



Original Research Article

A Clinico-Pathological Study and Demographic Profile of Japanese Encephalitis from a Tertiary Care Hospital in Assam, India

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ABSTRACT

Keywords

Japanese encephalitis, CSF, AES, IgM antibody, ELISA, Altered sensorium, Pleocytosis

Japanese encephalitis (JE), which is caused by infection with the JE virus (JEV), is one of the most important viral encephalitis in the world, especially in East and Southeast Asia. Approximately 35,000-50,000 people develop JE each year, demonstrating annual mortality of 10,000-15,000. About one-third of patients die, and half of all survivors develop severe sequelae. The study was done to observe clinic-pathological findings and demographic profile of Japanese encephalitis from a tertiary care hospital from Assam. CSF and serum samples were collected from the clinically suspected AES cases and tested for IgM antibody against JE by IgM Capture ELISA. JE positivity rate was 24.42% and adult group showed higher positivity than the paediatric group ($p < 0.05$). Study also observed that significant numbers of JE cases were in monsoon season. Fever was commonest symptom of all JE cases followed by altered sensorium 84.21%, headache 63.16%, neck rigidity 53.68%, seizure 47.39% and paralysis 18.42%. Pleocytosis was in 80.28% of positive cases and most of the positive cases showed high CSF protein level (> 60 mg/dl) and normal CSF sugar level (40-80mg/dl). CSF parameters and clinical features were also compared with the both group of people. The study implies a significant burden of JE with shifting of affecting group, as well as non JE-AES in Assam.

Introduction

Japanese encephalitis (JE), which is caused by infection with the JE virus (JEV), is one of the most important viral encephalitis in the world, especially in East and Southeast Asia. Approximately 35,000-50,000 people develop JE each year, demonstrating annual mortality of 10,000-15,000 [1-3]. About one-third of patients die, and half of all survivors develop severe sequelae [4-6]. JE is characterized by high fever, conscious

disturbance, seizures, focal neurological deficit, signs of meningeal irritation, *etc.* The Indian subcontinent has borne a considerable burden of the disease since the 1960s [7]. The disease has appeared in sporadic outbreaks or epidemic forms in Assam, since 1976. Climatic conditions, abundance of potential mosquito vectors, amplifying hosts, agricultural practices and the sociocultural behavior of the people are

conducive to spread JE in the state. During the rainy seasons, ie June to August, the incidence of the disease reaches its peak. JE virus cannot usually be isolated from clinical specimens because of low levels of viremia and rapid development of neutralizing antibodies [8]. The detection of JE virus specific IgM by IgM captive-enzyme linked-immunosorbent assay (IgM-Captive ELISA) has been accepted as the standard for serological diagnosis [9]. The presence of JE virus-specific IgM in cerebrospinal fluid (CSF) is considered to be a sign of JE virus infection of the central nervous system. CSF is the preferred sample for diagnosis of JE because if anti JE IgM is detected in the CSF this confirms infection of the central nervous system with JEV [10]. The study was carried out to observe the clinico-pathological findings and demographic profile of JE cases in this region.

Materials and Method

Seven hundred seventy eight clinically-suspected AES cases admitted in GMCH and different hospitals in Assam during the period January to December 2013 were included in the study. The medical history and clinical findings of each patient were recorded as per guidelines of National Vector Borne Disease Control Programme (NVBDCP), Ministry of Health and Family Welfare, India[11]. AES consists of patients who present with fever, altered sensorium (including symptoms such as confusion, disorientation, coma or inability to talk), and/or new onset of seizures[12].

A total of 778 cases comprising of both CSF and serum samples were collected after taking written consent from the guardians and family members in case of minor. CSF samples were collected for 757 cases and only serum samples were collected from 21 cases from other hospitals. Serum samples

were collected from patients in whom lumbar puncture was contraindicated or was not possible. Samples were collected in sterile vials and immediately transported to the laboratory of the Department of Microbiology, GMCH, Guwahati. All the samples were tested by the NIV JE IgM Capture ELISA Kit supplied by NVBDCP. If optical density (OD) value of sample tested exceeded the OD of negative control by a factor of 5, the sample was considered as positive. CSF cytological and biochemical analysis data were collected from the Pathology and Biochemistry Department of GMCH.

All collected data were statistically analyzed later. Chi square test was used for the analytic assessment using SPSS 11.5 version. The differences were considered to be statistically significant when the *P* value obtained was less than 0.05

Results and Discussion

Among the 778 cases 594 cases were from Gauhati Medical College and 184 cases from outside (private hospitals/different districts). JE specific IgM antibody was positive in a total of 190 cases . Among them 114 cases were from GMCH and 76 cases from outside. Out of 757 CSF samples 177 cases and out of 21 serum samples 13 cases turned out to be positive for JE IgM .

Age and sex distribution

The study included 492 male , 286 female patients and among them JE IgM antibody were positive in 117 male patients, 73 female patients . Statistically it was found to be insignificant ($P > .05$). Age distribution of cases was in <15 years(paediatric) and ≥ 15 years(adult) groups. Association of JE with these two groups was tested and found to be statistically significant ($P < 0.05$). The

prevalence of JE was higher in adult group (≥ 15 years) than the paediatric age group (< 15 years). [Table 1]

Geographic and seasonal variation

The geographic distribution of JE cases is depicted in Figure 1. Cases reported from Assam (mostly from lower Assam districts) and also neighbouring two states Arunachal and Meghalaya. Association of JE with seasonal variation was found to be highly significant ($P = 0.000$) with high incidence in July followed by August and June. [Table 2]

Clinical profile of JE positive cases

All the JE positive patients in our study had high grade fever (100%). Change in mental status was observed in 84.21% of positive patients followed by headache, neck rigidity, seizure and paralysis or hemiparesis. [Table 3]

IgM positivity and duration of illness

We also analysed the IgM positivity rate with the duration of illness. Among them 95 patients tested positive for JE IgM antibody in 5-10 days from the onset of illness, 61 patients tested positive in 0-4 days and 34 patients positive in > 10 days.

Cytological and biochemical analysis

Cerebrospinal fluid sugar and protein level was available for 71 cases out of 114 JE IgM positive cases from GMCH. The values were calculated in the Vitros 5600 international system. Reference values were taken according to the machine manual. Normal sugar level (40-80mg/dl) was found in 62 positive patients. CSF protein level was in normal range (12-60mg/dl) in 14 patients and 57 no of patients showed higher level of CSF protein (> 60 mg/dl). CSF cell

count data revealed 80.28% positive patients showed pleocytosis (> 5 cells/c.mm) and surprisingly 54.39% of patients showed mixed pleocytosis (lymphocytes+ polymorphocytes). [Table 4] CSF LDH level was available in 23 positive cases and all cases showed higher level (> 200 U/L).

Co-morbid conditions associated with JE positive cases

The study also revealed that 15 JE positive cases had evidence of associated Diabetes (blood sugar > 120 mg/dl). Renal dysfunction was associated with 8 positive cases (blood urea > 42 mg/dl and serum creatinine > 1.25 mg/dl). Liver dysfunction was associated with 2 cases and Pancreatitis was associated with 1 positive case. One positive case was associated with falciparum malaria.

Association of clinical features and laboratory findings in relation to age

Clinical features (change in mental status, seizure, headache, neck rigidity and paralysis) in 190 positive cases and laboratory findings (CSF cell count, CSF sugar and protein levels) in 71 positive cases were tested with two age groups using Chi-square test [Supplementary table 1]. Lymphocytic predominance and elevated protein levels (> 60 mg/dl) were significantly higher in the adult group. The study also revealed that seizure and mixed cellularity were significantly higher in paediatric group

Though JE is considered as a disease of children it has been more prevalent in adults in Assam [13,14]. Our study also supported this hypothesis with statistically significant difference between adult group and paediatric group. In lower Assam JE vaccination in children has been covered in some of the districts only.

Table.1 Distribution of respondents according to age and sex

Gender	JE positive	JE negative	Total	Chi-square P value
Male	117	375	492	$X^2=0.298$ $P=0.585$
Female	73	213	286	
Age in years				
<15 years	57	231	288	$X^2=4.92$ $P=0.026$
≥15 years	133	357	490	

Table.2 Seasonal distribution of JE

Month	JE positive	JE negative	Total	Chi-square P value
January	1	14	15	$X^2 =178.8$ $P=0.000$
February	0	17	17	
March	0	32	32	
April	1	23	24	
May	0	42	42	
June	23	57	80	
July	134	128	262	
August	20	97	117	
September	4	50	54	
October	2	39	41	
November	4	48	52	
December	1	41	42	

Table.3 Clinical features in JE positive cases (n=190)

Clinical features	Number	Percentage (%)
Fever	190	100
Headache	120	63.16
Change in mental status	160	84.21
Seizure	90	47.39
Neck rigidity	102	53.68
Paralysis or hemeparesis	35	18.42

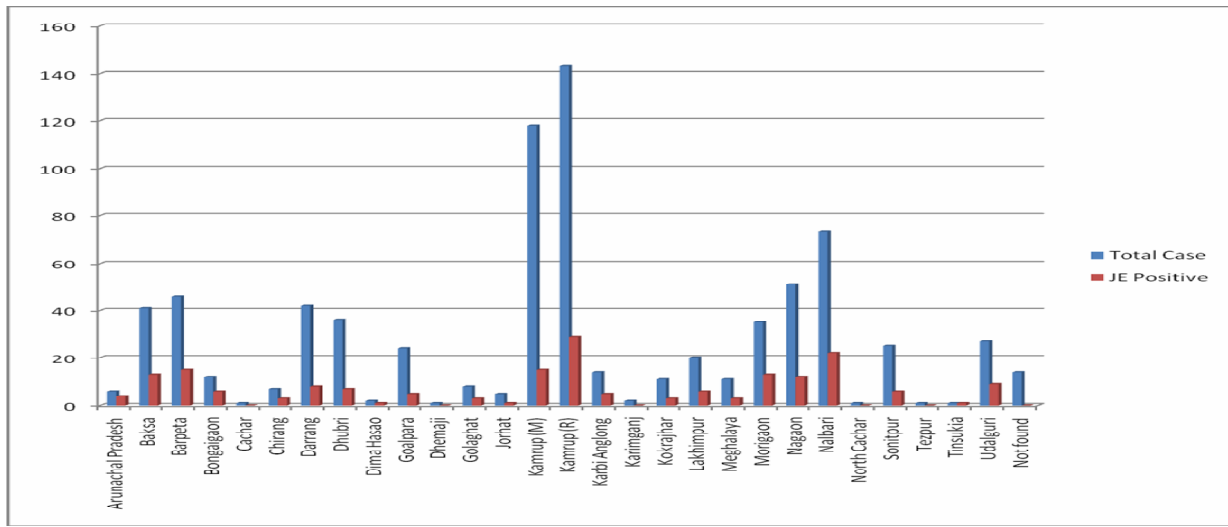
Table.4 Laboratory parameters in JE positive cases

CSF findings (n=71)	Number	Percentage (%)
Cell count		
0-5 cells/cumm.	14	19.72
>5 cells/cumm.	57	80.28
Pleocytosis(>5 cells/cumm)		
Lymphocytic	26	45.61
Mixed(lympho+polymorph)	31	54.39
Sugar level		
<40 mg/dl	1	1.41
40-80 mg/dl	62	87.32
>80 mg/dl	8	11.27
Protein level		
12-60 mg/dl	14	19.72
>60 mg/dl	57	80.29

Supplementary table.1 Association of clinical features and laboratory findings in relation to age

Parameters	Age<15 years	Age≥15 years	Chi-square P value
CSF cell count			
Pleocytosis	24	33	$X^2 = 0.862$
0-5 cells/cu.mm	4	10	$P = 0.353$
Pleocytosis			
Lymphocytic	7	20	$X^2 = 4.320$
Mixed	17	13	$P = 0.038$
CSF sugar level			
≤80 mg/dl	27	36	$X^2 = 2.739$
>80 mg/dl	1	7	$p = 0.098$
CSF Protein level			
>60 mg/dl	18	39	$X^2 = 7.473$
≤60 mg/dl	10	4	$p = 0.006$
Change in mental status			
Yes	51	109	$X^2 = 1.696$
No	6	24	$p = 0.193$
Headache			
Yes	30	90	$X^2 = 3.878$
No	27	43	$p = 0.049$
Seizure			
Yes	39	51	$X^2 = 14.476$
No	18	82	$p = 0.000$
Neck rigidity			
Yes	33	69	$X^2 = 0.581$
No	24	64	$p = 0.446$
Paralysis			
Yes	12	23	$X^2 = 0.375$
No	45	110	$p = 0.540$

Figure.1 Distribution of districts



So, higher prevalence of JE in adult is not only due to shifting of disease to adult population, as mentioned by some of the authors, it may be due to higher exposure to mosquito bite in paddy fields. The present study revealed significant number of JE cases in the monsoon season which is similar to the findings by Patgiri *et al*, Borthakur *et al* and Phukan *et al* [13,14,15].

The present study showed high grade fever is the commonest symptom of all JE positive cases. Similar observation were made by Patgiri *et al*, Phukan *et al* and Kumar *et al*[13,15,16]. Altered sensorium in 84.21% was the second most common symptom observed in the present study similar to the study of Kakoti *et al*[17]. Headache was observed in 63.16% and neck rigidity was observed in 53.68% and in Kakoti *et al* observed 41.79%, 55.22% accordingly[17]. Seizure was observed in 47.39% in our study which is comparable to observation made by Patgiri *et al*[13]. But Kumar *et al* and Kakoti *et al* observed a higher percentage (84.7% and 82.08%) than the present study[16,17]. Elevated cell count (>5 cell/mm³) in CSF was found in 80.28% and elevated CSF

protein level was recorded in 80.29% of positive cases in our study. Kakoti *et al* reported elevated CSF cell count in 77% with lymphocytic predominance and elevated protein level in 52.5%[17]. In contrary, Avabratha *et al* reported 45.06% and 74.67%, respectively [18]. In the present study, lymphocytic predominance was in 45.61% and mixed cells(lymphocyte +polymorph) was in 54.39% of positive cases.

The outcome of the patients could not be followed to assess the association of mortality with the co-morbid conditions. A study by Borah *et al* reported significantly higher percentage of paediatric group associated with altered sensorium, headache and seizure [19]. But the present study showed a significant association of paediatric age with seizure only. Higher percentage of adult group had high protein level(>60mg/dl) in CSF and associated with headache which were also reported by Patgiri *et al*[13]. Present study also observed lymphocytic predominance was significantly associated with adult group.

In conclusion, JE is a significant burden of lower Assam, more prevalent in adults, though JE vaccination in adults started in Assam from 2011. Laboratory parameters with clinical findings played a role to diagnose JE. And as there was significant number of non JE-AES cases in this region, the other causative agents of AES also need to be evaluated.

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