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Non Neurological Manifestations in Acute Encephalitis Syndrome in Uttar Pradesh, India

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Abstract

Non neurological features of Acute Encephalitis Syndrome (AES) or of Japanese Encephalitis (JE) have been scarcely reported.

Objective: To study hematological, hepatic and renal manifestations in patients of JE and compare with non JE.

Methods: Patients of AES admitted to hospital were screened for enrolment and testing for anti JEV IgM following inclusion and exclusion criteria. Enrolled patients were subjected to detailed history, physical examination and investigation according to a predesigned proforma. Cerebrospinal fluid or serum collected at admission was subjected to ELISA test for anti JEV IgM. If this was negative, a repeat test was done in serum after 7-10 days. Patients positive for IgM in CSF or serum were considered JE positive. Those negative for IgM when tested after one week of illness were considered non JE. Clinical and laboratory non neurological features were compared between JE and non JE.

Results: A total of 456 AES patients were enrolled and tested for JEV IgM, of which 224 were non JE and 110 were JE. Abnormal platelets and liver and renal function were found in both groups. Non JE AES patients were significantly more likely to have splenomegaly and lower platelet counts, but higher hepatic transaminases and International Normalized Ratio (INR).

Conclusions: Non neurological manifestations are common in both JE and non JE AES seen here and are more frequent in non JE.

Keywords:
Japanese encephalitis; Acute encephalitis syndrome; Non neurological; Thrombocytopenia; Hepatic derangement

Introduction

Japanese Encephalitis (JE) is presently the single most important cause of viral encephalitis in Asia. Besides producing an acute illness with high mortality, the disease may leave survivors with major permanent mental and physical disabilities. This poses an enormous load on the poor rural families who are least equipped to bear such a burden. Although originally found as summer epidemics in Japan in the early part of the last century, the disease has been almost eradicated from that country. Presently, it is seen as outbreaks and epidemics in large parts of Southeast Asia. In India, the first cases were reported from the southern state of Tamil Nadu in the 1950s [1]. However, the first major epidemic occurred in the state of West Bengal in 1973 [2]. Following this, there are annual monsoon and post monsoon outbreaks of the disease in most of the southern and eastern states, and the disease is also making inroads westwards [3,4].

India's northern state- Uttar Pradesh saw its first epidemic in 1978 [5] and since then the disease occurs in annual monsoon and post monsoon outbreaks affecting the northern and eastern districts of the state. Since 1978, it is estimated that JE has been responsible for about 8000 deaths in the state [6]. The districts which are the hardest hit are towards the eastern part of the state- Gorakhpur, Maharajganj, Deoria, Kushinagar and Sant Kabir Nagar. However there are at least 20 other eastern districts which are also affected and patients from here often reach our hospital in Lucknow. In 2005 a severe epidemic of JE occurred in these districts. Following the epidemic, the World Health Organization advocated surveillance of JE in affected countries and coined the term 'Acute Encephalitis Syndrome' (AES) for surveillance purposes. AES is a broad term and causes include JE as well as other viral encephalitides, non-viral meningoencephalitides and also metabolic and toxic encephalopathies.

We have been conducting a hospital based surveillance for JE from patients of AES presenting to our hospital and have observed various non-neurological manifestations which are worthy of dissemination.

Methods

Study site

The study was based in the pediatric and adult medicine wards of King George's Medical University (KGMU) hospital in Lucknow- the capital city of Uttar Pradesh, which is India's most populous state and also one of its poorest with lowest human development indices [7]. The hospital is a state run teaching hospital which caters mostly to the poor and severely ill from the city and surrounding districts extending up to Nepal.

From August 2012 to November 2013, we actively screened children and adults hospitalized with AES for testing for JE. 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 (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures) [8]. Exclusion criteria for testing were i) a firm alternate diagnosis made based on Cerebrospinal Fluid (CSF) examination or imaging ii) frank hepatic encephalopathy with jaundice. Attempt was made to enroll and test all patients satisfying the above criteria for JE, but many patients died very soon after admission before any testing was possible. All the enrolled patients were examined and worked up according to a pre-designed protocol that included demographic information, clinical history, complete clinical and neurological examination and investigations.

Laboratory tests

Enrolled patients were subjected to complete blood counts including platelets, peripheral blood smear for malarial parasite, rapid test for malaria and packed cell volume. Blood biochemical investigations included serum electrolytes, glucose, urea, creatinine and liver function tests (serum bilirubin, transaminases, prothrombin time and serum proteins). Results of Cerebrospinal Fluid (CSF) examination if done by the treating team were recorded. CSF was subjected to total and differential counts, protein and sugar estimation, bacterial culture and Gram stain. Neuroimaging was done by cranial Computerized Tomography (CT) or Magnetic Resonance Imaging (MRI) wherever possible.

In addition, CSF and serum were transported to the Indian Council of Medical Research (ICMR) Grade-1 Virology Laboratory of King George Medical University for IgM estimation against Japanese Encephalitis virus (JEV) using the National Institute of Virology (NIV) JE MAC ELISA kit (version 1.4) kit [9]. These tests were conducted and interpreted according to the manufacturer insert. The test was considered negative if optical density was less than twice that of negative control; it was positive if optical density was more than 5 times the negative control and equivocal if the optical density was between these values. In patients in whom the initial IgM was negative or equivocal, the test was repeated in serum after an interval of 7-10 days if the patient was alive and still admitted in the hospital.

Patients were classified as:

JE - Patients who were JE IgM positive at any time of testing.

Non JE - Patients with negative JE IgM results when done after 7 days of illness.

JE equivocal or unsure – i) Patients negative on initial testing in whom repeat testing could not be done and ii) patients with equivocal results even on repeat testing after 7-10 days.

Analysis

Clinical and laboratory non neurological manifestations were compared between JE and non JE patients by univariate analysis. Student '*t*' test was applied for continuous variables and Chi-square test was applied for categorical variables, using Epi-info software. The JE equivocal or unsure group was excluded from analysis.

Ethical approval for the study was obtained from the Ethics Committee of King George Medical University. Written informed consent was obtained from the guardians of the patients.

Results

Over a period of 14 months from August 2012 to November 2013, 456 AES patients were tested for JE IgM in CSF or serum or both. Of 397 CSF tested, 84 were positive, 272 were negative and 41 had an equivocal test result. Of the 65 sera tested, 5 were positive, 49 were negative and 11 gave equivocal result. So a total of 373 patients had negative or equivocal results on the first test. Of these 157 patients could have the test in a second sample taken after 7-10 days. Of these 157, 21 were positive, 134 were negative and 2 were equivocal. So a total of 110 (84+5+21) patients were confirmed JE and total 224 were non JE (134 who were negative on repeat test + 90 in whom the initial sample was taken after 7 days of illness) The remaining 122 patients were JE equivocal or unsure according to the criteria mentioned under Methods (Table 1).

Sample	AES patients tested	JE positive	Negative	Equivocal
CSF	397	84	272	41
Serum	65	5	49	11
Retested (n=157)	157	21	134	2

Table 1: Results of JE tests in 456 AES patients.

Table 2 compares various demographic features and clinical non neurological manifestations between the JE and non JE patients. Splenomegaly was significantly more common in the non JE group (p=0.046) while no significant differences were observed in mean age, sex, residence (rural or urban), and prevalence of vomiting, diarrhea, rash, swelling, hepatomegaly and bleeding episodes.

Table 3 compares the laboratory parameters between the 2 groups. Mean platelet count was significantly lower (p=0.017) and liver enzymes (p=0.010 for AST and 0.000 for ALT) and international normalized ratio (INR) (p=0.015) were significantly higher in the non JE group. No significant differences were observed in mean hemoglobin levels, total leucocyte count, percentage of neutrophils in the peripheral blood, mean serum bilirubin, mean serum protein, serum urea, creatinine or electrolytes.

Clinical parameters	JE N-=110 n (%)	Non JE N =224 n (%)	OR (95% CI)	P-value
Mean Age in years (SD)	14.7 (14.4)	17.3 (17.7)	-	0.838[1]
Male Sex	78 (70.9)	151 (67.4)	1.18 (0.7-2.0)	0.517[2]
Rural residence	101 (91.8)	199 (88.8)	1.59 (0.65-3.98)	0.273[2]
Vomiting	36 (32.7)	93 (41.5)	0.70 (0.43-1.13)	0.151[2]
Diarrhoea	2 (1.8)	14 (6.2)	0.28 (0.06-1.26)	0.062[2]
Rashes	8 (7.2)	31 (13.8)	0.49 (0.22-1.12)	0.088[2]
Swelling	15 (13.6)	44 (19.6)	0.65 (0.34-1.24)	0.198[2]
Hepatomegaly	26 (23.6)	61 (27.2)	0.84(0.49-1.43)	0.539[2]
Splenomegaly	3 (2.7)	19 (8.5)	0.3 (0.07-1.01)	0.046[2] *
Bleeding episode	8 (7.2)	23 (10.3)	0.69 (0.30-1.61)	0.401[2]

Table 2: Demographic and non-neurological clinical manifestations in JE positive and negative patients. 1: Student '*t*' test; 2: Chi square test. SD: Standard Deviation; OR : Odds Ratio; 95%CI : 95%Confidence intervals; *: significant.

Discussion

Earlier studies on JE in the last century did not report non neurological findings as neurological manifestations predominate throughout the illness. This study is an attempt to focus on non-neurologic findings like thrombocytopenia, bleeding, splenomegaly and liver and renal functions in JE and also compare with non JE AES patients seen in our hospital.

We followed the standard WHO definition of AES for screening patients and then excluded patients with frank hepatic encephalopathy and those with other diagnoses. IgM estimation was used for diagnosis of JE as it is convenient, simple and useful method with high sensitivity and specificity especially in CSF. Any patient with AES who was positive for anti JEV IgM in serum or CSF was considered as JE. However, IgM in CSF may be absent in the first week of illness in upto 30% of cases [10] and some JE patients who reach the hospital early may be negative for anti JEV IgM. So, as recommended [11]. we retested for IgM after one week and our non JE group was defined as those patients in whom the anti JEV IgM was negative when tested 7 days or more after onset of illness. The comparisons were made between JE and non JE groups and the remaining patients were excluded from the analysis.

Comparison between JE and non JE patients shows that platelet counts, liver enzymes and INR were significantly more deranged in non JE. This may be explained by the fact that our non JE group is probably a mixed bag of other viral meningoencephalitides as well as tropical disorders like dengue, severe malaria, enteric encephalopathy etc. which are known to show such derangements. In particular, dengue infection in this region often presents as encephalopathy [12]. In the only similar study, Rayamajhi et al. [13] presented a comparison of neurological and non-neurological manifestations and sequelae in JE and non JE patients seen in Nepal and found significantly higher mean serum aspartate aminotransferase (sAST) levels in non JE patients. Proportion of patients with elevated serum creatinine was also significantly higher in their non JE group.

Japanese Encephalitis Virus (JEV) is an enveloped RNA virus which belongs to the genus Flavivirus within the family *Flaviviridae.* This family also includes other genera like dengue and yellow fever viruses. The recently noticed abnormalities in platelet counts and liver functions in JE patients could mean that either i) these findings were not actively looked for in earlier studies and were missed or ii) there has been a change in the virulence of the virus or strain variation over time, with the virus developing properties similar to related flaviviruses like dengue which has hepatotropic properties. The latter possibility is supported by studies conducted by Chen et al. [18] which have shown a shift or change towards hepatic tropism in JE virus YL vaccine strain, different from other virulent strains which have neural tropism. Host genetic characteristics and environmental factors could also play some role. A recent study by Agrawal et al. [19] showed isolation of JE virus from kidney, liver and spleen in mouse models and showed infection of epithelial, primary endothelial and hepatocytes by JE virus.

It could also be argued that JE and other flaviviruses like dengue have cross reacting antibodies and our JE group is contaminated with some dengue patients. However, we have used ELISA test in CSF for anti JEV IgM for diagnosis, which is held to be more specific [10]. Specificity of the National Institute of Virology kit in differentiating these infections is high [9].

Renal functions were also deranged in this as well as several previous studies. This may be due to dehydration due to poor intake in an unconscious patient as repeat levels of urea and creatinine were normal or progressed towards normal in all patients.

Investigational parameter	JE positive	JE negative	P-value
Mean Hemoglobin (g per 100 ml)[1]	10.6 ± 1.8	10.6 ± 2.2	0.860[1]
< 10 (g per 100 ml)[2]	35/107 (32.7)	80/222 (36.0)	
Total leucocyte count (cell/mm^3)[1]	15979.4 (13589,6)	13501.5 ± 8336.9	0.257[1]
>11,000 (cell/mm^3)[2]	60/106 (56.6)	121/220 (55.0)	
Polymorphs in blood (percentage)[1] >75(percentage)[2]	66.6 ± 12.7 26/106 (24.5)	68.6 ± 15.7 79/219 (36.1)	0.082
Platelets (cell/mm^3)[1] <100,000(cell/mm^3)[2]	187566 ± 207098 21/103 (20.4)	149536 ± 113885 70/204 (34.3)	0.017[1*]
Serum Bilirubin (mg /100 ml)[1] >1 (mg per 100 ml)[2]	0.76 ± 0.36 21/89 (23.6)	0.95 ± 0.96 43/202 (21.3)	0.108[1]
Serum AST (U/L)[1] > 40 (U/L)[2] >100(U/L)[2]	70.2 ± 40.5 53/71 (74.6) 15/71 (21.1)	132.6 (217.3) 153/190 (80.5) 75/190 (39.5)	0.010[1*]
Serum ALT (U/L)[1] > 40 (U/L)[2] >100(U/L)[2]	57.0 (34.1) 53/85 (62.3) 3/85 (3.5)	116.9± 300.6 155/205 (75.6) 55/205 (26.8)	0.000[1*]
Serum protein (g /100 ml)[1] < 6(g per 100 mL)[2]	7.65 ± 7.1 11/75 (14.6)	7.24 ± 5.8 38/160 (23.7)	0.204[1]
Serum albumin (g /100 ml)[1] < 3(g /100 ml)[2]	3.43 ± 0.74 18/75 (24.0)	3.27 ± 0.7 49/157 (31.2)	0.098[1]
International normalised ratio (INR)[1] >1.5[2]	1.13 ± 0.34 3/49 (6.1)	1.33 ± 0.64 17/108 (15.7)	0.015[1*]
Serum Urea (mg per 100 ml)[1] >40.0 (mg per 100 ml)[2] >50.0 (mg per 100 ml)[2]	39.9 ± 15.6 36/104 (34.6) 20/104 (19.2)	44.2± 22.9 97/221 (43.9) 60/221 (27.1)	0.263[1]
Serum Creatinine (mg%)[1] >1.0 (mg%)[2]	1.04 ± 0.84 33/105 (31.4)	1.08 ± 0.75 72/221 (32.6)	0.716[1]
Serum Sodium (mmol/L)[1]	134.4 ± 6.0	135.5 ± 7.5	0.175[1]
Serum Potassium (mmol/L)[1]	3.8 ± 0.7	3.9 ± 0.7	0.177[1]

Table 3: Non neurological investigational parameters in JE positive and negative patients. 1: Mean ± standard deviation; Student *t* test applied; 2: Number (percentage) of patients; * significant.

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