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Original Article

Determinants of Acute Encephalitis Syndrome (AES) in Muzaffarpur district of Bihar, India: A case–control study

Girish Kumar Singh^a, Chandra Mani Singh^a, Alok Ranjan^{a,c,*},
Neeraj Agarwal^a, Sanjay Pandey^a, Pragya Kumar^a, Ghanshyam Setty^b

^aDepartment of Community and Family Medicine, All India Institute of Medical Sciences, Phulwarisharif, Patna 801507, Bihar, India

^bUNICEF Office, Patliputra Colony, Patna 800 003, Bihar, India

^cAssistant Professor, Department of Community and Family Medicine, All India Institute of Medical Sciences, Phulwarisharif, Patna 801507, Bihar, India

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ABSTRACT

Background: Muzaffarpur district in Bihar, India has witnessed several outbreaks of Acute Encephalitis Syndrome in the past decade with reportedly high case fatality in children under the age of 15 years. No study has documented the mode of transmission and nature of causative agent.

Methods: A case–control study was conducted to assess the risk factors associated with AES. All cases reported from two hospitals in the district were selected with four controls per case selected randomly. Questionnaires were administered using geographical positioning system (GPS) enabled Tablets.

Results: The median age was 4 years (IQR: 3–6.5) in both cases and controls. Case-fatality rate was 274 per 1000 AES cases. Logistic regression analysis showed significant association ($p < 0.05$) with literacy status, occupational status of parents, travel using public transport mode and presence of lychee orchard near the vicinity of households. Goodness-of-fit of model using chi-square statistic based on Hosmer–Lemeshow test was found to fit well for the final model ($p = 0.353$).

Conclusions: Outbreaks of acute encephalitis of unknown origin coinciding with the harvesting season of lychee strengthens hypothesis of oral–faecal transmission.

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* Corresponding author at: Department of Community and Family Medicine, All India Institute of Medical Sciences, Phulwarisharif, Patna 801507, Bihar, India. Tel.: +91 9471007028.

E-mail address: aranjan30@gmail.com (A. Ranjan).

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1. Introduction

Acute Encephalitis Syndrome (AES) is a complex and severe disease characterised by inflammatory condition of the central nervous system. The defining feature of AES is acute onset of fever with change in mental status, which may sometimes be associated with seizures. These clinical signs suggest that the patient has acute inflammation of the brain. Sometimes, AES can result in severe complications, such as impaired consciousness, limb paresis or death.

AES is thought to be caused by a wide variety of conditions. These include bacterial or viral infections in the brain, ingestion of toxic substances, complications of infectious diseases and underlying malignancy. However, infective causes are predominant factor for acute condition in the epidemic form. More than 100 different pathogens have been recognized as causative agents of AES. Among these, Japanese encephalitis (JE), Herpes simplex, Varicella-zoster, Epstein Barr virus, mumps, measles, Enteroviruses, Influenza, Adeno virus, Echo virus and *Mycoplasma pneumonia* are the most frequent pathogens. JE constitutes about 15% of AES. The aetiology of AES in 68–75% of the cases remains unknown and most of the AES secondary to viral infections (~90%) have no specific treatment.

The history of AES in India is documented in association with JE, with the virus first being reported from southern India in 1955. Recently, the Nipah and Chandipura viruses have also been identified in causing acute encephalitis outbreaks in Malaysia and India.^{1,2} The sources of viral infection resulting in AES may differ across regions.³ AES is a disease of public health importance because of its epidemic potential and high case-fatality rate. Various subsequent studies confirmed that most AES in India are due to JE, which has been considered as the only major cause of AES in India. In India, worst affected parts by AES outbreak are the border districts of eastern Uttar Pradesh and two districts (Muzaffarpur and Gaya) in Bihar. Irrespective of the region, the disease has mostly affected children (06 months – 15 years). The number of cases reported from Bihar in the previous years was 745 in 2012 and 821 in 2011.

It is unclear regarding the specificity of causative agents of AES in different parts of the country. AES disproportionately affects children and poses serious risk to life in the affected children. Barring JE, most of the underlying illnesses are rarely investigated for understanding the probable agent and mechanism of spread. It is therefore very important that detailed epidemiological investigations are carried out in the high burden areas. These analytical studies can contribute to the understanding of natural history of disease. Hence, we conducted a study to understand descriptive epidemiology of AES in Muzaffarpur. Our study also aimed at identifying various determinants of prevention by analysing in comparison with controls.

2. Materials and methods

2.1. Study area

This study population is from Muzaffarpur district of Bihar state, India. A case-control study was carried out recruiting all

the reported cases of AES in children ≤ 15 years of age admitted to Sri Krishna Medical College Hospital (SKMCH) and Kejriwal Hospital. The case definition was that the “person should have suffered from acute onset of fever and change in mental status and/or new onset of seizure”. The mental status change comprised of symptoms such as confusion, disorientation, coma or inability to talk. The exclusion criteria included simple febrile seizures or change in mental status due to other known conditions. The AES caseload was high in public as well as private health facilities during the epidemic. Since high proportion of the total cases from the district was reporting to both hospitals in the study, the chance of selection bias was minimized. Four children (without fever and sign of encephalitis and/or seizure in last one week) were selected randomly from the same village as that of the cases for the controls. Out of four control subjects per each case, one control was selected within the same family, one from the nearest neighbour, one from the central location of the village and the last control was chosen from the farthest household from the affected household. All the 135 cases registered at these two hospitals in Muzaffarpur district during the last week of May to third week of June, 2013 were included in the study.

2.2. Data collection

A comprehensive semi-structured questionnaire was used to collect information, such as socio-demographic factors, behavioural practices and the clinical history. Parents or guardians of the affected cases were interviewed at the hospital site for collecting socio-demographic and behavioural information. Clinical information was collected from the patient records of the hospital. The field team visited the house of every case for making on-site observation of surrounding area and completing the epidemiological investigation. These visits were used to collect information on ecological factors and neighbourhood conditions. For each affected AES cases, four controls were identified from a list of eligible controls from the same village. Field investigators visited the houses of randomly selected controls for collecting information and ecological conditions. The field investigators observed and collected the information, such as the type of house, fuel used for cooking, mode of mosquito protection, toilet facilities, source of drinking water, use of purified water, hand washing before eating and after defecation, washing material used for washing hands and utensils, presence of pigs, chickens, birds and other cattle, presence and location of cattle shed and presence of vegetation especially the lychee orchard in the vicinity of households of each case and control. Computerized questionnaires were administered in the field using geographical positioning system (GPS) enabled Tablets (Lava) by the field investigators. The data were transferred to the remote database subsequent to the completion of interview using Internet in the field. The aim of this was to create time and place distribution of the cases in the affected areas. The final data in Excel sheet were accessed from the database after the end of field survey work.

All the information was collected by a team of two trained investigators in each district. The team was assisted by local field workers from UNICEF. Informed consent was obtained from the parent of the affected cases and selected control

subjects. The study was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences (AIIMS), Patna, India.

2.3. Statistical analyses

The statistical analyses were conducted using SPSS, version 16 and STATA software (version 10.0; StatCorp LP, College Station, TX, USA). Continuous variables, such as age and per-capita income, were converted into categorical variables. Dummy variables were created for categorical variables having more than two categories. The strata having the lowest risk among the categories were taken as reference category for estimating odds ratio. Type of subjects (AES cases/Control) was included as dependent variable. The analysis contained the following steps. First, we performed two-way chi-square analyses for estimating association with types of subjects and independent variables. Crude odds ratio with 95% confidence interval for each potential risk factor for the occurrence of AES was calculated, and Mantel-Haenszel test statistic was used for assessing significance at 5% level. Logistic regression analysis was performed to model the dependent variable, i.e. occurrence of AES. The multivariate analyses included all the covariates with p value less than or equal to 0.2 in the univariate analyses, and a step-wise method was used to include variables in the model by deleting variables by Wald test until all variables in the final model had p values <0.05 . The adjusted odds ratio and the 95% confidence intervals were estimated after controlling the confounders in the final model. Likelihood ratio test was used for selecting the final model and Hosmer–Lemeshow test was used for checking the goodness-of-fit of the final model.

3. Results

A total of 147 cases in the age groups 0–14 years were reported during May–June in Muzaffarpur district. During the course of the study, a total of 135 children were admitted in the identified hospitals. Out of 135 cases, 101 (75%) were from Muzaffarpur district, and the rest 25% were from other districts like East Champaran, Vaishali, Sitamarhi, Sheohar, West Champaran and Samastipur, as per the ranking of incidence.

Out of 135 cases, 37 deaths were reported, i.e. a case-fatality rate of 274 per 1000 AES cases.

The epidemic curve of AES cases in Muzaffarpur and other adjacent districts during 2013 showed bimodal presentation. The onset of epidemic was in the last week of April, reaching its peak during the first and second week of June. The peak of the epidemic coincides with the onset of monsoon season in Bihar. The second peak following this had the maximum number of incidence of AES cases. The outbreak started to decline after the first spell of rain after third week of June (Fig. 1). The nature of the AES outbreak was found similar to the observed trend in previous years.

The median age of the cases was 4 years (interquartile range: 3–6.5), and was similar in the control group. The male to female ratio was 1.5:1. The proportions of Hindu and Muslim among the cases were 79% and 21% respectively, representing the demographic distribution of the religion in the district.⁴ Among the cases, 59% belonged to backward community followed by scheduled castes (32%). Compared to the general caste, the risk of developing AES in scheduled caste (OR = 2.18; 95% CI = 1.10–4.30; $p = 0.028$) and other backward caste (Crude OR = 1.91; 95% CI = 1.00–3.66; $p = 0.049$) was significantly higher. Low education level among the parents of AES cases was found to be significantly associated with AES (Crude OR = 2.03; 95% CI = 1.33–3.10; $p = 0.001$). The median per capita income was lower among the AES cases as compared to controls, but not statistically significant ($p = 0.154$). Agricultural-based occupation (own agricultural land, agricultural farmers, labourers) was found to be significantly associated with the occurrence of AES cases (Crude OR = 2.20; 95% CI = 1.33–3.79; $p = 0.0011$) (Table 1).

Children living in households having kutchha houses (mud plastered) were found to be at higher risk as compared to those living in brick and concrete houses (Crude OR = 1.93; 95% CI = 1.01–3.67; $p = 0.048$). Washing hands without soap after defecation was found to be significantly associated with the occurrence of AES (Crude OR = 1.80; 95% CI = 1.09–3.08; $p = 0.019$). We observed a significant increasing trend of risk (Mantel-Haenszel test for trend, $p = 0.001$) of getting AES among those using materials for washing hands after defecation (Water, Ash and Soap), indicating highest risk among those households using only water for washing hands after defecation (Table 2).

Fig. 1 – Time distribution of AES cases that occurred in Muzaffarpur and other adjacent districts during 2013.

Table 1 – Socio-demographic characteristics of AES cases and control.

Characteristics	Cases (135)	Control (539)	Total	Crude OR	p-Value
Age group category					
0–1	3 (2)	16 (3)	19	1	
1–5	75 (56)	301 (56)	376	1.17 (0.78–1.75)	0.43
5–10	43 (32)	182 (34)	225	0.92 (0.58–1.44)	0.70
10–14	14 (10)	40 (7)	54	1.53 (0.70–3.38)	0.28
Gender					
Male	81 (60)	291 (54)	372	1.27 (0.87–1.87)	0.2091
Female	54 (40)	248 (46)	302	1	0.4544
Religion					
Hindu	107 (79)	442 (82)	549	0.83 (0.52–1.34)	0.26
Muslim	28 (21)	97 (18)	125		
Castes					
SC/ST	44 (32)	148 (27)	192	2.18 (1.10–4.30)	0.028
OBC	79 (59)	303 (56)	382	1.91 (1.00–3.66)	0.049
General	12 (9)	88 (16)	100	1	
Education of parent					
Illiterate	100 (75)	315 (58)	415	2.03 (1.33–3.10)	0.001
Literate	35 (25)	224 (42)	259		
Per capita income	777 (IQR: 571–1000)	888 (IQR: 600–1200)			
≤Median	77 (57)	268 (49)	345	Chi-square = 2.03	0.154
Above median	58 (43)	271 (51)	329		
Occupation of parent					
Agriculture based	114 (84)	377 (70)	491	2.33 (1.39–4.05)	0.0005
Non-agricultural	21 (16)	162 (30)	183		

It was observed that travelling by public transport mode was found to be significantly associated with the risk of AES as compared to no travelling history (Crude OR = 2.18; 95%CI: 1.23–3.76; $p = 0.005$). Presence of lychee orchard in the vicinity of households was found to be highly significantly associated with the risk of AES as compared to non-presence of lychee orchard (Crude OR = 3.25; 95%CI: 2.10–5.00; $p = 0.001$) (Table 3).

We performed a step-wise multivariate regression analysis using logistic regression to model the association of significant factors on occurrence of AES. All the variables, such as castes, per capita income (below and above median), education and occupation status of parents, type of house, mosquito protection, washing hands after defecation, travel, cattle shed and presence of lychee orchard in the vicinity of households having a p value ≤ 0.20 in the bivariate analyses were put into the model after checking for co-linearity.

The final logistic regression model having four independent variables, i.e. literacy status and occupational status of parents, travel using public transport mode and presence of lychee orchard near the vicinity of households, were found to be independently associated with the occurrence of AES, after adjusting for other confounding variables into model. The adjusted odds ratios with 95% CI and p -value on the basis of z-score are presented (Table 4).

Likelihood ratio test using chi-square statistic was used for the model selection (log-likelihood = -310.10 , Chi-square = 53.43; $p = 0.0001$). We tested the goodness-of-fit of model using chi-square statistic at given degree of freedom using Hosmer–Lemeshow test, and was found to fit well for the final model (Chi-square = 2.083 at d.f. = 2; $p = 0.353$). We performed sensitivity-specificity classification at cut-off value of p less than

equal to 0.25, and the level of correct classification of the predicted value was nearly 82%.

4. Discussion

Muzaffarpur district has witnessed unexplained outbreaks of AES, also called non-JE acute encephalitis, since 2005. These outbreaks were limited to defined locations and were following seasonal trend with most of the cases occurring during the months of May–June. The epidemic used to start before the onset of monsoon season and was affecting very specific age group of subjects (<15 years old). The cases had similar clinical features, including abrupt onset of fever, rapid progression to coma, and extremely higher case fatality rate.

The high case-fatality rate of 274 deaths per 1000 cases showed is in conformity with evidence from geographical regions of Asia and North India. The case-fatality rate estimated during the year of study was lower compared to 310 per 1000 cases in 2011⁵ but was comparatively higher than in Gorakhpur district during 2012.⁶ In Vietnam, the case fatality rate during 2004–09 was found to be 250 per 1000 cases,⁷ similar to some parts of North India.⁸

The educational and occupational status of parents had significant association with occurrence of AES. The affected households had per capita income of INR Rs. 777 per month, and this was lower than the overall state per capita income, i.e. Rs. 20,069.⁹ This shows that the economic status of the affected families was far below compared to the rest of the state. Education, occupation and income are considered to be important factors directly influencing socio-economic status

Table 2 – Distribution of AES cases and controls based on Housing type and Personal Habit.

Characteristics	Cases (135)	Control (539)	Total	OR (95% CI)	p-Value
Type of house					
Kutcha	67 (50)	200 (37)	267	1.93 (1.01–3.67)	0.048
Semi Kutcha	55 (41)	264 (49)	319	1.20 (0.62–2.31)	0.83
Pucca	13 (9)	75 (14)	88	1	
Fuel used					
Choolha smoke	127 (94)	500 (93)	627	1.24 (0.56–2.71)	0.59
LPG	8 (6)	39 (7)	47	1	
Mosquito protection					
Yes	85 (63)	376 (70)	461	0.73 (0.48–1.11)	0.13
No	50 (37)	163 (30)	213	1	
Type of toilet					
Open filed	114 (84)	437 (81)	551	1.26 (0.75–2.11)	0.45
Personal	21 (16)	102 (19)	123	1	
Source of drinking water					
Open well	101 (75)	391 (73)	492	1.12 (0.73–1.73)	0.66
Deep bore well	34 (25)	148 (27)	182	1	
Use of purified water					
Non-purified	133 (99)	534 (99)	667	0.62 (.12–3.2)	0.63
Purified	2 (1)	5 (1)	7	1	
Water source used for washing					
Non-washed	59 (44)	215 (40)	274	1.16 (.79–1.72)	0.43
Washed	76 (56)	324 (60)	400		
Washing water					
Open + shallow well	101 (75)	389 (72)	490	1.14 (0.74–1.76)	0.58
Deep well	34 (25)	150 (28)	184		
Eating habit					
Non-vegetarian	130 (96)	515 (96)	645	1.21 (0.45–3.23)	0.81
Vegetarian	5 (4)	24 (4)	29		
Hand washing before eating					
No	27 (20)	99 (18)	126	1.11 (0.69–1.78)	0.71
Yes	108 (80)	440 (82)	548	1	
Washing material					
None	27 (20)	99 (18)	126	1.96 (0.67–7.00)	0.25
Water	103 (76)	404 (75)	507	1.83 (0.69–6.13)	0.30
Soap	5 (4)	36 (7)	41	1	
Hand washing with soap after defecation					
No	112 (83)	393 (73)	505	1.80 (1.09–3.08)	0.0160
Yes	23 (17)	146 (27)	169	1	
Washing material after defecation					0.001
Water	32	97	129	2.09 (1.10–3.98)	0.016
Ash/mud	80	296	376	1.71 (1.02–2.98)	0.043
Soap	23	146	169	1	

of the households in rural areas of India. The association of these variables clearly indicates that AES predominately occurs in the populations having low socio-economic status.

Our study reaffirms the importance of washing hands after defecation, as the lack of this was strongly associated with the occurrence of AES (Crude OR = 1.80; 95% CI (1.09–3.08); $p = 0.019$). A significant trend was seen ($p = 0.001$) with the highest risk in those who washed their hands only with water (OR = 2.01) followed by with mud or ash (OR = 1.71) in comparison to those washing their hands with soap after defecation. This reflects the possibility of oral-faecal transmission of the disease. However, the same was not observed after controlling for other confounding factors in multivariate analysis.

Using public transport (Adjusted OR = 2.18 95%CI: 1.26–3.78; $p = 0.005$) was significantly associated with the occurrence of AES and could be directly associated with hand hygiene practices in rural areas. Hand hygiene plays a very important role in spread of diseases transmitted through oral-faecal route. Similarly, lack of washing hands with soap was found to be significantly associated in case of diarrhoea among children <5 years of age in Bangladesh and other parts of the world.^{10,11,12} In rural areas of Bihar, hand hygiene practice is not very common due to lack of awareness and illiteracy. In the course of public transportation, the infected hands might get in contact with contaminated materials, which could play an important role in transmission of AES from one village located far away to another

Table 3 – Distribution of AES cases and controls based on travel and exposure to potential reservoir of infection.

Characteristics	Cases (135)	Controls (539)	Total	OR (95% CI)	p-Value
Travel history					
Public transport	25 (19)	52 (10)	77	2.18 (1.23–3.76)	0.005
Private transport	6 (4)	15 (3)	21	1.82 (0.56–5.09)	0.24
No travel	104 (77)	472 (87)	576	1	
Public stay					
Yes	20 (15)	36 (7)	56	1.56	0.38
No	11 (8)	31 (6)	42	1	
No travel	104 (77)	472 (87)	576		
Vaccination JE					
Yes	122 (91)	493 (94)	615	0.66 (0.33–1.32)	0.24
No	12 (9)	32 (6)	44	1	
Pigs surrounded					
Yes	43 (32)	180 (34)	223	1.079 (0.72–1.62)	0.76
No	91 (68)	353 (66)	444	1	
Chickens					
Yes	53 (40)	181 (34)	234	0.78 (0.53–1.15)	0.22
No	81 (60)	354 (66)	435	1	
Birds					
Yes	4 (3)	18 (3)	22	1.143 (0.38–3.434)	1.00
No	130 (97)	512 (97)	642		
Cattle shed					
Yes	118 (88)	432 (82)	550	0.63 (0.357–1.11)	0.11
No	16 (12)	93 (13)	109		
Location cattle shed					
Inside house	36 (27)	110 (20)	146	1.90 (0.95–3.90)	0.0504
Outside<25 ft	63 (47)	271 (50)	334	1.35 (0.73–2.63)	0.32
Outside>25 ft	20 (15)	63 (13)	83	1.85 (0.83–4.11)	0.13
No cattle shed	16 (11)	93 (17)	109	1	
Litchi orchard in vicinity					
Yes	46 (34)	74 (14)	135	3.25 (2.10–5.00)	0.0001
No	89 (66)	465 (86)	539	1	

Table 4 – Logistic regression model to predict AES incidence among the children.

Independent variables	Adjusted OR	95% confidence interval	p-Value
Public transport	2.18	1.26–3.78	0.005
Low education status of parents	1.73	1.10–2.74	0.018
Agriculture-based occupation	2.12	1.22–3.67	0.007
Presence of litchi orchard	3.31	2.12–5.17	0.0001
Log-likelihood test Chi-square = 53.43, $p = 0.0001$; Hosmer–Lemeshow lack of fit test, Chi-square = 2.083 at 2 d.f., $p = 0.353$; log-likelihood = –310.10.			

village in the same block or district. It has been observed that the spatial distribution of AES cases is very scattered and sporadic within the district. Usually cases are far away from each other, and one or two cases are reported from the same village during the outbreak. This epidemiological feature of AES supports the transmission by water, food and contact. Similar features were reported from Gorakhpur district.⁶

The presence of lychee (*Lychee chinensis*) orchard in the vicinity of affected households was found to be significantly associated with the occurrence of AES (Adjusted OR = 3.31; 95% CI: 2.12–5.17; $p = 0.001$). Muzaffarpur and other adjacent districts have large areas cultivating lychee, supplying to rest of the India and also exporting it worldwide. In particular, the

outbreak of AES coincides with the final phase of lychee cultivation during which fruits are ripened and ready for marketing and consumption. One of the behavioural factors that was observed during the field visit was that many of the inhabitants, including children, were visiting the lychee orchards frequently to escape from the high temperature in the villages. Many of them might pick up ripe fruits off the ground and eat them. Most of these fruits lying on the ground might be contaminated with faecal matter. This may also suggest oral-faecal transmission of the disease. Few studies have reported outbreak of acute encephalitis in children coinciding with lychee, as in Bac Giang Province in north Vietnam and in Muzaffarpur.^{5,7}

The strength of the study is that all the AEC cases that occurred during the reporting period were included in the study area. Controls were selected from the neighbourhood of the AES cases, thus making direct sampling of controls from the source population. This minimizes selection bias. However, our study has few limitations. Since controls are selected from the neighbourhood, it could obviously match to cases on many factors and some of them could be non-confounders. In this situation, there could be some bias due to overmatching.

In conclusion, our study of acute encephalitis of unknown origin in Muzaffarpur district strengthens our hypothesis of oral-faecal transmission. There could be some other ecological, environmental and behavioural factors directly or indirectly interacting with the high quantum of lychee harvesting season coinciding with the outbreak of AES, which needs further investigation.

Conflicts of interest

The authors have none to declare.

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