International Journal of Infectious Diseases (2010) **14**, e141—e146

http://intl.elsevierhealth.com/journals/ijid

Etiology and clinico-epidemiological profile of acute viral encephalitis in children of western Uttar Pradesh, India

Farzana K. Beig [a], Abida Malik [b,*], Meher Rizvi [b], Deepshikha Acharya [a], Shashi Khare [c]

[a] *Department of Paediatrics, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India*
[b] *Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India*
[c] *Department of Microbiology, National Institute of Communicable Diseases, New Delhi, India*

Received 10 May 2008; received in revised form 22 December 2008; accepted 31 March 2009
Corresponding Editor: William Cameron, Ottawa, Canada

KEYWORDS
Viral encephalitis;
Enterovirus 71;
Measles;
Mumps;
Varicella zoster virus;
Herpes simplex virus

Summary
Objectives: To study the etiology of viral encephalitis (VE) in the children of western Uttar Pradesh, India and to assess the clinico-epidemiological profile of these children in relation to VE.
Methods: Both cerebrospinal fluid and serum samples were collected from pediatric patients suffering from encephalitis hospitalized at Jawaharlal Nehru Medical College, Aligarh from July 2004 to November 2006. Viral isolation was done on RD cells, HEp-2 cells, and Vero cells from the cerebrospinal fluid samples of children with suspected VE. A microneutralization test was performed for enterovirus 71. An enzyme immunoassay for IgM antibodies was performed for measles virus, mumps virus, varicella zoster virus, herpes simplex virus 1, and Japanese encephalitis virus.
Results: Eighty-seven patients were enrolled in the study. The most common etiology of VE was enterovirus 71 (42.1%), followed by measles (21.1%), varicella zoster virus (15.8%), herpes simplex virus (10.5%), and mumps (10.5%). Japanese encephalitis virus was not found in any case. Enterovirus 71 infection caused significant morbidity in children; mortality occurred in 50%. A preponderance of cases occurred in December. In our study generalized convulsions along with altered sensorium were the significant findings in patients with VE.
Conclusions: Enterovirus 71, the major etiology of VE in our study, was associated with significant mortality and morbidity. Such studies should be conducted frequently to assess the role of emerging VE in different regions.

Introduction

Acute viral encephalitis (VE) is often an unusual manifestation of common viral infections and most commonly affects

* Corresponding author. Tel.: +009837007606; fax: +91 0571 2704498.
E-mail address: abidamalik2k@yahoo.com (A. Malik).

1201-9712/$36.00 © 2010 Published by Elsevier Ltd on behalf of International Society for Infectious Diseases.
doi:10.1016/j.ijid.2009.03.035

children and young adults; it can lead to considerable morbidity and mortality. Epidemiologic studies estimate the incidence of VE at 2.5—8.8 per 100 000 persons per year.[1,2] However the annual incidence of VE is most likely underestimated, especially in developing countries where there are problems with pathogen detection. Very few studies of VE have emanated from India, although it can be considered the epicenter of many emerging viral diseases.

Every day new viruses are being associated with encephalitis of varying severity. It would be useful to harbor a strong clinical suspicion for an unusual viral etiology in cases of both sporadic as well as epidemics of encephalitis. Japanese B encephalitis virus (JEV) is an emerging pathogen in North India and has entrenched itself firmly in the eastern parts of Uttar Pradesh. This study was undertaken to assess the viral etiology and epidemiology of acute encephalitis in children in western Uttar Pradesh. As a microbiological diagnosis of VE is not always possible in India, we attempted to identify surrogate markers for the diagnosis of VE. These included clinical signs and symptoms, radiological and cytological findings, seasonal variation, socioeconomic status, and other demographic variables. No such study has been conducted in this region, and so far no case of Japanese encephalitis has been reported from this area.

Materials and methods

This was a prospective cohort study of consecutive patients with acute febrile encephalopathy syndrome conducted at the Department of Paediatrics in collaboration with the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital and the Department of Microbiology, National Institute of Communicable Diseases (NICD) from July 2004 to November 2006. Patient age varied from 6 months to 12 years. Acute encephalopathy was defined as fever with alteration of consciousness and/or with neurological deficit, secondary to central nervous system involvement lasting more than 24 hours, and not more than a one-week history. Patients with a different final diagnosis (e.g., epilepsy, febrile convulsion, bacterial meningitis, tuberculosis, brain tumor, cerebral malaria, or metabolic disorder) were excluded from the study. Patients were managed symptomatically until a definite cause could be found.

A detailed clinical history was elicited and a rigorous systemic examination was performed. The outcome was noted, and any sequelae and residual neurological disability were observed and categorized. The investigations carried out were complete blood picture, peripheral smear for malaria parasite, and blood culture and sensitivity. Cerebrospinal fluid (CSF) examinations were carried out for viral isolation and serology, bacterial culture and sensitivity, cytology, and blood urea and creatinine. A computed tomography (CT) scan of the head was done whenever indicated. Both CSF and serum serology were done for the detection of antibodies to herpes simplex virus 1 (HSV-1), measles, mumps, varicella zoster virus (VZV), and JEV. Acute phase serum was used for the detection of IgM antibodies to enterovirus 71 (EV-71). The NICD, New Delhi monitors the nationwide etiology of VE and maintains a national registry of baseline and significant cut-off titers for serological diagnosis of encephalitis from acute phase serum. A positive case was defined as one that had a cut-off well above the baseline titer and varied according to the virus concerned. In the case of EV-71, on the basis of the national registry, the baseline titer was $\leq$32, while a titer $\geq$64 was taken as the positive cut-off.

Specimen collection

Acute phase samples of CSF and serum were collected with proper aseptic precautions from all patients. CSF was transported on ice and stored at $-70\,^{\circ}C$ until processed.

Viral isolation

All CSF specimens were inoculated into RD cells, HEp-2 cells, and Vero cells. Detection of viral growth in the cell lines was done by the characteristic cytopathic effect of some viruses.

Each tube of recently monolayered cultures was inoculated with 0.2 ml of specimen and incubated in the stationary sloped position at 36 °C. Observations of cytopathic effect (CPE) were recorded daily for 4 days. CPE was allowed to develop until 75% of the cells were affected. Negative cultures were discarded after 14 days.

Viral serology

A microneutralization test was performed for EV-71. IgM antibodies for HSV-1, measles virus, mumps virus, VZV, and JEV were estimated by capture ELISA as per the manufacturer's instructions (EIAgen Herpes Simplex Virus IgM kit supplied by Adaltis, IgM ELISA classic kit supplied by Virion, Parotite IgM EIA well kit supplied by Radim, Varicella Zoster kit supplied by Adaltis, and IgM antibody capture ELISA provided by the National Institute of Virology, Pune, India). Samples were considered positive for any of the viruses including HSV-1 when the optical density was above the cut-off specified by the manufacturer.

Wherever feasible both acute and convalescent sera were collected from the patient if the initial serological diagnosis was of EV-71.The background seropositivity of EV-71 is routinely monitored by the NICD so that distinction of acute from past infection is not a problem even if a single acute phase serum sample is tested.

Patient sera were inactivated at 56 °C for 30 min. A 1/8 dilution of each test serum was prepared in serum diluent; 0.025 ml of diluent was added to all wells except row A. A similar volume was added to another plate for back titration. Test sera — 2 × 0.025 ml of 1/8 dilution — were added to row A only of the test serum plates. Serial two-fold dilutions of the test sera were made until the serum dilutions ranged from 1/8 to 1/1024. Virus antigen — 0.025 ml of 100 $TCID_{50}$ (tissue culture infective dose) — was added to all wells in the test serum plate. Back titration of the virus was prepared.

The serum plates, virus back titration plates, standard serum control plates, and the cell control plate were wrapped in tin foil. These plates were gently shaken to ensure adequate mixing of the container and then incubated at 36 °C for 3 h in CO_2. Subsequently 0.1 ml of HEp-2 cell suspension was added to all the plates. They were then incubated at 36 °C in a CO_2 incubator for 5 days.

After 5 days the test was read microscopically and plates were stained with crystal violet. The presence of stained cells meant antibody was present or that there was normal

Table 1 Diagnosis of viral etiology

Viral agent causing encephalitis		Serology		Cell culture
	Total positive	Positive in CSF	Positive in acute serum	
Herpes simplex virus 1	2	2	2	0
Varicella zoster virus	3	3	2	0
Measles	4	4	4	0
Mumps	2	2	2	0
Enterovirus 71	8	-	8	0
Japanese encephalitis virus	0	0	0	0

growth of cells. The absence of stained cells meant that antibody was absent and that virus grew. The end point of neutralization was calculated using the Kärber formula, log 50% neutralization titer (log $CCID_{50}$ (cell culture infective dose)) = $TCID_{50}$ = L − d (S − 0.5), where L = log of lowest dilution used in the test, d = difference between log dilution steps, and S = sum of proportion of positive tests (i.e., cultures showing CPE).

The serum dilutions were transcribed into log terms. The serum antibody titer is the highest dilution of serum that protects 50% of the cultures against 100 $TCID_{50}$ of the challenge virus. Antibody titers were expressed as reciprocals.

Results

The study group comprised a total of 87 patients with a mean age of 4.35 ± 3.32 years, ranging from 6 months to 12 years. The median age was 3.5 years and the male to female ratio was 1.27:1. Almost two thirds of the cases of acute encephalopathy (n = 61 (70.1%)) were from Aligarh and the rest were from neighboring districts. Sixty-four (73.6%) belonged to the lower socioeconomic class according to the Kuppuswamy scale.[3] CSF and acute serum samples were collected for all these cases. Convalescent sera were collected from four cases.

A viral etiology was diagnosed in a total of 19 (21.8%) cases (Figure 1). Among these, the most common etiological agent identified was EV-71, which was found in eight (42.1%) cases, followed by measles in four (21.1%), VZV in three (15.8%), mumps in two (10.5%), and HSV-1 in two (10.5%). In all these cases the etiology was established by serology (Table 1). No virus was isolated in the cell lines in this study. EV-71 was detected by microneutralization test. Convalescent sera were positive in all the four surviving cases infected with EV-71. No Japanese B encephalitis virus was identified in this region.

A comparison of clinical features with viral etiology is given in Table 2. Generalized convulsions appeared to be

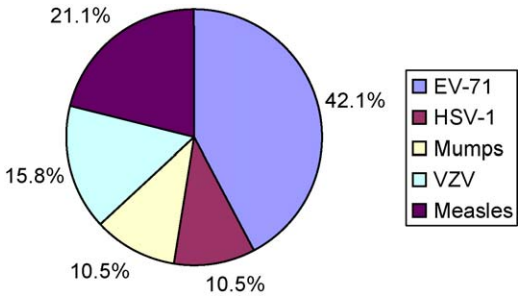

Figure 1 Viral etiology of encephalitis.

significantly associated with EV-71, measles, VZV, and HSV. No other clinical finding was significantly associated with VE. A skin rash was present in two patients, one with measles and the other with HSV infection. The other three cases of measles had a history of rashes when they presented at our center. Out of the two patients with mumps, one had neck swelling and both had a history of parotid swelling. The three patients diagnosed with VZV encephalitis gave a history of a centripetal distribution of dewdrop-like rashes, which subsided after a week or so. All three gave a history of chickenpox within the last 4—5 weeks. Patients diagnosed with EV-71 and HSV-1 gave non-specific histories. When a comparison was made between the above viral etiologies, differences between the epidemiological as well clinical features of the patients were not found to be statistically significant (p-values for age = 0.69, sex = 0.25, and clinical features = 0.17 (raised intracranial tension), 0.32 (meningeal signs and skin rashes), and 0.72 (neck swelling and focal convulsions)).

No diagnosis was possible in 34 (39.1%) cases. These cases were classified as patients with possible VE. Thus it can be presumed that there was a total of 53 patients with VE, 19 confirmed and 34 suspected cases.

There was a significant clustering of confirmed cases of VE in December (n = 13, 68.4%). On comparing the final diagnosis with socioeconomic status, the lower socioeconomic class was seen to be significantly associated with VE, $p < 0.001$.

The mean CSF cell count in the 53 patients was $<5 \times 10^6$ cells/l, mean CSF protein was 43.39 ± 33.39 mg/dl, and mean CSF glucose was 66.21 ± 25.21 mg/dl. No statistical significance was observed between CSF findings and VE.

A CT scan was done in 25 of the 53 patients with confirmed and suspected VE; six (17.6%) patients with unknown etiology had abnormal CT findings, while one case each of EV-71, HSV, and VZV had abnormal findings as shown in Table 3.

The mean age was lower in the unknown etiology group. Although death was more common in the unknown etiology group (35.3%) as compared to the known etiology group (26.3%), the difference was not statistically significant. The mortality in children infected with EV-71 was 50%. Convalescent sera were available in two cases with EV-71 infection, which showed a four-fold rise in titers in comparison to acute phase titers. Sequelae were more common in the known etiology group (10.5%) as compared to the unknown etiology group (5.9%) as seen in Table 4. Children with a Glasgow coma scale (GCS) score >12 had no sequelae, i.e., the outcome was good. When the day-to-day progress of symptoms was recorded, the patients who had a GCS of >12 on day 1, improved without any sequelae. When the

Table 2 Comparison of clinical features of patients with enterovirus, mumps, measles, herpes simplex virus, and varicella zoster virus infections

Clinical features	Number of cases				
	EV-71 (n = 8)	Mumps (n = 2)	Measles (n = 4)	HSV (n = 2)	VZV (n = 3)
Generalized convulsions	6 (75%)	1	4 (100%)	2 (100%)	3 (100%)
Focal convulsions	1	0	0	0	0
Meningeal signs	0	0	1	0	1
Raised ICT	0	1	1	0	1
Focal deficit	0	0	0	0	0
Cranial nerve palsy	0	0	0	0	1
Skin rashes	0	0	1	1	0
Malaise	0	0	0	0	0
Mild URTI	0	0	0	0	0
Neck swelling	0	1	0	0	0
Mean CSF cellularity, $\times 10^6$/l	<5	260	<5	<5	<5
Mean CSF protein, mg/dl	40	60	34	38	48
Mean CSF glucose, mg/dl	68	70	77	80	62
Death	4	0	1	0	0
Sequelae	0	0	0	1	1

EV-71, enterovirus 71; HSV, herpes simplex virus; VZV, varicella zoster virus; ICT, intracranial tension; URTI, upper respiratory tract infection; CSF, cerebrospinal fluid.

Table 3 Computed tomography features of patients with suspected viral encephalitis

Etiology	Normal CT (n = 16)	Abnormal CT (n = 9)	Abnormal CT finding	CT not done (n = 28)
Unknown	11	6	Loss of gray—white differentiation Hypodensity in basal ganglia Infarcts in fronto-temporal region Global ischemia with diffuse brain edema	18
EV-71	3	1	Hypodensities in thalamus and parietal lobes	4
Mumps	2	0	-	1
Measles	0	0	-	3
HSV	0	1	Hypodensity in basal ganglia	2
VZV	0	1	Prominence of temporal horns	0

CT, computed tomography; EV-71, enterovirus 71; HSV, herpes simplex virus; VZV, varicella zoster virus.

Table 4 Comparison of patients with suspected viral encephalitis with known etiology and unknown etiology

Parameter	Known viral etiology (n = 19)	Unknown viral etiology (n = 34)	p-Value
Mean age, years	4.39 ± 3.36	3.34 ± 2.85	0.042
GCS score			
<6	8	10	0.026
>6 to <14	7	22	
Death			
<48 h	3	3	0.003
>48 h	2	9	
Sequelae	2 (13.3%)	2 (6.3%)	0.069

GCS, Glasgow coma scale.

final outcome of patients with suspected VE was correlated with age, coma scale, and death it was found that the correlation was statistically significant (p-values of 0.042, 0.026, and 0.003, respectively). Correlation with respect to sex was found to be statistically insignificant (p-value of 0.375).

Discussion

The most common etiological agent of VE in our study was EV-71, which occurred in 42.1% of cases, followed by measles in 21.1% cases, VZV in 15.8%, HSV in 10.5%, and mumps in 10.5%. JEV was not found in any case.

HSV-1 is the most common cause of acute sporadic encephalitis in the USA. Owing to its ubiquitous worldwide distribution it should represent an important etiology of encephalitis in India too. However, data pertaining to its prevalence in India are lacking, thus making its inclusion in the study essential. Similarly VZV, measles, and mumps are important causes of encephalitis and their prevalence in this region is high, making them likely etiological agents of encephalitis. EV-71 is emerging as an important encephalitogenic virus. Given the poor sanitation services, overcrowding, and lack of awareness of good hygiene habits in this area we felt EV-71 could be an important but little sought for etiological agent in encephalitis. Since JEV has already established itself firmly in eastern Uttar Pradesh, surveillance to determine if it is gaining a foothold in western Uttar Pradesh is absolutely essential.

In a study conducted in Beijing on childhood encephalitis, enterovirus was most commonly identified (15.4%), followed by mumps (7.2%), rubella (6.1%), JEV (5.1%), human herpes virus (2%), and Epstein—Barr virus (1%).[4] In contrast, in a Thai study, dengue virus was identified in a maximum number of cases, followed by JEV, HSV, human herpes virus 6, enterovirus, VZV, and rabies.[5] In a European study conducted in Finland, varicella predominated (25%), followed by mumps, HSV, and measles.[6] In Slovenia, Central European tick-borne encephalitis topped the list with 28.8% of cases, followed by VZV (17%), HSV (10%), rubella (2.9%), mumps (2.3%), and measles (1.1%).[7] Thus a wide variation exists in the viral etiological agents across the globe and even in the same continent. Several factors such as age, geographic location, season, climate, and host immune competence affect the epidemiology of VE. However measles, mumps, and VZV appear to feature quite prominently in both Asian and European countries. Interestingly this is despite effective vaccination programs against mumps, measles, and rubella in the developed countries.

In our study, a preponderance of cases was noticed in December (68.4%). A similar variation was observed in a multicenter study in Finland where most cases occurred from September to January, with the lowest number seen from May to August.[8] However in other studies the highest rate of infection was seen in summer.[4,7] In another study no seasonal pattern was noticed.[5] Thus no clear pattern emerges. However in the Indian context, the index of suspicion should be higher in winter.

Generalized convulsions along with altered sensorium were the significant 'duo of clinical features' in our study in patients with VE. In other studies, focal neurological signs with alteration of consciousness have been the major findings.[2,5] A significant association was noticed among VE patients with age, GCS, and death. Of the patients with VE in our study, 32.1% died, which is higher than in other studies (17%, 1.2%).[5,9] The lower rate in these studies may be due to the higher incidence of VZV encephalitis.

This study used elevated levels of IgM antibodies to HSV-1 as an indicator of herpes simplex encephalitis. Given the poor Indian population this center caters for, the use of more expensive and sensitive molecular assays for HSV, like PCR, was not possible. It is possible that some cases of HSV-1 may have been missed by the enzyme immunoassay technique. However, since we detected IgM antibodies to HSV-1 in CSF, the sensitivity was proportionately higher. In resource-poor countries serology is still the more cost effective option.

Microneutralization testing for EV-71 though time consuming is cheaper than the more sensitive molecular tools. Since we used both acute and convalescent phase sera, the sensitivity for detection of EV-71 increased. In the cases that demonstrated a four-fold rise in titer, the evidence for acute EV-71 is excellent; however in the remaining EV-71 serologic positive cases, the serologic data are suggestive of EV-71, but not confirmatory.

Enteroviruses appear to be significant pathogens in the East as seen in the studies undertaken in Taiwan and China, as well as in our study.[3,10,11] The pathogenesis of EV-71 in causing encephalitis is not very clear. Some believe that particularly virulent strains of EV-71 lead to encephalitis and subsequent mortality. Others believe that it could be a case of hypersensitivity to EV-71 just like there is in dengue shock and dengue hemorrhagic syndrome.[10] Whatever the pathogenesis, we need to be aware of its capacity to cause large epidemics that may lead to death. EV-71 is emerging as a significant pathogen in this part of the country. It is usually associated with sporadic VE. In this study too, EV-71 was sporadic in nature. However it was quite virulent, as 50% of children infected with EV-71 died. A significant finding in this infection was generalized convulsions with altered sensorium.

Herpes simplex encephalitis remains the most common and important cause of fatal sporadic VE in the industrialized world.[12] However in the Indian as well as Asian context it does not appear to play a major role.

Japanese encephalitis, although a big public health problem in eastern Uttar Pradesh, has not made any inroads into our region of western Uttar Pradesh. However we need to be extremely vigilant because the primary host and vector are present in abundance here, not to mention a large susceptible human population. Vector control should be practiced stringently to prevent the onward march of JEV.

The microbiological diagnosis of VE is usually difficult. Despite using various cell lines, no virus could be isolated from the CSF. Similar results were reported in another study.[13] This is the first study of its kind from this region. EV-71 emerged as the major pathogen in our study. There is a need for more such studies to further delineate the etiologies of viral encephalitis.

Conflict of interest: No conflict of interest to declare.

References

1. Davison KL, Crowcroft NS, Ramsay ME, Brown DW, Andrews NJ. Viral encephalitis in England, 1989—1998: what did we miss? *Emerg Infect Dis* 2003;**9**:234—40.
2. Rantala H, Uhari M. Occurrence of childhood encephalitis: a population-based study. *Pediatr Infect Dis J* 1989;**8**:426—30.
3. Mishra D, Singh HP. Kuppuswamy's socioeconomic status scale—a revision. *Indian J Paediatr* 2003;**70**:273—4.
4. Xu Y, Zhaori G, Vene S, Shen K, Zhou Y, Magnius LO, et al. Viral etiology of acute childhood encephalitis in Beijing diagnosed by analysis of single samples. *Pediatr Infect Dis J* 1996;**15**(11): 1018—24.
5. Chokephaibulkit K, Kankirawatana P, Apintanapong S, Pongthapisit V, Yoksan S. Viral etiologies of encephalitis in Thai children. *Pediatr Infect Dis J* 2001;**20**:216—8.
6. Rautonen J, Koskiniemi M, Vaheri A. Prognostic factors in childhood acute encephalitis. *Pediatr Infect Dis J* 1991;**10**(6): 441—6.

7. Cizman M, Jazbec J. Etiology of acute encephalitis in childhood in Slovenia. *Pediatr Infect Dis J* 1993;**12**:903—8.
8. Koskiniemi M, Korppi K, Mustonen H, Rantala M, Herrgard E, et al. Epidemiology of encephalitis in children. A prospective multicentric study. *Eur J Pediatr* 1997;**156**:541—5.
9. Jemsek J, Greenberg SB, Taber L, Harvey D, Geshon A, Couch RB, et al. Herpes zoster-associated encephalitis: clinicopathologic report of 12 cases and review of the literature. *Medicine (Baltimore)* 1983;**62**:81—97.
10. Monto H, Chen ER, HSu KH, Twu SJ, Chan KT, Tsai SF, et al. An epidemic of enterovirus 71 infection in Taiwan. *N Engl J Med* 1999;**341**:929—35.
11. Chang LY, Huang YC, Lin TY. Fulminant neurogenic pulmonary oedema with hand, foot and mouth disease. *Lancet* 1998;**352**: 367—8.
12. Ho M, Chen ER, Hsu KH, Twu SJ, Chen KT, Tsai SF. An epidemic of enterovirus 71 infection in Taiwan. *N Engl J Med* 1999;**341**: 929—35.
13. Glaser CA, Gilliam S, Schnurr D, Forghani B, Honarmand S, Khetsuriani N, et al. In search of encephalitis etiologies: diagnostic challenges in the California encephalitis project, 1998—2000. *Clin Infect Dis* 2003;**36**:731—42;
Wong V, Yeung CY. Acute viral encephalitis in children. *Aust Pediatr J* 1987;**23**:339—42.