REVIEW

Japanese viral encephalitis

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One of the leading causes of acute encephalopathy in children in the tropics is Japanese encephalitis (JE). Transmitted by the culex mosquito, this neurotropic virus predominately affects the thalamus, anterior horns of the spinal cord, cerebral cortex, and cerebellum. It mainly affects children <15 years and is mostly asymptomatic. The occasional symptomatic child typically presents with a neurological syndrome characterised by altered sensorium, seizures, and features of intracranial hypertension. Aetiological diagnosis is based on virus isolation or demonstration of virus specific antigen or antibodies in the cerebrospinal fluid/blood. Though no antiviral drug is available against JE, effective supportive management can improve the outcome. Control of JE involves efficient vector control and appropriate use of vaccines.

Japanese encephalitis (JE) is one of the leading causes of acute encephalopathy affecting children¹ and adolescents in the tropics. Nearly 50 000 cases of JE occur worldwide and 15 000 of them die.^{2,3} Considerable information on epidemiology and clinical features of this dreaded disease is available, yet much more needs to be understood in terms of pathophysiology, clinical management, and prognostication.

THE PAST

Though outbreaks of encephalitis attributed to JE virus (JEV) were reported in Japan as early as 1871, it wasn't until 1924 that JEV was isolated from a clinical case in the first recorded epidemic in Japan (see box 1).⁴ The Nakayama strain of JEV, used in development of mouse brain inactivated virus vaccine was first isolated in 1935. The mode of transmission by mosquito vector was elucidated only 25 years after recognition of JEV. Until 1970, the temperate zone of Asia was the principal site of JE transmission. In the last three decades, the focus of viral epidemics has switched over to South and Southeast Asia.

Box 1: History

1871: First outbreak of JE attributable to JE.
1924: Isolation of JEV.
1934: Isolation of Nakayama strain.
1950: Elucidation of the route of transmission.
1970: Change in geographical location of viral transmission.

EPIDEMIOLOGY

Epidemics and sporadic cases of JE occur in many Asian countries (see fig 1), including Cambodia, China, Indonesia, India, Japan, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Republic of Korea, Sri Lanka, Thailand, Vietnam, and the south eastern Russian federation. Gradual spread to other non-Asian regions—for example, Torres strait of Australian mainland has been reported recently.⁵

Patterns of JE transmission vary within individual countries and from year to year (table 1).⁶ In endemic areas, the annual incidence of disease ranges from 10–100 per 100 000 population. An endemic situation, with occurrence of sporadic cases throughout the year, is present in tropical zones. In temperate regions of Asia and the northern tropical region, JEV is transmitted seasonally. A probable explanation could be the prolonged mosquito larval development time and longer extrinsic period of JEV at cooler temperatures in temperate regions, which can reduce the viral transmission.^{7,8} In some instances, outbreaks have been associated with rainfall, floods, or irrigation of rice fields.

The risk of travellers acquiring JE is very low (monthly incidence is less than one per million travellers among short term and urban travellers, 0.25 to 1 per 5000 travellers among rural travellers to endemic regions).^{9–11} Travellers living for prolonged periods in rural areas where JE is endemic or epidemic are at greatest risk. Travellers with extensive unprotected outdoor, evening, and night-time exposure in rural areas might be at high risk even if their trip is brief.

JEV is transmitted in a zoonotic cycle among mosquitoes and vertebrate-amplifying hosts, chiefly pigs and wading birds. The presence of prolonged and high titres of viraemia without clinical symptoms, the ability to produce many uninfected offspring, and amplification of the virus by multiplication makes pigs the most important natural hosts for human transmission.¹² The mosquito vector of JE differs in different regions. The major mosquito vector of JE in South East Asia is *Culex tritaeniorhynchus*. *Culex vishnui* complex is also incriminated as a vector in India.¹³ JEV has been isolated from 10 different species of culex, four species of anophelids, and three species of mansonia mosquitoes.^{14,15} Humans are considered as the

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Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; EEG, electroencephalogram; JE, Japanese encephalitis; JEV, Japanese encephalitis virus; Mac-ELISA, IgM antibody capture ELISA; M/GSS, monoclonal antibody/immunogold/silver staining; PCO₂, carbon dioxide tension

Figure 1 Map showing the distribution of Japanese encephalitis.

Box 2: Cellular characteristics

- Single positive stranded RNA genome: length 11 kb.¹²⁰
- Spherical virion: 30 nm core with a surrounding lipid envelope.
- RNA genome encodes a single polypeptide that is cleaved into:
 1. Structural capsid, member (M) and envelope (E) proteins.
 2. Non-structural proteins (NSI, 2A, 2B, 3, 4A, 4B, and 5).¹²¹

dead end host, as the brief periods of viraemia and low titres of virus do not facilitate transmission.

THE PATHOGEN

JEV is a member of the flaviviridae. The cellular characteristics of JEV are given in box 2. The M protein containing hydrophobic domains presumably serves as a transmission anchor.¹⁶ E protein constitutes the major immunogen and is also expressed on the plasma membrane of infected neurons.¹⁷ It is thought to be the cell receptor binding protein, and a mediator of membrane fusion and cell entry.¹⁸ This protein is the major target of the host antiviral immune response.^{19–20} JEV is related antigenically to St Louis, Murray Valley, West Nile encephalitis viruses, and dengue fever virus.

PATHOLOGY

Grossly, the brain appears oedematous with changes mainly involving grey matter. The areas most commonly affected are the thalamus, substantia nigra, anterior horns of the spinal

cord, cerebral cortex, and cerebellum. Microscopy reveals pan-encephalitis with abundant glial nodules, perivascular cuffing, and necrosis with or without characteristic circumscribed necrotic foci.^{21–23} Neuronal inflammation is typically associated with mononuclear cell infiltration. Diffuse microglial proliferation and formation of gliomesenchymal nodules in brain parenchyma dominate the histological picture in acute encephalitis. In the post-encephalitic phase, lesions become linear and tend to localise in thalamus, substantia nigra, and Ammon’s horn. Histopathological examination of these focal lesions show rarefied areas with few cellular and fibrous elements surrounded by dense gliomesenchymal scarring.^{24–25}

Pathological changes described in extraneural tissues include hyperplasia of germinal centres of lymph nodes, enlargement of malpighian bodies in spleen, interstitial myocarditis, swelling and hyaline changes in hepatic Kuffer’s cells, pulmonary interalveolitis, and focal haemorrhages in the kidney.

PATHOGENESIS

After transmission to man by an infected vector mosquito, JEV multiplies locally and in regional nodes.²⁶ After a phase of transient viraemia, invasion of the central nervous system (CNS) occurs. JEV is thought to invade brain via vascular endothelial cells by endocytosis.²⁷ In the neurons, JEV replicates and matures in the neuronal secretory system, mainly the rough endoplasmic reticulum and Golgi apparatus, eventually destroying them.²⁸ Experimental studies in mammalian hosts have shown JEV tropism to neurons in the CNS,

Table 1 Transmission patterns

Transmission pattern	Transmission season	Countries
Sporadic	Year round	Brunei, Malaysia, Singapore
	June to September	Japan
	July to October	South Korea
Endemic/hyperendemic	July to December May to October	Bangladesh, Northern India, Nepal Myanmar, Cambodia, Vietnam, Thailand, southern India, Laos, northern China

Table 2 Clinical features

Feature	No of cases	Altered sensorium (%)	Convulsion (%)	Meningeal signs (%)	Fever (%)	Headache (%)	Mortality (%)
Lincoln and Silverstone (1952) ⁴⁵	201	Majority	–	–	100	100	8.5
Webb and Perriera (1956) ⁴⁴	16	100	87.5	75	100	56.7	–
Sengupta <i>et al</i> (1976) ⁴⁶	340	100	50.3	80	100	42.3	45.2–72
Mohan Rao <i>et al</i> (1983) ⁴⁷	26	84.6	27.3	70	96.2	37.3	26.8
Gourie Devi (1984) ⁴⁸	133	85	67.3	35.2	94.7	24.0	50.0
Poneprasert <i>et al</i> (1989) ¹²²	59	100	59	61	96	76	17
Kumar <i>et al</i> (1990) ⁴⁹	92	100	84.7	13	94.5	20.6	36.9
Desai <i>et al</i> (1994) ¹²³	72	77.6	59.7	–	98.5	62.7	32

indirectly indicating the presence of specific receptors with strong affinity for the virus.²⁹

IMMUNOLOGY

Both humoral and cellular arms of immune system are involved in immunity to JEV. But the relative contribution of individual components has not been well understood. After primary infection with JEV, a rapid and potent monotypic IgM response occurs in serum and cerebrospinal fluid (CSF), usually within seven days.³⁰ The role of antibodies in protection is not yet clearly understood. Passive administration of anti-JEV antibodies has shown to confer protection in mice.³¹ Disappearance of neurological signs has been noted in the presence of IgM antibodies.³² Presence of CSF IgM antibodies has been correlated with a favourable outcome in JE.³³ However, the significance of this protection remains unknown, since neurovirulence of JEV has been enhanced by administration of virus specific antibodies.³⁴ An anamnestic antibody response with an early rapid IgG and delayed slow IgM response has been noted in patients previously infected with antigenically related flaviviruses.

The importance of cell mediated immunity is being recognised of late. In vitro studies using macrophage and leucocyte migration inhibition tests have suggested development of cell mediated immunity in mice.^{35–36} Though, cytotoxic T lymphocyte response to flaviviral infection has been noted in man and mice, their role in JE is not very clear.^{37–38} Life long cell mediated immunity could be conferred by passive transfer of immune spleen T cells in mice.³⁹ Immunisation with inactivated JE vaccine induces T cell activation in vivo.⁴⁰ These studies indicate that cell mediated immunity probably has a greater role than has been reflected so far.

CLINICAL FEATURES

Children under 15 years of age are principally affected in endemic areas. When JEV first affects a nascent population, adults are also affected.⁴¹ Seroprevalence studies in endemic areas indicate nearly universal exposure by adulthood. Approximately 10% of the susceptible population is infected every year. Infection with JEV is often asymptomatic. The ratio of asymptomatic to symptomatic infection varies between 25:1 and 1000:1.^{9–42} Man to man transmission in JE has not been reported. However, the risk of acquiring JE in laboratory settings does exist and laboratory acquired cases have been reported.⁴³ The exact risk of acquiring JE in the laboratory, particularly in research settings, is not known. The incubation period in man, after a mosquito bite, is not exactly known. In general, it varies from 1–6 days. However, it can be as long as 14 days.

Onset of the illness can be abrupt, acute, subacute, or gradual. The course of the disease can be conveniently divided into three stages: (i) a prodromal stage preceding CNS features, (ii) an encephalitis stage marked by CNS symptomatology, and (iii) a late stage noticeable by recovery or persistence of signs of CNS injury.⁴⁴

The prodromal stage is characterised by high grade fever with or without rigors, headache, general malaise, nausea, and vomiting. During this stage, a definitive clinical diagnosis is not possible. This is followed by the encephalitis stage (third to fifth day), which manifests with altered sensorium, convulsions, neck stiffness, muscular rigidity, mask-like facies, and abnormal movements. The relative frequency of various symptoms in different studies is given in table 2.^{45–49}

Abnormal oculocephalic reflex, acute onset hemiparesis with hypertonia and decorticate and decerebrate posturing are important CNS signs, which help in early clinical identification of intracranial hypertension. Features of extraneural involvement reported in JE include gastric haemorrhage in absence of bleeding diathesis and pulmonary oedema. Death usually occurs due to neurological illness in the first week. The reported mortality rate varies between 8.5% and 72% (table 2).

Children who survive slowly regain neurological function over several weeks. Residual neurological impairment includes thick, slow speech, aphasia, and paresis. Only one third of cases recover normal neurological function. Intellectual involvement may be noted in 30% of cases, speech disturbance in 34%, and motor deficits in 49%. Secondary infections, especially pneumonia, urinary tract infection, and stasis ulcers are frequent complications during recovery period.

Apart from the classical presentation described above, other atypical presentations of JE have been reported. In a few children, sensorium may recover rapidly after initial an episode of seizure leading to a false clinical diagnosis of “atypical/ typical febrile seizures” or “fever associated seizure disorder”. Isolated acute onset abnormal behaviour can be the initial presentation and is seen in adolescents. A small proportion of children may present with features of aseptic meningitis with no other clinical features of encephalopathy.⁵⁰ An acute flaccid paralysis-like illness has been recently reported as the initial presenting feature.⁵¹

LABORATORY DIAGNOSIS

Clinical diagnostic studies

In JE, the leucocyte count is often raised (total counts 10–34 × 10⁹/l). Differential counts often reveal neutrophilia ranging between 51% and 90%. CSF examination shows a raised opening pressure, cell count of 10–980 × 10⁶/l, protein <900 mg/l, and normal glucose concentration. Features on an electroencephalogram (EEG) are non-specific and include diffuse theta and delta waves, burst suppression, epileptiform activity, and alpha coma.^{52–53} The generalised changes in an EEG may help in differentiating JE from herpes encephalitis.⁵⁴

Computed tomography show non-enhancing low density areas in the thalamus, basal ganglia, midbrain, pons, and medulla. Cortical atrophy has been noted in some children in the post-encephalitic phase. Magnetic resonance imaging is more sensitive than computed tomography in revealing neural lesions. Magnetic resonance imaging on T2-weighted images shows extensive hyperintense lesions of the thalamus, cerebrum, and cerebellum.^{55–56} These neuroimaging techniques

Box 3: Aetiological diagnosis**Culture**

- Intracerebral inoculation in suckling mouse brain.
- Primary chick, duck embryo cells, and lines of Vero, LLCMK₂, C6/36, and AP61 cells.
- Mosquito inoculation techniques.

Antigen detection

- Reverse passive haemagglutination.
- Immunofluorescence.
- Staphylococcal coagglutination tests using polyclonal or monoclonal antibodies.
- Monoclonal antibody/immunogold/silver staining (M-IGSS).

Antibody detection

- IgM antibody capture ELISA.
- Avidin biotin system.
- Biotin labelled immunosorbent assay.
- Nitrocellulose membrane based immunoglobulin M capture dot enzyme immunoassay.
- Haemagglutination inhibition.
- Complement fixation test.
- Single radial haemolysis.
- Neutralisation test.

may be useful in distinguishing JE from herpes encephalitis. In JE, the regions mainly affected are the diencephalon and basal ganglia, whereas in herpes encephalitis, the changes are characteristically frontotemporal. Single photon emission tomography in acute encephalitic phase reveals hyperperfusion of thalamus and putamen in some cases.⁵⁷ In the post-encephalitic phase, hypoperfusion of the thalamus, frontal cortex, and lentiform area has been demonstrated.⁵⁸ Though these neuroimaging techniques demonstrate significant changes, they are too non-specific to be used for aetiological diagnosis.

Aetiological diagnosis

Aetiological diagnosis of JE is based on virus isolation or demonstration of virus specific antigen or antibodies in CSF/blood (see box 3). The laboratory diagnosis of a confirmed case of Japanese encephalitis is based on one of the following.

1. Fourfold or greater rise in serum antibody titre, *or*
2. Isolation of virus from or demonstration of viral antigen or genomic sequences in tissue, blood, CSF, or other body fluid, *or*
3. Specific IgM antibody by enzyme immunoassay antibody captured in CSF or serum.

1. Culture

Isolation of JEV was conventionally carried out by intracerebral inoculation of clinical specimens in suckling mouse brain. Various cell cultures that are being used more recently include primary chick, duck embryo cells, and lines of Vero, LLCMK₂, C6/36, and AP61 cells. Virus can be isolated from the blood of patients in preneuroinvasive and neuroinvasive phases of the illness, usually not later than six or seven days after the onset of symptoms.⁵⁹⁻⁶⁰ However, isolation of virus from clinical specimens is generally considered a rare occurrence⁶¹ probably because of low viral titres, rapid production of neutralising antibodies, and the logistic difficulty in transportation of specimens in developing countries and frequent freezing and thawing of clinical material.⁶²⁻⁶³ Recently sensitive mosquito inoculation techniques have been described for isolation of JEV.⁶⁴ Identification of JEV in culture substrates was traditionally carried out by the complement fixation test and agar gel diffusion. The neutralisation test, monoclonal based immunofluorescence technique, and enzyme immunoassay are presently being used.⁶⁵

Table 3 Comparison of various tests

Test	No studied	Cases (%) detected
Antigen detection		
Reverse passive haemagglutination ¹²⁴	22	5 (22.7)
Reverse passive haemagglutination ¹²⁵	92	30 (32.6)
Immunofluorescence ¹²⁶	17	15 (88.2)
Immunofluorescence ⁶⁸	120*	53 (44.2)
Immunofluorescence	92	37 (40.2)
Antibody detection		
Microneutralisation ¹²⁷	200	156 (78.0)
Mac-ELISA	22	15 (68.2)
Mac-ELISA	200	94 (47.0)
ABC Mac-ELISA	72	53 (73.6)
MacDOT ⁷²	60	59 (98.3)
Polymerase chain reaction	52*	45 (86.5)
ACRIA ⁷³	12	11 (91.7)

*Suspected cases of JE. In all other studies, cases have been proved by other tests.
For explanation of tests see text.

2. Antigen detection

Various studies have proved the efficacy of antigen detection in CSF using reverse passive haemagglutination,⁶⁶ immunofluorescence,⁶⁷ and staphylococcal coagglutination tests using polyclonal or monoclonal antibodies⁶⁸ in rapid diagnosis of JE. Modified techniques such as use of M-IGSS have been successfully tried in the detection of antigen in mononuclear cells of peripheral blood and CSF of patients.⁶⁹

3. Antibody detection

IgM antibody capture ELISA (Mac-ELISA) is the method of choice to demonstrate virus specific antibody in both blood and CSF. However, when serum IgM antibodies are used for confirming JE, the co-presence of IgG antibodies should be demonstrated by another serological assay. Avidin biotin system (ABC Mac-ELISA),⁷⁰ biotin labelled immunosorbent assay to sandwich ELISA,⁷¹ nitrocellulose membrane based IgM capture dot enzyme immunoassay (Mac DOT),⁷² and antibody capture radioimmunoassay (ACRIA)⁷³ are some of the newer modifications of Mac-ELISA that have been used in antibody detection. Other serological tests such as haemagglutination inhibition, the complement fixation test, single radial haemolysis, and the neutralisation test are still in use in some laboratories. A relative comparison of the ability of the various tests to detect JE is given in table 3.

The molecular fine mapping of important antigenic regions in JE over the last few years has paved way for the future development in laboratory diagnosis. The reverse transcriptase polymerase chain reaction amplification of viral RNA may help in specific and rapid detection of JEV in various samples.⁷⁴⁻⁷⁵

DIFFERENTIAL DIAGNOSIS

Many common illnesses masquerade as JE. They include both infective and non-infective conditions affecting the CNS (table 4). Among the various infectious causes, viruses that mimic JE include arboviruses (West Nile virus, western equine virus, eastern equine virus, etc), enteroviruses, herpesvirus, and Nipah virus. Nipah virus, a recently described paramyxovirus, resembles JE significantly except for a few features like a shorter incubation period, segmental myoclonus, and tendon areflexia.⁷⁶

MANAGEMENT

No specific antiviral therapy is available for JE. Treatment is mainly supportive and symptomatic. In vitro utility of isoquinoline compounds,⁷⁷ monoclonal antibodies,⁷⁸ and recombinant interferon alfa⁷⁹ in JE models has been demonstrated,

Table 4 Differential diagnosis of viral encephalitis

(A) Bacterial infections	
Pyogenic meningitis	Leptospirosis
Suppurative brain lesions	Tuberculosis
Typhoid encephalopathy	Mycoplasma encephalitis
(B) Viral infections	
Arboviruses (West Nile, eastern equine, western equine)	Enterovirus (Coxsackie virus, echovirus)
Herpesvirus	Nipah virus
Acute disseminated encephalomyelitis	
(C) Parasitic infections	
Cerebral malaria	Toxoplasmosis (HIV patients)
Amoebic encephalitis	Neurocysticercosis
(D) Rickettsial disorders	
Rocky Mountain spotted fever	Ehrlichiosis
(E) Fungal meningitis	
(F) Inflammatory conditions	
Systemic lupus erythematosus	Behçet's disease
(G) Primary CNS tumours: non-Hodgkin's lymphoma in HIV infected patients	
(H) Cerebral infarction	

but they should not be used in routine practice without further clinical studies. Corticosteroids have been used empirically, but a recent double blind controlled placebo trial failed to demonstrate any benefit.⁸⁰

Though we are handicapped by the non-availability of a specific drug against JE, mortality and morbidity can be decreased appreciably by control and treatment of factors causing secondary deterioration such as raised intracranial pressure and convulsions. The value of this approach has been documented in traumatic coma,⁸¹ and has been effectively applied in our centre in management of JE (table 5 and boxes 4 and 5).

Reduction of intracranial pressure, optimisation of systemic arterial pressure so as to maintain adequate cerebral perfusion pressure, and prevention of secondary complications are the main objectives of treatment. The concept of cerebral perfusion pressure is an important practical guide to management of intracranial hypertension (which often accompanies JE). Cerebral perfusion pressure is defined as the difference between mean arterial pressure and cerebral venous pressure. As cerebral venous pressure is equal to intracranial pressure in most circumstances, this equation may be rewritten as follows:

Cerebral perfusion pressure = mean arterial pressure – intracranial pressure⁸²

In presence of intracranial hypertension, autoregulation of cerebral blood flow is impaired, if cerebral perfusion pressure is less than 40 mm Hg.⁸³ When autoregulation is impaired, the degree of cerebral ischaemia is significantly worse due to lack of functional integrity of cerebral blood vessels, especially in conditions like viral encephalitis. Even minor degrees of hypotension or intracranial hypertension may markedly aggravate cerebral ischaemia. Focal cerebral lesions may impair autoregulation in affected areas alone. Other uninvolved areas with intact autoregulation may be affected during treatment, due to the intracranial steal phenomenon, as the induced vasoconstriction will result in shunting of blood away from these areas.

Maintenance of cerebral perfusion is essential to prevent secondary cerebral ischaemia. In conditions where cerebral autoregulation is impaired, cerebral perfusion depends exclusively on cerebral perfusion pressure. The normal cerebral perfusion pressure in infants is in the range of 30 mm Hg.⁸⁴ Hence it is important that cerebral perfusion pressure should be maintained above 30 mm Hg in infants less than 6 months, above 40 mm Hg in older children,⁸⁵ and above 60 mm Hg in adolescents.⁸⁶ Considering the fact that clinical signs appear when intracranial pressure is usually above 15–20 mm Hg,

mean arterial pressure should be maintained above 75 mm Hg in mild and moderate grade coma and more than 85 mm Hg in severe grade coma.

The most important aspect of managing intracranial hypertension is early identification and initiation of appropriate therapeutic measures. Most therapeutic measures fail if instituted late, as irreversible cerebral damage often occurs before these agents start their action. The control of intracranial hypertension involves a two pronged approach: (1) careful avoidance and control of situations that exacerbate intracranial pressure and (2) therapeutic measures to decrease intracranial hypertension, if present.

(1) Control of factors aggravating intracranial pressure

(A) Positioning of patient

A 15–30° head up tilt with head in midline position decreases intracranial hypertension and improves cerebral perfusion pressure.⁸⁷ Elevation enhances CSF drainage and maximises cerebral venous output. Midline position prevents any obstruction of jugular venous drainage.

(B) Temperature control

Aggressive treatment of fever is essential as fever worsens intracranial hypertension by increasing cerebral metabolism, cerebral blood flow, and cerebral oedema.⁸⁸ Antipyretics and other physical measures like tepid sponging and cooling blankets should be employed to reduce temperature effectively. As physical measures cause shivering, which can aggravate intracranial hypertension, they should always be used in conjunction with antipyretics.

(C) Role of sedation

Pain and arousal cause raised intracranial pressure by increasing cerebral blood flow. Sedatives have a huge role in prevention of the worsening of intracranial hypertension by this mechanism.⁸⁹ Conventionally, sedatives were avoided, due to fear of “clouding the neurological examination”. Such an ill supported prejudice should not be allowed to supersede proper control of intracranial pressure through sedation. In a clinical study conducted to identify the effect of management parameters on outcome in JE, it was found that sedation positively influenced immediate survival irrespective of severity of coma (unpublished data).

(D) Seizure control

Seizures increase intracranial pressure by increasing cerebral metabolism and cerebral blood flow and by Valsalva. Hence anticonvulsants should be administered prophylactically or therapeutically.

Table 5 Management protocol

Mild grade	
Intravenous fluids	Three quarters of maintenance (100 ml/100 kcal/day), with allowances for temperature, hyperventilation, and excess urine output
Electrolytes	Sodium: 3 mmol/100 kcal/day; potassium: 2 mmol/100 kcal/day
Temperature control	Paracetamol (60 mg/kg/day: divided doses every 4–6 hours)
Sedation	Physical measures: tepid sponging, cooling blankets
Anticonvulsants	Diazepam 0.1–0.3 mg/kg/dose as and when required
Moderate grade	
Intravenous fluids, electrolytes, temperature control	Similar to management of mild grade
Sedation	Diazepam every 6–8 hours
Head end elevation by 15°–30°	
Severe grade	
Intravenous fluids	Three quarters of maintenance: initial and when CVP is not available
	Full maintenance: if hypovolemia is present or if diuretic therapy is employed or after 48–72 hours, if no signs of ICT
Sedation	Intravenous diazepam every 6–8 hours
	Phenobarbitone 3–5 mg/kg/day in two divided doses
Temperature control, head end elevation, electrolytes	Similar to management of moderate grade
Seizure control	Step 1: Intravenous diazepam; followed by intravenous phenytoin
	Step 2: Phenytoin: loading dose 15–20 mg/kg infused at 0.5–1.5 mg/kg/min. Maintenance dose 5–8 mg/kg/day in two divided doses
	Step 3: Phenobarbitone: for uncontrolled seizures, 15–20 mg/kg at a rate not exceeding 1 mg/kg/min. Maintenance dose: 5–8 mg/kg/day
	Step 4: Diazepam infusion: if seizures still persist, then diazepam infusion at a rate of 0.1–0.4 mg/kg/hour or midazolam infusion: loading dose of 0.05–0.2 mg/kg followed by infusion at 1–5 µg/kg/min. Diluents to be used: sterile water, normal saline
	Step 5: Thiopentone infusion as mentioned below
Management of ICT	
<i>Stabilisation of vital functions and control of factors aggravating ICT should be instituted before the following medications are used</i>	
Mannitol (only if serum osmolality is ≤300 mmol/kg)	0.2–1 g/kg given over 30 min. ¹²⁸ Repeat doses (0.25–0.5 g/kg) can be given every 4–6 hours, after reassessing ICP status
Furosemide (furosemide)	Persistent ICT after mannitol therapy: 1 mg/kg/dose every 12 hours (potassium levels to be monitored)
Hyperventilation	Bag and mask ventilation or after endotracheal intubation. 4% lignocaine as local spray or intravenous lignocaine at 1 mg/kg/dose (slow intravenous) to be used to avoid further rise in ICP during intubation
Thiopentone	Used when above measures fail. Loading dose of 5 mg/kg over 30 min with maintenance dose of 1 mg/kg/hour as infusion. Maximum maintenance dose: 5 mg/kg/hour. Whenever maintenance dose is increased by 1 mg/kg/hour, a loading dose of 5 mg/kg to be given
Paralysis and ventilation	Pancuronium: 0.05–0.1 mg/kg intravenously to be used along with a loading dose of thiopentone 5 mg/kg. Succinyl choline and ketamine should be avoided. While tracheal suctioning, boluses of intravenous lignocaine 0.5–1 mg/kg may be used to prevent raise in ICP
Indications for antibiotics:	
1. When lumbar puncture is with held or traumatic, then crystalline penicillin at a dose of 400000 unit/kg/day and chloramphenicol 100 mg/kg/day in divided doses to be given till CSF findings are available.	
2. For aspiration pneumonia, crystalline penicillin at a dose of 200000 units/kg/day to be given.	
Mild: 13–15, moderate: 9–12, severe: ≤8. CSF, cerebrospinal fluid; CVP, central venous pressure; ICP, intracranial pressure; ICT, intracranial hypertension.	

(E) Fluid management

Traditionally, fluid and sodium restriction have been advocated in hope of preventing cerebral oedema. The value of such an approach has been seriously questioned in traumatic coma.⁹⁰ Dehydration has been found to increase risk of cerebral infarction in patients with subarachnoid haemorrhage.⁹¹ Hypovolaemia often accompanies viral encephalitis due to decreased intake and increased loss (vomiting, sweating). In our centre, fluid restriction is used only for

those children with mild and moderate grade coma. For those with severe grade coma, central venous pressure is used to guide fluid therapy.

(F) Electrolyte management

Full maintenance of sodium should be given as hyponatraemia impairs cerebrovascular reactivity. In our centre, we provide normal sodium maintenance and vigilantly treat any

Box 4: Monitoring

- Coma scale score.
- Seizure type and frequency.
- Clinical signs of intracranial hypertension.
- Blood pressure (continuous/hourly): mean arterial pressure >75 mm Hg in mild and moderate grade and >85 mm Hg in severe grade.
- Urine output every four hours: maintained at 0.5 ml/kg/hour.
- Calculated serum osmolality: every 24 hours (preferably every 12 hours).
- Continuous oxygen saturation monitoring, if not possible at least hourly monitoring.
- Central venous pressure for severe coma.

Box 5: Management of intracranial hypertension**Control of factors aggravating intracranial pressure**

- Positioning of head.
- Temperature control.
- Sedation.
- Seizure control.
- Appropriate fluid therapy.
- Appropriate electrolyte therapy.

Therapeutic measures to decrease intracranial pressure

- Hyperventilation.
- Mannitol.
- Furosemide (furosemide).
- Barbiturates.

Table 6 Prognostic factors

Poor prognostic factors	Good prognostic factors
Age less than 10 years	High levels of neutralising antibodies in CSF
Low Glasgow coma scale	High levels of JEV IgG in CSF
Hyponatraemia	
Shock	
Presence of immune complexes in CSF	
Presence of antiNFP or antiMBP antibodies	
Increased levels of tumour necrosis factor	
Coexisting evidence of neurocysticercosis ¹²⁹	

CSF, cerebrospinal fluid; JEV, Japanese viral encephalitis; MBP, myelin basic protein; NFP, neurofilament protein.

degree of hyponatraemia, as and when it arises and maintain serum sodium above 140 mmol/l.

(2) Therapeutic measures to decrease intracranial pressure

(A) Hyperventilation

Reduction in intracranial pressure induced by hyperventilation is the result of a decrease in cerebral blood flow secondary to cerebral vasoconstriction caused by carbon dioxide wash-out. In normal subjects, cerebral blood flow decreases by 4% per mm Hg decrease in carbon dioxide tension (PCO_2). The ceiling limit of cerebral blood flow reduction is 40%, which corresponds to a PCO_2 2.67–3.33 kPa (20–25 mm Hg). Further reduction in PCO_2 level has no beneficial effect on cerebral blood flow. Intracranial pressure begins to diminish 10–30 seconds after inception of hyperventilation, reaches a nadir in 30 minutes, and returns to the original value in less than hour.⁹² The potential deleterious effects of hyperventilation include an elevation of mean airway pressure and diminished cardiac filling pressures with resultant barotrauma and hypotension respectively. Although hyperventilation is useful in acutely decreasing intracranial pressure, prolonged hyperventilation, in fact, worsens the outcome. This could be probably due to production of oligoemia in marginally perfused brain tissue by chronic aggressive hyperventilation.⁹³ Hence, hyperventilation should be gradually withdrawn (rapid withdrawal causes rebound intracranial hypertension) after a period of 30–60 minutes so as to raise PCO_2 level by 0.2–0.29 kPa/hour, with institution of other modes of therapy simultaneously.

(B) Mannitol

Mannitol is the most commonly used agent for control of intracranial hypertension. Response to mannitol depends on original intracranial pressure, dose given over the previous three hours (the lesser, the better effect),⁹⁴ and rate of administration. Rapid administration of mannitol is more effective in reducing intracranial pressure, but the action has a much shorter duration. A slower infusion rate produces a lesser degree of decrease that lasts longer.^{95–96} The protracted effect of mannitol is due to water reduction from the intravascular compartment (diuresis), whereas vascular mechanisms explain the acute effect of mannitol. Intravenous administration of mannitol decreases blood viscosity and cerebral blood flow increases transiently.⁹⁷ In the presence of autoregulation, this causes a reflex vasoconstriction and decrease in cerebral blood flow and intracranial pressure. If autoregulation is impaired, as in the case of diffuse neuronal injury in encephalitis, then the effect of mannitol is not maximal.⁹⁸ Though mannitol is a very useful drug, significant side effects can occur if used inappropriately. Mannitol tends to accumulate in oedematous white matter and cause a hyperosmolar state, which is injurious to the brain.⁹⁹ Hence overdosing should be avoided to prevent formation of this hyperosmolar state. When prescribing mannitol, both standing and prophylactic orders should be avoided. Use of mannitol should be limited to patients with a

serum osmolality of less than 300 mmol/l.¹⁰⁰ This is because administration of mannitol at a dose of 1 g/kg over a period of 20 minutes increases serum osmolality by 11%–15%,¹⁰¹ and there is a high risk of tubular damage and renal failure when serum osmolality exceeds 330 mmol/l. Chronic continuous therapy should be avoided as the brain adapts to the sustained hyperosmolality of plasma with an increase in intracellular hyperosmolality by increased concentrations of cations, sodium and potassium and free amino acids, which contribute to “idiogenic” osmoles (that appear in brain in its adaptation to hyperosmolality and contribute to rebound cerebral oedema after withdrawal of anticerebral oedema measures).¹⁰²

(C) Frusemide (furosemide)

It acts by interfering with the formation of CSF and preferential excretion of water over solute in the distal renal tubule.¹⁰³ Frusemide (furosemide) alone causes a slow reduction in intracranial pressure, but when combined with mannitol, the fall in intracranial pressure is rapid and is sustained for a considerably longer period than when either agent is used alone.¹⁰⁴

(D) Barbiturates

Short acting barbiturates like thiopentone, as a continuous infusion, evoke a decrease in intracranial pressure by decreasing cerebral blood flow and cerebral metabolic oxygen demand.¹⁰⁵ The therapeutic objective is either to elicit a satisfactory decrease in intracranial pressure or to produce burst suppression in a continuously monitored EEG. Barbiturate therapy has the added advantage of causing sedation, which by itself can contribute to reduction of intracranial hypertension. Unfortunately, barbiturate therapy is associated with major adverse effects, including precipitate hypotension,¹⁰⁶ and hence should be reserved only for cases in which other measures have failed.

PROGNOSTIC FACTORS IN JE

Certain factors have been identified which can be used to predict outcome in terms of mortality and morbidity. These are listed in table 6 (unpublished data).^{107–108}

CONTROL OF JE (SEE BOX 6)

Vector control

Information regarding prevalence, density, and insecticide susceptibility of known and potential vectors of JE is essential for control of vectors. Surveillance of the adult mosquito

Box 6: Prevention

- Control of mosquito vectors.
- Vaccination of humans.
- Prevention of mosquitoes from biting humans.
- Measures against reservoirs.

Table 7 Vaccines against Japanese encephalitis

Vaccine	Strain	Efficacy
Inactivated mouse brain	Nakayama, Beijing-1	91% ¹³⁰
Inactivated primary hamster kidney cell	P3	85% ⁴¹
Live attenuated primary hamster kidney cell	SA 14-14-2	>95% ¹⁰⁹

population should be carried out throughout the year. Spraying of an appropriate insecticide should be carried out in the resting places of mosquitoes. Thermal fogging with ultra low volume insecticides such as pyrethrum or malathion has been recommended for the prevention of local transmission during epidemics, particularly in periurban areas with marshes. The vastness of breeding places make larvicidal measures currently impracticable. Effective measures undertaken in some countries to prevent or inhibit larval development include novel water management and irrigation practices such as periodic lowering of the water level, intermittent irrigation, and constant flow systems. Vector control alone cannot be relied upon to prevent JE.

Vaccine

Vaccination of the population at risk is the method of choice for prevention of JE. The earliest controlled field trials with a vaccine against JE were carried out in Japanese schoolchildren in the latter half of the 1940s. Three JE vaccines are in use worldwide (table 7).

Inactivated mouse brain vaccine had been used in Japan for 30 years and Asian field trials have shown that two doses (one to weeks apart) provide an efficiency of 91%. However three doses (0, 7, and 14/30 days) appear to be necessary to produce a protective immune response in persons living in non-endemic regions. In China, 75 million doses of inactivated primary hamster kidney cell derived vaccine are distributed each year. The vaccine's efficacy is only 85% and a live attenuated vaccine, made from the SA-14-14-2 strain, has been developed as a potentially more effective alternative. This vaccine is cheap (60 cents). Extensive efficacy trials have demonstrated >95% protection with two doses.¹⁰⁹ The vaccine appears to be safe and neuroattenuation evidently is stable.¹¹⁰

The decision on the population to be vaccinated should be decided by local epidemiological factors. The use of JE vaccine in travellers should be decided after adequate consideration of the risk-benefit ratio for the various factors on the risks for exposure to the virus and for developing illness, the availability and acceptability of repellents and other alternative protective measures, and the side effects of vaccination.¹¹¹ However, it is better to individualise the considerations regarding vaccination, particularly in travellers who are likely to spend less than 30 days in an endemic area, as it requires just a single bite by an infected mosquito to cause JE in a particular individual. Risk assessments should be interpreted cautiously since risk can vary within areas and from year to year and available data are incomplete. Infants need not be vaccinated, though as a precautionary measure they should be protected from mosquito bites. In general, vaccination is indicated in following groups.

1. People living in endemic areas.
2. Travellers spending 30 days or more in an endemic area.
3. Travellers spending less than 30 days during epidemics or extensive outdoor activity in rural areas is expected.
4. Laboratory workers with potential risk of exposure to JEV. JE vaccination is associated with local side effects (20%: tenderness, redness, swelling), sometimes with systemic adverse reactions (10%: fever, headache, malaise, rash), occasionally with hypersensitivity reactions (0.5%: generalised

Box 7: Key references

1. Solomon T, Dung NM, Kneen R, et al. Japanese encephalitis. *J Neurol Neurosurg Psychiatry* 2000;**68**:405–15.
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urticaria, angio-oedema, respiratory distress, and anaphylaxis), and very rarely major neurological side effects (1–2.3 per million recipients: encephalitis, seizures, and peripheral neuropathy).^{112–114} However, a report from Europe (Denmark) has suggested that the incidence of major neurological side effects might be higher. It is possible that the difference in the incidence of neurological side effects may due to differences in surveillance or ethnic or geographical difference.¹¹⁵ Type I hypersensitivity reactions have been recognised among European, American, and Australian vaccine recipients in the last decade.¹¹⁶

Second generation recombinant vaccines are being developed with the aim of improving immunogenicity and decreasing adverse reactions seen with current vaccines. In these vaccines, signal sequences of PrM along with genes encoding PrM and E proteins are packaged into viral vector like *Escherichia coli* and vaccinia.^{117–119} The immunogenicity and protective efficacy of these expression systems has been demonstrated in animal models.

Prevention of mosquito bite

Use of nets and mosquito repellents by the population at risk and avoidance of outdoor sleeping in the tropics in evening hours, staying in screened houses, and wearing long sleeved shirts and long trousers¹¹¹ reduces the risk of exposure to vector mosquitoes.

Protection of reservoirs

Building of piggeries away from human dwellings in countries where pigs are reared near human settlement and making them mosquito proof would be desirable. Spraying of piggeries and mixed dwelling with residual insecticides, wherever there is an alarming rise in vector species, should be carried out promptly. Vaccination of pigs has also shown encouraging results.

MULTIPLE CHOICE QUESTIONS (ANSWERS AT END OF PAPER)

Q1: From which of the following species of mosquitoes, has JEV not been isolated?

- (A) Culex
- (B) Anopheles
- (C) Mansonia
- (D) Aedes

Q2: The major immunogen in JEV is

- (A) Capsid protein
- (B) Member protein
- (C) Envelope protein
- (D) Non-structural proteins

Q3: In which of the following organelles does JEV multiply intracellularly—I, Golgi apparatus; II, rough endoplasmic reticulum; III, mitochondria; IV, lysozyme?

- (A) I and II
- (B) I and III
- (C) II and III
- (D) II and IV

Q4: What is the method of choice for detection of IgM antibodies in blood and CSF?

- (A) Reverse passive haemagglutination
- (B) Immunofluorescence
- (C) Staphylococcal coagglutination tests
- (D) Mac-ELISA

Q5: Who among the following do not require JE vaccine?

- (A) People living in endemic areas
- (B) Travellers spending 30 days or less in an endemic area
- (C) Travellers spending less than 30 days during epidemics
- (D) Laboratory workers with potential risk of exposure to JEV

Q6: One of the following statements regarding JE is true

- (A) Sedation of children with JE should be avoided
- (B) Mannitol can be given irrespective of serum osmolality
- (C) Chronic aggressive hyperventilation can be used in the management of intracranial hypertension in JE
- (D) A 15–30° head up tilt with the head in midline position decreases intracranial pressure

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ANSWERS

1: D; 2: C; 3: A; 4: D; 5: B; 6: D.

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