	9.1
14	Siddharth Nagar (26)	55	15	0	20	10	10
15	Gorakhpur (27)	38.1	9.5	9.5	4.8	4.8	9.5

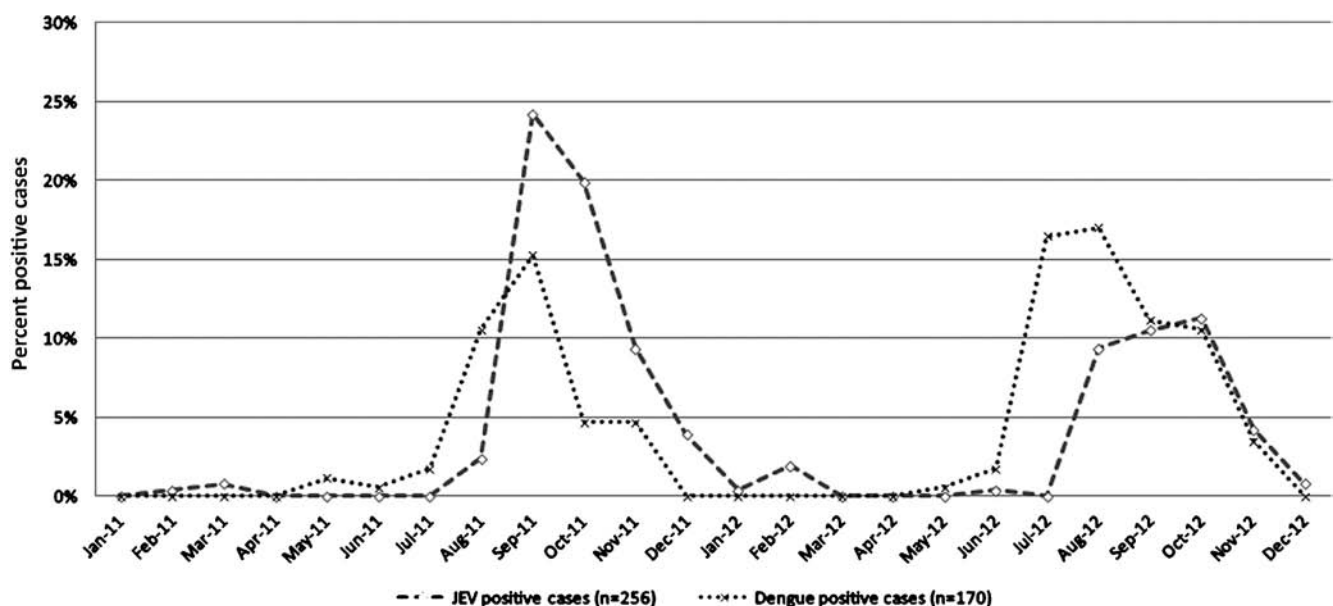


Fig. 2. Seasonal distribution of AES cases (January 2011–December 2012).

shown in Table 3. The mean age of the participants was 11 years (range 1–70 years; S.D. = 14.69), and 544 (34.5%) of the patients were females. Among pediatric patients ($n = 1045$, age < 15 years), the etiology of AES could be confirmed in 632 (60.48%) patients, while among adults ($n = 533$, age ≥ 15 years), the etiology could be confirmed in 289 (54.22%) patients. JEV most commonly affected children aged between 5 and 15 years, while DV infection most commonly affected children between 1 and 5 years. No child of < 1 year of age was affected by HSV, while all the other age groups were almost equally affected. Mumps was most commonly seen in young adults (15–50 years of age) but not in infants, while measles most commonly affected children between 1 and 5 years of age. Cases of VZV were not observed in patients of extreme age groups, i.e., < 1 year or > 50 years of age. Mumps virus and HSV were significantly more common among males, whereas VZV

was significantly more common among females. The sex distribution of all other etiologic groups was more or less even.

The district-wise distribution of AES patients is shown in Table 4 and Fig. 1. Districts from where less than 20 cases were referred are not shown.

A definite seasonality was observed for DV and JEV infections; the highest positivity was observed from July through November, which includes the monsoon and post-monsoon seasons in this part of the country. Such seasonal distribution was not evident for the other viruses (Fig. 2).

DISCUSSION

The present study describes the epidemiological characteristics of acute viral encephalitis prevalent in the vicinity of Lucknow in UP from January 2011 to De-

cember 2012. JEV, the most common causative agent of AES detected in this study, has also been reported as the chief cause of encephalitis in tropical countries, which occurs both sporadically and in outbreaks and has mortality rates ranging from 20% to 40% (9–11). A large proportion of AES patients were found to be positive for DV. Unusual manifestations of dengue including encephalopathy, have been reported earlier from North India, although the prevalence of DV as a causative agent of AES in this study (8.92%) is lesser than that in the previous study (23.4%) (12). The pathogenesis of dengue encephalopathy has been attributed to multisystem derangement, including liver failure, shock, and coagulopathy, that accompanies severe infections; however, direct viral encephalitis resulting from dengue viral neurotropism has also been suggested (13). HSV remains the causative agent of acute-onset sporadic viral encephalitis in the developed world (14); however, data pertaining to its prevalence in India is limited (15). Before the start of nationwide MMR vaccination programs in countries like Finland in 1980s, measles and mumps viruses were most commonly associated with encephalitis in children (16). These organisms have almost disappeared from developed countries (17); however, they continue to be important causative agents of AES in developing countries like India (18–20), probably because of suboptimal immunization rates. Enteroviruses were not detected in the current study, although EVs were detected in 21.6% and 41.11% patients in 2006 (21) and 2008 (22), respectively, during outbreaks of AES in UP; the serotypes involved in both the outbreaks were different, with similarity to EV-89 and EV-76 in 2006 and to coxsackievirus B5 and echovirus 19 in 2008. Co-positivity for anti-JEV and anti-DV IgM antibodies was detected in 6 cases in the current study. Both JEV and DV are endemic in UP, are mosquito borne viruses, with same seasonality, and have cross-reacting antigens. To differentiate co-infection from cross-reactivity, we previously investigated cases co-positive for both these viruses (23,24), and concluded that co-infection with more than 1 flaviviral species could occur in hyperendemic areas. Similarly, antigens of measles and mumps viruses are well known to have cross-reactivity (25), rendering it difficult to differentiate co-infections from cross-reactivity, on the basis of only IgM-positivity. Three patients were positive for both HSV and mumps virus; whether these were co-infections or sequential infections could be determined only by nucleic acid detection techniques, which could not be employed for these patients.

The age distribution of patients in the current study revealed that both children and young adults in India comprise the population chiefly affected by JEV, in accordance with previous findings. The attack rates are 5–10 times lower in adults (>15 years) than in children (3–15 years) (4). Similarly, the association of other flaviviral infections with age indicates that early childhood is the most susceptible age for these infections (26). Mumps was found to predominantly affect older teens and young adults, which include the unvaccinated population; this population has probably not developed immunity through exposure to the mumps virus because of decreased circulation of the virus after the implementation of childhood immunization program (27).

Globally, measles is still ranked fifth among killer diseases in children under 5 years of age. It is estimated that 1 in 1,000 measles patients develops encephalitis. Acute post-infectious measles encephalitis generally occurs during the exanthematous stage in children and adolescents (28). Age predisposition for HSV and VZV infections was not observed in the present study, in accordance with various other studies where similar infection rates were obtained for both children and adults (15,29). Male preponderance for JEV, DV, and measles was observed in earlier studies; in contrast, no sex predominance was evident in the current study, thereby ruling out increased susceptibility of males to these infections. On the other hand, HSV and mumps were significantly more common in males than in females. Previous studies have shown a similar trend of male predominance in HSV (15) and mumps (30) encephalitis.

Mass vaccination program against JEV was launched by the Government of India in 7 highly endemic districts of UP in 2006; a single dose of SA14-14-2 vaccine was given to children of up to 15 years age (31). The districts covered were Gorakhpur, Deoria, Kushinagar, Maharajganj, Kheri, Sant Kabir Nagar, and Siddharth Nagar. The vaccination program was expanded in 2007 to 11 more districts, including Ambedkar Nagar, Behraich, Balrampur, Barabanki, Basti, Gonda, Mau, Rae Bareilly, Saharanpur, Sitapur, and Shravasti. The program was further extended in 2008 and 2009, with the inclusion of an additional 9 (Azamgarh, Ballia, Bareilly, Faizabad, Hardoi, Lucknow, Muzaffarnagar, Sultanpur, and Unnao) and 7 (Allahabad, Pratapgarh, Kanpur Nagar, Shahjahanpur, Fatehpur, Jaunpur, and Ghazipur) districts, respectively (32). In the current study, children and young adults from vaccine-covered districts were also found to be positive for JEV; this could be attributable to low vaccination coverage in the corresponding districts, or because of changes in the genotype of the circulating strains. A higher percentage of JEV-positive patients was recorded from non-vaccinated districts like CSM Nagar, with the highest JEV load reported from Amethi district. The average vaccination coverage of infants in Lucknow district for measles and JEV was 58% and 50%, respectively, for the year 2011 (source: Ministry of health, UP); similar vaccination data could not be obtained for other districts. Despite low vaccination coverage in Lucknow the percentage of patients with AES attributable to both JEV and measles significantly declined over a period of 12 years (10.4% and 3.9%, respectively, in 2012) from the previous value of 23% and 13.7%, respectively (20).

A mortality rate of 15% was observed among JEV patients in the current study, similar to previous studies (20%–40%), with a majority of the deaths occurring within the first week of illness. Mental retardation and neurological deficits were observed among survivors of JEV infection, as has been previously reported (5). Mortality rate of 22% and 11.7% were found to be associated with dengue encephalopathy in a previous and the current studies, respectively, while complete recovery without any residual illness was observed in both the studies (33). Empiric acyclovir administration was initiated for all patients with AES at our center. Therefore, a lower rate of mortality due to HSV was observed

in this study, although neurological sequelae are common even with therapy, particularly in patients of >35 years of age (34,35). The patients were not monitored after discharge from the hospital, although permanent sequelae, including cerebellar ataxia and behavioral abnormalities, have also been reported in patients with mumps encephalitis (36).

The highest number of patients with JEV and DV encephalitis was observed during monsoon and post-monsoon seasons, which coincide with increased population density of vector mosquitoes. However, variation in temperature pattern has also been considered a possible explanation; rise in temperature during the summer season also corresponds to increased numbers of patients with encephalitis (37).

The results of PCR analysis have not been included in the manuscript because studies show that detection of viruses by isolation or RT-PCR is not sensitive enough for laboratory diagnosis using clinical specimens; this is because of low counts of circulating viruses, rapid clearance of transient viremia soon after the onset of illness, and rapid production of neutralizing antibodies (38,39). For instance, in 90% of the cases, anti-JEV or anti-DV IgM antibodies are detectable in CSF by 4 days and in serum, by 7–9 days following the development of clinical illness (40,41). Similar observations have been made in our laboratory, when only occasional samples which were serologically-positive were also positive by RT-PCR analysis (data not shown). In addition, RT-PCR requires experienced technicians and specialized laboratory equipment.

The current study is laboratory-based, with a focus on patients referred to a tertiary care health center. A limited number of etiologic agents were tested, which did not include non-viral causative agents of AES. The patients were not monitored after discharge from the hospital; hence, long-term sequelae, which are known among survivors of viral infection, have not been examined (5,35,36).

To conclude, the etiologic panorama of acute viral encephalitis varies with time and geographical location. JEV followed by DV still remain the chief causative agents of AES, although ongoing epidemiological surveillance is essential for establishing preventive strategies.

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Conflict of interest None to declare.

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