ORIGINAL ARTICLE

Role of Chandipura virus in an “epidemic brain attack” in Andhra Pradesh, India *

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Abstract

This is the first report of epidemic stroke and epidemic reversible ischemic neurological deficit. Objectives of this study were to confirm that strokes can occur in epidemics, to identify the association of any pathogen, to study its clinical characteristics, to study its pathology by neuroimaging, to know the arterial territory involved, to understand the effect of symptomatic treatment and to know if some ischemic cases are reversible. This is a cross sectional and case-control study. It included 55 stroke cases. It was performed in hospitals of Andhra Pradesh from 1st June 2003 to 12th August 2003. The cases were analyzed for age, sex, symptoms and signs, investigations done, treatment given and course of the disease. There was a 13-fold increase in the incidence of pediatric strokes. Diagnostic symptoms and signs included abdominal colic in 28 (50.91%), diarrhea without dehydration or dyselectrolytemia in 26 (47.27%), focal symptoms and signs in 33 (60%) and meningeal irritation signs in 0%. Cerebrospinal fluid, except for increased pressure, was normal in 100%. Computerized tomography revealed hypodensities restricted to middle cerebral artery territory. Twenty-eight

(50.91%) cases had evidence of Chandipura infection. Strokes do occur in epidemics. Though infection is the cause, etiologic role of *Chandipura virus* is doubtful. Enterovirus 71, Varicella and any other yet unidentified endotheliotropic virus should be investigated for. Epidemic stroke has characteristic features. Middle cerebral artery territory is involved. Early treatment of raised intracranial pressure significantly reduced Case Fatality Rate. Twenty-three (85.19%) of 27 survived cases recovered totally within 3 months. Two (7.41%) cases developed late onset refractory epilepsy and four (14.81%) continued to have hemiplegia after 8 months. (J Pediatr Neurol 2004; 2(3): 131-143).

Key words: Chandipura virus, Chandipura stroke, Chandipura encephalitis, epidemic brain attack, epidemic stroke, epidemic reversible ischemic neurological deficit, neuroepidemiology.

Introduction

Neuroepidemiology

Coma epidemics continue to pose considerable challenges to neurologists in establishing the diagnosis and unraveling the pathogenesis. During epidemics, mass hysteria of parents of hundreds of cases overloads the available few rural basic doctors. Media, bureaucratic, political and public criticism is always less if a diagnosis is made and something is done to show that doctors are taking all possible measures to contain the epidemic and treat the cases. This results in the tendency of medical personnel to label any acute epidemic coma as epidemic encephalitis or Reye's syndrome to tide over the crisis since other neurological diseases that can cause coma were never reported to have

* This article is dedicated to those children who succumbed to this new epidemic.

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presented in epidemic form. Epidemic encephalitic coma in India was reported only with Japanese encephalitis virus so far (1-3), though another unidentified virus causing epidemics of coma has long been suspected (1). There were more than 10 coma epidemics of undetermined etiology in India during the last 15 years.

The recent epidemic, reported as Chandipura encephalitis (4-6), was neither encephalitis nor the role of *Chandipura virus* (CHPV) confirmed. Reye's syndrome was suspected because of the rapid response to antiedema therapy (7), but was excluded. It was, in fact, an “epidemic of brain attacks” masquerading as an encephalitis epidemic, which played havoc in 10 districts of Andhra Pradesh, a state in India situated between 77°-84° East and 13°-19° North. Adjacent districts of the neighboring Maharashtra state were also affected. This is the first report of both epidemic brain attacks (EBA) or epidemic stroke and epidemic reversible ischemic neurological deficit reported in the middle cerebral artery (MCA) territory.

The term “brain attack” for stroke not only conveys urgency, but also builds on the public's understanding that just like a heart attack, stroke also requires emergency management (8). Cerebral ischemia initially causes cytotoxic and subsequently vasogenic edema (ischemic edema). Cytotoxic edema predominantly involves the gray matter and vasogenic edema predominantly involves the white matter. Wedge shaped hypodense area involving both gray and white matter indicates ischemic edema and follows vascular territory. Cytotoxic edema is caused by swelling of glia, neurons and endothelial cells and begins within minutes of the hypoxic insult. This is a result of Na⁺ K⁺ pump failure, which can recover if the blood supply is reestablished. Within several minutes of onset of ischemia, cells begin to swell. Swelling is more prominent in astrocytes than neurons. Pericapillary edema compresses the capillaries and edema aggravates the ischemia and so progression to infarction results. The faster the blood flow restoration, the less the brain tissue damage. Intracellular accumulation of calcium and sodium causes depolarization of the transmembrane potentials resulting in local ionic shifts and reduction of seizure threshold. Another likely mechanism is glutamate excitotoxicity operating during acute ischemic injury. Large infarcts are associated with early onset post stroke seizures due to a large penumbra zone where the neurons are still active to produce an epileptic discharge (9). Following the onset of cerebral ischemia there is an increase in both tissue-type plasminogen activator (tPA) activity and neuroserpin expression in the area surrounding the necrotic core (ischemic penumbra) and treatment with neuroserpin following ischemic stroke or over expression of the neuroserpin gene

results in a significant decrease in the volume of the ischemic area as well as in the number of apoptotic cells. TPA activity and neuroserpin expression are also increased in specific areas of the brain by seizures, and treatment with neuroserpin slows the progression of seizure activity throughout the CNS and results in significant neuronal survival in the hippocampus (10).

CHPV is a vesiculovirus, a member of the Rhabdoviridae family, related to, but phylogenetically distinct from Vesicular Stomatitis Virus. It was first isolated in 1967 from Chandipura, Maharashtra, India, as chance isolation during arboviral epidemic surveillance (11). Though initially thought to be an orphan virus (not associated with disease), it was later known to cause sporadic cases of fever with arthralgia or Reye's syndrome and now it is a suspected cause of epidemic strokes. CHPV was isolated from sand flies (12).

Objectives

1. To confirm that “brain attacks” (strokes) can occur in epidemics,
2. To identify the association of any pathogen,
3. To study its clinical characteristics,
4. To study its pathology by neuroimaging,
5. To know the arterial territory involved,
6. To understand the effect of symptomatic treatment and
7. To know if some ischemia cases are reversible.

Materials and Methods

Design

This is a cross sectional and case-control study.

Sample selection

Three hundred and twenty two cases admitted to Government hospitals of Andhra Pradesh from 1st June to 12th August 2003, satisfied the World Health Organization definition of stroke (“rapidly developing clinical signs of focal {or global} disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin”) (13). The cases were analyzed for geographical distribution, age, sex and Case Fatality Rate. Only 55 cases satisfied the inclusion and exclusion criteria and so were analyzed for clinical presentation, investigations done, treatment given and outcome.

Inclusion criteria

1. Coma with onset to peak duration <12 hours.
 - i. Onset is determined by headache/fever/abdominal pain/diarrhea/seizure/weakness/loss of consciousness.
 - ii. Peak is determined by minimum Glasgow coma scale score or death.

2. Tested for known causes of encephalitis/encephalopathy/stroke and Chandipura viral infection.

Exclusion criteria

1. Clinical/laboratory evidence of meningitis/encephalitis/encephalopathy (Japanese encephalitis, West Nile, dengue, Herpes simplex, enteroviruses, paramyxoviruses {measles, mumps, nipah virus etc.}, corona viruses, Varicella, Influenza, Chikungunya, rabies, malaria, typhoid, mycoplasma, leptospirosis, Reye's syndrome, metabolic disorders).
2. Any predisposing factor for stroke other than infection.
3. Meningeal irritation signs.
4. Abnormal cerebrospinal fluid (CSF).

Investigations

These included complete blood count, erythrocyte sedimentation rate, C-Reactive protein, fasting blood sugar, serum ammonia, blood urea nitrogen, serum creatinine, serum electrolytes, screening for malaria (peripheral blood smear and serological test), liver function tests, coagulation profile (bleeding time, clotting time, prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen level), platelet counts, cytokines, homocysteine, lipid profile, complete urine examination, CSF analysis, electrocardiogram, electroencephalogram (EEG) and neuroimaging. Since *clinically similar epidemics* were observed in Warangal District earlier (1998, 2002) with inconclusive investigations, a total of 54 blood samples, 22 throat swabs and 10 CSF samples from 55 "epidemic brain attack" cases; five blood samples from five fever cases; 10 blood samples from 10 family contacts were collected and transported in dry ice for virological and serological studies to four different Indian National Laboratories-National Institute of Communicable Diseases, New Delhi, India, National Institute of Mental Health and Neurological Sciences, Bangalore, India, National Institute of Virology (NIV), Pune, India and Veterinary Biological Research Institute, Hyderabad, India. Aspirated brain specimen from two fatal cases (using Vim-Silverman needle through nose and cribriform plate) were transported in vaccine carrier to NIV. Reverse transcriptase polymerase chain reaction (PCR) test was used to detect respiratory viruses. *The samples were investigated for CHPV at only NIV, since other laboratories did not have the facilities.* Paired sera could not be collected due to either death or patients not returning for medical check up as they had recovered totally. So, antibody titers in samples drawn on different days after onset of disease were compared. Virus was isolated using throat swabs and brain suspension in madin-darby canine

kidney cell line, vero, rhabdomyosarcoma cell lines and peripheral blood mononuclear cell lines co-cultures. Identity was investigated by electron microscopy, immunofluorescence, complement fixation, neutralization, PCR and sequencing of the amplicons. PCR was used to compare the nucleic acid of presently isolated CHPV with that of originally isolated CHPV at NIV. One-day old Swiss-albino suckling mice were inoculated by intra-cerebral route with CHPV isolates for testing neurovirulence. Serum tumor necrosis factor-alpha and interleukin-2 were estimated (14). Symptoms and signs of EBA cases were compared with those of encephalitis and Reye's syndrome. Cases with evidence of CHPV infection were compared with those without evidence of CHPV infection since these cases were reported as Chandipura viral encephalitis (4-6).

Statistical methods

Student's *t* test for proportions was done for comparison of symptoms, signs and response to treatment.

Results

There were 322 cases of stroke during the study period (Figure 1). They all occurred during monsoon following hot summer. Cases presented within 2 days of heavy rain through the monsoon season. Serial interval between primary and secondary cases was 2-3 days. Age group affected was 5 months-15 years with maximal involvement at the age of 2-9 years. Boys were affected more than girls 172:150 (1.15:1). However, Case Fatality Rate was lower in boys 51.16% (88/172) than in girls 59.33% (89/150) (Figure 1).

Clinical findings

Symptoms and signs were similar in both groups with or without evidence of CHPV infection. Fever and altered sensorium without meningeal signs of irritation were present in all 55/55 (100% cases). Other signs included seizures in 33/55 (60%), asymmetric quadriparesis in 23/55 (41.82%), hemiparesis in 21/55 (38.18%), aphasia in 20/55 (36.36%), upper motor neuron type of facial palsy in 17/55 (30.91%), unilateral dilated and nonreacting pupil on admission in 13/55 (23.64%), gaze palsy in 7/55 (12.73%), homonymous superior quadrantanopia (as checked by Menace reflex) in 1/55 (1.82%), transient extrapyramidal symptoms (dystonia, choreoathetosis) in 1/55 (1.82%), papilledema in 0/55 (0%) and focal deficit in 33/55 (60%) cases. No patient had shock or cardiovascular abnormality. Fundus examination was normal. Abdominal colic was present in 28/55 (50.91%), vomiting in 43/55 (78.18%) and diarrhea without

Figure 1. District wise, sex wise distribution of “epidemic brain attack” cases and deaths in various districts of Andhra Pradesh.

Figure 2. Relationship between time of giving mannitol after onset of disease and Case Fatality Rate.

dehydration or dyselektrolytemia in 26/55 (47.27%). Abdominal colic or diarrhea (without dehydration or dyselektrolytemia) was more frequent in fatal cases 16/28 (57.14%) when compared to recovered cases 12/27 (44.44%). The cases that received mannitol early improved rapidly (Figure 2). 2/27 (7.41%) cases developed late onset refractory epilepsy 6 months after the stroke. 4/27 (14.81%) continued to have hemiplegia after 8 months. EBA cases differed from those of encephalitis (Table 1) and Reye's syndrome (Table 2).

Laboratory results

a. Routine tests: They revealed leukocytosis, neutrophilia, elevated erythrocyte sedimentation rate and C-Reactive protein, but normal serum electrolytes, coagulation profile, platelet counts and

electrocardiogram. Plasma homocysteine levels and lipid profile were normal in 3/3. CSF examination except for increased pressure was normal.

b. Cytokines: There was significant elevation of tumor necrosis factor-alpha and interleukin-2 after the 8th day (14).

c. EEG: EEGs were done in nine cases. They revealed bilateral slowing in 6/9 cases and periodic lateralized epileptiform discharges in 3/9.

d. Neuroimaging: 25/55 (45.45%) CT scans showed hypodensities without any hemorrhages involving both the gray matter and the white matter and restricted to the MCA territory, with a midline shift. CT scans done in 8/8 (100%) within one day of onset were normal. 21/25 cases (84%) CTs done between 1-5 days and 4/22 (18.18%) done on 6th day or later showed hypodensities. Hypodensities were bilateral

Table 1. Characteristics of encephalitis and “epidemic brain attack” (*Words in italics are the findings seen in the current epidemic*)

Characteristics	Evidence favoring encephalitis *	Evidence favoring “epidemic brain attack” (present series)
Epidemic occurrence	<i>Yes</i>	<i>This is the first report</i>
Fever	+	+
Focal symptoms and signs	+	+
Onset	Acute (hours to days)	<i>Abrupt (minutes)</i>
Course	Worsens over days	<i>Worsens over hours</i>
Seizures	Usually after 1-2 days and are difficult to control	<i>Immediate (within 48 hours-early onset poststroke seizures) and easily controlled.</i>
Meningeal irritation signs	Present in 13% (15) to 80% (16)	<i>Absent</i>
Cerebrospinal fluid	Abnormal. Lymphocytic pleocytosis with normal glucose	<i>Normal except for raised intracranial pressure</i>
Virus in cerebrospinal fluid	May be present	<i>Absent. All 10 cerebrospinal fluid samples were negative for Chandipura virus</i>
Viral RNA in cerebrospinal fluid	May be present	<i>Absent</i>
Neuroimaging	In 50% cases of Japanese encephalitis, CT shows bilateral non-enhancing low density areas in one or more of the thalamus, basal ganglia, midbrain, pons and medulla (17). Acute disseminated encephalomyelitis shows demyelination	<i>Ischemia restricted to middle cerebral artery territory</i>
Response to antiedema treatment	Slow (> 2 days)	<i>Rapid improvement (< 2 days)</i>
Progression to death	Slow (> 2 days)	<i>Very rapid (< 2 days) if not treated with antiedema measures</i>
Rapidity of recovery	Slow (months to years)	<i>Rapid. Usually < 1 week 20/27 (74.07%) recovered within 1 week 2/27 (7.41%) recovered within 3 weeks 1/27 (3.7%) recovered within 3 months</i>
Persistent neurological deficit	Frequent and severe in 25% (18) to 52.5% (19) of survivors	<i>Less frequent. Only 4/27 (14.81%) had persistent neurological deficit after 8 months</i>

* Japanese Encephalitis findings are used for comparison since it presents as an epidemic coma and confused the clinicians in this epidemic.

Table 2. Characteristics of Reye’s syndrome and “epidemic brain attack”. (*Words in italics are the findings seen in the current epidemic*)

Characteristics	Evidence in favor of Reye’s syndrome	Evidence in favor of “epidemic brain attack”
Epidemic presentation	<i>Yes</i>	<i>This is the first report</i>
Clinical course	Biphasic	<i>Monophasic</i>
Focal symptoms and signs	Not seen except when there is herniation	<i>Present</i>
Gaze palsy	Never	<i>Present</i>
Aphasia	Never	<i>Present</i>
Fever	Never	<i>Present</i>
Glucose	Low	<i>Normal</i>
Ammonia	High	<i>Normal</i>
Liver function tests	Abnormal	<i>Normal</i>
CT scan	Diffuse edema	<i>Edema is restricted to MCA territory</i>

in 15 (60%) cases and unilateral in 10 (40%) (six on the left side and four on the right side) cases. Two children with initial abnormal CT scans (edema) had normal magnetic resonance imaging (MRI) after recovery 3 months later and four hemiplegic children had chronic infarcts in MCA territory on MRI scans done after 8 months.

e. Virology: All 55 samples were negative for known pathogens.

i. Transmission electron microscopy of brain aspirate showed a rhabdovirus.

ii. Identification of virus: Identity was confirmed as CHPV by electron microscopy, immunofluorescence, complement fixation, neutralization, PCR and sequencing of the amplicons. The nucleic acid of the isolated virus gave 96.1% homology with the earlier identified CHPV.

iii. Neurovirulence: All five mice, inoculated intracerebrally with cultured virus died with convulsions within a day.

iv. Of the 55 EBA cases examined, 28/55 (50.91%)

Table 3. Laboratory results of samples tested for CHPV *

	Samples positive ^a /total no. of samples tested	Fever cases without nervous system involvement: positive/total cases tested	Contact samples: positive/total family contacts' samples tested
Brain aspiration	1/2 ^b	-	-
Immunoglobulin M	14/46	3/5	2/10
Immunoglobulin G	9/46	2/5	3/10
Neutralizing antibodies	17/47	2/5	7/10
PCR for CHPV-RNA			
Throat swabs	4/21	-	-
Serum	5/25	-	-
Cerebrospinal fluid	1/7	-	-
Brain aspirate	1/1	-	-
Virus Isolation			
Throat swabs	3/22	1/8	-
Blood clot	2/10	-	-
Brain	1/1	-	-
Cerebrospinal fluid	0/10	-	-

* Data was received from NIV; ^a Total Number of cases with evidence of CHPV infection, 28/55; ^b One brain aspirate sample was damaged during transport.

Figure 3. The risk of EBA is much more in serologically negative cases rather than positive cases suggesting that CHPV is unlikely to be the cause.

had evidence of CHPV infection (Table 3).

Treatment

Acute stroke treatment was aimed at preserving the ischemic penumbra and protecting the neurons against further ischemia to maximize recovery. It included symptomatic treatment and nursing care of the comatose patients at the earliest in the nearest hospital since earlier experience in Andhra Pradesh with coma cases over the last 20 years was that most deaths were occurring during transportation of cases due to preventable causes like aspiration. After noticing rapid improvement of cases that received mannitol, special care was taken to treat

raised intracranial pressure (Figure 2). Aspirin/ anticoagulants/thrombolytics were not used since they are still experimental in pediatric strokes. Oral frusemide (1 mg/kg), though found useful to reduce raised intracranial pressure when administered at home before shifting to a hospital by paramedical workers as a first aid in cases of Japanese encephalitis (1), was not used here because of the risk of public attributing the death induced by the then undetermined disease to the drug.

Discussion

The usual number of strokes is ~ 1 per month

Table 4. Comparison between cases with and without evidence of CHPV infection

Findings	CHPV positive cases with the characteristic	CHPV + cases n1	% of the characteristic in CHPV + cases p1	CHPV negative cases with the characteristic	CHPV - cases n2	% of the characteristic in CHPV - cases p2
Interval between onset of illness and hospitalization < 1 day ^a	28	28	100	27	27	100
Onset to peak illness <1 day ^a	28	28	100	27	27	100
Abdominal colic ^b	15	28	53.57	13	27	48.15
Vomiting ^b	22	28	78.57	21	27	77.78
Diarrhea without dehydration /dys electrolytemia ^b	14	28	50	12	27	44.44
Meningeal irritation signs ^a	0	28	0	0	27	0
Focal symptoms and signs ^b	17	28	60.71	16	27	59.26
Seizures (immediate-early onset poststroke seizures) ^b	17	28	60.71	16	27	59.26
Generalized seizures ^b	15	17	88.24	14	16	87.5
Early onset poststroke focal seizures ^b	2	17	11.77	2	16	12.5
Early onset poststroke seizures controlled in <5 minutes with 1 dose of diazepam+phenytoin ^a	17	17	100	16	16	100
Late onset post stroke (partial) refractory seizures ^b	1	14	7.14	1	13	7.69
Papilledema ^a	0	28	0	0	27	0
Case Fatality Rate ^b	14	28	50	14	27	51.85
If death occurred, it was within 1 day ^b	14	28	50	14	27	51.85
Leukocytosis ^b	8	28	28.57	7	27	25.93
Neutrophilia ^b	9	28	32.14	7	27	25.93
C-Reactive protein elevated in ^b	1	4	25	1	4	25
Elevated erythrocyte sedimentation rate ^b	3	28	10.71	2	27	7.41
Normal CSF (except for increased pressure) ^a	28	28	100	27	27	100
Hypodensities in MCA territory ^b on neuroimaging ^b	13	28	46.43	12	27	44.44

^b $p > 0.05$. Variation is not significant. ^a Both values are similar.

Table 5. Serology results on various days after onset of the disease ^a

Serology results	Day of sample collection after onset of illness	
	(0-4 days)	(>4 days)
IgM(+), IgG(-)	2/30	4/16
IgM(+), IgG(+)	1/30	7/16
IgM(-), IgG(+)	0/30	1/16
IgM(-), IgG(-)	27/30	4/16
Neutralizing antibodies	2/29	15/18
Total cases tested	30	18

^a Data was received from NIV.

per district and is evident from the statistics of Ananthapur and Hyderabad districts (which were not affected by this epidemic) during the study period (Figure 1). The number of strokes seen in a comparable period in the state of Andhra Pradesh is usually 20-25. It has risen to 322 cases showing that there was a 13-fold increase in cases (epidemic) of stroke in some districts (Figure 1). Presence of fever, acute phase responses and cytokine elevation (14) suggested infarction or thrombosis or inflammation or foreign body response and epidemic presentation in many districts all over the state suggested infective etiology.

Our patients presented with fever and 33/55 (60%) had focal neurological signs indicating

Table 6. Evidence in favor and against CHPV as the cause of “epidemic brain attacks”

Characteristics	Evidence in favor	Evidence against
MCA involved	<ol style="list-style-type: none"> 1. Hematogenous dissemination occurs with CHPV. CHPV is cultured from 2/10 blood clots. 2. CHPV RNA is demonstrated in 5/25 sera samples. 3. Since CHPV is known to produce transient low-grade viremia in mice and Langur monkeys (11), it may be the pathogen. 	<ol style="list-style-type: none"> 1. CHPV could not be cultured from 8/10 blood clots. EBA may be due to an unidentified virus. 2. 20/25 sera samples did not have CHPV RNA. 3. CHPV is not known to cause strokes.
Antibodies	<ol style="list-style-type: none"> 1. Demonstrated in sera of 28/55 cases. Some samples may have been negative due to very early collection timing of samples. 2. Immunoglobulin M, immunoglobulin G and neutralizing antibodies were significantly higher in samples collected after 4 days of illness (69%) when compared with those collected before 4 days (10%) (Table 5). These findings suggest strong association of CHPV with EBA outbreak. 	<ol style="list-style-type: none"> 1a. 27/55 did not have any antibodies. 1b. CHPV-reactive antibodies were also found in 3/5 fever cases without CNS involvement and 7/10 asymptomatic contacts. 1c. Antibodies were not demonstrated in CSF. So direct involvement of meninges/brain by CHPV is unlikely. 2a. The sera belonged to different patients and so may not indicate seroconversion. 2b. It may be an anamnestic reaction. <p>3. Figure 3 shows that EBA were more common in serologically negative cases rather than in positive cases suggesting that CHPV is unlikely to be the cause.</p>
Immunoglobulin M antibodies (indicate acute infection with CHPV)	30.43% sera tested positive indicating an acute infection	<ol style="list-style-type: none"> 1. Immunoglobulin M antibodies were absent in 69.57% cases. 2. Specificity and sensitivity of the Immunoglobulin M capture enzyme-linked immunosorbent assay has to be tested by other laboratories.
Neutralizing antibodies	Were present in 17/47 (Table 3)	<p>Neutralization tests function of antibodies and NOT class of antibodies. Serological surveys in asymptomatic humans indicated that the virus was widespread and present at least since 1955 all over India (Visakhapatnam, A.P. 23/33, Delhi 41/74, Banni, Gujarat 62/74, Vellore, Tamilnadu 22/42, Madras, 38/59, Nagpur 73/94, Bangalore 16/106, Lucknow 25/28, Calcutta 5/73) (11). Therefore, presence of neutralizing antibodies does not indicate disease.</p>
Demonstration of CHPV RNA in the brain and CSF by PCR	Confirmed direct involvement of brain and CSF	<ol style="list-style-type: none"> 1. Demonstrated in a single case only. 2. Does not prove pathogenicity. Firstly, it may be an artifact. Secondly, in spite of the presence of CHPV RNA, there were no meningeal irritation signs and CSF was normal but for raised intracranial pressure indicating that meninges have not reacted. Thirdly, virus in CSF need not cause disease (25).
Virus isolation from throat swabs and blood clots	Virus is isolated from four throat swabs and two blood clots	<ol style="list-style-type: none"> 1. Prevalence of neutralizing antibodies (11) suggested asymptomatic cases. So viremia does not mean disease. 2. Infected adults were asymptomatic.
Virus isolation from brain	CHPV was isolated and identified in brain aspirate	<ol style="list-style-type: none"> 1. Since clinical and neuroimaging evidence of pathology is localized to MCA territory, this

Pure culture on artificial laboratory media	Done	<p>CHPV obtained by brain aspiration is unlikely to be from the affected part of the brain because MCA or its branches cannot be accessed by Vim-Silverman needle aspiration. <i>The part of the brain that is aspirated is supplied by anterior cerebral artery, which is not affected in the current epidemic.</i></p> <p>2. Brain aspiration was done, when this child was in rigor mortis. CHPV might have been carried into brain from nasopharynx during passage of the needle. Alternatively, since all protective barriers break down immediately after death, virus could have entered the brain after death.</p> <p>May be a passenger virus May be a concomitant virus May be a contamination.</p>
Neurovirulence	Cultured organism killed 5/5 mice on intracerebral inoculation.	<p>1. Original isolated virus did not kill mice. Only the culture killed it. Contamination of virus medium might have killed the mice.</p> <p>2. Death within 24 hours raises doubt about the possibility of traumatic death.</p> <p>3. Pathological examination of brain of dead mice was not done.</p>
Cytopathic effect	Was seen on the 6th post inoculation day.	Earlier strain induced cytopathic effect within 3 hours (11). Is this strain less virulent?
Inflammatory response in brain	-	Inflammatory response has not been demonstrated so far.
Wrap up	-	Currently available evidence is not enough to confirm the etiological role of CHPV in EBA.

CHPV: Chandipura virus; MCA: Middle cerebral artery; EBA: Epidemic “brain attack”; CSF: Cerebrospinal fluid; PCR: Polymerase chain reaction.

direct involvement of brain by an infection. The clinical symptoms and signs reported in a single patient out of the two confirmed cases of CHPV infection in 1965 (during Dengue and Chikungunya viral epidemic) were different from the current presentation and included sudden onset of fever with chills, constipation, body aches and arthralgias which lasted about 3 days (11). A confirmed case reported in 1980, had fever without chills, vomiting, loose motions without blood or mucus, generalized seizures, coma, bipyramidal signs, no meningeal irritation signs and a total leukocyte count of 14,000/ μ L. The CSF was normal and so a diagnosis of Reye’s syndrome was made (20).

Clinical and laboratory findings of our series were in favor of EBA rather than encephalitis (Table 1) or Reye’s syndrome (Table 2). One epidemic has one cause. The similarity of symptoms and signs of 28 cases with evidence of CHPV infection and 27 without any evidence not only makes CHPV an unlikely cause but also raises the possibility of another yet unidentified virus (Table 4). Figure 3 shows that the risk of an EBA is much more in

serologically negative cases rather than in positive cases suggesting that CHPV is unlikely to be the cause. Table 5 shows characteristics of serology results on various days after onset of disease. As seen in Table 5, IgM IgG and neutralizing antibodies were significantly higher in samples collected after four days of illness (69%) when compared with those collected before 4 days (10%).

Pathogenesis

Involvement of MCA without CSF changes and identical incubation period in all cases suggests hematogenous dissemination of the pathogen since axoplasmic spread, firstly, will not lead to arterial involvement and secondly, must have presented with a wide variation in incubation period (as in other Rhabdoviruses like Rabies) depending upon whether face or upper or lower limbs are bitten.

Hematogenous spread of virus to MCA occurred probably due to its large size and direct continuity with internal carotid artery. In addition, there is a possibility of collateral circulation through communicating arteries in the circle of Willis

protecting the anterior and posterior cerebral arterial territories from ischemia.

Pathology

CHPV was shown to cause cytopathic effect within 6 days now, but as early as 3 hours in an earlier study (11). Present observation of cases presenting within 1-2 days of heavy rain and a serial interval of 2-3 days does go with a short incubation period and brain damage. This epidemic was reported as Chandipura encephalitis (4-6). However, neither was it encephalitis nor the role of CHPV confirmed. Encephalitis was excluded by the clinical and laboratory findings (Table 1). Thalami and basal ganglia were typically spared in all the cases in contrast to Japanese encephalitis where they are involved. Others reported it as Reye's syndrome (7) but this was excluded by the clinical and laboratory differences (Table 2).

CT scans and standard MRI examinations cannot confirm the presence and location of a stroke until eight or more hours after its onset. Therefore, initial 8/8 scans might have been normal. Breakthrough for pathology came when 21/25 CT scans done between 1-5 days revealed asymmetrical hypodensities (edema) in MCA territory. Though CT cannot differentiate edema from ischemic infarct, disappearance of hypodensities on repeat scans suggested edema as the cause of hypodensities. Edema must have resulted from transient ischemia due to vasospasm of MCA for few seconds to few minutes in the majority of cases. Longer lasting vasospasm or vasculitis must have been responsible for the cases which had sequelae. Coma and death must have been the result of either bilateral MCA ischemia or unilateral MCA ischemia with massive cerebral edema compressing upper brainstem as evidenced by unilateral dilated nonreactive pupils in 23.64% cases. Alternately, large infarcts are also known to cause immediate drowsiness or stupor due to an ill-defined effect on the Reticular Activating System.

Recovery of symptomatically managed cases within a few days, especially those that were given mannitol early in the course (Figure 2) without any specific treatment and the presence of hypodensities in only 4/22 CTs done on or after 6 days indicates that raised intracranial tension due to ischemic cytotoxic edema was the cause of death and the ischemia was transient in most cases. The cerebral cytotoxic edema must have disappeared resulting in normal scan in two recovered cases. The majority (85.19%) of survived cases recovering without any sequelae within 3 weeks indicated that they had reversible ischemic neurological deficit. Reversible cerebral lesions on MRI have been reported in posterior circulation (21,22). Whether some children had transient ischemic attacks or not was

not recorded.

Exclusion of other causes of infarct

Systemic shock causes infarcts in border zone between major cerebral arterial territories (resulting in hypoxic ischemic encephalopathy) and not in MCA territory alone. Venous infarcts are multiple with hemorrhages and are not restricted to arterial territories. Embolic infarcts are patchy, multiple, hemorrhagic. Though rapid recovery suggested embolic infarcts, absence of intracerebral hemorrhage and embolic manifestations elsewhere excluded them. Lack of shock, asymmetrical infarcts, absence of evidence of bleeding elsewhere and normal cardiac status excluded peripheral vascular, hematological and cardiac causes of cerebral infarction.

Experience from epidemics of mysterious disease in Siliguri, India (23) and so-called Plague in Surat, India (24) cautioned us to review the evidence critically.

a. Is it an infection? Epidemic situation, occurrence within 2 days of monsoon after a hot summer, association of fever, leukocytosis, neutrophilia, elevated C-Reactive protein and cytokines (14) suggested acute infection.

b. What is the pathogen? As per the claims of NIV, (4-6) the lead for probable etiology came from demonstration of CHPV from the brain aspirate by electron microscopy and its identification by serology.

However, the clinical and epidemiological features did not support CHPV as the cause of EBA. Findings in favor and against CHPV as the cause of EBA are discussed in Table 6. There is no conclusive evidence regarding the pathogen.

Relationship of infection and stroke

This is the first report of infection related epidemic stroke. Sporadic cases of strokes precipitated by infection have been reported earlier. The pathogenetic linkage between infection and stroke is insufficiently understood (26). Both bacterial (especially *Mycobacterium tuberculosis*, *Helicobacter pylori* and *Chlamydia pneumoniae*) and viral infection contribute to increased risk of stroke. Coagulation abnormalities and immunological reactions are among possible pathogenic pathways that link infections and stroke (27). Infection elevates the risk for cardioembolism and tends to increase the risk for arterio-arterial embolism that is ruled out in our cases. Recent infection is an independent risk factor for acute cerebrovascular ischemia. Its role appears to be more important in younger age groups. Stroke patients with and without preceding infection are not different with respect to factor VII and factor VIII activity, fibrin monomer, fibrin D-dimer, von Willebrand factor, C4b-binding protein,

protein S, anticardiolipin antibodies, interleukin-1 receptor antagonist, soluble tumor necrosis factor- α receptor, interleukin-6, interleukin-8 and neopterin (28). Varicella-zoster (28-31), Human Immunodeficiency Virus (32,33), parvovirus B19 (34), Enterovirus 71 (35), Epstein-Barr virus (36), and Human herpes virus 6 (37) are reported to cause stroke. The role of hypoxic/ischemic damage to oligodendrocytes has to be investigated (38).

Although proof of a correlation between infection and human vasculitis would aid in patient management, it is difficult to confirm causality. Transient arterial block (whatever the cause is) is yet to be demonstrated. The fact that severe acute respiratory syndrome (SARS) was confirmed only when it was investigated by the network of laboratories spread over many countries indicates the necessity for investigation in more laboratories (39). International experience with SARS (only 17/22 laboratories confirmed SARS) tells us that unless the result comes from multiple laboratories it is better to play safe and take the results with a pinch of salt.

Incubation period

Has to be <2 days because the epidemics (1998, 2002, 2003) occurred within 2 days of heavy rains after a hot summer and the serial interval between primary and secondary cases was 2-3 days.

Vector: The short time period between rain and disease suggests that a vector is unlikely to play any role in EBA.

If the cause is not CHPV, what is it? Ten throat swab samples, three CSF samples and six rectal swab samples are under process for virus isolation including enteroviruses. Some additional observations made in cases other than the 55 cases included in this paper suggest looking at three other possibilities.

i. *Enterovirus 71:* Diarrhea suggested feco-oral route of spread. Though many characteristic clinical findings like exanthem, acute flaccid paralysis cases/brainstem encephalitis, were conspicuously absent, the short incubation period, the age group involved did suggest Enterovirus 71. Features like vomiting, absence of mouth ulcers, atypical presentation and raised total white cell count are known to result in a fatal course with Enterovirus 71 infection (40). Screening for enteroviruses group by PCR test was negative in 1998, but in 2003, seven samples had evidence of Enterovirus 71 infection.

ii. *Varicella:* Varicella-zoster virus is known to cause aseptic meningitis, encephalitis, transverse myelitis, Guillain-Barre syndrome, Reye's syndrome and sporadic strokes but not epidemic strokes. Five cases had chickenpox 2 months before. Rapid response to empirical acyclovir in five cases suggested a role but serology excluded it. The fact

that this epidemic occurred about 2 months after an epidemic of Varicella suggests the need to look for Varicella zoster virus with tests that are more sensitive.

iii. Another yet unidentified endotheliotropic virus similar to equine arteritis virus needs to be investigated for (41).

Limitations of the study

a. In view of CHPV antibodies in fever cases and contacts and coexistent epidemics of diarrhea and complex febrile seizures, it is likely that subclinical cases, cases with only diarrhea or fever or complex febrile seizures or other mild and transient symptoms have gone unnoticed. Further, not all cases might have come to Government hospitals. It is possible that EBA represents only the tip of the iceberg. Therefore, the clinical spectrum needs to be further investigated.

b. Small quantity of available sera and CSF samples requiring large number of tests resulted in not investigating all 322 cases fully.

c. Lack of availability of transcranial Doppler, functional MRI or magnetic resonance angiography in the rural areas and the difficulty in shifting the critically ill children to centers where they were available restricted the study of cerebral vasculature.

d. *Anatomic Localization:* Clinical findings and neuroimaging localize the pathology to the MCA territory. But it must be remembered that involvement of intracranial portion of the Internal Carotid Artery also may produce a clinical picture like that of MCA involvement. Further neuroimaging studies are essential to differentiate them.

e. Studying only 55/322 cases may not have represented the disease correctly and completely. For example among the cases which could not be included in this study (because CHPV was not tested), CT scans showed 5 cases with both anterior cerebral artery and MCA territories' hypodensities and 3 cases with hemorrhagic infarction.

f. Investigation of an epidemic requires a Rapid Action team of doctors of various specialties, maintenance of thorough records and provision of finances for immediate and expensive investigations (42).

g. Lack of postmortem examination of complete brain specimen (essential to differentiate infarction from encephalitis).

h. Role of inflammatory mediators implied in the pathogenesis of stroke in acute inflammation must be investigated (43).

Conclusions

It was neither a mystery disease nor a missed disease. However, what we know is less and what

we have to know is more.

i. "Brain attacks" and reversible ischemic neurological deficits can occur in epidemics. This is the first report. EBA are the second important cause of epidemic coma in India after Japanese encephalitis.

ii. Though infection is the cause, *etiological role of Chandipura virus is not confirmed. The role of Enterovirus 71, Varicella* and any yet unidentified *endotheliotropic virus* similar to equine arteritis virus needs to be investigated (41).

iii. Epidemic coma, fever, lack of meningeal signs of irritation, normal CSF and asymmetric hypodensity in MCA territory on neuroimaging are characteristic of EBA. Abdominal colic/diarrhea may be present.

iv. Ischemia in MCA territory is the cause.

v. Unlike routine stroke cases where cerebral edema reaches its peak by about 3-4 days, rapid deterioration of EBA cases due to very rapid elevation of intracranial pressure to critical level within 12 hours of onset makes it a medical emergency requiring initiation of treatment at home. Using oral frusemide (1 mg/kg) as a first aid measure and then transporting the child on its side with head end elevated without any flexion of neck may save many precious lives and reduce the unacceptably high Case Fatality Rate.

vi. Mannitol must be administered at the earliest.

vi. Finding out the mechanism by which mannitol promoted recovery (reduction of raised intracranial pressure/scavenging free radicals/attenuating increase in regional cerebral blood flow) may help us in improving the treatment.

vii. Role of tumor necrosis factor-alpha and interleukin-2 needs to be investigated.

viii. Some ischemic cases are reversible. This is the first report of epidemic reversible ischemic neurological deficits in MCA territory.

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