Original Article

Etiological Profile and Clinico Epidemiological Patterns of Acute Encephalitis Syndrome in Tamil Nadu, India

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Abstract

Introduction: Establishing the etiological cause of acute encephalitis syndrome (AES) is challenging due to the distinct distribution of various etiological agents. This study aims to determine the etiological profiles of both viruses and bacteria and their associated clinico-epidemiological features among the AES suspected cases in Tamil Nadu, India. **Methods:** Samples of 5136 suspected AES cases from January 2016 to December 2020 (5 years) were subjected to the detection of etiological agents for AES through serological and molecular diagnosis methods. Further, the clinical profile, age- and gender-wise susceptibility of cases, co-infection with other AES etiological agents, and seasonality pattern with respect to various etiological agents were examined. **Results:** AES positivity was established in 1480 cases (28.82%) among the 5136 suspected cases and the positivity for male and female groups were 57.77% and 42.23%, respectively. The pediatric group was found to be more susceptible than others. Among the etiological agents tested, the Japanese encephalitis virus (JEV) was the predominant followed by *Cytomegalovirus*, Herpes Simplex virus, Epstein–Barr virus, Varicella Zoster virus, and others. Co-infection with other AES etiological agents was observed in 3.5% of AES-positive cases. Seasonality was observed only for vector-borne diseases such as JEV, dengue virus, and West Nile virus infections in this study. **Conclusion:** AES was found to be a significant burden for Tamil Nadu with a diverse etiological spectrum including both sporadic and outbreak forms. Overlapping clinical manifestations of AES agents necessitate the development of region-specific diagnostic algorithm with distinct etiological profiles for early detection and effective case management.

Keywords: Acute encephalitis, diagnosis, endemic, etiology, India, leptospirosis, scrub typhus, viruses

Introduction

Acute encephalitis syndrome (AES) is characterized by an acute onset of fever and neurological manifestations such as disorientation, mental confusion, delirium, or coma. Viruses are the major causative organisms of AES, though other sources such as bacteria, fungus, parasites, and chemicals or toxins have been reported and definitive diagnosis remains elusive and challenging in most cases.[1] Occurrences of sporadic or outbreak forms are the most common epidemiological patterns of AES resulting in high mortality rates, especially among children aged below 12 years and the incidence rate was found to be about 1.4 per lakh pediatric population.[2]

The causative agents of outbreaks of encephalitis largely depend on the geographic distribution of the etiological agent in addition to the environmental, seasonal, virus, and host factors. AES is a major public health problem in India necessitating its surveillance and monitoring through a diagnosis of causative agents indicated in AES. Since 1955, sporadic cases and outbreaks of AES in India have been attributed mainly to the Japanese encephalitis virus (JEV), which is also the leading cause of viral encephalitis in Asia resulting about 50,000 cases of death each year, especially among the pediatric population. It is reported that about 7,500 cases of Japanese encephalitis (JE) occur annually in India during epidemic periods with a morbidity rate ranging

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between 0.3 and 1.5 in 1 lakh population.[3] Indian states such as Uttar Pradesh (UP), Bihar, Assam, West Bengal, and Tamil Nadu are identified as JE endemic zones. However, other viruses such as *Chandipura virus*, Nipah virus, *Enteroviruses*, dengue, chikungunya, West Nile virus (WNV), Varicella Zoster virus (VZV), Parvovirus B4 and Herpes Simplex virus (HSV) are also reported to be the causative agents of AES in India suggesting that AES cases have shifted toward the JE etiology post-2012, especially in North-Eastern, Northern, and Southern India.[1,4-7] Recent outbreak investigations and surveillance studies have increasingly reported non-JE and non-viral etiologies in AES, indicating a changing epidemiological pattern or the use of efficient diagnostic tests.[5] In India, most of the AES cases have been investigated during outbreak investigations where the focus is only on one virus. Even in hospital-based studies, cases of dual infection have either been underreported or not been investigated properly.

These findings suggest the necessity to adopt definitive diagnostic methods for treatment and management as well as to explore newer strategies for the prevention of AES beyond vector control and JEV vaccination. In the absence of vaccines against various AES etiological agents as well as considering the fact that AES in India has not been restricted to the JE etiology, diagnosis of infectious agents developing AES is necessary.

Clinical and neurological tests can usually diagnose encephalitis condition but do not establish the etiologic cause that often remains unknown. Confirmative diagnosis of etiological viral agents of AES using improved detection methods supports surveillance and effective management of illness due to AES as some of these infections are treatable or preventable. Though cases of AES have been reported from several states of India such as Rajasthan, Odisha, Uttar Pradesh, West Bengal, and Maharashtra, the etiological agents have been identified in only 20%–30% of cases.[8] The profile of agents causing AES varies widely in the country.[1,9] Reports on the incidence and seasonality of AES across diverse geographical regions of India are available. However, the incidence of AES with respect to various etiological agents and the associated burden in Tamil Nadu is not characterized as reports on AES etiology are scarce.

Hence, this study focuses on the diagnosis of AES causative agents in suspected samples collected during the years 2016–2020 with special reference to the context of Tamil Nadu state of India. In addition, this study provides the clinico-epidemiological features of AES due to common etiological agents for not only understanding the trend and status of AES in this tropical region but also for finding out the need of developing region-specific diagnostic algorithm for AES.

Methods

Study region and case criteria

This study was taken up to elicit the common causative agents of AES in a clinical setting in Tamil Nadu, India, from January 1, 2016, to –December 31, 2020. The cerebrospinal fluid (CSF) and serum samples for the routine viral diagnosis of AES cases from tertiary care Government Hospitals as well as Private Hospitals in various districts of Tamil Nadu were obtained and processed at the Department of Health Research (DHR)/Indian Council of Medical Research Grade 1 Viral Research and Diagnostic Laboratory (VRDL), King Institute of Preventive Medicine and Research (KIPMR), Chennai, which is also the State Apex Lab for Viral Diagnosis (DHR, Government of India). The study was approved by Institutional Ethical Committee. Inclusion criteria include samples that were collected from patients with AES as defined by the World Health Organization.[10] 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 status and/or new onset of seizures (sudden violent attack of an illness). Patients suffering from other febrile illnesses or simple febrile seizures were excluded from the study.

Blood and CSF samples were collected from suspected cases of AES with prior informed consent from the patients/parents/guardians. Proforma was filled in detail with patient information and signs and symptoms including fever, seizures, change in mental status, rashes, trauma, breathlessness, etc. Samples were labeled and transported to laboratory in the cold chain, serum separated and stored at –20°C till tested for enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) tests. Samples were processed in accordance with established standard operating procedures in the laboratory.

Serological studies

Antibody detection from the suspected clinical samples is the most effective method of diagnosing AES. Nearly all AES-infected individuals are seropositive for immunoglobulin (Ig)M antibody within 14 days of the onset of symptoms. The National Institute of Virology, Pune, IgM antibody Capture ELISA is used for the diagnosis of JEV, Dengue, and Chikungunya infection. IgM ELISA is used for the detection of WNV and Scrub typhus (InBios International, USA). The HSV IgM antibodies in serum are detected using DIALAB kit. VZV, Epstein-Barr virus (EBV), *Cytomegalovirus* (CMV), Parvo B19, and Mumps were detected using IgM ELISA (NovaTec Immundiagnostica GmbH). Rubella was detected by the Anti-Rubella virus Glycoprotein IgM ELISA (Euroimmun). The IgM ELISA (PanBio, Australia) is used for the detection of Leptospira. ELISA experiments were performed following the Manufacturer's Instructions.

Polymerase chain reaction experiments

PCR is a sensitive technique for identifying the viral genome of HSV, CMV, and *Enterovirus* in CSF. The CSF samples collected within 5 days from the date onset of illness were subjected to Conventional PCR and reverse transcription (RT)-PCR. Briefly, viral RNA and viral DNA from AES suspected samples were extracted using QIAmp Viral RNA mini kit and QIAmp DNA mini kit (QIAGEN, Germany) according to the Manufacturer's Instructions. PCR (Pan HSV and CMV and RT-PCR (Pan *enterovirus*) experiments were performed as per the methods reported in the previous studies[11-13] [Table 1]. Each assay was run using a positive control and a negative

Table 1: Primers used for the detection of viruses causing acute encephalitis syndrome by polymerase chain reaction and reverse transcription-polymerase chain reaction

Virus	Gene target	Primer	Amplicon size	Sequence 5' - 3'
Pan HSV (nested PCR)	Glycoprotein D	Forward	382 bp (round 1)	ATCCGAACGCAGCCCCGCTG
		Reverse		TCCGG (G/C) GGCAGCAGGGTGCT
		Forward	289 bp (round 2)	GCGCCGTCAGCGAGGATAAC
		Reverse		AGCTGTATA (G/C) GGCGACGGTG
CMV	Hind III-X fragment	Forward	406 bp	GGATCCGCATGGCATTCACGTATGT
		Reverse		GAATTCAGTGGATAACCTGCGGCGA
Pan *Enterovirus*	5 NCR	Forward	440 bp	CAAGCACTTCTGTTTCCCCGG
		Reverse		ATTGTCACCATAAGCAGCCA

HSV: Herpes simplex virus, PCR: Polymerase chain reaction, CMV: *Cytomegalovirus*, NCR: Noncoding region

control. Nucleic acid was either processed immediately for PCR amplification or stored at −80°C for further use.

Statistical analyses

The retrospective statistical analysis was carried out using GraphPad Prism version 5.0, GraphPad Software, San Diego, CA. For studies on gender-wise distribution, intergroup comparison of variables was performed using Fisher's exact test through Chi-square analysis. For age group distribution studies, regression analysis was performed for various etiological agents in AES. $p < 0.05$ was considered statistically significant.

Results and Discussion

Patient characteristics

The present study describes the etiological and clinico-epidemiological characteristics of AES in Tamil Nadu from January 2016 to December 2020. Samples from a total of 5136 suspected AES cases during the study were screened for the detection of etiological agents for AES through serological and molecular diagnosis. Among the screened cases, male and female groups respectively had 2911 (56.68%) and 2225 (43.32%) cases. AES positivity was established in 1480 cases (28.82%) and the positivity for AES etiological agents in male (57.77%; $n = 855$) and female (42.23%; $n = 625$) groups was not statistically significant ($p = 0.009$). Further, Fisher's exact test on gender group with respect to total AES positivity showed that the groups exhibit significance ($p < 0.05$) though the groups showed insignificant p value for all other etiological agents except JEV indicating JEV was the predominant etiological agent among others in this study [Table 2]. It was observed that AES positivity was higher in males than females and the male group had more cases than females with respect to all the etiological agents studied except chikungunya virus (CHIKV), which was diagnosed equally among the gender groups. The study observations on 28.82% AES positivity among AES suspected cases could be correlated or compared with reports of other studies conducted in India. These studies have reported AES positivity in the range of 17.20%–29.81% in different parts of India, at Odisha (17.2%),[14] Uttar Pradesh (21.83%),[15] Rajasthan (22.73%),[9] and West Bengal (29.81%);[9] however, in contrast, other studies from India reported higher positivity ranging between 58.4% and 71.9% in some states, at Uttar Pradesh (58.4%),[16] at Karnataka (59.9%)[17] and at New Delhi (72%).[18] Our findings are also in agreement with earlier studies across the world, especially in developed countries as AES with unidentified etiology were observed among AES-suspected patients from the United States (59.5%), United Kingdom (60%), and Australia (69.6%) suggesting that this trend seems to occur even in the presence of extensive laboratory expertise.[19] It has to be noted that the number of AES cases varies from year to year. This variation in the sample size shown here directly corresponds to the number of samples that were sent to our laboratory for analysis. We have taken care to include all the samples in this study. The variation also depends on the number of patients that were diagnosed in that particular year and their samples sent to our laboratory for further analysis.

Age-wise distribution for different etiological agents in positive cases in Table 2 reveals that the age group of 0–12 years has more cases than the other groups indicating that the pediatric group is more susceptible than others. Among pediatric suspects ($n = 2757$, age <12 years), the etiology of AES could be confirmed in 633 patients (22.96%), while among adults ($n = 1352$, age ≥12 years) the etiology could be confirmed in 322 patients (35.60%). The statistical analysis of overall AES positivity and individual etiological agents in the AES-positive cases with respective to AES suspects indicates statistical significance ($p < 0.05$) except the agents such as dengue virus (DENV), WNV, CHIKV, and *Enterovirus* suggesting that these viruses are not very significant causative agents of AES in this study. This observation is further confirmed by the data on the diagnosis of etiological agents in AES suspects and the study shows that JEV (11.84%) is the most predominant causative agent among AES suspects, which is followed by CMV (4.34%), HSV (3.66%), EBV (2.43%), VZV (1.79%), Leptospirosis (1.21%), Scrub typhus (1.01%), DENV (0.64%), Mumps (0.62%), CHIKV (0.58%), *Enterovirus* (0.27%) and WNV (0.21%) [Tables 2 and 3]. Several of the etiological agents were found predominantly in 0–12 years of group when compared to other age groups except CHIKV, WNV, and *Enterovirus*, which were detected more in adults. Besides, AES cases having co-infection with other etiological agents of AES (3.85%) were also observed in this study. Among the co-infection, notable cases are JEV with either HSV ($n = 7$)

or CMV (n = 5), EBV with VZV (n = 9), and HSV with CMV (n = 7). It is also important to note that co-infection with more than two etiological agents (0.61% of total AES positives) were also observed. One sample was found to be positive for three different arboviruses such as JEV, CHIKV, and DENV. Among the cases of co-infection, the predominant agent was JEV which is followed by EBV and HSV in the study. A study reported JEV positivity (16.2%) among AES suspects and JEV was the predominant causative agent followed by DENV and HSV.[16] Co-infection of more than one AES etiological agent was reported by few studies. A report observed the co-infection of arboviruses such as JEV with WNV in an AES patient.[20] In another study, co-positivity of AES agents such as JEV and DENV, HSV and Mumps, and Measles and Mumps was found in 1.3% of AES-confirmed cases.[16]

Viral and bacterial etiological profiles of acute encephalitis syndrome and their clinical spectrum

Among the causative organisms identified, JEV was predominant in all the study years except 2017, which had more EBV positives, and the total number of JEV-positive cases in the study period was 608 (41.08%) among the total AES positives [Figure 1]. The highest number of JEV (n = 270) was observed in the year 2019 and this was two- to three-fold higher than the cases of previous years suggesting the increased incidence of JEV in recent years. JEV has been reported as the main cause of encephalitis in tropical countries including India wherein it occurs both sporadically and in outbreaks with high mortality. An earlier study from Tamil Nadu reported that JEV etiology was confirmed in 27.3% of the hospitalized encephalitic children.[21] In another study conducted in Assam, JE etiology was confirmed in 30% of total AES suspects.[22] However, there are studies from India that report the predominant causative agent was not JEV but HSV,[9,14] EBV,[17] *Enterovirus*-71,[15,23] scrub typhus,[24] and co-infections reflecting the changing landscape of AES in India. Although HSV is the chief causative agent of acute-onset sporadic encephalitis in developed countries, data on its significance on AES is largely unknown.

Apart from JEV, herpesviruses such as EBV, VZV, and CMV were identified as other important AES causative agents in all the study years whereas HSV was the significant causative agent in the year 2020 followed by JEV. Scrub typhus and Leptospirosis were observed in AES suspects during the years 2016–2018 but absent in the years 2019 and 2020 and this might be due to a reduction in referral of cases for these agents or low incidence of AES cases due to these pathogens. The positivity for these agents was low (1.01%–1.21%) among the AES positives in this study. These nonviral agents present a range of AES symptoms including neurological manifestations such as seizures and change in mental status. It was reported that about 20% of AES cases were due to scrub typhus (*Orientia tsutsugamushi*

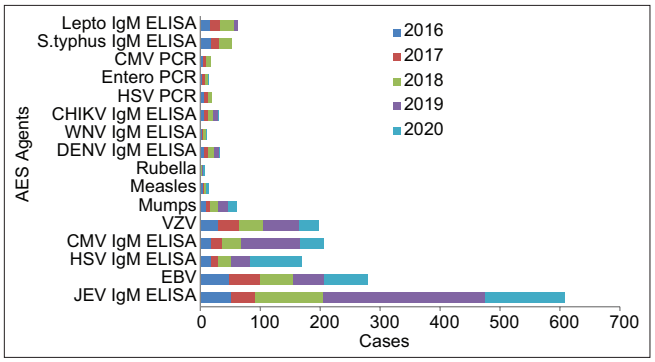

Figure 1: Year-wise distribution of AES cases. AES: Acute encephalitis syndrome

Table 2: Age- and sex-wise distribution of acute encephalitis syndrome cases with established etiology

Agent	Age-wise distribution, positive (%)						Gender-wise distribution, positive (%)		
	0-12 years (n=2757)	13-18 years (n=353)	19-30 years (n=557)	31-55 years (n=998)	>56 years (n=471)	p	Males (n=2768)	Females (n=2368)	p
JE (n=608)	236	66	94	128	84	0.0001	364	244	0.005
EBV (n=125)	58	17	19	22	9	0.01	74	51	0.27
HSV (n=188)	96	17	18	42	15	0.59	98	90	0.68
CMV (n=223)	109	24	40	39	11	0.0005	123	100	0.76
VZV (n=92)	22	13	22	24	11	<0.0001	52	40	0.69
Mumps (n=32)	25	1	0	4	2	0.068	23	9	0.06
Measles (n=5)	2	1	0	0	2	0.08	4	1	0.47
Rubella (n=5)	4	0	0	0	1	0.53	3	2	0.86
Dengue (n=33)	15	5	8	4	1	0.02	21	12	0.34
WNV (n=11)	4	1	2	3	1	0.81	7	4	0.73
Chikungunya (n=30)	4	3	5	8	10	<0.0001	15	15	0.80
EV (n=14)	5	3	3	3	0	0.09	8	6	0.98
Scrub typhus (n=52)	21	7	5	11	8	0.12	29	23	0.89
Leptospirosis (n=62)	32	4	6	9	11	0.21	34	28	0.98
Total	633	162	222	297	166	<0.0001	855	625	0.009

EBV: Epstein-Barr virus, HSV: Herpes simplex virus, CMV: Cytomegalovirus, VZV: Varicella-zoster virus, JE: Japanese encephalitis, WNV: West Nile virus, EV: Enterovirus

Table 3: Clinical profile of patients diagnosed with varied acute encephalitis syndrome etiology (percentage positivity) and unknown etiology among acute encephalitis syndrome suspected samples

Symptoms	JE (*n*=608; 11.84%), *n* (%)	EBV (*n*=125; 2.43%), *n* (%)	HSV (*n*=188; 3.66%), *n* (%)	CMV (*n*=223; 4.34%), *n* (%)	VZV (*n*=92; 1.79%), *n* (%)	Mumps (*n*=32; 0.62%), *n* (%)	Measles (*n*=5; 0.1%), *n* (%)	Rubella (*n*=5; 0.1%), *n* (%)	Dengue (*n*=33; 0.64%), *n* (%)
Fever	473 (77.80)	90 (72)	162 (86.17)	144 (64.57)	63 (68.48)	15 (46.88)	3 (60)	3 (60)	31 (93.94)
Seizure	271 (44.57)	66 (52.8)	99 (52.66)	109 (48.88)	40 (43.48)	15 (46.88)	0	2 (40)	12 (36.36)
Headache	122 (20.06)	3 (2.4)	13 (6.91)	1 (0.45)	2 (2.17)	0	0	0	4 (12.12)
Myalgia	56 (9.21)	2 (1.6)	7 (3.72)	3 (1.35)	0	0	0	0	3 (9.09)
Arthralgia	40 (6.27)	0	0	0	0	0	0	0	2 (6.06)
Altered sensorium	265 (43.59)	45 (36)	73 (38.83)	59 (26.46)	23 (25)	18 (56.25)	3 (60)	4 (80)	9 (27.27)
Neck rigidity	156 (25.66)	27 (21.6)	94 (50)	36 (16.14)	10 (10.87)	11 (34.38)	1 (20)	2 (40)	9 (27.27)
Irritability	208 (34.21)	42 (33.6)	62 (32.98)	72 (32.29)	18 (19.57)	16 (50)	0	3 (60)	11 (33.33)
Change in mental status	240 (39.47)	36 (28.8)	60 (31.91)	50 (22.42)	27 (29.35)	18 (56.25)	2 (40)	5 (100)	9 (27.27)
Somnolence	75 (12.34)	12 (9.6)	18 (9.57)	22 (9.87)	7 (7.61)	12 (37.5)	1 (20)	1 (20)	1 (3.03)
Vomiting	10 (1.64)	4 (3.2)	0	7 (3.14)	1 (1.09)	0	0	0	3 (9.09)
Diarrhoea	5 (0.82)	2 (1.6)	7 (3.72)	0	0	0 (0)	0	0	0

Symptoms	Dengue (*n*=33; 0.64%), *n* (%)	WNV (*n*=11; 0.21%), *n* (%)	CHIKV (*n*=30; 0.58%), *n* (%)	EV (*n*=14; 0.27%), *n* (%)	Scrub typhus (*n*=52; 1.01%), *n* (%)	Leptospirosis (*n*=62; 1.21%), *n* (%)	Unknown etiology (*n*=3656; 71.18%) *n* (%)	*p*
Fever	31 (93.94)	6 (54.55)	29 (96.67)	8 (57.14)	51 (98.07)	56 (90.32)	2572 (70.35)	0.52
Seizure	12 (36.36)	5 (45.45)	13 (43.33)	5 (35.71)	22 (42.31)	16 (25.81)	1489 (40.73)	0.68
Headache	4 (12.12)	0	0	2 (14.29)	3 (5.77)	30 (48.39)	119 (3.25)	<0.0001
Myalgia	3 (9.09)	0	5 (16.67)	1 (7.14)	0	18 (29.03)	107 (2.92)	<0.0001
Arthralgia	2 (6.06)	0	9 (30)	0	0	7 (11.29)	24 (0.66)	<0.0001
Altered sensorium	9 (27.27)	0	9 (30)	2 (14.29)	16 (30.77)	1 (1.61)	1438 (39.33)	<0.0001
Neck rigidity	9 (27.27)	0	5 (16.67)	1 (7.14)	16 (30.77)	0	838 (22.92)	<0.0001
Irritability	11 (33.33)	4 (36.36)	6 (20)	1 (7.14)	19 (36.54)	0	590 (16.14)	0.0017
Change in mental status	9 (27.27)	3 (27.27)	4 (13.33)	2 (14.29)	21 (40.38)	6 (9.68)	798 (21.83)	0.0011
Somnolence	1 (3.03)	0	2 (6.67)	0	3 (5.77)	0	189 (5.17)	0.0023
Vomiting	3 (9.09)	1 (9.09)	1 (3.33)	1 (7.14)	0	17 (27.42)	156 (4.27)	<0.0001
Diarrhoea	0	0	1 (3.33)	1 (7.14)	0	11 (17.74)	82 (2.24)	<0.0001

JE: Japanese encephalitis, EBV: Epstein-Barr virus, HSV: Herpes simplex virus, CMV: Cytomegalovirus, VZV: Varicella zoster virus, WNV: West Nile virus, CHIKV: Chikungunya virus, EV: Enterovirus

infection) in Assam, India.[25] Another study reported that 62.7% of AES patients from Gorakhpur of Uttar Pradesh had *O. tsutsugamushi* IgM, with a case-fatality rate of 16.2%.[26] Neurological manifestations were indicated in substantial scrub typhus patients from Tamil Nadu.[27] These findings suggest that Scrub typhus can be considered as part of the surveillance algorithm for AES cases in India. It is to be noted that the AES positivity varies across different states of India and it depends on various factors such as the geographical location, type of etiological agents included in the diagnosis panel; study samples, diagnostic methods included in the study; regions characterized for etiological endemicity, significance of vector distribution and their increased density, especially for agents with definite seasonality, occurrence of any epidemic during the study, etc.

The most common clinical presentation was fever followed by seizure, altered sensorium, irritability, and change in mental status through other symptoms such as headache, vomiting, neck rigidity, and diarrhea were also present in several cases [Table 3]. Observations on clinical presentations of AES cases will also support syndromic case management in situations lacking a laboratory investigation facility. However, several of the etiological agents produce similar symptoms in AES suspects, definitive diagnosis is essential for case management [Figure 2].

Seasonality of acute encephalitis syndrome

A definite seasonality was observed for JEV, DENV, and WNV infections in this study; the high positivity was observed from October to December including North East Monsoon and Winter seasons in this tropical region of India coinciding with increased population density of vector mosquitoes [Figure 3]. Mean rainfall and temperature profiles were presented for this tropical study region [Figure 4]. Such seasonal distribution was not clearly observed for other etiological agents. In many states, AES outbreaks occur during the rainy season and are associated with high mortality rates. Seasonality was reported for JEV and dengue infections and high positivity was observed from July to November in the Northern states, which includes monsoon and post-monsoon seasons.[16] However, such seasonal distribution was not evident for other viral agents of AES.[1] With the onset

of winter, JE incidence declined substantially.[28] Reports also showed that there was a seasonal variation, AES becomes epidemic between March and July and peak incidence was seen in June.[29] The study also reported that AES becomes epidemic in Lychee season, April–June. In the present study, AES-suspected samples were received from several districts of Tamil Nadu, and AES positivity was observed in samples from the districts such as Chennai, Thiruvallur, Kanchipuram, Tiruvannamalai, Vellore, Krishnagiri, Dharmapuri, Villupuram, Cuddalore, Thanjavur, Thiruvarur, Tiruchirapalli, Pudukkottai, Perambalur, Nagapattinam, Erode, Salem, Tiruppur, Coimbatore, Theni, Tirunelveli, Madurai, Tuticorin, and Pondicherry (Union Territory) suggesting the magnitude of burden due to AES as well as distribution of various AES causative agents in this state. AES can occur as both vector borne or sporadic, however, the vector-born accounts for the maximum cases in tropical and subtropical countries[30] such as India/Tamil Nadu. This is particularly challenging because control of vector population, such as mosquitoes has a direct impact on the prevalence of AES and the number of cases that are detected each year.

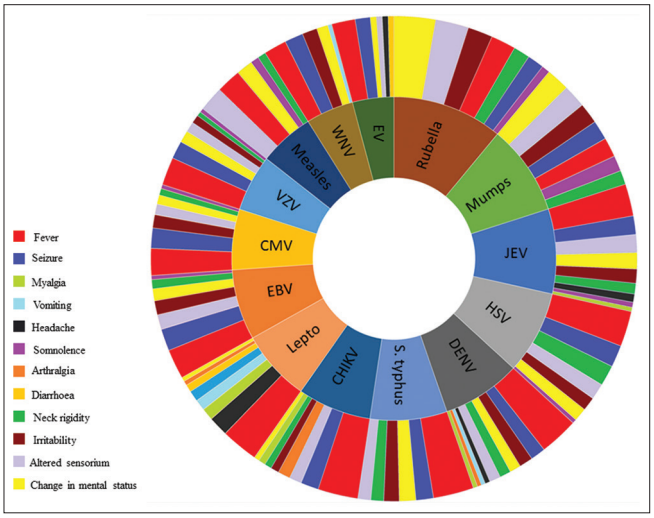

Figure 2: Symptoms of AES cases. AES: Acute encephalitis syndrome

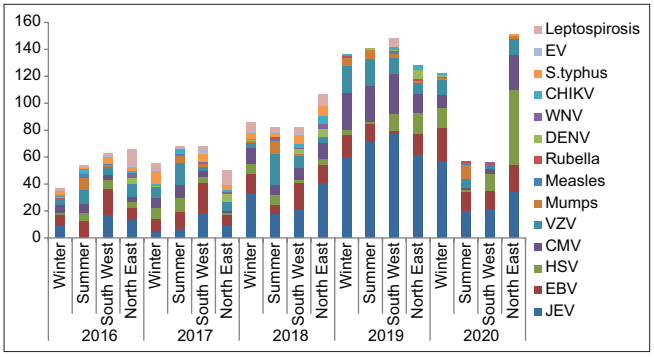

Figure 3: Seasonal distribution of AES cases (January 2016–December 2020). AES: Acute encephalitis syndrome

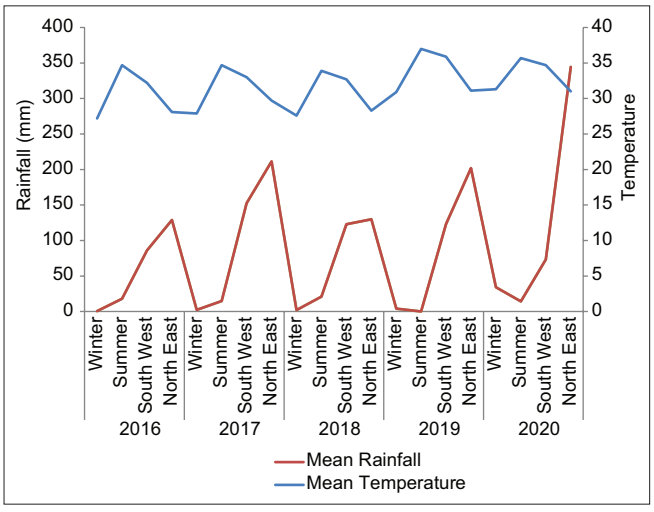

Figure 4: Rainfall and Temperature profiles of the study period

Encephalitis is of public health importance worldwide because it has high morbidity and mortality. The National Vector-borne Disease Control Program reported more than 50,000 cases of AES in India during 2013–2017 with mortality rate of 12.23% and states such as Uttar Pradesh, Assam, West Bengal, Odisha, Tamil Nadu, Karnataka, and Manipur accounted for most cases in these recent years.[31] Tamil Nadu has accounted for 6.36% among these reported cases and the state has shown a substantial increase in the number of cases reported during the years. In the year 2017 alone, the state has witnessed 10% of total AES cases reported in the country and among the total AES cases, JEV etiology is established in 8.87% suggesting the importance of the detection of non-JE etiology in AES. Although AES cases are frequently being reported in India, examining the actual burden as well as establishing the etiology is associated with diagnostic challenges even in an established resource set up beside the fact that a wide range of Central nervous system disorders, both infectious and noninfectious, may present the illness alike. The symptoms and clinical presentations manifested by a range of pathological agents in AES often look similar, which is a major diagnostic challenge in establishing the etiology. There is a paucity of data on the regional epidemiology and etiology of AES in India. Though epidemics have a singular etiology, sporadic cases could be due to multiple etiologies requiring the diagnosis of the spectrum of agents for effective surveillance. Currently, there is no single method available for facilitating the simultaneous detection of all pathogens causing AES.

The study has a limitation in terms of not accounting for the mortality among the AES suspects as well as long-term sequelae of patients. It is also limited by the fact that other possible etiologies and co-infections have not been addressed in this study. Notwithstanding these limitations, the study has focused in detail on establishing both the important viral and nonviral etiologies of AES with a high number of AES suspect samples. Besides, the study has given the etiologic and epidemiological spectrum of AES relevant to the region which would be helpful for the policymakers to take specific action not only for prevention and control of AES but also for definitive management of the patients that may improve the outcome both in terms of morbidity and mortality.

Conclusion

The diverse etiological and clinical spectrum of AES including both sporadic and outbreak forms underscores the prevailing burden in Tamil Nadu, a state in South India. Overlapping clinical manifestations of AES agents and varying AES epidemiological profiles across India substantiate the need of developing region-specific surveillance algorithm for AES based on the distribution of etiological agents and prioritization of diagnostic tests to advance the confirmation of etiology in more AES suspects and reporting of AES more effectively than before.

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Research quality and ethics statement

This study was conducted after obtaining ethical permission from the King Institute for Preventive Medicine and Research Institutional Review Board and the approval number is IRB# KIPM/IEC/017. The authors followed applicable EQUATOR Network (https://www.equator-network.org/) guidelines during the conduct of this research project.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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;25:212-20.
2. Kakkar M, Rogawski ET, Abbas SS, Chaturvedi S, Dhole TN, Hossain SS, *et al.* Acute encephalitis syndrome surveillance, Kushinagar district, Uttar Pradesh, India, 2011-2012. Emerg Infect Dis 2013;19:1361-7.
3. Ravi V, Mani RV, Govekar SR, Desai A, Lakshman L, Ravikumar B. Aetiology and laboratory diagnosis of acute encephalitis syndrome with special reference to India. J Commun Dis 2014;46:12-23.
4. Reuben R, Gajanana A. Japanese encephalitis in India. Indian J Pediatr 1997;64:243-51.
5. Kumar R. Viral encephalitis of public health significance in India: Current status. Indian J Pediatr 1999;66:73-83.
6. Benjamin LA, Lewthwaite P, Vasanthapuram R, Zhao G, Sharp C, Simmonds P, *et al.* Human parvovirus 4 as potential cause of encephalitis in children, India. Emerg Infect Dis 2011;17:1484-7.
7. Ghosh S, Basu A. Acute encephalitis syndrome in India: The changing scenario. Ann Neurosci 2016;23:131-3.
8. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, Roos KL, *et al.* The management of encephalitis: Clinical practice guidelines by the infectious diseases society of America. Clin Infect Dis 2008;47:303-27.
9. Tiwari JK, Malhotra B, Chauhan A, Malhotra H, Sharma P, Deeba F, *et al.* Aetiological study of viruses causing acute encephalitis syndrome in North West India. Indian J Med Microbiol 2017;35:529-34.
10. WHO. WHO Recommended Standards for Surveillance of Selected Vaccine Preventable Diseases. Geneva: WHO; 2003.
11. McIver CJ, Jacques CF, Chow SS, Munro SC, Scott GM, Roberts JA, *et al.* Development of multiplex PCRs for detection of common viral pathogens and agents of congenital infections. J Clin Microbiol 2005;43:5102-10.
12. Cotte L, Drouet E, Bissuel F, Denoyel GA, Trepo C. Diagnostic value of amplification of human cytomegalovirus DNA from gastrointestinal biopsies from human immunodeficiency virus-infected patients. J Clin Microbiol 1993;31:2066-9.
13. Zoll GJ, Melchers WJ, Kopecka H, Jambroes G, van der Poel HJ, Galama JM. General primer-mediated polymerase chain reaction for detection of enteroviruses: Application for diagnostic routine and persistent infections. J Clin Microbiol 1992;30:160-5.
14. Rathore SK, Dwibedi B, Kar SK, Dixit S, Sabat J, Panda M. Viral aetiology and clinico-epidemiological features of acute encephalitis syndrome in eastern India. Epidemiol Infect 2014;142:2514-21.
15. Beig FK, Malik A, Rizvi M, Acharya D, Khare S. Etiology and clinico-epidemiological profile of acute viral encephalitis in children of western Uttar Pradesh, India. Int J Infect Dis 2010;14:e141-6.
16. Jain P, Jain A, Kumar A, Prakash S, Khan DN, Singh KP, *et al.* Epidemiology and etiology of acute encephalitis syndrome in North India. Jpn J Infect Dis 2014;67:197-203.
17. Ramamurthy M, Alexander M, Aaron S, Kannangai R, Ravi V, Sridharan G, *et al.* Comparison of a conventional polymerase chain reaction with real-time polymerase chain reaction for the detection of neurotropic viruses in cerebrospinal fluid samples. Indian J Med Microbiol 2011;29:102-9.
18. Karmarkar SA, Aneja S, Khare S, Saini A, Seth A, Chauhan BK. A study of acute febrile encephalopathy with special reference to viral etiology. Indian J Pediatr 2008;75:801-5.
19. Huppatz C, Durrheim DN, Levi C, Dalton C, Williams D, Clements MS, *et al.* Etiology of encephalitis in Australia, 1990-2007. Emerg Infect Dis 2009;15:1359-65.
20. Khan SA, Dutta P, Chowdhury P, Borah J, Topno R, Mahanta J. Coinfection of arboviruses presenting as acute encephalitis syndrome. J Clin Virol 2011;51:5-7.
21. Kabilan L, Ramesh S, Srinivasan S, Thenmozhi V, Muthukumaravel S, Rajendran R. Hospital- and laboratory-based investigations of hospitalized children with central nervous system-related symptoms to assess Japanese encephalitis virus etiology in Cuddalore District, Tamil Nadu, India. J Clin Microbiol 2004;42:2813-5.
22. Kakoti G, Dutta P, Ram Das B, Borah J, Mahanta J. Clinical profile and outcome of Japanese encephalitis in children admitted with acute encephalitis syndrome. Biomed Res Int 2013;2013:152656.
23. Joshi R, Mishra PK, Joshi D, Santhosh SR, Parida MM, Desikan P, *et al.* Clinical presentation, etiology, and survival in adult acute encephalitis syndrome in rural Central India. Clin Neurol Neurosurg 2013;115:1753-61.
24. Jain P, Prakash S, Tripathi PK, Chauhan A, Gupta S, Sharma U, *et al.* Emergence of *Orientia tsutsugamushi* as an important cause of acute encephalitis syndrome in India. PLoS Negl Trop Dis 2018;12:e0006346.
25. Khan SA, Bora T, Laskar B, Khan AM, Dutta P. Scrub typhus leading to acute encephalitis syndrome, Assam, India. Emerg Infect Dis 2017;23:148-50.
26. Murhekar MV, Mittal M, Prakash JA, Pillai VM, Mittal M, Girish Kumar CP, *et al.* Acute encephalitis syndrome in Gorakhpur, Uttar Pradesh, India – Role of scrub typhus. J Infect 2016;73:623-6.
27. Murhekar MV. Acute encephalitis syndrome and scrub typhus in India. Emerg Infect Dis 2017;23:1434.
28. Kumari R, Joshi PL. A review of Japanese encephalitis in Uttar Pradesh, India. WHO South East Asia J Public Health 2012;1:374-95.
29. Sudhir SK, Prasad MS. Acute encephalitis syndrome (AES) associated with sociocultural and environmental risk factors in infants/children of Muzaffarpur, Bihar hospital based, prospective study. J Evid Based Med Health 2018;5:23-6.
30. Chen YH, Huang KY, Liu CC, Weng YM. Who is at risk? A critical case of Japanese encephalitis. J Acute Med 2022;12:122-5.
31. National Vector Borne Disease Control Program. Ministry of Health and Family Welfare, Government of India. State wise Number of AES/JE Cases and Deaths from 2010 to 2017