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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

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A collaborative investigation of hospitalized encephalitic children in south India, between July 2002 and February 2003, has indicated that Japanese encephalitis was confirmed in 27.3% of these children. In developing countries, assessment of actual Japanese encephalitis disease burden requires strengthening of diagnostic laboratory capacities at hospitals.

Japanese encephalitis (JE) is a serious pediatric public health problem in a few areas of southeast Asia (1). In India, sporadic cases of JE are known to occur in children in some provinces (1, 2). In Tamil Nadu (southern India), though JE is highly endemic in some districts (Cuddalore), the actual JE-related morbidity and mortality remain to be assessed due to lack of a surveillance system. A prospective study in some villages of this district revealed high rates of seroconversion in sentinel pigs against flaviviruses and high vector densities with high infection frequency for JE virus (JEV) (minimum infection rate for JEV = 10.4%). Despite the fact that the estimated probability of a child receiving an infective mosquito bite during the JEV transmission season was reported to be between 0.50 and 0.75, the number of JE cases reported to the local hospitals was low and thus did not reflect the actual disease burden. This information is necessary to identify vulnerable areas and the target population to be protected.

It was understood that most of the acute encephalitis syndrome (AES) case patients (patients with encephalitis and related central nervous system disorders) were attending the two nearby referral hospitals (Rajah Muthiah Medical College and Hospital [RMMCH], Chidambaram, and Jawaharlal Institute of Postgraduate Medical Education and Research [JIPMER], Pondicherry) for want of better treatment facilities. Between July 2002 and June 2003, a collaborative study was undertaken to estimate the number of pediatric AES cases of JE etiology in patients attending the hospitals. The study was interrupted in March 2003. Therefore, we analyzed AES case patients reporting between July 2002 and February 2003. We also investigated whether these cases represent the areas of JE endemicity in and around this district.

Both the hospitals are 1,000-bed teaching hospitals, where a provisional diagnosis of JE has been arrived at based mainly on the clinical manifestations. These peripheral hospitals are not equipped with enough laboratory diagnostic facilities for JE and thus tend to underestimate the disease rate. Therefore, during the study period, clinical specimens collected from the AES children were transported under cold conditions to the Center for Research in Medical Entomology (CRME) (200 km away) and tested for JEV infections by using a panel of diagnostic tests for JE (Table 1).

Between July 2002 and February 2003, a total of 58 hospitalized children (42 from RMMCH and 16 from JIPMER) (0 to 15 years of age) with AES (clinical criteria consisting of three or more of the following symptoms: headache, vomiting, seizure, coma, motor deficit, and neck rigidity) were investigated for JE etiology. Details of clinical data, results of laboratory investigations of clinical specimens (cerebrospinal fluid [CSF], blood, urine, etc.), clinical events, the treatment given, and the prognosis of the patients during the hospitalization were recorded. We could not collect paired sera from all the cases due to early deaths of patients within 72 h of admission or the patients being taken home against medical advice.

Since the patients admitted were in different clinical phases, CSF and sera were analyzed at CRME for JE confirmation by five laboratory tests, namely, (i) JEV antigen detection in CSF by immunofluorescence assay (IFA), (ii) JEV-specific antibody detection in CSF, (iii) JEV-specific antibody detection in serum by immunoglobulin M antibody capture (MAC) enzyme-linked immunosorbent assay (ELISA), (iv) virus isolation from CSF in *Toxorhynchitis splendens* larvae (insect bioassay; toxo-IFA) (1), and (v) virus genome detection in CSF by reverse transcriptase PCR (RT-PCR) (5). A patient was declared pos-

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TABLE 1. Laboratory diagnosis of JE among patients with AES hospitalized at JIPMER and RMMCH between July 2002 and February 2003[a]

Sample no.	Patient no.	Serum, MAC ELISA	CSF			
			Cell IFA	MAC ELISA	Toxo-IFA	RT-PCR
1	CDM-4	−	−	+	−	−
2	CDM-5	−	−	−	−	+
3	CDM-7	−	−	−	+	+
4	CDM-9	−	−	−	−	−
5	CDM-13	−	−	−	−	−
6	CDM-14	−	−	−	+	−
7	CDM-15	−	−	−	+	+
8	CDM-20	−	+	−	+	+
9	PY-1	−	+	+	+	+
10	PY-2	−	−	−	−	−
11	PY-3	+	+	+	+	−
12	PY-4	−	−	−	−	−
13	PY-5	−	−	−	−	−
14	PY-6	+	−	+	−	−
15	PY-8	−	−	−	−	−
16	PY-9	−	−	−	−	−
17	PY-10	−	−	−	+	+
18	PY-12	+	−	+	−	−
19	PY-13	−	+	−	+	+

[a] A total of 19 patients were examined by all five tests. CDM, RMMCH, Chidambaram; PY, JIPMER, Pondicherry; +, diagnosed as JE by the test; −, negative by the test.

itive by clinical and laboratory result criteria (1). Out of the 58 study subjects, we could get CSF and sera from 37, sera alone from 11, and CSF alone from 10 patients.

Out of 58 AES patients enrolled in the study, a total of 19 patients were examined by all five tests, and the results from these assays are presented in Table 1. The JE confirmation by different assays varied. CSF samples from 47 patients were tested by cell IFA, insect bioassay, and CSF MAC ELISA, and 35 samples were tested by RT-PCR assay. Serum samples from 38 patients were analyzed by MAC ELISA. The positivity rate of each assay with different denominators is shown in Table 2. Out of the tests used here, RT-PCR and virus isolation by insect bioassay have shown higher positivity rates, indicating their superior sensitivity (1, 5). However, the insect bioassay is cumbersome and time-consuming and requires maintaining a host mosquito colony. Studies elsewhere have reported a higher positivity rate for CSF MAC ELISA (3). It is likely that the patients reported to the hospitals in the initial stages of the infection before the appearance of anti-JE immunoglobulin M (IgM) antibodies. Due to the nonavailability of serum specimens from 30% of the JE-confirmed patients, results for anti-JE IgM antibodies in serum were not considered.

Few samples positive by toxo-IFA, cell IFA, and CSF MAC ELISA (Table 1) showed negative results with RT-PCR. This may be explained by the likelihood that the viral RNA had been destroyed by RNases (no RNase inhibitors were added in the CSF samples) or may be due to a low virus titer in the sample. Toxo-IFA, a biological system, can amplify the virus even at low titers. However, it remains to be elucidated whether it is possible to detect JEV RNA in CSF along with anti-JE IgM antibodies. Considering the advantages and disadvantages of each assay and the different clinical categories of the patients, it would be ideal to apply at least a minimum of two laboratory tests (one for detection of viral antigen-viral genome and one for detection of anti-JE IgM antibodies) to confirm JE diagnosis in the hospitals.

JE was diagnosed in 17 (29.3%) out of 58 pediatric AES cases. Nearly half (7 of 16, 43.75%) and one-quarter (10 of 42, 23.8%) of these AES-JE children were reporting from JIPMER and RMMCH, respectively. In a recent study carried out in Pondicherry, South India, JE was confirmed in 70.7% (212 of 300) of pediatric patients hospitalized with AES, and all these cases were coming from Tamil Nadu districts bordering Pondicherry (4). In comparison, we report a lower incidence of JE (43.75%, 7 of 16) in Pondicherry. However, we investigated only 16 AES patients from Pondicherry.

The JE incidence recorded was highest among the children aged 3 to 8 years. In our earlier studies, the attack rate of JE was highest among the children aged 4 to 5 years (2). However, the number of cases decreased with rising age in this study, which is in accordance with our earlier observations (2). The distribution was equal between the sexes. The seasonal distribution of AES-JE cases has shown (Fig. 1) that JE occurrence appears to be perennial, with a peak in the month of December. In general, the distribution of AES cases followed that of JE cases, suggesting that JE may constitute a major component of hospitalized children with AES reporting to both these referral hospitals. Next to JE, tuberculosis was most often reported to be responsible for pediatric AES of non-JE etiology. Analysis of the locations of these cases revealed that all these cases represent provinces in (10 of 17, 59%) and around Cuddalore District near these hospitals, where JEV activity has been demonstrated in vector mosquitoes and in animal hosts.

Our investigation revealed that 29.3% (17 of 58) of pediatric patients with AES were confirmed as having JE. Although

TABLE 2. Positivity rate (percent) of diagnostic assays for JE

Type of positivity rate[a]	No. positive/no. total (%)				
	Serum, MAC ELISA	CSF			
		Cell IFA	MAC ELISA	Toxo-IFA	RT-PCR
A	3/38 (7.9)	6/47 (12.8)	6/47 (12.8)	9/47 (19.1)	11/35 (31.4)
B	3/12[b] (25)	6/17 (35.3)	6/17 (35.3)	9/17 (52.9)	11/17 (64.7)
C	3/12 (25)	4/12 (33.33)	5/12 (41.67)	8/12 (66.7)	7/12 (58.3)

[a] A, positivity rate (%) (number positive by the test/number of encephalitis patients examined [$n = 58$]) × 100. B, positivity rate (%) = (number positive by the test/number positive by any test [$n = 17$]) × 100. C, clinical specimens from a total of 19 AES patients were examined by all five tests (for details please see Table 1). Positivity rate (%) = (number positive by the test/number positive [out of 19] by any test) × 100.

[b] Sera were not available from five patients.

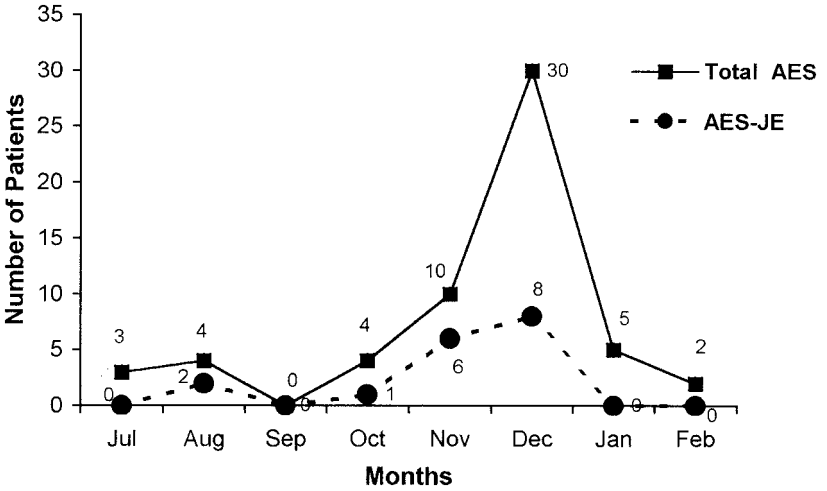

FIG. 1. Monthwise distribution of pediatric AES and AES due to JE infection seen in JIPMER and in RMMCH between July 2002 and February 2003.

surveillance data collected on JE in Tamil Nadu, India, are limited, our study suggests that the incidence of JE was high among hospitalized children with AES in JIPMER and RMMCH. All these JE cases belonged to areas of JE endemicity in and around Cuddalore District, Tamil Nadu. The clinical outcome report from JIPMER stated that one-third of these children (35.86%) died (4) and two-thirds (63.44%) survived (Pondicherry) (6). JEV infections in children may pose a serious public health problem in some districts of southern India, considering the proportion of JE infections among the hospitalized pediatric AES cases, the widely scattered areas with JE vector potentials and animal reservoirs, and the numbers of the susceptible pediatric population

In general in developing countries, nonreporting of cases from areas of JE endemicity to nearby small hospitals does not rule out JE occurrence, since the clinical cases may be reported to the nearby referral hospitals in view of the seriousness of the disease. Therefore, in order to know the true incidence and to reduce JE-related mortality in children in areas of JE endemicity, it will be ideal to (i) strengthen diagnostic facilities (inclusion of more than one diagnostic test for JE) and management capabilities for JE in the peripheral hospitals and (ii) also maintain contact with the nearby referral hospitals to know the particulars and locations of JE cases. This will help to identify JE-prone areas where control measures can be intensified.

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