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A retrospective study of acute encephalitis syndrome with special reference to Japanese encephalitis in tertiary care centre, Tezpur, Assam

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Abstract
The present study was conducted to find out the clinico-epidemiological profile of the AES (Non JE and JE) during its outbreaks from the year 2014 to 2016 in tertiary care centre, Tezpur (Assam). It was a retrospective study. The AES cases were initially tested for antibodies against JE by using IgM Mac ELISA (National Institute of Virology, Pune). Out of 553 AES cases, 12 patients excluded as death occurred. A total of 541 sera samples from the patients clinically suspected with acute encephalitis syndrome (AES) were collected from the year 2014 to 2016. Out of which 54/541 (9.98%) were JE positive by IgM Mac ELISA kit, where 30/54 (55.56%) from male and 24/54(44.44%) was female. The remaining 487/541 (90.02%) patients were grouped as non JE AES. This study give the scenario of AES (Non JE and JE) in this part of the state and further efforts has to be done to find out the etiology of Non JE AES cases.

Keywords: Japanese encephalitis, syndrome, ELISA, acute encephalitis syndrome

1. Introduction
Acute Encephalitis Syndrome (AES) is defined as the acute-onset of fever with change in mental status including symptoms such as confusion, disorientation, coma or inability to talk and/or often with new onset of seizures (excluding simple febrile convulsion) in a person of any age at any time of the year [1].

The causative agent for AES is generally considered wide variety of neurotopic viruses but there are also other probable causes such as bacteria, fungus, parasites, certain chemicals and toxins that have been reported over the past few decades [2]. Japanese encephalitis is one of the leading causes of viral neurologic disease and one of the most common causes of AES in India. It is the most prevalent and significant mosquito borne viral encephalitis of man occurring with an estimated 35,000 to 50,000 of cases and 15,000 deaths annually [3]. About 20% to 30% of JE cases are fatal and 30–50% result in permanent neuropsychiatric sequelae [4].

Japanese encephalitis virus (JEV) belongs to the family flaviviridae and genus flavivirus; it is a single stranded positive sense polarity RNA genome of approximately 11kb in length [5]. It was first reported from Japan, and subsequently characterized for the first time in the year 1943. The disease at present is reported from South Asia, South- East Asia, East Asia and the Pacific region [3]. Three billion people live in countries where JE virus is endemic [6].

In India JE Virus has been reported from different parts of the country [7, 8]. It was first isolated in humans in Vellore (North- Arcot) district of Tamil Nadu in 1955 [9]. Large outbreak occurred in the districts of Bankura and Burdwan in West Bengal in 1973 [10]. Since then, the virus is active in many parts of India and outbreaks have been reported from the states of Bihar, Uttar Pradesh, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa and union territories of Goa and Pondicherry [11].

In North east (NE) region of India, the disease was first observed in 1976 in Assam and since then the disease has appeared in endemic forms or in sporadic outbreaks [12]. The Assam state, particularly the upper Brahmaputra valley has been experiencing recurrent JE episodes during

July to October every year [13]. Several outbreaks were reported from Assam in 3 consecutive years from August 2000-2002 [14]. Besides Assam, JE cases have been reported from NE states like Manipur and Nagaland [15] Arunachal Pradesh [16].

JE virus is maintained in a cycle involving mosquitoes and vertebrate hosts, mainly pigs and wading birds. It is important to note that eco-epidemiological factors interact with the less well-defined socio-cultural drivers in a complex continuum to influence the overall disease epidemiology [17, 18].

Serological surveys (non-human) carried out during 1955 to 1972 showed that JE infection occurred in different parts of Assam and Arunachal Pradesh (AP) besides other states [19]. JE cases from two districts of Arunachal Pradesh viz, Papum pare and East Siang was reported during 2001[20].

Confirmation of JE is usually done by specific titres of IgM antibodies in serum or CSF during acute illness of a suspected AES case [4]. JE is causing significant burden in Assam, more prevalent in adults which may be due to higher exposure to mosquito bites, although JE vaccination in adults started in Assam from 2011 but still it is occurring in endemic form.

The objective of the present study was to find out the clinico-epidemiological profile of the AES (Non JE and JE) during its outbreaks from the year 2014 to 2016 in tertiary care centre, Tezpur (Assam).

2. Materials and Methods

The present study was a retrospective study and data was collected from the hospital record book. Based on clinical criteria of AES cases patients were included. Collection of sera samples from patients suffering from acute encephalitis syndrome and admitted to hospital during the period of 2014 to 2016 was taken into consideration. The medical history and clinical findings of each patient was recorded. Consents of the patients were taken for collection of blood samples.

Samples were tested for JEV specific IgM antibody by using a MAC ELISA kit (NIV, Pune) developed by National Institute of Virology, Pune, India.

2.1 Statistical analysis

Statistical significance was measured using SPSS software package version 16.0. Chi square test and p value of less than 0.05 was considered statistically significant.

3. Results

During the present study period of three years a total of 553 AES cases were reported, out of which 12 patients were excluded as death occurred. A total of 541 sera samples from the patients clinically suspected with acute encephalitis syndrome (AES) were collected from the year 2014 to 2016. Out of which 54/541 (9.98%) were JE positive where as 30/54 (55.56%) from male and 24/54(44.44%) was female. The remaining 487/541 (90.02%) patients were grouped as non JE AES.

During the study period from 2014-2016 it was observed that out of all the cases males were affected with AES (62.29%) showing both non-JE and JE symptoms and females were observed to be less effected with 37.70% of all the cases. The chi-square value for this analysis is 0.281, r=1 it was not statistically significant (Table 1).

Table 1: Gender wise distribution of AES cases (Non JE and JE) from the year 2014 to 2016

Gender	Non JE (%)	JE (%)	Total (%)
Male	307 (63.04)	30 (55.56)	337 (62.29)
Female	180 (36.96)	24 (44.44)	204 (37.71)
Total (%)	487 (100)	54 (100)	541 (100)

Chi square = 0.281, r =1

Year wise distribution of AES (2014-2016) stated that the numbers of patients suffered with non-JE AES were 88.70% (204 cases out of 230) in the year 2014, 94.92% (168 cases out of 177) in the year 2015 and 85.82% (115 cases out of 134) in the year 2016 respectively. JE positivity in 2014 was 11.30% (26 cases out of 230), 2015 it was 5.08% (9 cases out of 177) and in 2016 was 14.18% (19 cases out of 134).The chi-square value for this analysis is 0.020, r=0.3, p= 0.806 (Table 2; Fig 1).

Table 2: Year wise distribution of AES case (Non JE and JE)

Year	Non JE (%)	JE (%)	Total (%)
2014	204 (41.89)	26 (48.15)	230 (42.51)
2015	168 (34.50)	9 (16.67)	177 (32.72)
2016	115 (23.61)	19 (35.19)	134 (24.77)
Total (%)	487 (100)	54 (100)	541 (100)

Chi square =0.020, r=0.3, p=0.806 not significant

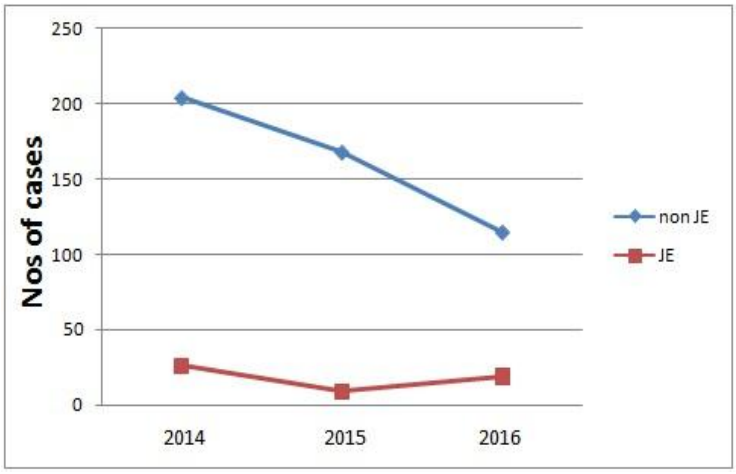

Fig 1: Year-wise distribution of AES cases (Non JE and JE) 2014-2016

Age wise distribution of AES cases were 5 distinct age groups were taken. Maximum number of AES cases was reported in the age group 0-15 yrs with 185 (34.20%) and JE positive 14.05% (26 cases out of 185) while most of the JE case was found in age group 16-30 yrs 16.22% (12 cases out of 74). The chi-square value for this analysis is 0.001, r=0.45, p=0.10 (Table 3, Fig 2a and b).

Table 3: Age wise distribution of AES case (Non JE and JE)

Age group (years)	Non JE (%)	JE (%)	Total (%)
0-15	159 (32.65)	26 (48.15)	185 (34.20)
16-30	62 (12.73)	12 (22.22)	74 (13.68)
31-45	61 (12.53)	8 (14.81)	69 (12.75)
46-60	32 (6.57)	3 (5.56)	35 (6.47)
>60	173 (35.52)	5 (9.26)	178 (32.90)
Total (%)	487 (100)	54 (100)	541 (100)

Chi square=0.001, r=0.45, p=0.10 not significant

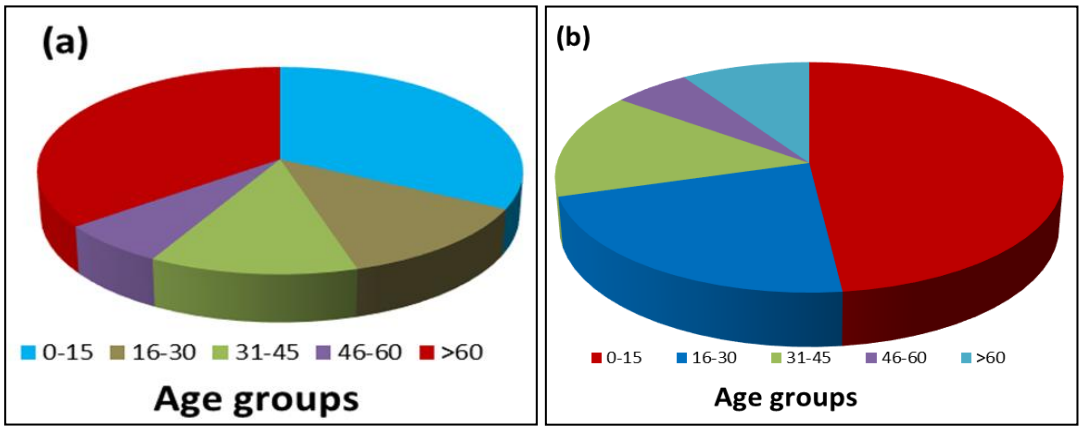

Fig 2: The figure represents the (a) Non JE and (b) JE cases of AES distributed among the 5 different age groups for the year (2014-2016).

Again, when the availability of AES cases was investigated area wise near Tezpur town of Assam, it was observed that AES cases were more prevalent in rural sites which was 58.96% and JE positive cases in rural area was 8.77% (28 out of 319), teagarden 19.75% (16 out of 81), semi-urban 7.09% (10 out of 141). Location wise distribution of AES cases in rural area 219/319 (68.65%), Tea garden 65/81 (80.25%), semi-urban 131/141 (92.91%). JE case in rural area 28/319 (8.77%), teagarden 16/81 (19.75%), semi-urban 10/141 (7.09%) (Table 4, Fig 3).

Table 4: Location wise distribution of AES (Non JE and JE)

Location	Non JE (%)	JE (%)	Total (%)
Rural	291 (59.75)	28 (51.85)	319 (58.96)
Teagarden	65 (13.35)	16 (29.63)	81 (14.97)
Semi- urban	131 (26.90)	10 (18.52)	141 (26.06)
Total (%)	487 (100)	54 (100)	541(100)

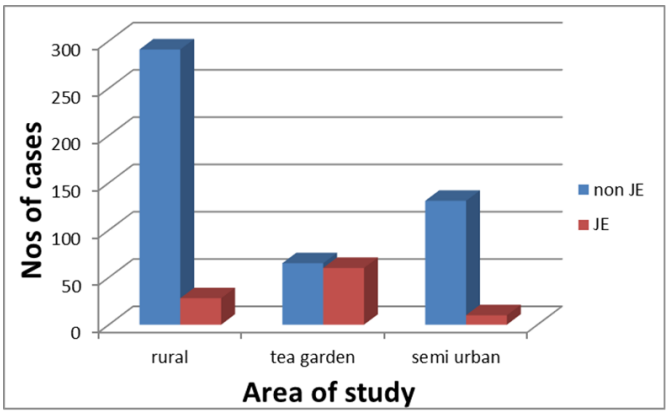

Fig 3: Area wise distribution of non-JE and JE cases of AES.

4. Discussion

In this study it was reported that a total of 541 sera samples from the patients clinically suspected with acute encephalitis syndrome (AES) were collected from the year 2014 to 2016. Out of which 54/541 (9.98%) were JE positive diagnosed by IgM Mac ELISA kit, where 30/54 (55.56%) from Male and 24/54(44.44%) was Female. Death occurred in 12/54 (22.22%) patients. The remaining 487/541 (90.02%) patients are grouped as non JE AES.

Study carried out by (Sarkar *et al*., 2012) [21] reported that out of 648 specimens, only 175 (27.0%) specimens were reactive to JE IgM antibody, of which 60.0% were from the male individuals and 40.0% from the female population. A study conducted in nepal reported 4.7% patients were JE serology positive [22]. In a study from Assam, India, JE positive cases were found to be 33.6% [23]. In a study done in Patna medical college it was reported that children diagnosed with Japanese Encephalitis positive were 66% of them were discharged, whereas overall discharge rate of children admitted with the clinical diagnosis of AES was 51% [24].

In our study the incidence of AES and JE was found to be more common in males compared to females, similar findings were also reported were AES and JE cases were more prevalence in male as compare to female [25]. JE outbreak in UP 2005 also reported more cases in males (61%) and less in female (39%) [26]. In our study gender differences were not found to be statistically significant, this is similar to the findings from other Indian studies [26, 27, 28].

The male predilection for AES (and JE) may be explained by an increased amount of outdoor activities in males that includes playing outside and also working in rice fields which is the main crop in this region, and hence having a higher chance of being bitten by the *Culex tritaeniorhynchus* mosquito [28]. The vector usually breeds in the stagnant water in the cultivation fields and the majority from this age group gets exposed to the vector directly. The intermediate hosts, i.e. pigs and cattle egrets are found in abundance in rural areas near paddy field ecosystems in Assam, which play an undeniably important role in the transmission of JE.

In our study age group 16- 30 yrs JE cases reported was 16.22% and age group 0-15 yrs (14.05%), which may be due to children and young age group are more exposed to mosquito bites due to outdoor activities. This is similar to other studies carried out earlier in the subcontinent [27, 29].

Children from rural areas were found to be affected more as compared to those from urban area. This finding are supported by the increase breeding and density of mosquitoes during monsoon water logged paddy fields, piggery in close proximity to home, not using of bed nets [30].

Area wise distribution of non JE cases in this study showed most of the case from rural population followed by next semiurban and teagarden respectively. The reason might be that majority of them involve in agriculture and farming occupation and are in constant contacts with the various vectors of AES cases. In the present study most of the JE cases was reported from tea garden people as they are mostly involve in day activities in teagarden and the vector of JE is mostly day biters culex mosquitoes.

Year wise distribution of cases did not show any significant difference in our study. This is in contrast to the study conducted by (Bandyopadhyay *et al*., 2013) [31] from West Bengal where they found a significant reduction in JE cases from 2011-2012 which had been attributed to better awareness, mass vaccination against JE.

Occurrence of JE in India is attributable to several factors which include large number of people living in the vicinity of irrigated lands, high vector densities in endemic areas, dependence on pig farming, and meteorological conditions [32, 3]. Other socio-economic factors (occupation, education, environment, income, place of residence) plays a major role in incidence and mortality and likely to cause disease burden in the form of mortality and morbidity which is unnoticed. Education regarding it can play a major role in control of this disease.

5. Conclusion

The present study gives insight regarding the presence of AES (Non JE and JE) in this part (Tezpur) of the state (Assam). It needs to be mentioned that AES cases cannot be presumed to be only JE as most of the cases were of Non JE origin which is a matter of concern as Assam is endemic for AES, so further elaborate study has to be done to know the multifactorial etiology of Non JE so that proper intervention can be taken to lower the disease burden in this part of the state. Sero surveillance ELISA can be used as rapid test so that effective measures can be taken for minimizing the disease burden in form of mortality and morbidity. In short it could be controlled by effective surveillance systems, an integrated vector control approach, vaccination and segregations of pigs as well as health awareness and educating the local population. Further research plans for understanding the pattern of AES cases are warranted in this part of the state with more sophisticated and molecular biological approach so as to reduce the disease burden.

6. Conflict of interest statement

We declare that we have no conflict of interest.

7. Acknowledgements

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