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LETTER TO THE EDITOR

Acute encephalitis syndrome in Gorakhpur, Uttar Pradesh, India – Role of scrub typhus

Sir

With reference to the recent communication to this Journal by Ranjan and colleagues,[1] it is notable that outbreaks of acute encephalitis syndrome (AES) with high fatality have been occurring in Gorakhpur division, Uttar Pradesh, India since several years. These outbreaks occur during rainy season, peak during August–September and predominantly affect children aged ≤14 years.[1–3] Annually, approximately 1500–2000 AES patients get admitted to BRD Medical College (BRDMC), Gorakhpur - the only tertiary care hospital in the region, with a case-fatality of 20–25%.[3] In the past, AES patients have been investigated for viral and non-viral etiologies including Japanese encephalitis (JE), herpes simplex, enteroviruses, Chandipura, measles, mumps, dengue, varicella, Parvovirus, West Nile, malaria, and typhoid.[1–4] However, except for a small contribution (<10%) from JE, the etiology of AES has largely remained unknown. We investigated AES patients to explore role of scrub typhus (ST).

We enrolled 370 AES patients (defined as acute onset of fever and change in mental status and/or new onset of seizures, excluding simple febrile seizures[5]) and 109 patients of acute febrile illness (AFI; defined as fever of ≤2 weeks duration, without localizable signs) admitted at BRDMC during September–October 2015 (Table 1). Blood samples were collected from these patients (1 ml in EDTA, 2 ml in plain tube without anticoagulant for serum). EDTA blood was centrifuged; buffy coat was applied on four spots of Whatman FTA classic card (GE Healthcare, UK) and air-dried. DNA was extracted from one spot using Qiagen protocol.[7] A quantitative real-time PCR for 47 kDa gene was performed using primers, probes and protocol described by Jiang et al. and validated by Kim et al.[8,9] A cycle threshold value ≤38 was considered positive. All samples were tested in duplicate and ST positives were repeat-tested. DNA quality and sample integrity was tested by performing RNase P qPCR.[10] Sera were tested for IgM antibodies against *Orientia tsutsugamushi* (OT) using commercial ELISA (Scrub Typhus Detect, InBios International Inc., Seattle, USA). In the absence of any data about antibody titers in healthy individuals from the local area, an OD value of >0.5 was considered as positive.[6]

Of the 370 AES patients, results of CSF examination were available for 240. Out of these 240 patients, 222 had CSF pleocytosis (>5 cells/cmm) indicating CNS inflammation (meningitis and/or encephalitis). The clinical and laboratory features of these patients with CSF pelocytosis are described below. About half of these patients (54.1%) were hospitalized in other health facilities for a median duration of 2 days (IQR: 1–3) prior to admission to BRDMC. The reported median duration between fever onset and hospitalization at BRDMC was 7 days (IQR: 5–10). Other symptoms included seizures (68%), vomiting (59%), altered sensorium (56.3%), up-rolling of eyes (54.5%), headache (36.5%), twitching of face (31.1%), abdominal pain (27%) and diarrhea (13.1%). The median interval between fever onset and CNS manifestations was 6 days (IQR: 4–9). The salient findings on physical examination included hepatomegaly (54.1%), conjunctival congestion (50%), cervical and/or inguinal lymphadenopathy (46.9%) and peri-orbital edema (44.6%). Seventeen had maculo-papular rash, while two had eschar. The common hematological and biochemical abnormalities are summarized in Table 2. The patients were managed with supportive therapy including anti-epileptic drugs, inotropes, and mechanical ventilation, as required. Ceftriaxone (61.3%) and azithromycin (41.4%) were the commonly used antibiotics. Thirty-six AES patients died (CFR:16.2%).

Of the 370 AES patients, 365 were tested for IgM antibodies against OT and 229 (62.7%) were positive (Table 1). Antibody positivity was not different by age-groups (<1 year: 17/25 = 68%; 2–5 years: 95/146 = 65.1; 6–14 years: 105/169 = 62.1%; ≥15 years: 12/25 = 48%; $\chi^2 = 2.98$, $p = 0.38$) and sex (female: 109/166, 65.7%; male: 120/199, 58.8%, $\chi^2 = 1.11$, $p = 0.29$). Six (1.6%) of the 370 patients were positive for ST-PCR.

Among the 109 hospitalized AFI patients, 59 (54.1%) and four (3.7%) were positive for IgM antibodies against OT and ST-PCR, respectively. The quality of DNA was adequate, with RNase P qPCR positive in 98.1% of samples from AES patients and 97.2% of samples from AFI patients.

The presence of IgM antibodies against scrub typhus in 63% of patients with AES and 54% of patients with AFI suggests that scrub typhus could be an important etiology for AES and AFI. This is also supported by the presence of multisystem involvement among the patients. Similar findings of high IgM positivity for OT were observed during an

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Table 1 Age and sex distribution of AES and AFI case-patients and results of laboratory investigations for scrub typhus, Gorakhpur division, Uttar Pradesh, India, 2015.

	Patients with acute encephalitis syndrome (n = 370)	Patients with acute febrile illness (n = 109)
Age group (years)		
≤1	25 (6.8)	4 (3.7)
2–5	151 (40.8)	48 (44.0)
6–14	169 (45.7)	48 (44.0)
≥15	25 (6.8)	9 (8.3)
Median	6 years (IQR: 3.6–10)	6 years (IQR: 3–9)
Sex – Male	204 (55.1%)	54 (49.5)
District		
Gorakhpur	89 (24.1)	44 (40.4)
Kushinagar	85 (23.0)	18 (16.5)
Deoria	48 (13.0)	18 (16.5)
Maharajgunj	31 (8.4)	6 (5.5)
Siddharth Nagar	28 (7.6)	7 (6.4)
Others	89 (24.1)	16 (14.7)
Laboratory investigations for scrub typhus		
	No. positive/no. tested (%)	No. positive/no. tested (%)
	AES patients (n = 370)	AES patients with CSF pleocytosis (n = 222)	Patients with AFI (n = 109)
IgM scrub typhus (OD > 0.5)	229/365 (62.7)	135/219 (61.6)	59/109 (54.1)
Scrub typhus PCR	6/370 (1.6)	3/222 (1.4%)	4/109 (3.7)

Table 2 Hematological, biochemical and CSF investigations among AES patients with CSF pleocytosis (n = 222), Gorakhpur division, Uttar Pradesh, India, 2015.

Parameter		Number (%)
Total WBC count	>11,000 per cmm	138 (62.2)
SGOT (IU/L)	≤40	76 (34.2)
	41–120	73 (32.9)
	121–200	30 (13.5)
	>200	43 (19.4)
SGPT (IU/L)	≤56	130 (58.6)
	57–168	65 (29.3)
	>168	27 (12.2)
Serum urea (mg/dl)	≤20	39 (17.6)
	21–40	166 (74.8)
	>40	17 (7.7)
Serum creatinine (mg/dl)	≤1.3	209 (94.1)
	1.4–2.6	5 (2.3)
	>2.6	8 (3.6)
Blood sugar	<70	0
CSF appearance	Clear	214 (96.4)
	Turbid	8 (3.6)
CSF cell count (per cmm)	6–10	100 (45.0)
	11–100	103 (46.4)
	>100	19 (8.6)
% Mononuclear cells	>50%	209 (94.1)
CSF proteins (mg/dl)	≤45	88 (39.6)
	46–100	87 (39.2)
	>100	47 (21.2)

earlier investigation conducted on AES patients in BRDMC in 2014 (Arunkumar G, unpublished data) as well as retrospective analyses of samples collected during 2009, 2011 and 2014 outbreaks (Gupte MD, unpublished data).

However, certain findings are not consistent with scrub typhus as the main etiology of AES. First, the PCR positivity among AES and AFI patients was very low. PCR positivity is affected by the time of sample collection, prior use of antibiotics and sample quality. The sensitivity of PCR starts falling by 8 days after onset of disease.[11] In our study, 65% (238/370) of the AES patients and 83% (90/109) AFI patients were hospitalized within 8 days of fever onset. Patients received azithromycin only after admission to BRDMC. The sample quality in our study was adequate, as we could amplify RNase P gene in most samples tested. Very low PCR positivity therefore could not be solely explained on account of delay in sample collection, prior antibiotic administration or poor sample quality. Second, the eschar – which is pathognomonic of ST – was observed in only two patients.[12] Third, the age distribution of AES cases observed in Gorakhpur is not typical of ST, with most AES patients being aged ≤14 years; about half of them aged ≤5 years. Fourth, the response to azithromycin among AES patients with CSF pleocytosis was not dramatic, with case-fatality among patients treated with azithromycin (13/92, 14.1%) not different that those untreated (23/130, 17.7%, p = 0.48). However, the lack of dramatic response to azithromycin may be because the patients received the drug at the later stage of illness after CNS involvement.

In conclusion, the presence of IgM antibodies in about 63% of patients with AES suggests a role for scrub typhus in the etiology of AES in the Gorakhpur region. The presence of features of multisystem involvement also supports this.

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However, very low PCR positivity, low prevalence of eschar, age distribution of cases, and lack of dramatic response to azithromycin cast doubt on this assumption. Further, lack of data on IgM antibody titers in healthy population of children and adults makes interpretation of serological tests difficult. Further studies need to focus on detecting children with AES and AFI early in the course of illness and identify the relative contribution of scrub typhus, using both serology and PCR. Serosurveys to estimate the sero-prevalence of scrub typhus in adult and pediatric population as well as entomological studies to identify the reservoirs and vectors in the Gorakhpur region are also needed.

Conflict of interest

Nil.

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