TOWARDS IMPROVING THE MANAGEMENT OF ACUTE ENCEPHALITIS SYNDROME IN NEPAL

by

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ABSTRACT

Acute Encephalitis Syndrome (AES) is a group of clinical symptoms and signs, used by the World Health Organisation (WHO) and clinicians, to screen for acute encephalitis. Viruses are the most important cause of AES worldwide. Japanese encephalitis virus (JEV), which causes Japanese encephalitis (JE), accounts for approximately one-quarter of AES cases in Nepal. In the absence of definite treatments for JE and many other viral enecpahlitides, improvements in supportive management are vital.

In my thesis, the predictors of bad outcome (neurological sequelae or death) among patients with AES and JE were investigated. The relationships between weight-for-age (WFA), hydration status, intravenous fluids and outcome were studied. In addition, a preliminary randomised double blind placebo controlled trial of intravenous immunoglobulin (IVIG), as a novel adjunctive treatment for JE, was conducted.

Prolonged fever duration was identified to be a significant predictor of bad outcome in both AES and JE patients. Prolonged fever, low Glasgow coma score (GCS) and focal neurological deficit at hospital admission were significantly associated with bad outcome in AES patients. AES patients with focal neurological deficit were significantly more likely to have a final diagnosis of JE. JE patients presented with a significantly lower body weight and higher respiratory rate. They also presented with a trend for higher urea and potassium levels compared to other AES patients. These findings led me to investigate further whether children with JE were more likely to suffer from dehydration during acute illness.

When children are grouped into different weight categories by WFA (Z score), low WFA can indicate dehydration or malnutrition. I found a significant association between frequency of bad outcome and low WFA among both AES and JE patients at hospital admission. To help distinguish dehydration and malnutrition in low WFA children, I then studied additional indicators of malnutrition and dehydration status, including mid-upper arm circumference, blood lactate levels and fluid status at admission and during hospital stay. I found AES patients suffering a bad outcome had significantly higher admission serum lactate levels, drunk a lower volume of oral fluids, and were more likely to be prescribed a restricted regimen of intravenous fluids. These results suggest AES patients with bad outcome were more likely to be dehydrated. The implications of my findings are that earlier hospital admission during the course of the illness and better in hospital administration of adequate and appropriate fluids may improve outcome among AES and JE patients. Since the majority of families self-refer to hospitals, provision of this simple message into the community, could help improve the lives of people living in high risk areas for JE, like Nepal.

Improvement in the treatment of JE is necessary to improve the outcome of the disease in Nepal. Intravenous immunoglobulin, which contains anti-JE virus neutralising antibodies and has anti-inflammatory properties, may be a useful adjunctive treatment. In a preliminary Phase II study, I showed IVIG could be safely administered to JE patients, without any significant increase in drug related adverse events. JE patients treated with IVIG exhibited higher levels of neutralising antibodies and higher IL-4 and IL-6 cytokine levels compared with placebo (saline) treated patients. Although, there was no difference in clinical outcome, the data from this small pilot study suggests IVIG may be an appealing adjunctive treatment option for a phase III trial in the future.

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DECLARATION

Except for the assistance as outlined in the acknowledgements above, the work described is my own work and has not been submitted for a degree or other qualification to this or any other university.

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Publication and presentations arising from this work

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1. **Rayamajhi A, Ansari I., Ledger E, Bista KP, Impoinvil DE, Nightingale S, Kumar R, Mahaseth C, Solomon T, Griffiths MJ.** Clinical and prognostic features among children with acute encephalitis syndrome in Nepal; a retrospective study. *BMC Infectious Diseases.* 2011;11:294 DOI: 10.1186/1471-2334-11-294.
2. **Rayamajhi A, Nightingale S, Bhatta NK, Singh R, Ledger E, Bista KP, Lewthwaite P, Mahaseth C, Turtle L, Robinson JS, Galbraith SE, Wnek M, Johnson BW, Faragher B, Griffiths MJ, Solomon T.** A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for Japanese encephalitis in Nepal. *PLoS ONE*. 2015;10(4):e0122608.

Other publications indirectly connected to this work

1. **Impoinvil DE, Solomon T, Schluter WW, Rayamajhi A, Bichha RP, Shakya G, Caminade C, Baylis M.** The spatial heterogeneity between Japanese encephalitis incidence distribution and environmental variables in Nepal. *PLoS ONE*. 2011;6:e22192.
2. **Griffiths MJ, Lemon JV, Rayamajhi A, Poudel P, Shrestha P, Srivastav V, Kneen R, Medina-Lara A, Singh RR, Solomon T.** The functional, social and economic impact of acute encephalitis syndrome in Nepal - a longitudinal follow-up study. *PLoS Neglected Tropical Diseases*. 2013;7: e2383.
3. **Baylis M, Barker CM, Caminade C, Joshi BR, Pant GR, Rayamajhi A, Reisen WK, Impoinvil DE**. Emergence or improved detection of Japanese encephalitis virus in the Himalayan highlands? *Trans R Soc Trop Med Hyg*. 2016;110:209-11.

Invited presentations

1. **Ajit Rayamajhi** Developing Paaediatric Neurology as a Subspecialty in Nepal *British Paediatric Neurology Association 2011 Annual Meeting* 26-28 January 2011, Edinburgh, UK.
2. **Ajit Rayamajhi** Progress on Research in Acute Encephalitis Syndrome and Japanese Encephalitis *Government of Nepal/National Committee on Immunisation Programme joint Consultative meeting on JE, Maternal Neonatal Tetnus and Polio* 17-18 November 2011, Kathmandu, Nepal
3. **Ajit Rayamajhi** Validation of Predictors of Bad Outcome of Acute Encephalitic Syndrome of Suspected Viral Aetiology and Japanese Encephalitis in Nepali Children *Infection & Global Health Day* 30 October 2012, Liverpool, UK.
4. **Ajit Rayamajhi** Japanese encephalitis: Clinical features, Investigation and Follow Up of Confirmed JE Cases *Immunisation for Vaccine Preventable Diseases: First Aid Training and Quarterly Review Meeting of WHO-IPD* 11th April 2012, Kathmandu, Nepal.
5. **Ajit Rayamajhi** Brain Infections in Nepal *Brain Infection UK Research Update Day* 16th May 2012, Liverpool, UK.
6. **Ajit Rayamajhi** Progress on AES and JE Research in Nepal *Quarterly review meeting of WHO-IPD Nepal* 24 January 2013, Kathmandu, Nepal.
7. **Ajit Rayamajhi** Intravenous Immunoglobulin to Treat Japanese Encephalitis- A Phase II Randomised Double Blind Placebo Controlled Trial *The 27th Congress of the International Pediatric Association* 24-29th August 2013, Melbourne, Australia.
8. **Ajit Rayamajhi** Improving outcome of AES and JE in Nepali Children *Workshop on Emergence of Japanese Encephalitis in the highlands of Nepal* April 9-10th 2014, Nagarkot, Nepal.

LIST OF ABBREVIATIONS

AE	Acute encephalitis
AES	Acute encephalitis syndrome
ADH	Anti-diuretic hormone
AFRIMS	Armed Forces Research Institute of Medical Sciences
BBB	Blood brain barrier
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebralspinal fluid
CT	Computed Tomography
ELISA	Enzyme-linked immunosorbent assay
F- 75 diet	Diet of 100 grams provide 75 Kilocalories
GCS	Glasgow coma score
ICP	Intracranial pressure
IL	Interleukin
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IVF	Intravenous fluids
IVIG	Intravenous immunoglobulin
JE	Japanese encephalitis
JEV	Japanese encephalitis virus
LOS	Liverpool outcome score
LP	Lumbar puncture
MAC-ELISA	IgM antibody capture-Enzyme Linked Immunosorbent Assay
MRI	Magnetic Resonance Imaging

MUAC	Mid-upper arm circumference
Non-JE	Non-Japanese encephalitis
NPV	Negative predictive value
OR	Odds ratio
ORS	Oral rehydration salts
PAID	Paroxysmal autonomic instability with dystonia
PPV	positive predictive value
RCT	Randomised control trial
RNA	Ribonucleic acid
RT-PCR	Reverse-transcriptase polymerase chain reaction
SES	Socioeconomic status
SIADH	Syndrome of inappropriate ADH secretion
SPECT	Single-Photon Emission Computed Tomography
TNF	Tumour necrosis factor
VE	Viral encephalitis
WFA	Weight for age
WHO	World Health Organisation
WHO-IPD	World Health Organisation Programme for Immunisation Preventable Diseases

Chapter 1: Introduction

1.1 Acute Encephalitis Syndrome (AES)

Acute Encephalitis Syndrome (AES) is defined as acute onset of fever, with an acute change in mental status (including confusion, disorientation, coma or inability to talk) and /or new onset of seizures (excluding simple febrile seizures) in a person of any age (1). It is a group of clinical symptoms and signs, used by the World Health Organisation (WHO) for surveillance purposes, to screen patients with viral encephalitis, including Japanese encephalitis (JE). These clinical features suggest the patient has acute inflammation of the brain. These features are also used by clinicians to help identify patients with suspected encephalitis. Viruses are regarded as the most important cause of encephalitis worldwide. However, encephalitis can also be caused by bacteria or parasitic infections, as well as immune mediated processes in the brain. Despite intense diagnostic testing, recent studies in England, Vietnam and Papua New Guinea did not identify a specific aetiology among 37%, 59% and 63% of encephalitis patients respectively (1-3). Specific aetiological agents differ considerably depending on geographical location and age of the patient. In the United Kingdom (UK), where there are adequate resources, specific aetiologies were identified in 33% children and 45% adults, with herpes simplex virus (HSV) the most common diagnosis (4). In North America, enteroviruses were found to be the most common aetiology in children and HSV-1 in adults (5,6). In Southeast Asia, Japanese encephalitis is the most common cause in children, accounting for 31%- 45% of cases

(6). Emerging viruses, such as Nipah virus, avian influenza H5N1 and bat lyssavirus, have recently been linked to encephalitis in humans. Discovery of known pathogens in new regions, such as West Nile virus in Romania, and greater recognition of antibody mediated encephalitis, where antibodies develop against neuronal components, such as voltage gated potassium channels (VGKC) or N-methyl-D-aspartate receptors (NMDAR) are of an increasing global concern (7).

1.2 Aetiology of AES

In the UK, common aetiologies of viral encephalitis are herpes simplex virus 1 and 2, varicella zoster virus, enterovirus, parechovirus, adenovirus, human herpesvirus-6, mumps virus, measles virus and influenza A and B viruses (2). Other rarer viral aetiologies include cytomegalovirus, Epstein-Barr virus, hepatitis viruses, human T-cell lymphotropic virus, parainfluenza virus, parvovirus B19, lymphocytic choriomeningitis virus, polio virus, rabies virus, respiratory syncytial virus and human immunodeficiency virus (2). Other viruses suspected to cause AES in other settings include Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, Hendra virus, Kyasanur forest disease and St Louis encephalitis virus (8). Bacteria more often cause meningitis than encephalitis. However, bacterial meningitis can present as AES when patients exhibit altered consciousness or seizures. Bacteria known to cause AES include; *Streptococcus pneumoniae, Hemophilus influenza* type B, *Niesseria meningitidis, Streptococcus pyogenes, Bacillus anthracis, Bartonella henselae, Chlamydia trachomatis, Chlamydophila psittaci*, Leptospira, *Listeria*

monocytogenes, *Borrelia burgdorferi, Mycoplasma pneumoniae*, Salmonella and Mycobacterium tuberculosis (7). Other infectious aetiologies, include Coxiella burnetii, Rickettsia rickettsii, Toxoplasma gondii and Histoplasma capsulatum (7). Acute disseminated encephalomyelitis (ADEM) is the second most common diagnosed encephalitis and first most common immune-mediated encephalitis in the UK (2). Other immune-mediated encephalitis include NMDA receptor antibodies, voltage-gated potassium channel antibodies, multiple sclerosis and secondary to systemic vasculitis (2).

A variety of factors influence viral pathogens, particularly arboviruses, associated CNS infection in Asia. It includes presence of a variety of viral vector mosquitoes that bite humans; close interaction between humans and animals, such as pigs or birds, which act as intermediate hosts for JEV and H1N1 influenza virus; poor housing having open windows and thatch roofs, which provide access and suitable habitats for mosquitoes to live inside home; and wet, warm climates that can promote mosquito breeding and survival. Other important contributory factors include, poverty, overcrowding, poor sanitation and lack of education. Clinicians often have limited access to diagnostics in resource poor settings. AES patients are ideally investigated by lumbar puncture. Depending on the laboratory set-up, cerebrospinal fluid (CSF) may, but not always, undergo microscopy for total cell count, white cell differential, staining for bacteria (Gram and Acid Fast Bacillary) and bacterial culture. CSF may also undergo protein and glucose measurement. In part, due to lack of viral culture, molecular assays and limited serological testing, aetiology is often unknown. As a result, patient treatment is often undirected.

Even hospitals in densely populated areas can have limited diagnostic facilities. In these areas, based on clinical features, national surveillance data, and reports from population screening programmes, viruses are suspected to be the most common cause of AES. Reported viral pathogens include: Japanese encephalitis, enteroviruses (e.g. Coxsackie, echovirus or polio), herpes simplex, measles, West Nile, varicella-zoster, dengue, Nipah, Chandipura, and Murray valley. Other reported causes include; pyogenic bacteria, mycobacteria, fungi, malaria, leptospirosis, rickettsia, toxoplasma and rabies. Many non- infectious conditions can cause encephalopathy, overlapping the clinical features for AES. Such conditions include: hypoglycemia, shock, head injury, poisoning, diabetes ketoacidosis, epilepsy, cerebral vasculitis, glomerulonephritis, cerebral tumour or Reye's syndrome. Excluding these conditions poses a challenge to clinicians, particularly in resource poor settings (7). In Nepal, aetiology of AES has not been extensively studied.

1.3 Incidence of AES

Since there are many aetiological agents causing encephalitis, and the diagnosis is difficult to make, acute encephalitis (AE) has been described as a whole disease syndrome. Where population based studies have been undertaken, the incidence of AES is reported to be 6.34 and 7.4 per 100,000 populations in tropical and Western industrialised country settings, respectively. In relation to age group, the incidence of AES is reported to be 10.5 per 100,000 AES cases in children and 2.2 per 100,000 cases in adults (9).

1.4 Clinical features of patients of AES

Clinical presentation depends on the aetiological agent, age of the patient, site of lesion in the central nervous system (CNS) and immune status of the patient. There is substantial overlap between clinical presentations of AE with other infections of the CNS. In general, involvement of cerebral cortex, midbrain, pons, medulla oblongata and cerebellum cause upper motor lesion types of symptoms and signs. Younger children may present with fever, headache, vomiting, diarrhoea, cough, sore throat, running nose, shortness of breath, lethargy, irritability, urinary retention, urinary incontinence, skin rash, impaired consciousness, seizures, change in behaviour and personality, photophobia, motor or sensory deficits, speech or movement disorder (10). Older children have milder symptoms such as mild irritability, subtle speech and personality or behavioural abnormalities. Sometimes patients may present with characteristic clinical features such as tremors and dystonia in JE, emotional instability and confused state in herpes simplex virus encephalitis, cerebellar ataxia in varicella-zoster virus encephalitis and deafness in mumps encephalitis. On rare occasions, JE, Murray Valley encephalitis, St. Louis encephalitis, tick-borne encephalitis, West Nile virus, non-polio enteroviruses, Louping ill and Powassan virus illness may present with acute flaccid paralysis (11).

1.5 Diagnosis of AES

Investigation depends on the available facilities and likely local pathogens. The cerebral spinal fluid (CSF) contains from a few to a thousand cells. Initially neutrophils and later lymphocytes predominate. The CSF protein level may be normal or elevated. Glucose level may be normal. However, characteristically,

CSF protein may be elevated in herpes simplex virus encephalitis and CSF glucose low in mumps virus encephalitis (12). CSF culture may show viruses but to increase aetiological diagnosis, it should be combined with specimens collected from nasopharyngeal swab, urine and faeces. CSF polymerase chain reaction (PCR) can also identify viruses for JE, dengue, Nipah encephalitis, Murray Valley encephalitis, St Louis encephalitis, poliomyelitis, rabies encephalitis and West Nile encephalitis. CSF serology is a cheap alternative to diagnose JE, dengue, tickborne encephalitis, Nipah encephalitis, Murray Valley encephalitis, St Louis encephalitis, rabies encephalitis, herpes simplex encephalitis and West Nile encephalitis. CSF antibodies against voltage-gated potassium channels and NMDA receptor could help diagnose immune-mediated forms of encephalitis (2).

1.6 Treatment of AES

Management is predominantly supportive. Treatment for AES, in high risk areas, include appropriate antibiotics for bacterial meningitis, quinine for cerebral malaria and fluoroquinolones with high dose dexamethasone for typhoid encephalopathy. Aciclovir is the only definite treatment available for viral encephalitis, which is administered for herpes simplex and herpes zoster encephalitis. All other viral encephalitis have no definite treatment. Acetaminophen is administered for fever, codeine for nausea and non-aspirin containing analgesics for headache. Severely ill patients require hospitalisation, intensive care, anticonvulsants for control of seizures and administration of intravenous fluids. The patients need to be monitored for complications such as convulsions, cerebral oedema, raised intracranial pressure, electrolyte imbalance, adequate breathing and aspiration pneumonia.

1.7 Clinical outcome of AES

Outcome of AES in children differs based on the aetiology of AES, virulence of the organism, stage of illness, immunity status of the host, available definite treatment and supportive management. Since a large majority of AES do not have definite treatment, estimating the outcome becomes important in explaining the prognosis to the parents at the time of admission. In the past, different diagnostic criteria have been used to define AES or acute encephalitis cases. Some cases have been based on the WHO International Classification of Disease (ICD) (13-15). Some cases have been reported based on the final diagnosis by consulting physician (16, 17). Encephalitis cases have been reported based on a variety of inclusion criteria (18). Some publications have failed to provide exclusion criteria (4, 18, 19). Accepting the wide variety of case definitions for children with AES, mortality rates range between 4- 29%, with 14- 60% having neurological sequelae at discharge (3,6,10,20-25). Studies that have followed up AES cases in the community report between 2.8- 30% of cases exhibit residual neurological sequelae 6 months post-discharge (16,26,27).

1.8 Sequelae of AES

Residual neuro-psychiatric sequelae are seen in quarter of children with AES. Sequelae are more common in JE as compared to other viral encephalitides (28). Behavioural problems, such as hyperactivity, are the most common sequelae. Other sequelae described include; development delay, expressive language difficulties, receptive language problems, visual impairment, hearing impairment, seizures, cranial nerve palsies, bulbar palsy, ataxia, urinary

retention or incontinence, monoparesis and hemiparesis. Given the aetiologies of AES exhibit seasonal and geographical variation, outcomes are also likely to vary between studies conducted in different locations and at different times of the year.

1.9 Prognostic indicators of AES

In resource-rich settings (29,30), clinical predictive scores are now a cornerstone of the management of a range of clinical conditions (30-32). Efforts have been made to identify indicators of poor outcome in developing countries as well (33, 34). These can help focus attention on the most severe patients, who are likely to do badly, as well as providing important clues about disease mechanisms. Similarly, indicators of bad outcome in AES and JE have been reported in the past. A previous study conducted in Nepali children has identified, prolonged duration of fever, as a predictor of bad outcome in AES and JE (10). However, these results were from a single location and the authors expressed uncertainty regarding its application to other settings.

1.10 Recovery of sequelae in AES

Since there is no definite treatment for majority of patients of AES, knowing the recovery profile of patients of AES would help in counselling and explaining to parents about the prognosis of AES. In children with viral encephalitis other than JE, motor sequelae is seen to decrease from 8/36 (22%) at the time of discharge to 5/36 (14%) at 6 weeks (10). Paucity of data and lack of definite treatment necessitates more research in this area of AES.

1.11 Japanese encephalitis (JE) and other Flaviviruses

Although epidemics of encephalitis were described in Japan from 1871, the Nakayama strain of JEV was first isolated from a fatal human case in 1935. At least five genotypes of JEV circulate in Asia. Although the geographical boundaries may overlap between different genotypes, they are of same serotype and similar virulence and host preference. Presently genotype 1 is the most widely circulating genotype in the world (35). JEV causes JE. JE affects over 68,000 people annually, leading to 20-30% mortality and 30-50% disability, with children bearing the brunt of the disease burden (20, 36-38). JEV (genus *flavivirus*, family *Flaviviridae*) is a neurotropic *flavivirus,* transmitted by bite of an arthropod (*Culex* mosquito) because of which it is also classified as Group B arbovirus. All neurotropic *flaviviruses* are antigenically related and derived from common ancestor (Figure 1.1). They have small positive sense RNA in icosahedral shaped capsid. The vectors are either ticks or mosquitoes.

Flavivirus encephalitis occurs as a result of complex interaction between environment, virulence of the virus, human activity, entomology, bird and host immunity. These mosquito borne viruses are transmitted naturally between birds in an enzootic cycle by bird biting mosquitoes. Humans become incidental dead-end hosts by crossing path in this cycle and because of their inability to transmit the virus further (exception being Yellow fever and Dengue viruses). Every year, mosquito-borne, St Louis encephalitis in North America, West Nile encephalitis in Africa, Murray Valley encephalitis in Australia and JE in Asia occur as a result of this interplay. In cooler climates of northern Europe and Asia, ticks are more abundant. The tick-borne neurotropic

flavivirus encephalitis that occur more frequently in the region are; Russian spring-summer encephalitis in Russia, Western tick-borne encephalitis in Germany and Austria and Louping Ill viral encephalitis in UK.

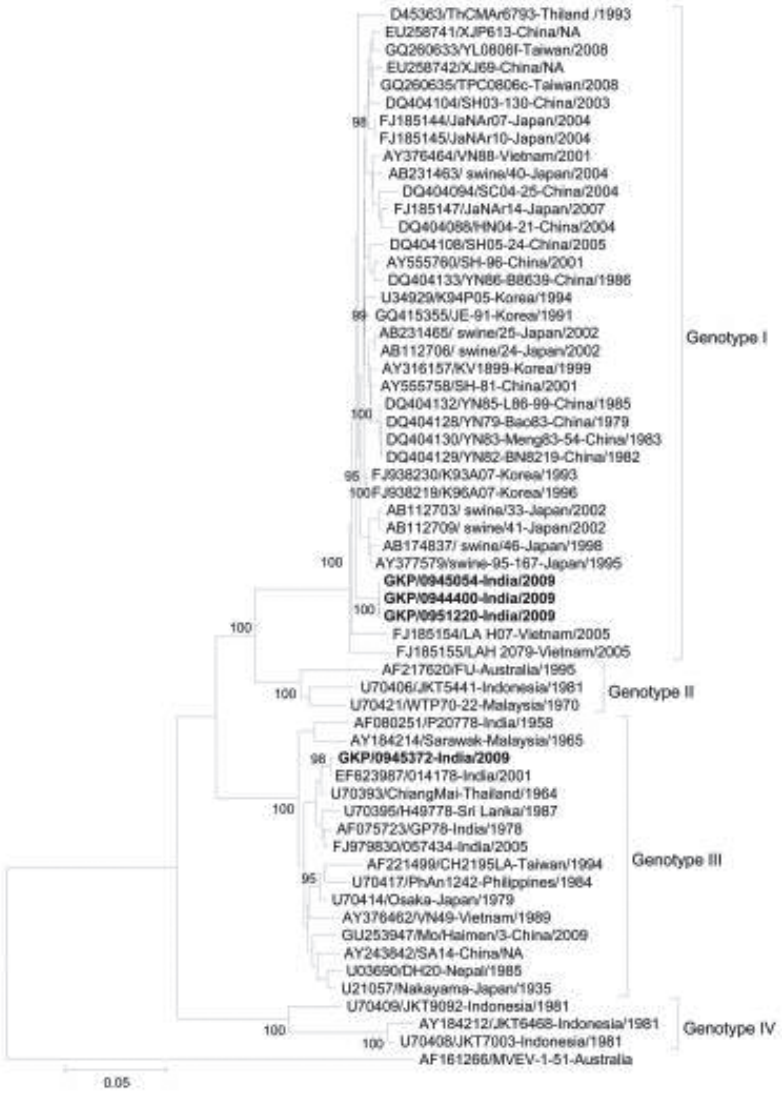


Figure 1.1: Phylogenetic tree directly amplified from CSF collected in acute stage of illness of patient of AES using a 1,381-nt JEV envelope sequence between September and November, 2009. Multiple sequence alignment and phylogenetic analysis were conducted by using ClustalW software (www.ebi.ac.uk/Tools/clustalw2/ index.html) and MEGA version 4 (www.megasoftware.net). The tree was rooted within the JE serogroup by using Murray valley virus (GenBank accession No AF 161266). The robustness of branching patterns

was tested by 1000 bootstrap pseudo replicates. Each strain is abbreviated with accession number, strain name followed by country of origin and year of isolation on the right of each tree. Bootstrap values are indicated above the major branch; 43 taxa comprised the in-group, and all taxa were rooted with Murray valley encephalitis virus (39).

1.12 Morphology of JEV

Morphologically JEV has a 50 nm lipoprotein envelope surrounding a nucleocapsid comprised of core protein wrapped around 11 KB of single-stranded positive-sense RNA. The RNA has three regions: a short 5' and longer 3' untranslated regions (UTR) and a single open reading frame. The single open reading frame encodes for three structural proteins (core – C, pre-membrane – prM and envelope – E) and 7 non-structural (NS) proteins (40, 41). The E protein is critical for viral attachment and entry into cells and along with NS1 and NS3 is a major target of the immune response.

1.13 Enzootic cycle of JE

Although JEV transmission occurs in many animals, those that have high viremia are important in the natural cycle. JEV is transmitted naturally between birds and pigs by *Culex* mosquito (Figure 1.2). Collected rain water in paddy fields for sewing rice during Monsoon provides suitable breeding site for female *Culex tritaeniorhynchus* mosquito an important vector that transmits JE in human. The mosquito is known to be infective 14 days after entry of JEV from a viraemic host and fly up-to 5 kilometres; important information during epidemics (42). Rice-fields are important sites for JEV transmission to susceptible birds (cattle egrets and herons) and humans. Pigs are important

amplifier host for the virus because of high and prolonged viraemia, continuous source of nascent hosts through off springs and are raised in close proximity to human dwelling causing viral transmission and risk of encephalitis. Apart from abortions in pigs, the virus does not cause any illness in its natural hosts. Although JEV has been recovered from human blood, the low level of viraemia is not sufficient to infect a mosquito. When the mosquito population becomes adequately high, JEV transmission spills over from the mosquito-bird-pig cycle into human populations through mosquito bite.

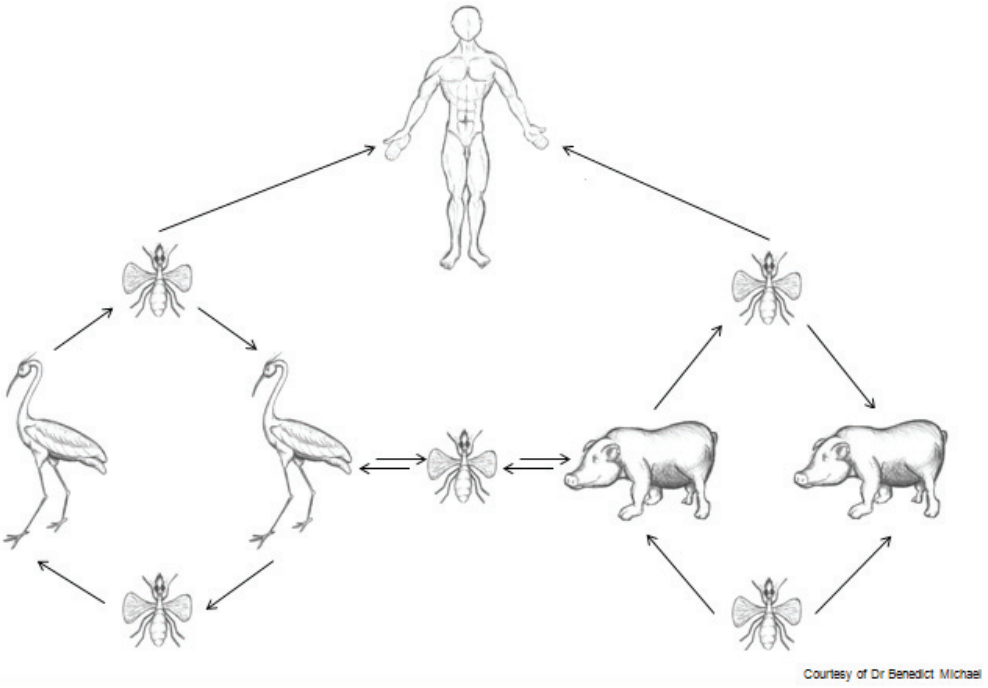


Figure 1.2: JE transmission occurs naturally in an enzootic cycle between birds and pigs by *Culex* mosquitoes. Humans are accidental dead-end hosts.

1.14 Pathogenesis of JE

The pathogenesis of JE is incompletely understood. The immune status of the host and integrity of blood brain barrier (BBB) are important in JE pathogenesis. After an effective bite of a mosquito the virus enters the body and multiplies locally in dermal tissue and draining lymph nodes causing a

transient period of viraemia. The virus reaches the CNS probably through T-lymphocytes where the virions bind to vascular endothelial cells of the CNS and internalize by endocytosis (43). JE typically develops after an incubation period of 5-15 days. It is postulated that during this time JEV resides and multiplies inside host leukocytes, which act as a vehicle to transport the virus into the CNS. Any therapy which could clear the virus from the lymphatic tissue and spleen during this period could prevent JE pathogenesis and prevent illness. Infected astrocytes, a component of the BBB, also help in passage of JEV from peripheral tissues to CSF. After entering the neuronal cell, JEV can cause neuronal death directly by multiplication within the rough endoplasmic reticulum and Golgi apparatus.. JEV can also cause cell death indirectly by uncontrolled over activation of the microglia's inflammatory response, with microglial releasing of pro-inflammatory cytokines such as interleukin 6 (IL-6), tumour necrosis factor α (TNF α), regulated upon activation, normal T cell expressed and secreted (RANTES) and monocyte chemotactic protein 1 (MCP 1). These inflammatory mediators released into the CSF then trigger a massive leukocyte influx across the BBB into the CNS compartment. Interferon-inducible protein 10 secreted by activated astrocytes also causes infiltration of natural killer cells and monocytes causing neuronal death. Improving understanding of JE pathogenesis is the key to exploring treatment option.

1.15 Cytokines in JE

Inflammatory response to flavivirus encephalitis suggests inflammation could cause vascular endothelial damage and contribute to JE pathogenesis (44). CSF levels of IL-6, tumour necrosis factor (TNF)- α and IL-8 are higher at the time of admission in those who died of JE than those who survived. In the plasma,

RANTES is higher in those who die of JE as compared to survivors (Table 1.1). However, correlation of anti-inflammatory (IL- 4) and pro-inflammatory (IL- 6) markers at the time of admission and again at discharge with death and recovery would provide better insight into the role of cytokines in disease pathogenesis.

Table 1.1: Cytokine, chemokine, nitric oxide (NO) and immunoglobulin levels on admission to hospital in non-survivors and survivors of JE (45)

CSF			
Mediator	Non-survivor (n=8)	Survivor (n=51)	P-value
TNF-α	25.3 (11.6-43.7, 0-60)	0 (0-13.0, 0-116.45)	0.093
IL-4	36.5 (14.6-72.9, 0-175)	58.3 (0-87.5, 0-218.8)	0.535
IL-6	1377 (418-1648, 193-2190)	174.1 (38-849, 0-2821)	0.006
IL-8	1140 (420-1656, 190-3823)	286 (78-799, 0-4714)	0.04
IFN-α	4.4(0.4-13.9, 0-35.6)	0 (0-2.2, 0-53.3)	0.044
IFNγ	60 (0-240, 0-255)	13 (0-180, 0-320)	0.511
RANTES	32.2 (13-50.1, 3.1-95.4)	18.5 (6-47.6, 0-353.7)	0.486
NO mmol/l	97.6 (25.9-67.5, 18-503.5)	54.9 (30.7-69, 14.6-170)	0.875
IgM units	60.5 (33.5-60.5, 4-274)	162 (67-229.5, 0-412)	0.035
IgG units	5.5(1.5-13, 0-17)	23 (7.5-60, 0-273)	0.003
Plasma			
Mediator	Non-survivor (n=13)	Survivor (n=105)	P-value
TNF-α	0 (0-17.5, 0-180)	0 (0-32.5, 0-3970)	0.91
IL-4	23.9 (0.6-89.6, 0-478.4)	15.9 (0-90.9, 0-1530.4)	0.691
IL-6	1.5 (0-6.5, 0-997.7)	7.4 (0-26.4, 0-2466.2)	0.16
IL-8	29.2 (21.3-92.4, 8-182)	54.0 (16.4-252, 0-5632)	0.506
IFN-α	0 (0-0, 0-31.9)	0 (0-2.6, 0-31.7)	0.201
IFNγ	0 (0-5, 0-20.1)	0 (0-40, 0-2510)	0.306
RANTES	11312 (8823-15704, 357-17536)	8269 (6158-10113, 1-29224)	0.0314
NO mmol/l	79.4 (41-61.5, 3.3-436.5)	119.6 (42.8-93.1, 18.8-631.6)	0.121
IgM units	31.5 (12.5-41.5, 2-187)	90.5 (36-148.5, 1-145)	0.009
IgG units	10 (4-13, 0-40)	11 (4-28, 0-188)	0.463

Data are median and interquartile range (IQR) in picograms per millilitre, unless otherwise stated in the table. *(Winter et al, 2004)*

1.16 Immunology of JE

Both humoral (against E and NS1 protein) and cellular (cytotoxic T lymphocytes) immunity play an important role in protection against JE (46). After primary infection (JEV as the first *f*lavivirus infection), a rapid and potent IgM response occurs in serum and CSF usually within 7 days of infection (47). This probably protects the host by arresting viral replication before it crosses the blood brain barrier. In established encephalitis, it may limit further damage by neutralising extracellular virus and lysing infected cells by antibody-dependent cellular cytotoxicity (ADCC). Attempts to isolate virus during such raised immune response is usually negative and is associated with good outcome. Within 30 days in surviving patients immunoglobulin class switch occurs and most have IgG in serum and CSF. Patients of asymptomatic infection with JEV have raised IgM in the serum, but not in CSF (47). An anamnestic antibody response with early rise in IgG and slow rise in IgM has been described in patients with secondary infection (previously infected with antigenically related *f*laviviruses). Animal models of JE have also shown cellular immune response to restrict viral replication in acute infection and prevent disease progression. Further, administration of inactivated JE vaccine has also shown to activate T-cell response (48). However, like other neurotropic *f*lavivirus, for JEV, antibody-mediated protection is more efficient than cytotoxic T cell responses for viral clearance (49-51). Hence, presence of antibodies in the serum and CSF is associated with survival and recovery (Table 1.1)

1.17 Clinical features of JE

In humans, most JEV infections are asymptomatic or cause a mild flu-like illness. The symptomatic to asymptomatic ratio following JEV infection ranges between1/25 to 1/1000. The clinical features depend on the site of the lesion in the brain and stage of illness. First stage, called prodromal stage, lasts for 2-3 days and is characterized by non specific febrile illness such as fever with or without rigors, coryza, nausea and diarrhoea. The second stage, called encephalitic stage, lasts for 3-5 days and is characterised by headache, vomiting, reduced level of consciousness, seizures, abnormal mental status, change in speech, abnormal movements, motor deficit and cranial nerve involvement. Signs of meningeal involvement such as neck stiffness, Brudzinski sign and Kernig's sign may also be seen at this stage. The typical features of JE seen include; Parkinson syndrome features such as a dull mask like face, wide unblinking eyes, hypophonia, tremors and cogwheel rigidity. Other extrapyramidal signs include lip smacking, pill rolling movement of hands, dystonia, opsoclonus and myclonus. Opisthotonus posturing and generalized spasms on touch suggest severe illness. In fatal cases when the brainstem is involved, the patients develop change in respiratory pattern, change in oculocephalic and pupillary reflexes, decerebrate or decorticate posture and deep coma.

The movement of an individual depends on the effective function of the upper motor neurons (UMN) originating from the motor cortex and descending on midbrain, pons, medulla oblongata to spinal cord. In the spinal cord the UMN synapses with the lower motor neurons (LMN) which innervate skeletal muscle

for contractions and movements. Basal ganglia and thalamus regulate control of motor and premotor area of cerebral cortex so that voluntary movement can occur smoothly. In JE, the involvement of cerebral cortex, midbrain, pons, medulla oblongata and cerebellum causes upper motor lesion type of signs. However, the virus has also been reported to affect the anterior horn of the spinal cord causing poliomyelitis like acute flaccid paralysis (11). Other movement abnormalities are predominantly because of involvement of basal ganglia and thalamus. Most of the deaths during the illness occur in this stage. All those who survive regain neurological function and progress to the third stage called recovery stage. In resource poor settings, where confirmatory testing is not easily available, knowing typical clinical features could help in the diagnosis and early referral.

1.18 Diagnosis of JE

On investigation, peripheral leukocytosis and increased erythrocyte sedimentation rate are seen in most of the patients. Hyponatremia may occur because of syndrome of inappropriate antidiuretic hormone secretion (SIADH). Lumber puncture will show CSF pleocytosis of 10- 1000 cells/mm^3 with lymphocyte predominance. The protein is slightly elevated of 50- 200 mg/dl with normal sugar. Computer tomography (CT) scan of the brain shows bilateral non-enhancing hypodensity in the thalamus, basal ganglia, midbrain, pons and medulla oblongata. Magnetic resonance imaging (MRI) of the brain is more sensitive and shows high signal intensity on T2 weighted images of the thalamus, cerebrum and cerebellum. Mixed signal intensity in T1 and T2 weighted scan in thalamus would suggest haemorrhage. Bilateral asymmetrical

haemorrhagic lesion in one or more area of thalamus in a patient living in an endemic area would suggest JE. Acute single photon emission tomography (SPECT) studies show hyperperfusion in the thalamus, frontal cortex and lentiform area. However, follow up studies have shown hypoperfusion in the same areas (52). Electroencephalogram (EEG) abnormalities reported are nonspecific slowing, burst suppression pattern, epileptiform discharges, theta, delta and occasional alpha coma (52,53). It is difficult to isolate the virus from blood because of low viral titres, rapid production of protective antibody and transient viraemia. Intracerebral inoculation in suckling mouse is the conventional method to isolate the virus. However, it has rarely been isolated from human CSF and brain tissue.

Viruses cannot grow in non-living culture material. Their isolation thus requires inoculation into susceptible living cells, Different cell cultures, such as primary chick embryo cells and duck embryo cells can be used to culture the virus. Different cell lines such as Vero, LLCMK2, C6/36 and API cells are also often used to isolate the virus. Isolation of JEV is also possible by inoculation into mosquitoes (54). Isolation of virus directly from serum or CSF is rare. This is believed to be due to the low viral titres and rapid production of neutralising antibodies in the body. In resource poor settings, frequent freezing or thawing of clinical specimens, logistic difficulty of timely transport, lack of skilled human resources and lack of laboratory facilities suitable for virus culture impairs successful virus isolation. Other methods of JE diagnosis, include demonstration of virus specific antigen or antibodies in the pateint's CSF and/or serum. Antibody based serological diagnostics are a key method

for JE diagnosis (55). Reverse haemagglutination, immunofluorescence and staphylococcal co-agglutination tests using polyclonal and monoclonal antibodies are some of the serological approaches used to diagnose JE (56, 57).

Serological diagnosis of JEV relies on the identification of an anti-JEV antibody response. In the past, the following approaches have been used; haemagglutination inhibition (HI), complement fixation (CF), plaque reduction neutralisation test (PRNT) and indirect fluorescent antibody (IFA) detection. These approaches have now largely been replaced by antibody capture enzyme linked immunosorbent assays (ELISAs). ELISAs have become the accepted standard for the diagnosis of JE (58, 59). ELISAs typically recommend using paired sera, demonstrating a four-fold rise in antibody titres (between acute and convalescent samples), to confirm the diagnosis.

IgM and IgG ELISAs have been used to detect JEV since the early 1980s. Anti-JEV IgM can be detected in almost all patients 7 days after acute onset of illness. IgM antibody capture ELISA (MAC-ELISA) is the method of choice to demonstrate JEV specific antibody in the serum or CSF. It is reported to be the most reliable indicator of JEV infection (60, 61). A single sample of serum or CSF is adequate to confirm presence or absence of anti-JEV antibody if conducted after 7 days of illness. ELISA is a highly sensitive, relatively rapid and reproducible method for detection of JEV. The sensitivity and specificity of ELISA for JEV has been reported as 89% and 91% respectively (62). Their use has increased since their development into commercial kits. However, they require a relatively sophisticated laboratory that includes an

ELISA reader. Avidin biotin system (ABC MAC-ELISA) (63), biotin labeled immunosorbent assay to sandwich ELISA (64), nitrocellulose membrane based IgM capture dot enzyme immunoassay (MAC DOT) (11), and antibody capture radioimmunoassay (ACRIA) (59) are some newer modifications of MAC-ELISA. A commercially available, relatively new IgM/IgG antibody capture ELISA for JEV has shown sensitivity of 88% with serum, and 81% with CSF (65).

Another simple and inexpensive method to detect anti-JEV antibody is by hydroxyapatite coated nylon beads (Ha-Ny beads) (66). The molecular mapping of JEV has paved the way for newer diagnostic techniques (67). The reverse transcriptase polymerase chain reaction (RT PCR) amplification of viral RNA is an useful test to not only detect but also quantify JEV load in the CSF (68). MassTag PCR is a form of multiplex PCR that uses primers with tags of different molecular weights to identify the product. The MassTag PCR allows a rapid and sensitive diagnostic platform that can screen up to 35 bacterial, viral and fungal agents including JEV, using a combination of PCR and mass spectrometry (69).

Whole-genome sequencing is another method of identifying pathogens not identified from PCR (70). Enterovirus 75 has recently been detected using this technique in cases of aseptic meningitis in Europe (71). Where direct pathogen detection is not possible, host- response gene expression patterns can be used to asses body's response to discern whether it has been infected by bacterial or viral pathogens (including JEV).The immune cells, circulating in

the blood stream, detect and respond to bacterial and viral infection in distinct and specific manner. These responses can be observed by the activation of host genes (expression patterns). This approach has been used to distinguish different types of infective pathogens among acutely unwell children in Kenya (72).

1.19 Principle of MAC-ELISA developed by AFRIMS

This ELISA has been designed to detect anti-JEV IgM antibody in serum or CSF. IgM antibodies are captured by goat anti-human IgM previously coated on the solid phase (wells of ELISA plate). Then the JEV antigen, which only binds to anti-JEV IgM, is applied over the captured IgM. The non specific antibodies are then removed by washing. The presence of JEV antigen bound to anti-JEV specific antibody is detected by addition of enzyme linked conjugate (horse redox peroxidase enzyme and human anti-flavivirus IgG). This enzyme changes the substrate into coloured compound. The intensity of the colour is then detected with the help of ELISA reader.

1.20 Outcome of JE

Around 4- 25% of patients of JE die acutely and 30- 69% have sequelae at discharge (10, 20-25). Around half of the children with JE recover completely. It may take up to 3 months before the stable outcome following acute encephalitis emerges.

There is paucity of data on the long term outcome of JE. Some studies have reported neurological sequelae and death to be more common in JE compared

with other viral encephalitis at the time of discharge (6,10). Even after 27 years, JE patients have been reported to have significantly more neurological sequelae, less independent life style and lower IQ than patients affected by other forms of encephalitis (73).

1.21 Sequelae of JE

Patients with neuro-psychiatric sequelae exhibit cognitive, language and motor impairments. These impairments can lead to a significant social and financial burden (28). Cognitive defects include impaired consciousness, altered behaviour, inability to recognize family members, amnesia, altered personality, reduced concentration span, depression, impairment in IQ. Motor sequelae include flaccid or spastic weaknesses (monoparesis, hemiparesis, quadriparesis,), focal or generalized abnormal limb tone (hypertonia, hypotonia), focal or generalized abnormal limb reflexes (hyperreflexia, hyporeflexia), cranial nerve palsies or movement disorders. Other problems include seizures, movement disorders, bowel or urinary incontinence, difficulty in feeding, poor school performance, difficulty in speaking, visual or hearing impairment or autonomic disturbance (21, 73-76).

1.22 Recovery profile of patients of JE

Knowing a JE patient's likely recovery profile helps clinicians prioritise treatment, helps them build a team of specialists, plan rehabilitation strategies and explain to the parents or patients the sequence of estimated recovery to better prepare them for the patient journey and eventual outcome.

The recovery profile of JE has not been well studied. The few studies which have followed up JE patients in the community have shown that outcome often

changes between hospital discharge and community follow-up, with many patients improving and some worsening after discharge (21,74). Complete recovery has been observed in patients initially exhibiting severe motor sequelae at discharge (10, 21, 74). Occurrence of sequelae at hospital discharge does not appear to accurately predict long term outcome. In one study, one in every five patients had deteriorated post discharge. Presence of neurological sequelae at later community assessment (6 months post-hospital discharge) has been reported to better predict long term outcome in JE patients (21).

1.23 Prognostic indicators of JE

Previous studies have identified prolonged illness, prolonged fever, prolonged duration of altered sensorium, poor Glasgow coma score (GCS) below 8, multiple convulsions, presence of focal neurological deficits, abnormal breathing, decerebrate posturing and hyponatremia at the time of admission as predictors of bad outcome in JE. Often patients present with more than single high risk clinical features. A combination of poor perfusion, GCS ≤ 8 and $\geq$ 2 witnessed seizures has previously been reported to predict bad long term outcome (21). However, these predictors were identified in studies recruiting patients from distinct geographic locations. The authors were uncertain whether their findings could also represent other locations or countries (10, 21, 37, 77, 78).

1.24 Immunization against JE

Vaccination of high risk population is the key strategy for JE prevention. There are currently 15 vaccines in use. All the vaccines are based on genotype 3 strains of JEV. There are five main classes of JEV vaccine. First is a live

attenuated vaccine clone, SA-14-14-2. It was generated by serial passage of the SA 14 strain in primary hamster kidney cell cultures. Attenuated vaccines are generated by passing a virus repeatedly through various cell culture systems until the virus becomes non-pathogenic. This live attenuated SA-14-14-2 vaccine has been licensed for use in China since 1988. It is administered to over 20 million children each year. It is commercially manufactured in Korea and used in Nepal, Sri Lanka and India. It currently accounts for over 50% of the global production of all JE vaccines (79). Second is a live attenuated recombinant chimeric vaccine, based on the genes of yellow fever 17D backbone spliced with Vero cell propagated SA 14-14-2 strain. Recombinant vaccines are based on splicing viruses with other virus protein or nucleic acid sequences (80). It has recently been licensed for routine use in Europe, America, and Asia (81). Third is an inactivated mouse brain derived JE vaccine. It is derived from mouse brain infected by the Nakayama or Beijing-1 JEV strain. It was initially licensed for use in Japan in 1954 and now being produced in Taiwan, India, Russia, Korea, Thailand, China, and Vietnam (79). Fourth is an inactivated primary hamster kidney cell derived vaccine based on the Beijing-3 strain. It was developed and widely used in China since 1968 (82). Fifth is an inactivated Vero cell culture vaccine based on the Beijing-1 JEV strain. It was initially manufactured for local use in Japan and now routinely used in Europe, America, and Asia (79, 81).

1.25 JE as a cause of AES in Nepal

JE is an important cause of AES in Nepal. It is associated with considerable morbidity and mortality (10). Syndromic surveillance, conducted between 1978 and 2003, reported 26046 suspected JE cases in Nepal. More recently,

laboratory surveillance based on anti-JEV IgM ELISA testing of serum or cerebrospinal fluid (CSF) from suspected AES cases, collected between 2004 and 2015, has identified a total of 17,875 AES cases and 3067 JE cases (Table 1.2). The actual numbers of JE cases may still have been under represented in the latter study due to logistical issues, deaths before hospital admission, and difficulties in confirming the diagnosis of JE without paired sera or CSF samples.

Table 1.2: Table showing total AES and JE patents after starting laboratory based surveillance in Nepal

YEAR	AES	JE
2004	1533	368
2005	2290	584
2006	1471	293
2007	1650	442
2008	1988	339
2009	1515	146
2010	1572	197
2011	1337	128
2012	952	79
2013	1228	127
2014	1402	226
2015	937	138
Total	**17875**	**3067**

(WHO-IPD, Nepal)

A Nepalese hospital based study conducted in a JE endemic region in 2006 reported JEV accounted for 50% of AES patients and 62% of patients with probable viral encephalitis (37). The mortality from JE is reported at 20-60% in Nepal (42). Immunisation programmes against JEV began in humans in 1999 (83,84) and in pigs in 2001. Both programmes began in high risk districts

(42). Since introduction of the JEV immunisation in Nepal, numbers of JE and AES patients have steadily fallen. These findings suggest that the majority of AES cases are due to JEV (Figure 1.3). Nevertheless, cases of JE continue to be reported. Recent sero-surveillance studies in Nepal among animal hosts for JEV reported high prevalence of JEV. Anti-JEV antibody responses were detected in almost half of the pigs and horses and a quarter of ducks tested (85). JEV remains a major public health problem in Nepal. There is a pressing need to develop better treatment.

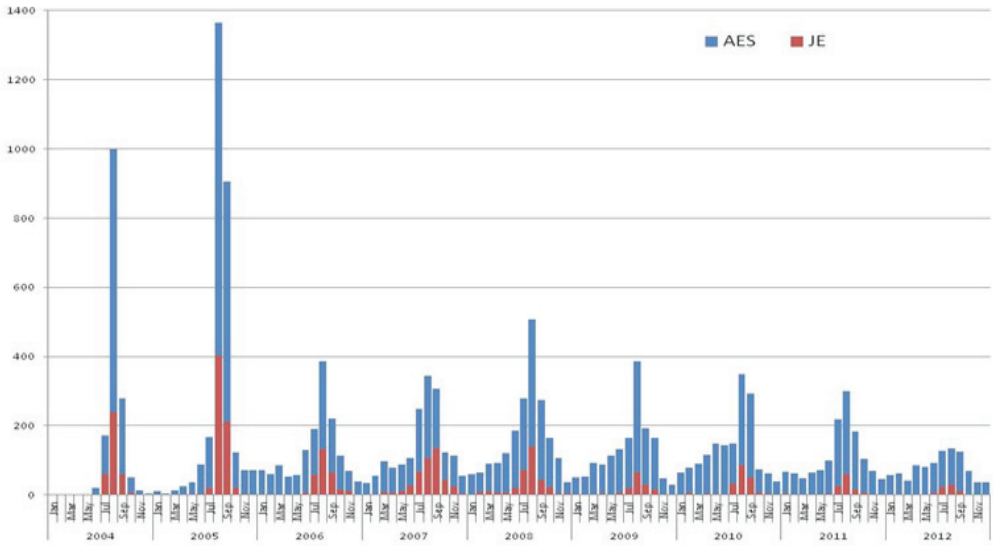


Figure 1.3: Distribution of AES and JE cases on laboratory based surveillance *(Child Health Division, Ministry of Health, 2014)*

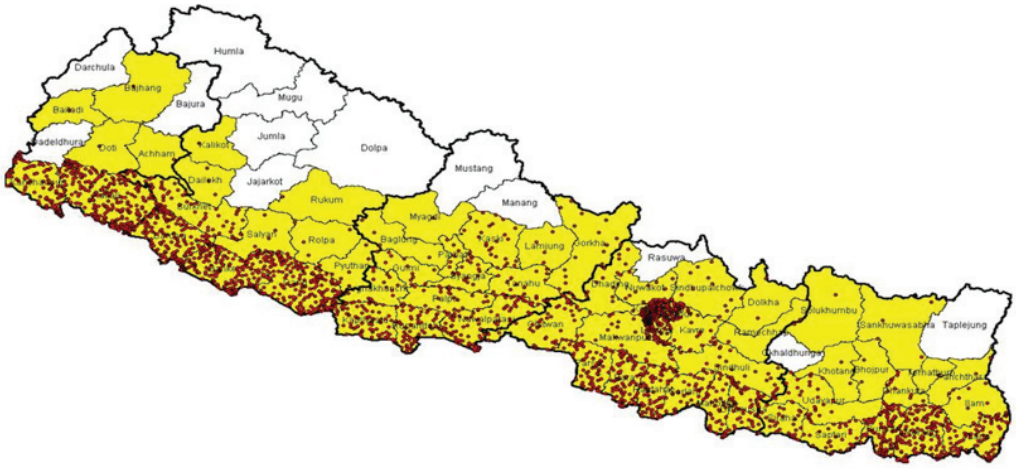


Figure 1.4: Geographical distribution of JE patients in Nepal. *(Child Health Division, Ministry of Health, 2014)*

Out of a total of 75 districts in Nepal, JE exhibits a high prevalence in 24 low lying Southern districts. Sporadic cases have been reported from an additional 41 districts located in the mountainous districts (Appendices A & B). Between 2004-and 2012, JE cases were detected in 62 districts (Figure 1.4). Most JE patients (60%) are children (Figure 1.5). Sporadic cases occur throughout the year in endemic areas. Every year epidemics occur during the monsoon season. An increase in JE cases starts in April, peaks in August, and declines in October (Figure 1.6). The tropical climate, paddy ecosystem, abundance of *Culex* mosquitoes, pigs near human dwellings in Southern Nepal provides suitable setting for JEV. Although *Culex gelidus, Culex vishnui, Culex pseudovishnui* and *Culex fuscocephala* have been suspected, JEV has been isolated from female *Culex tritaeniorhynchus* which is the considered the principle vector for JEV in Nepal (42). Three strains of JEV namely Nep- 1/90, B- 2524 and B- 9548 are found in Nepal. Pigs, ducks and horses are natural reservoirs of JEV in Nepal (85).

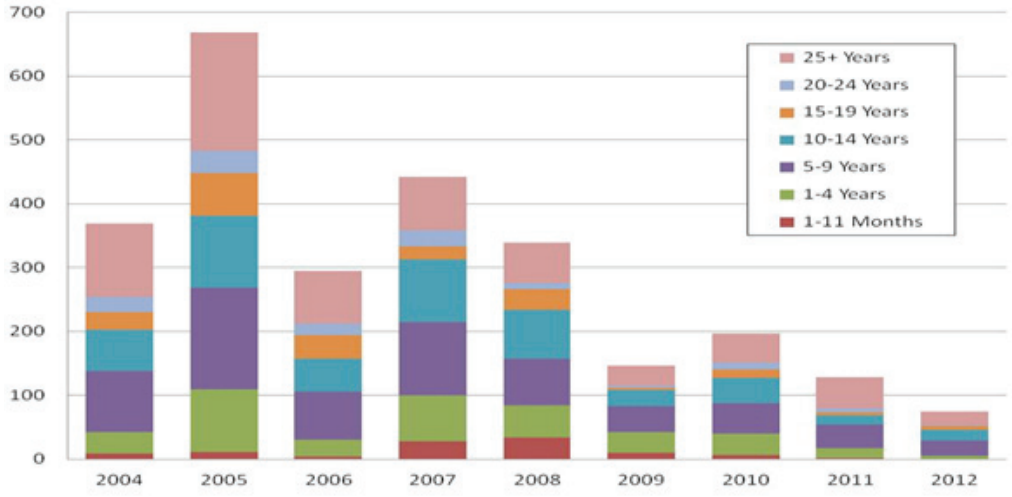


Figure 1.5: Age distribution of JE cases in Nepal. *(Child Health Division, 2014)*

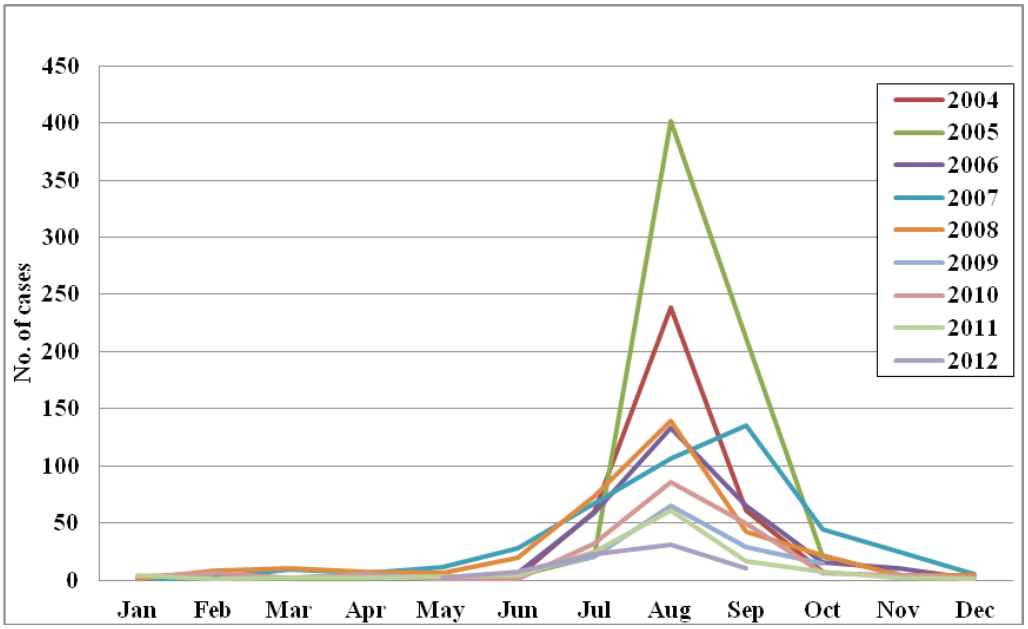


Figure 1.6: Distribution of JE cases by months in Nepal. *(Child Health Division, 2014)*

1.26 JE control strategies

Through extensive knowledge on the epidemiology of JE it is clear that rice cultivation, vector densities, and pig rearing close to human habitation is important for disease control (36). Human vaccination, pig and horse vaccination, use of pesticides in rice fields, effective management of pig breeding locations relative to human habitation have all contributed to a marked reduction of JE cases (36).

JE is predominantly reported in Asia. According to recent JE epidemiological studies and disease surveillance and vaccination strategies, affected countries have been divided into 4 groups. First group is represented by Japan, Korea and Taiwan, which report less than 30 cases annually and have effective JE control measures in place. Second group contitutes of China, India, Nepal, Malaysia, Cambodia, Vietnam, Thailand and Srilanka, which have high disease burden, effective sentinel surveillance programmes, national and high risk JE vaccination programmes and report on disease burden and outbreaks. However, these countries still need to improve their JE control strategies. Third group constitutes of Indonesia, Burma, Bhutan, Myanmar, Bangladesh, North Korea, Pakistan, Papua New Guinea, Philippines and Timor-Leste, which report fewer than 100 JE cases annually. They do not have JE sentinel surveillance or immunization programmes. There are plans to conduct JE surveillance through their national surveillance programmes in the future. Fourth group constitutes of Australia, Guam, Russia (Siberia), Singapore and Saipan, which report less than 3 cases annually. Maintaining effective surveillance and immunisation strategy designed for one-time campaign in target populations are the base of JE control strategies in these countries (86).

Nepal's JE control strategy is based on effective surveillance, improved case management, improved vector management and immunisation of at-risk population. Although syndromic surveillance based on signs and symptoms of JE began from 1978 (Appendix C), laboratory based surveillance was initiated by the Government with support of World Health Organisation-Programme for Immunisation Preventable Diseases (WHO-IPD) from 2004 (Table 1). The objective of the surveillance is to monitor morbidity and mortality, characterise epidemiology, identify high risk geographical area and population, guide intervention programme and find out the impact of control measure. Currently two epidemiological surveillance and reporting systems exists. Health Management Information system (HMIS) is used for routine monthly reporting of clinical cases of JE from peripheral health facilities to District Health Office and HMIS division of Department of Health Services (DOHS). There is also a laboratory based surveillance of JE integrated with acute flaccid paralysis, measles and neonatal tetanus surveillance. Technical assistance and quality control of this surveillance is being provided by WHO-IPD.

As an effort to control JE, for JE surveillance, the WHO has developed a set of clinical symptoms and signs to capture patients of AES, who are later confirmed and classified, on the basis of laboratory reports, to have JE or other causes of AES (Appendix D). The Ministry of Health and Population of Nepal, supported by WHO-IPD, has integrated JE surveillance with Acute Flaccid Paralysis, Neonatal Tetanus and Measles surveillance, in its National surveillance network since 2004.

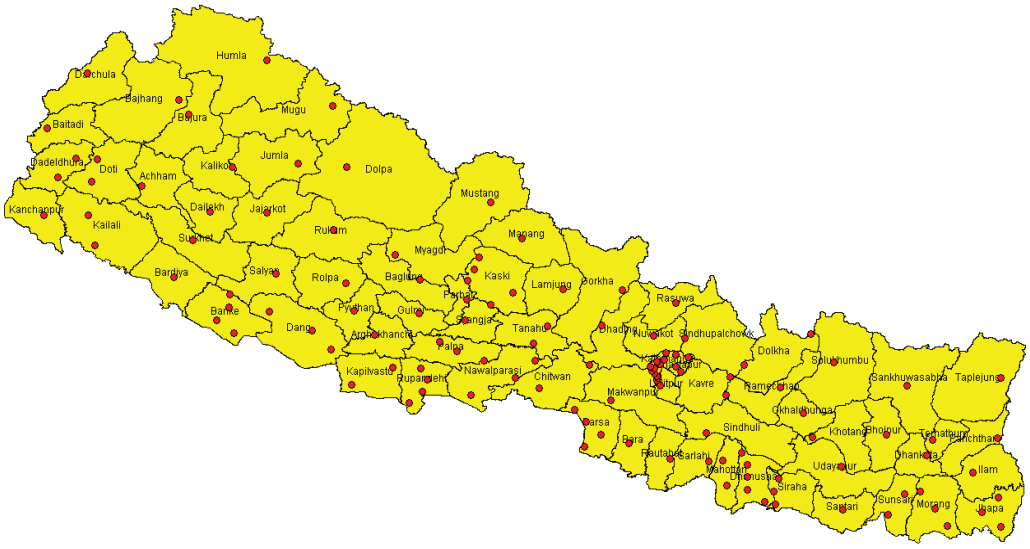
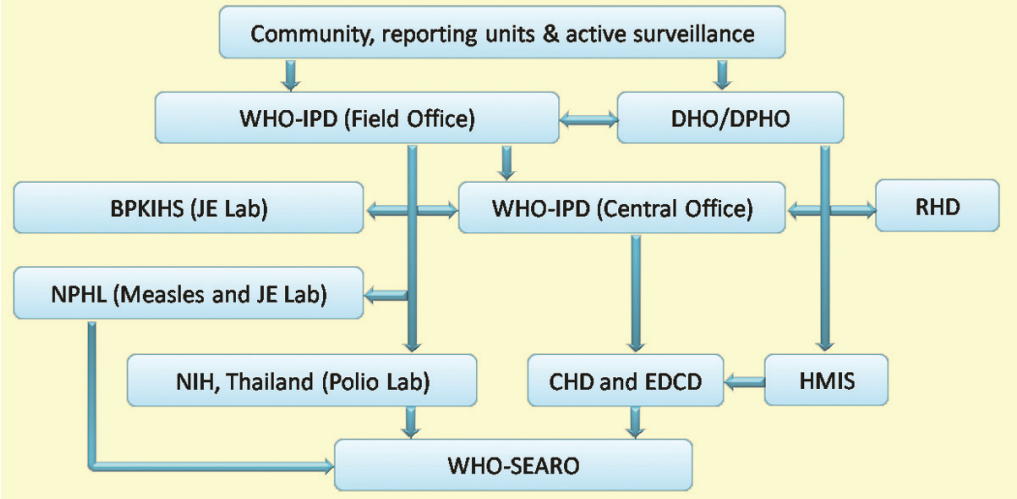


Figure 1.7: AES reporting sites in Nepal *(Child Health Division, 2014)*

Through a national network of 127 sentinel sites under the surveillance (Figure 1.7), reports on demography, clinical features and outcome of AES (suspected JE) patients and collected CSF and/or serum samples, are delivered to the surveillance medical officer (SMO) of WHO-IPD, who then transfer them to Child Health Division of the Department of Health Services (DOHS) on weekly or monthly basis (Figure 1.8). The samples are transferred and tested for the presence of anti-JEV IgM antibodies by ELISA in this programme in two referral laboratories namely National Public Health Laboratory (NPHL), Kathmandu and BP Koirala Institute of Health Sciences (BPKIHS), Dharan, using Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, kit or National Institute of Virology (NIV), Pune, India, kit, with external quality assurance performed by The National Institute of Mental Health and Neuroscience, Bengaluru, India. Cases are classified as confirmed JE, proven non-JE and JE-status unknown if they are ELISA positive, ELISA negative and ELISA negative when tested within the first 7 days of illness or never tested for anti-JEV IgM levels by ELISA, respectively.



(WHO-IPD, World Health Organisation-Programme for Immunisation Preventable Diseases; DHO, District Health Office; DPHO, District Public Health Office; BPKIHS, BP Koirala Institute of Health Sciences; RHD, Regional Health Directorate, NPHL, National Public Health Laboratory, NIH, National Institute of Health of Thailand; CHD, Child Health Division; EDCD, Epidemiology and Disease Control Division; HMIS, Health Management Information System; WHO SEARO, World Health Organisation South-East Asia Regional Office)

Figure 1.8: AES and JE surveillance system in Nepal *(Child Health Division, 2014)*

Clinical and laboratory features that distinguish confirmed JE from other AES cases would be highly useful to allied health professionals, who otherwise may have to wait for weeks for an ELISA result (samples are batch tested to reduce cost causing delay in reporting). The ongoing Nepali National Immunisation Schedule has recently included JE vaccination for children of one year of age. Improved clinical and laboratory diagnosis of JE would also complement local

monitoring of effectiveness of this schedule. Improved clinical and laboratory diagnosis could also be useful to help rationalise antibiotic treatment of AES cases.

Vaccination against for JEV in Nepal has occurred in multiple phases. Sporadic vaccination campaigns were conducted between 1999 and 2000 in districts highly endemic for JE. In 2006, Nepal initiated it's JE control programme. This involved a phased JE vaccination campaign with live attenuated SA-14-14-2 vaccine. People living in districts with high rates of confirmed JE transmission (≈16.0/ 100,000 population) received vaccine first. Vaccine was administered to everyone above 12 months of age. Districts with intermediate rates of JE transmission (≈1.6/ 100,000 population) were vaccinated next. Children between 12 months and 15 years of age were vaccinated then. JE vaccine was introduced in the routine immunisation programme in the post campaign districts from 2009. In 2010, an independent evaluation of vaccine impact found JE incidence had significantly decreased to 72% in post campaign districts (87). The efficacy of a single dose of this vaccine in preventing JE, when administered only days or weeks before exposure to infection, has been reported as 99.3% (83). By 2014, the JE immunisation campaign had been completed in all 31 high and intermediate risk districts and the vaccine had been introduced into the national routine immunisation programme in these regions. In May 2015, JE vaccine campaign was conducted in the remaining 44 low risk hill and mountain districts. Following this, all 75 districts in Nepal had JEV vaccine introduced into the routine immunisation schedule. Mass vaccination campaign is planned in response to an increase in the incidence

of JE. Public private partnership is also being sought to cover cost of JEV vaccine for people of all ages at risk of JE. All adverse effects following JE immunisation are being investigated in a comprehensive manner (including casualty assessment). Research on efficacy of JE vaccine in post campaign districts has been prioritized. In 2001, 200,000 pigs were also vaccinated by live attenuated JE vaccine by Department of Livestock, Ministry of Agriculture.

There is no insecticide spraying programme for JE in Nepal. An ongoing awareness programme conducted in high risk districts aims to improve hygiene and encourage personnel application of mosquito repellents. Mosquito breeding around residential areas is prevented by cleaning around homes, draining stagnant water and filling in ditches. The National Health Education, Information and Communication Centre of the DOHS prepare and distribute materials regarding JE awareness.

1.27 National Guideline for the diagnosis of JE in Nepal

The definition of a case of JE, in the National surveillance program, was provided on the basis of a guideline developed by a team of experts attending the National workshop on Vector-Borne Diseases at the Ministry of Health of Nepal in 1997. According to this guideline, a patient having an elevated temperature (> 38˚C) and altered consciousness is considered to be "possible case of meningitis/encephalitis" or AES case. He or she is then advised a lumber puncture (LP). Hence, diagnosis of AES does not require LP but confirmation of CNS infection does require a LP and CSF analysis. If the

suspected case has CSF cells < 1000 cells/mm^3, he or she is defined as "probable viral encephalitis". If a case of probable viral encephalitis tests positive for anti-JEV IgM in CSF and/or serum, he or she is diagnosed as a "confirmed case of JE".

1.28 Clinical and laboratory features as a predictor of JE from other AES

There is a tendency to treat all patients with fever and seizure as bacterial meningitis. This results in excessive misuse of antibiotics. This in turn leads to rapid development of bacterial resistance against those antibiotics. Ultimately, this presents a grave threat to safe, affordable and effective treatment options. Use of a simple clinical and laboratory based predictor score (or clinical decision tool), such as the Bacterial Meningitis Score, during initial patient evaluation, has been reported to differentiate aseptic meningitis from bacterial meningitis with approximately 100% accuracy in children (88). Diagnostic tests for JE are not routinely available or affordable in developing countries. Hence, a similar tool that accurately differentiates JE from other cause of AES could help rationalise antibiotic use in Nepal.

Prognostic indicators can also be developed by relating clinical information available at the time of initial patient evaluation to outcome at discharge. Identifying prognostic indicators for residual neuropsychiatric sequelae or death at the time of admission among AES cases would help guide management and be useful for counselling patients and parents on likely outcomes.

Prognostic indicators could help encourage early referral to tertiary care centres, which in turn may improve outcome. Such indicators would also help prevent referral delays, unnecessary wastage of financial resources and support clinical management. The use of predictors could have vital implications to clinicians treating children with AES and JE. However, there is a need identify and test the validity of different clinical predictors in different settings to assess their robustness and effectiveness.

1.29 Hydration and nutrition status in AES

Poor hydration status may be associated with morbidity among children with AES. Since AES can be associated with brain swelling, clinicians have recommended fluid restriction so that brain swelling is not exacerbated by cerebral oedema secondary to fluid overload or associated shifts in electrolytes (89, 90). However, AES patients may be unwell and vomiting for many days. They may be unable to eat or drink fluids on their own which could cause dehydration. Restricting fluid in such cases may be detrimental. Assessing hydration status is therefore essential in patients with AES. Hydration status can be assessed indirectly by daily measurement of weight. However, the total weight of a person is contributed by muscle, fat, water and bone. Consequently, weight is also an indicator of nutritional status. In children, weight corresponds to age and gender. It increases with increase in age. Weight-for-age (WFA) metrics reflect body weight relative to the child's age and gender. Weight loss or retardation in a children can be assessed by comparing how a child's individual WFA relates to a relevant reference population. Z scores equate to standard deviations (SD) around the reference median. In a population with a normal WFA distribution, you would expect 15.9%, 2.3%, or 0.13% of

children to have a weight for age Z score of -1, -2 or-3 respectively. No single baseline WFA distribution chart is available for all age groups for the Nepali population. To assess whether weight is lower than expected in children below 5 years, the WHO child growth standards, can be used to estimate expected WFA [http://www.who.int/nutgrowthdb/about/introduction/en/index5.html (accessed on 20 February, 2016)]. For older children, aged between 5and 14 years, the 'Nepalese Growth Standard for the School-aged Children' developed by the Public Health and Infectious Disease Research Centre, Kathmandu, Nepal can be used. Children are categorised with a a standard deviation score or Z score (-3, -2, -1, 0, +1, +2, +3) based on their actual weight compared to their expected weight based on the WFA distribution among males or females. Z scores of -1, -2 and -3 are considered low WFA (91). Low WFA can indicate malnutrition and/or dehydration. Other clinical and laboratory features, such as capillary refill, acute changes in serum urea and electrolyte levels can be used to help distinguish acute dehydration from malnutrition. Similarly, other clinical measures, such as body mass index, mid upper arm circumference (MUAC) and skin fold thickness can be used to help identify malnutrition. MUAC is a very simple measure that can be used to assess nutritional status in children (http://motherchildnutrition.org/early-malnutrition-detection/detection-referral-children-with-acute-malnutrition/muac.htm). Other indices such as Dugdale's index, Rao's index and Kanawati index require multiple measurements of different body parts. In a low WFA patient with AES or JE, MUAC and other supportive laboratory findings may help differentiate dehydration from malnutrition. Additional clinical and laboratory features of dehydration are presented in Appendix E.

My previous work has shown that JE patients who die or have severe neurological sequelae have significantly prolonged illness, prolonged altered sensorium and presence of focal neurological deficit including hemiparesis at admission than those who recover completely (10). Such patients would not be able to feed or drink on their own, making them dependent on others and vulnerable to dehydration. Many AES patients, including those with JE, do not have definite treatments. Appropriate fluid therapy to adequately hydrate patients is an important part of supportive therapy. Appropriate fluid therapy may limit death or development of neurological sequelae.

Children admitted with an altered sensorium generally receive intravenous fluids in Nepal. The most common type of fluid used is 0·18% saline with 5% dextrose. It is typically administered at a maintenance rate of 100 ml/kg for the first 10 kg of bodyweight, 50 ml/kg for the next 10 kg, and 20 ml/kg of bodyweight exceeding 20 kg (92). There is a practice, in developing countries, to modify and restrict fluids to two-thirds of maintenance requirements in AES and JE patients (93, 94). There is a general fear that intravenous fluids given at maintenance rates may be unsafe, particularly where serum sodium concentrations cannot be closely monitored, putting the child at risk for developing cerebral oedema. As a consequence, AES and JE patients may suffer dehydration, which in-turn may aggravate metabolic acidosis. Excessive fluid restriction is independently associated with a poor outcome among traumatic brain injury patients (89). Fluid and sodium restriction has also been associated with a poor outcome in JE patients (93). Administration of adequate

fluid to maintain cerebral perfusion pressure without worsening cerebral oedema is important when managing brain injury.

Serum lactate level is raised in patients with both brain injury (95) and seizures (96, 97). CSF lactate level is also elevated in patients of JE with seizures (20). However, currently with no definite anti-viral or immunomodulatory treatment, there is a need to increase knowledge of fluid balance, change in weight and acidosis status which may be contributing to elevation of intracranial pressure and convulsions, causing secondary deterioration of patient's health after the primary insult from viral brain infection.

1.30 Treatment trials for JE

Since JE is the most common cause of AES, the treatment of JE would help improve outcome of AES. However, there is no definite treatment of JE at the moment. The management is largely supportive. The pathogenesis of JE involves combination of viral cytopathology and immunopathology (98-101). Both have been explored while seeking definite treatment in humans. As an adjunctive treatment of JE, a randomised-placebo controlled trial of dexamethasone (initially 0.6 mg/kg followed by 0.2 mg/kg intravenously every 6 hours for 5 days) was conducted in Thailand. The trial showed a reduction in CSF opening pressures and white cell counts but no overall benefit in terms of outcome (102). Similarly, a placebo controlled trial of Interferon α-2a, produced as part of the innate response to JEV infection and had antiviral activity against the virus in vitro (103), administered at 10 million unit/m^2 daily for 7 days, also proved to be unhelpful to improve outcome in

Vietnam (38). A controlled trial of oral ribavirin (1-β-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide), proven effective against JEV in vitro (104, 105), at 10 mg/kg/day in four divided doses for 7 days in India, was also not seen to affect outcome in children with JE (106). Intravenous immunoglobulin (IVIG) has been postulated as a potential treatment of JE because of its anti viral and anti inflammatory properties. Currently IVIG is used as standard treatment for a number of childhood immune mediated diseases including Guillain-Barré syndrome, Kawasaki's disease, immune thrombocytopenia and dermatomyositis in Nepal.

IVIG works by its anti-inflammatory properties, such as down regulation of T cell and B cell activation in Kawasaki disease, blockade of FCγ receptors causing down regulation of secretory cytokines in thrombocytopenic purpura and inhibition of complement binding and prevention of membranolytic attack complex formation in Guillain-Barré syndrome and dermatomyositis. In addition, IVIG has been used to treat, and later to provide prophylaxis, for children with hypo- or agammaglobulinamia with chronic enteroviral meningoencephalitis (107). Children with X-linked agammaglobulinaemia and enteroviral meningoencephalitis treated with intensive and prolonged IVIG therapy have achieved long-lasting clinical and viral remission. However, the role of neutralizing antibodies in IVIG has always been speculative. It would be interesting to find out if locally manufactured IVIG, could have antibodies against this neurotropic virus, which together with its anti-inflammatory properties, may be a useful adjunctive treatment option.

1.31 Scope of this thesis

AES is a considerable cause of morbidity, mortality, and a disease of public health priority in many countries, including Nepal. In the majority of AES cases a specific infectious aetiology is not found. Even for known aetiologies, there is considerable geographical and age specific variation. JEV is the most common cause of AES in children in Nepal. Currently, active AES and JE case surveillance, case management and immunisation with SA-14-14-2 vaccine of at risk population are the key strategies for AES and JE control in Nepal. However, we are likely to continue to see cases because, unlike poliovirus, there are animal hosts for JEV, which means the virus will never be eradicated. In addition, we will continue to see the many cases of JE negative AES. Improved insight into clinical features specific for JE, or that predict poor outcome, would not only help in identify and explain the prognosis for children at risk of severe disease, death or sequelae but also help guide management. Although some clinical features have been reported as proxy markers for JE or poor outcome in the past, the robustness and generalisability of these findings are uncertain and needs validation. When there is no definite treatment, identifying inadequate supportive management in AES and JE patients could be an important strategy to reduce morbidity and mortality. Since JEV is the most common known pathogen of AES, also exploring adjunctive treatment options could behelp reduce death or neurological sequelae. This thesis is therefore directed at improvement of the management and outcome of AES, especially JE, in Nepal.

My thesis has the following objectives:

1. Describe the clinical features, laboratory parameters of AES and JE in Nepali children, and investigate how these features relate to patient outcome.
2. Assess the clinical features, laboratory parameters and outcome of AES in Nepali children of different weight-for-age categories.
3. Describe the relationship between fluid balance, change in weight, blood lactate level and outcome of AES and JE patients.
4. Conduct a pilot randomised double blind placebo controlled trial of intravenous immunoglobulin in Japanese encephalitis.

Chapter 2: General Methods

2.1 Study Location

Nepal is a landlocked country in Asia which lies between China and India. It is located at latitudes 26°22' and 30°27' North and longitudes 80°4' and 88°12' East in the globe. Topographically, the 147,181Km^2 area is divided into three ecological regions, namely northern mountain region which is 4877-8848 metres above sea level and stretched over 51,817 Km^2 area, middle hill region, which is 610- 4877 metres above sea level and stretched over 61,345 Km^2 area and southern plane lowlands called "Terai" region, which is lower than 610 metres above sea level and stretched over 34,019 Km^2 area (Figure 2.1). In the mountains, temperature ranges from [(-1^{0}C)- 12^{0}C] in summer and between 0^{0}C - (-13^{0}C) in winter, in the hills temperature ranges from 19.5^{0}C - 28.11^{0}C in summer and 3^{0}C - 19.3^{0}C in winter and in Terai, temperature ranges between 24^{0}C - 44^{0}C in summer and 10^{0}C - 27^{0}C in winter. The country has hugely diverse nature and climate. The monsoon occurs between June and September with precipitation between 180- 225 cm. Water logging is commonly practiced for effective use of rain or irrigated water to grow crops. Therefore, these climatic conditions are most suitable for the breeding and high density of mosquitoes and sand flies which are transporters of various vector borne diseases such as JE, dengue, malaria and kala-azar.

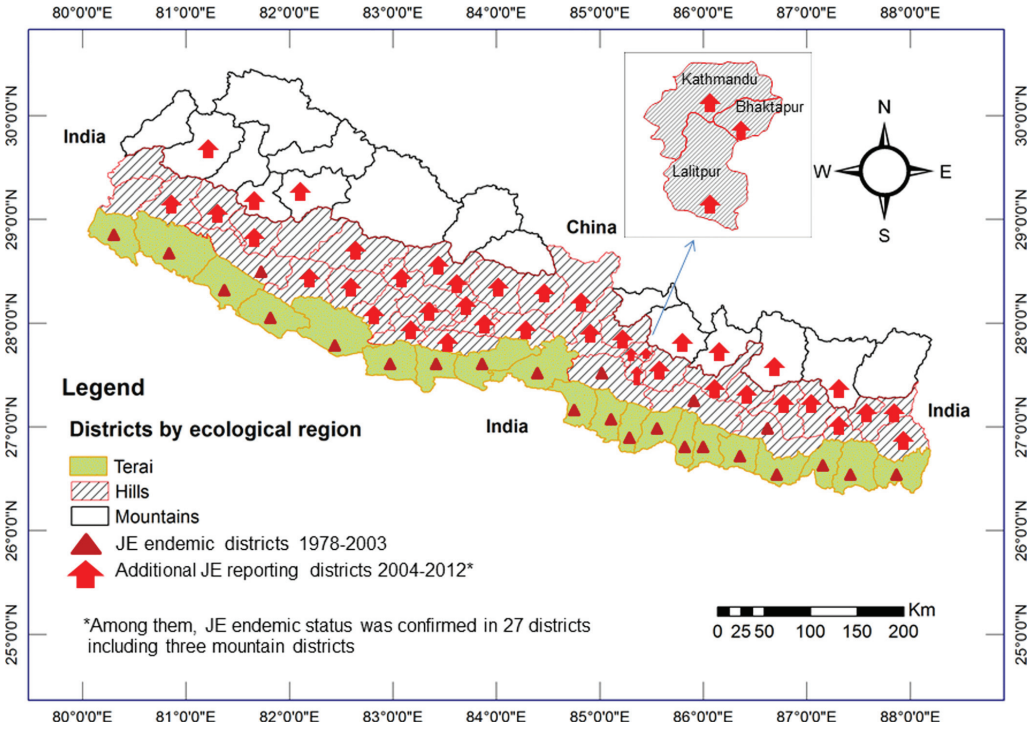


Figure 2.1: Geographical map of Nepal showing mountainous region in the north, hilly region in the middle and plains of Terai in the south.

The figure shows spatiotemporal relationship of JE cases in Nepal from 1978-2014. It also shows climatic conditions conducive of transmission of JE to mountain region of Nepal. Additional JE reporting districts represents those districts where JE is not endemic. *(Dhimal M et al, 2015)*

Nepal has a population of around 26.5 million, including 34.9% children below 14 years of age. The population growth rate is 1.35%. With callous terrain and limited access to roads and effective communication, the mountain region is inhabited by only 6.7% of the total population. The hill region, including valleys such as Kathmandu and Pokhara, centres of national resource, is inhabited by 43% of the population. The warm, humid, fertile and more industrialized Terai region, although only 23% of the total land, is inhabited by 50.3% of the population. About 80% of the population continues to rely

on agriculture for their livelihood. The major crops are rice, maize, wheat and millet. Paddy ecosystem, predominantly agriculture base for livelihood and large population density make Terai region most suitable setting for vector borne diseases.

There are 125 ethnic groups, who speak 123 languages making it a multi-ethnic and multi-lingual country. Chhetri (16.6%) form the largest ethnic group. Nepali is the most commonly (44.6%) spoken language as the mother tongue followed by Maithili (11.7%). Hinduism (81.3%) is the most commonly practiced religion followed by Buddhism (9.0%).

It is a low income country with gross domestic product per capita at $ 694.10 in 2013 which is 15 times lower than the global average. The country's political instability is being reflected in its economy. The majority (80.7%) of the households are rural based with significant health inequalities between rich urban and poor rural communities. The Gini-Coefficient, which purports inequality in income distribution, is 0.328, where 0 would be perfect equality and 1 as maximal inequality (Child Health Profile, Child Health Division, Ministry of Health, Nepal).

Administratively, Nepal is divided into 5 regions, 14 zones and 75 districts. Districts are further divided into smaller Village Development Committees (VDCs, 30- 100 in a district) and urban municipalities. A VDC is the lowest political unit and consists of nine wards, while the number of wards in an urban municipality depends on the size of the population. At present, there are

3,915 VDCs and 58 municipalities. Government facilities are organised in a hierarchical structure. A community and village health worker in each ward is the frontline health workers for the government. Each VDC has one Sub-health Post (manned by Auxillary Health Worker), the lowest Government health service unit, which refers patients to Health Post (manned by a Health Assistant and 1 in every 8-10 VDC) in turn refers patients to Primary Health Centre (manned by clinician and 3- 5 in each district) which reports to the District Hospital and Public Health Office. The referral ladder then is zonal hospital, regional hospital and central hospital (Figure 2.2).

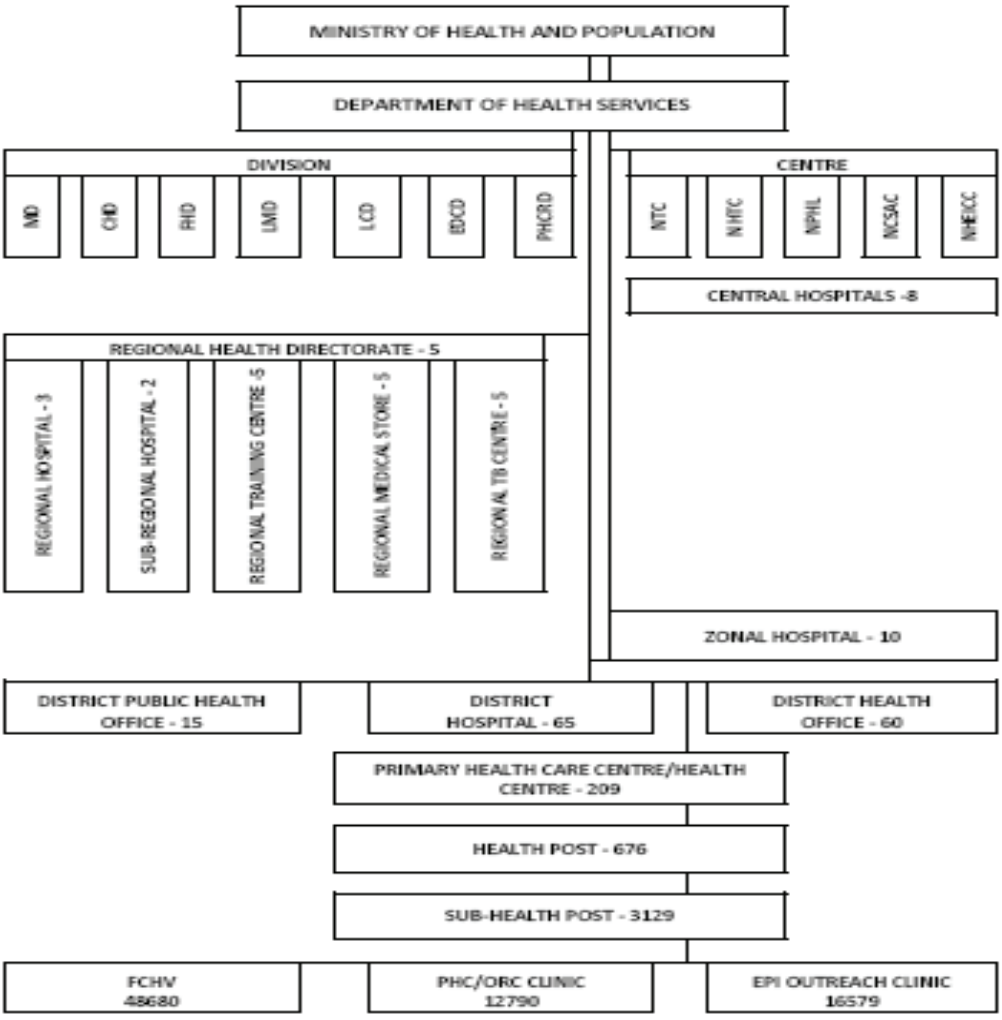


Figure 2.2: Organisation structure of the health system of Nepal

Health care is provided by the Government and private sector. Most of the cities in Nepal have both private and public hospitals. The private hospitals cater primarily to the socio-economically affluent population and the public hospitals to the marginalised. There are no health insurance schemes, so the cost for any expenditure on health is out of pocket for the entire public. The government provides most of the preventive, promotive and curative health services for rural population free of cost.

The country's child health policy is guided by WHO's millennium Development Goal (MDG) 4 which is to reduce child mortality. Although in the past 16 years, the infant mortality rate has reduced significantly from 78.5 to 46/1000 live births and the under 5 mortality reduced from 118 to 54/ 1000 live births, it is still very high. Disability is prevalent in 2% of the population, of which physical disability constitutes 36.3%, blindness or low vision (18.5%), deaf or hard of hearing (15.4%), speech abnormality (11.5%), intellectual disability (2.9%) and others 15.4%. AES is a preventable cause of under-five mortality and disability in Asia including Nepal.

2.2 Study Sites

The study was based at three hospitals in Nepal:

1. Kanti Children's Hospital (KCH), Kathmandu
2. Patan Hospital (PH), Patan
3. BP Koirala Institute of Health Sciences (BPKIHS), Dharan

Kanti children's Hospital is a 300 bed tertiary care referral centre and the only children's hospital with super-specialties in Nepal. It is a busy hospital located

in Kathmandu valley and sees 1000- 1500 patients per day in the Emergency Department, and another 300 patients per day through outpatient services. It is funded by the government. The hospital fees are minimal, although families are made to pay from their pocket. Those who cannot afford receive free treatment as judged by the director. Amongst others KCH has a related neurology specialty clinical service, pediatric intensive care facilities and electroencephalogram (EEG) and Computer Tomography (CT) Scan facilities. It receives referral of paediatric patients from all over Nepal.

Patan Hospital is a large multidisciplinary not- for- profit general hospital situated in Patan which is a neighbouring city to Kathmandu, the national capital. It is located at the southern end of Kathmandu valley. It serves as a major teaching hospital for Patan Academy of Health Sciences. There are 450 beds (including 60 paediatric beds) and the hospital sees around 1000 patients every day. It predominantly caters to population of Lalitpur districts and also accepts referrals.

BP Koirala Institute of Health Sciences is a public University, with four colleges namely medical, dental, nursing and public health and a central 700 bed teaching hospital. It is renowned for its constantly innovative educational programme. It is located in a city called Dharan which is at the Southeastern end of Nepal, in the Terai region, around 300 Km from Kathmandu. The paediatric ward has 60 beds. It also has pediatric intensive care facilities and neurology specialty clinic.

2.3 Study design

This research was conducted in four parts from 2009- 2016.

i. The first part of the research was a **retrospective study** aimed at looking at the clinical features, laboratory parameters and outcome of AES and JE.

ii. The second was a **prospective cross-sectional study** to validate prospectively clinical features which predicted bad outcome in AES and JE identified by the first study and also find out the outcome of children with AES and JE in relation to low weight for age at the time of admission.

iii. The third part was a **prospective cross-sectional study** to identify the role of fluid management in outcome of AES.

iv. The fourth part was a **pilot interventional study** (treatment trial) of IVIG in JE.

2.4 Timeline of the study

The total duration for all studies in my thesis was from 2009 until 2016.

Patients were recruited into the studies between January, 2006 - October, 2012.

1) Firstly, the retrospective study was conducted between February and March 2009. The patient records were analysed using admission data from January 2006 to January 2008 for patients admitted to Kanti Children's Hospital (KCH) and BP Koirala Institute of Health Sciences (BPKIHS).

2) Secondly, the prospective study was conducted between March 2009 and March 2011. The patients were recruited between April, 2009 and November, 2010 at KCH and BPKIHS.

3) Thirdly, the next prospective study was conducted between April 2011 and April 2013. Patients were recruited from September, 2011 to October, 2012 in KCH

4) Fourthly, the IVIG treatment trial was conducted between April, 2009 and September, 2009. A separate group of patients from the second prospective study (chapter 4) were recruited in May and June 2009 in KCH and BPKIHS.
5) All the four studies were compiled together and written up until March 2016

2.4.1 Contribution

For the first three studies, I wrote the research proposal, acquired ethical clearance, organized and conducted the study with help from research assistants; analyzed the data and wrote up the findings. For the fourth study, I wrote the research proposal, acquired ethical clearance, organized and conducted the study with help from research assistants, analyzed the data and wrote up the findings. Laboratory tests such as cytokine levels, Dengue and JE virus ELISA, plaque reduction anti-JEV antibody neutralizing titres and real-time reverse transcription-polymerase chain reaction tests were conducted by my supervisors.

2.5 Patient recruitment and diagnostic criteria

2.5.1 First part, retrospective study (chapter 3): The hospital records charts of all children aged between 1 and 14 years admitted in KCH, Maharajgunj, Kathmandu and PH, Lalitpur, Nepal between January, 2006 to January, 2008 were retrieved from the record sections. Between February and March, 2009, 13397 records of admitted patients in the paediatric medicine wards (medical ward, paying ward, special cabin) and paediatric intensive care (PICU) in

KCH and 4532 patients admitted in the paediatric ward and PICU in PH were reviewed. Altogether, 17929 records of admitted patients in the two hospitals were reviewed with the help of staff working in the record section.

All hospital in-patients aged 1- 14 years admitted between January 2006 to January 2008 with a discharge diagnosis or clinical features and investigations fulfilling the WHO definition for AES based on the recorded information in the clinical notes from Kanti Children's Hospital and Patan Hospital [http://apps.who.int/iris/bitstream/10665/68334/1/WHO_V-B_03.01_eng.pdf?ua=1 (assessed on 3rd March 2016)] were included in the study. Relevant demographic data, clinical history, investigation reports, treatment provided, hospital stay and immediate outcome were recorded in a standardized proforma of 225 patients (Appendix F).

Based on available records, patients who had incomplete information on clinical history about AES case definition, lacked relevant investigation reports, no discharge outcome, fever duration of more than 14 days or age below 1 year or above 14 years were excluded from the study. Those with diagnosis of JE and concomitant infections, bacterial meningitis, cerebral malaria, typhoid encephalopathy were also excluded from the study and analysed separately. Those without LP reports were also excluded and analysed seperately.

Pertinent points noted in history were information on date of admission, duration of illness, age, fever duration and seizure. Pertinent information of examination findings noted, included: fever, altered sensorium, seizure and

focal neurological deficits. haemoglobin, total and differential white cell count, platelets count, random blood sugar, total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, urea, creatinine, sodium and potassium, peripheral blood smear and/or rapid diagnostic test for malaria and widal test. Date and result of CSF investigation such as total and differential cell count, protein, sugar, Gram's stain, culture and sensitivity after LP was also recorded. Information on chest x-ray, computerized tomography (CT) scan and magnetic resonance imaging (MRI) scan of the brain were also recorded if they were available.

The anti-JEV immunoglobulin-M (IgM) antibodies measured in serum and/or CSF of suspected JE patients admitted in these two hospitals was being conducted, as part of the national surveillance of JE, at the National Public Health Laboratory (NPHL), Teku, Kathmandu. The NPHL at Teku in Kathmandu was then approached with the inpatient number, name and address of all the included patients of the two hospitals for the result of serum and/or CSF anti-JEV immunoglobulin-M (IgM) antibodies. The JE result acquired from NPHL was further verified from the records of the office of WHO-IPD. Anti-JEV immunoglobulin-M (IgM) antibodies were measured in serum and/or CSF on admission and if possible on day 7 of admission by enzyme-linked immunosorbent assay (ELISA) using the Armed Forces Research Institute for Medical Sciences (AFRIMS) JE MAC IgM ELISA, which was developed at AFRIMS in Bangkok. Therefore, only 145 AES patient of suspected viral aetiology were included in the study. These patients were included because there was no definite treatment for most of the patients and identifying clinical

features and prognostic factors could help in patient management. These patients were further classified as confirmed JE and AES of unknown viral aetiology (which included non-JE and JE status unknown) as defined under general case definitions in this chapter and analysed. Since significantly higher proportion of patients without LP results died or had bad outcome as compared to those who had LP, an additional analysis was undertaken, whereby patients without LP results were classified into AES categories based on their hospital discharge diagnosis supported by their JE serological results.

Emphasis was given to retrieve information on treatment with mannitol, steroids and anti-epileptic drugs. Information on course of the illness in the hospital, duration of hospital stay and outcome was also recorded. The immediate outcome was classified and recorded as bad outcome and good outcome as defined in the case definitions section of this chapter. The bad outcome group were not further classified as mild, moderate or severe residual neurological sequelae because of the limited breadth and quality of information available in the hospital notes, a pitfall of a retrospective study.

2.5.2 Second part, prospective study (chapter 4): The study was conducted in KCH and BPKIHS, from April 2009 to November 2010. 217 patients aged 1 year to 14 years, fulfilling clinical history consistent with AES as defined by the WHO surveillance standard admitted to the Paediatric Ward (Medical Ward, Paying Ward, Special Cabin) and PICU in KCH were approached by me; and the Paediatric Ward and PICU in BPKIHS were approached by my research assistant during the study period for recruitment.

After brief introduction, a detailed history including demographic data, duration of the fever, seizure and level of consciousness were inquired. The character of the seizure was asked and noted. Other relevant history included headache, photophobia, vomiting, ear discharge, running nose, cough and loose motion. Examination findings included the conscious level, vital signs and other relevant systemic findings including signs of meningeal irritation and bulged anterior fontanel. Importantly, weight was measured and recorded at the time of admission using a digital weighing scale. The quality control of the weighing machine was conducted every morning using standard weights. After initial stabilisation, investigations sent were haemoglobin, red blood cells, total white blood cells, differential white blood cells, platelet count, random blood sugar, blood for bacterial culture, total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, urea, creatinine, sodium, potassium, peripheral blood smear and/or rapid diagnostic test for malaria and widal test. Serum was also sent to NPHL, Teku to test for anti-JE IgM antibodies. To identify the cause of illness, further investigations conducted included chest X-ray, urine routine and culture, stool routine, ultrasonography (USG) of brain, CT scan of brain, MRI of brain.

After a separate informed written consent, LP was performed with aseptic precaution with patient in lateral position with a disposable needle of different gauge and length varying from 22-24 according to age. Around 3 millilitres of CSF was collected in 3 sterile vials (aliquots) and sent for investigations.

One aliquot containing the CSF was sent for total white blood cell count, differential cell count, sugar and protein. The second aliquot was sent for Gram's stain and culture. The samples were transported to the emergency laboratory and tested for cell count and biochemical analysis within 1 hour.

The CSF was analyzed as per standard laboratory methods by laboratory technicians. The colour and transparency of the CSF was first noted. For glucose estimation, an enzymatic method was employed. CSF was kept in a water incubator with glucose oxidase for 10 minutes. If pink colour developed, it was quantitated by the colorimeter based on its optical density. To quantitate the protein content, equal amount of CSF was mixed with 3% sulfosalicyclic acid and later at 10 minutes, if there was turbidity, it was compared with standard solution.

The cells were counted on a Neubauer chamber after dilution with Turk's fluid. For differential count, sediment of the centrifuged CSF sample was stained with Wright's stain and cells were counted. The CSF sent for microbiology (bacterial culture) was kept in the incubator at 37°C until it was plated on blood agar, chocolate agar and Mc Conkey agar for 48 hours. From the colonies, Gram's stain and biochemical tests were conducted to identify the organism. The third aliquot of 1 millilitre CSF was sent to NPHL, Teku along with serum for testing anti-JE IgM antibodies as part of the National Surveillance of JE.

Following LP, patients who were classified as probable viral encephalitis if they had CSF cell count < 1000 cells/mm^3, and a CSF/Plasma glucose ratio >

40% and absence of organisms on a Gram stain and culture were included in the study (37). Since there are no treatments for most viral encephalitis, only patients of suspected viral encephalitis were included in the study and analysed.

All the patients who had not undergone LP were excluded from the study. In those who had LP, patients having bacterial meningitis, tubercular meningitis, typhoid encephalopathy and simple febrile seizure were excluded from the study because they were treatable illness. Patients without appropriate admission weight records, below 1 year and above 14 years of age, fever duration of more than 14 days, failure to provide consent, history of recent head trauma and past history of seizure disorder on antiepileptic drugs were also excluded from the study.

A total of 152 patients of suspected viral aetiology after the availability of LP result, as defined in general case definitions in this chapter, were recruited in the study. Informed written consent was taken from the parents or accompanying caretaker of the included patients. Detailed history, examination findings, investigation results were then recorded in a standardised proforma (Appendix G). Since the normal weight of children differed with age and sex, during analysis, weight for age (WFA) was used instead of just weight. WFA was defined according to WHO definition for children below 5 years of age (Appendices H & I) and "Development of the Nepalese Growth Standard for the School-aged Children" (Appendix J).

All the recruited patients of AES including JE were reassessed by me at the time of discharge in KCH and a research assistant in BPKIHS. During

assessment at discharge, parents were interviewed and patients examined clinically with emphasis on neurological system using LOS- discharge [http://www.liv.ac.uk/infection-and-global-health/research/brain-infections-group/education (assessed on 3rd March 2016)] (Appendix K). They were then prospectively followed-up twice between 6- 24 months (intended at one year and 2 years) after discharge in order to find out the long term outcome and recovery profile of the recruited patients. During follow up, they were assessed or inquired by phone about recovery, deterioration or death based on LOS- follow-up (Appendix L). Those who did not come for follow-up were interviewed by telephone.

2.5.3 Third part, fluid study (chapter 5): A total of 143 children, aged 1 month- 14 years, from September 2011- October 2012 attending emergency department, paediatric ward and paediatric intensive care unit of KCH with clinical features consistent with AES, as defined by WHO surveillance standard were seen by me or my research assistant. The parent / caregiver were explained about the study when they were first seen in hospital. They were explained that in order to be eligible for the study the results of the routine LP was needed. At this stage any questions or concerns were addressed. After confirming with the entry criteria, a written consent from the parents or accompanying guardians were obtained. A detailed history was then taken from the accompanying parents or guardian. First, demographic information was inquired and recorded. Then clinical history was inquired and findings recorded. Then complete clinical examination including neurological examination was performed and findings recorded. Special attention was paid

to child's weight, mid upper arm circumference (MUAC), clinical signs of dehydration, capillary refill time, peripheral oxygen saturation and axillary temperature, presence of seizures and clinical signs of raised intracranial pressure and brain stem herniation.

After initial stabilisation, investigations were sent which included blood count, liver function test, renal function test, electrolytes, blood for bacterial culture including antibody titers against specific bacteria measured via a commercial kit (PASTOREX MENINGITIS, BIO-RAD, France, 2011), peripheral blood smear and/or rapid diagnostic test for malaria and widal test. Aliquot of serum was also sent to NPHL, Teku for anti-JE IgM antibodies as part of the National Surveillance of JE. To aid diagnosis, further investigations conducted included chest X-ray, urine routine and culture, stool routine, ultrasonography (USG) of brain, CT scan of brain, MRI of brain. The results of the investigations were also recorded.

After a separate written consent, LP was performed with aseptic precaution as described above in the second study. Around 3 millilitres of CSF was collected in 4 sterile vials (aliquots) and sent for investigation. One aliquot containing the CSF was sent for total white blood cell count, differential cell count, sugar and protein. The second aliquot was sent for Gram's stain and culture. The samples were transported to the emergency laboratory and tested for cell count and biochemical analysis within 1 hour as described above in the second study. The third aliquot of 1 millilitre CSF was sent to NPHL, Teku for testing anti-JE IgM antibodies. The fourth aliquot was tested with Pastorex meningitis kit.

The following procedure was conducted with both blood and CSF samples. A drop of the pre-treated sample supernatant in each circle of the agglutination was placed on a card. The latex reagent bottles were gently shaken. Holding the bottles upright, one drop of each latex reagent was placed on the disposable card following the indicated distributable pattern: R9, R6, R7, R1 and R2 in the white circles and R8, R3, R4 and R5 in the black circles. The latex reagents and the sample was mixed gently with a rod, changing the rod for each latex. The card was rotated (~120 rotation per minute) gently for 10 minutes and watched for the appearance of any agglutination visible to the naked eye within 10 minutes. While interpreting the result, positive reaction for the aetiological diagnosis of meningitis was indicated by fine agglutination, visible to the naked eye, compared to negative controls at the button hole of the agglutination card. A negative reaction (absence of meningitis) was indicated by homogenous suspension in the absence agglutination.

Once the LP results were available, I or my research assistant informed the parents/ caregiver of the results and what they meant. If 1 month to 14 years aged child was eligible for the study; met the inclusion criteria for AES; on LP was diagnosed as AES of suspected viral aetiology (not meeting the exclusion criteria); and MUAC $\geq$ 11.5 cm; then the parents or caregivers were informed and the patient included in the study (10, 37).

However, patients diagnosed bacterial meningitis, tubercular meningitis, typhoid encephalopathy, cerebral malaria, children with age below 1 month and above 14 years, fever duration of more than 14 days, simple febrile seizure,

fever more than 2 weeks, MUAC < 11.5 cm and traumatic head injury were excluded from the study. Families were also advised that they could withdraw consent at any time and that participation was voluntary. If they chose not to participate then their child/ teenager received the usual standard supportive care provided by the treating clinician. I included infants over one month of age in this study because JE cases decreased markedly after mass vaccination campaign with JE vaccines in the region. I increased the age range with the aim of increasing recruitment of JE cases.

Of the 92 included patients, total fluid input (oral, nasogastric and intravenous including drug diluents) and output (urine, vomit) was monitored daily after admission until discharge or death by me or a research assistant. They were specially assessed for weight, blood lactate, oxygen saturation (Spo2) and capillary refill time at the time of admission, at 48 hours after admission and at discharge or death. Use of other supportive management procedures such as head positioning, sedative (including anti-epileptic drugs), mannitol and analgesia provision was also monitored. The immediate outcome at discharge was assessed by LOS- discharge (Appendix K). All the information was recorded in a standardised proforma (Appendix M).

The normal weight of children differ with age and sex. Therefore, we used sex matched weight for age (WFA) to find out the actual weight of patients included in the study. During analysis, WFA was defined according to WHO definition for children below 5 years of age (Appendices H & I) and "Development of the Nepalese Growth Standard for the School-aged Children" for older children (Appendix J).

2.5.4 Fourth part, IVIG treatment trial (chapter 6): During the monsoon period in May and June in 2009, patients aged between 1 and 14 years with AES as defined by WHO surveillance standard such as fever or history of fever (<14 days), change in mental status and/ or new onset of seizures (excluding simple febrile seizure) were considered for recruitment in the study in KCH and BPKIHS (37, 38). Following LP, patients were classified as suspected JE (or probable viral encephalitis) if they had CSF cell count < 1000 cells, and a CSF/Plasma glucose ratio > 40% and absence of organisms on a Gram stain and culture, and included in the study (37). In instances where a LP needed to be delayed, because of clinical contraindications, children were entered into the study, and LP performed as soon as possible. Summary of the trial protocol is shown in Appendix N.

Patients who had asexual *Plasmodium falciparum* parasites in blood, documented antibiotic treatment before admission and *bacterial meningitis appeared more likely than encephalitis,* simple febrile seizure, pregnant or breastfeeding female adolescents, age below 1 year and above 14 years and fever duration of more than 14 days were excluded from the study. Patients with a GCS of 3/15, who were receiving artificial ventilation without signs of spontaneous respiration and with absent oculocephalic reflex were also excluded from the study (20,37).

The primary outcome assessed was evidence of side effects of study drug such as infusion site reaction, diarrhoea, rise in blood pressure and change in urinary output every 12 hours from first day of commencing treatment until death or

discharge. Patient on average were administered the study drug on the first day of admission. The study drug was administered daily for 5 days. Patients were discharged on an average on eighth day (192 hours) of hospital admission.

The secondary outcome assessed was death or neurological sequelae at the time of discharge, an expected average of eighth day of admission and again at 6 months after discharge. At the time of discharge from the hospital they were assessed for time to death, to recover from coma, to sit independently, to stand independently, to walk at least 5 metres independently, and to leave hospital (Appendix K). At 6 months after discharge they were assessed for history of further seizures, behavioural changes, evidence of recovery of neurological sequelae such as sit independently, stand independently, walk at least 5 metres independently (Appendix L).

After inclusion, a detailed history was taken from the accompanying parent or guardian by me or research assistant. Patients were then examined including full neurological examination. Attention was focused on presence of seizures, and clinical signs of raised intracranial pressure and brainstem herniation because of their association with bad outcome.

Blood was sent for complete blood count (CBC), renal function test, liver function test and aliquot of serum sample sent to NPHL to test for anti-JE IgM antibodies. Urea and electrolytes were repeated during treatment to exclude renal toxicity. LP was done and CSF opening pressure recorded using a CSF manometer. CSF was also sent for total cell count, differential cell

count, glucose, protein, Gram's stain, bacterial culture and aliquot of sample sent to NPHL to test for anti-JE IgM antibodies. Aliquot of CSF and serum samples were frozen on site at -20°C, and subsequently transported for further investigations to Centers for Disease Control and Prevention (CDC), Division of Vector-Borne Diseases in Fort Collins, USA for confirmatory testing for JE and neutralising antibodies levels and the Institute of Infection and Global Health, University of Liverpool (UOL), UK, for cytokines levels. Classification of the patients as JE negative or positive was by initial JEV IgM ELISA testing in Nepal measured in serum on admission and day 7 of admission, and in CSF using the AFRIMS JE MAC IgM ELISA as part of national surveillance programme.

Confirmatory testing was subsequently performed at the CDC, USA using the following testing algorithm. Further in the CDC, the last serum sample collected from each patient was tested by JEV and DENV IgM capture ELISA. Positive or equivocal results were confirmed by JEV and DENV $PRNT_{90}$, with a 4-fold or greater difference in titre interpreted to be virus specific. If the final sample was positive or equivocal for JEV and/or DENV IgM all samples for that case were tested. In addition, if the last serum sample from a patient was collected at less than 7 days post onset of disease and had an IgM ELISA negative result, the CSF from that patient was also tested by JEV and DENV IgM ELISA.

In addition, if the last serum sample collected from a case had a negative JEV and DENV IgM ELISA result and was collected <7 days from illness onset,

CSF was tested for the presence of JEV ribonucleic acid (RNA) by two real-time reverse transcription-polymerase chain reaction (RT-PCR) assays, one designed in-house at CDC (JEV239F: GGCTCTTATCACGTTCTTCAAGTTT; JEV344R: ACTAGTAAGATGTTTCATTGCCACACTCT; JEV269probe: ATTAGCCCCGACCAAGGCGCTTT) and one developed by Parida et al (108). Both assays had sensitivities of 0.001 equivalent plaque forming units for SA-14-14-2 reference viral RNA. Viral RNA was extracted from 100µl of CSF, eluted in 50 µl using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA); 20 µl RNA template was used in a 50µl reaction using the iScript One-Step RT-PCR kit for Probes (BioRad, CA).

To study in more detail the effect of IVIG on the neutralising antibody titres, JEV $PRNT_{50}$ was done on serum from all patients at pre-treatment (immediately prior to the first dose), and post-treatment (1hour after the 5^{th} dose). Interleukin (IL) -6 and IL-4 cytokine measurements were undertaken on aliquots of IVIG and on patients' serum pre-treatment (before first dose) and post-treatment (1 hour after 5^{th} dose), using ELISA kits following the manufacture's protocol (Bender MedSystems GmBh, Wien, Austria). Samples were measured in duplicate (50ul) at each time-point. Quality control was based on concurrent measurement of negative and positive controls supplied in the kits. Other investigations such as blood for bacterial cultures, electrocardiogram (ECG), chest radiograph, CT scan of brain, MRI scan of brain was performed at the discretion of the admitting physician if clinically indicated.

Participants were randomly allocated treatment with IVIG or placebo. Randomisation was stratified by centre using a variable block size of 4, 6 or 8, selected on a random basis similar to those schemes used previously (38). Each centre was provided with duplicate sealed envelopes with its own randomisation schedule. The randomisation code for each patient was kept in a sealed envelope in a locked cupboard on a separate ward. Once a patient was enrolled, technicians who were not otherwise connected to the study opened the envelope, drew up the study drug, covered the syringe in opaque tape (because of subtle differences in colour and viscosity of the two substances) and delivered it to the ward, where the nurses gave the treatment. Of the 23 considered during the study period, 22 patients with suspected JE were randomised to receive either IVIG [ImmunoRel™ (batch 20081217)] at a dose of 400mg/kg/day for 5 days or an equivalent volume of 0.9% normal saline (placebo). The infusion rate was initially started at 0.01 to 0.02 ml/kg body weight/minute and if tolerated gradually increased to 0.08 ml/kg body weight/minute over 30- 60 minutes. Empty vials were concealed in paper bags and disposed of. Hence, all investigators, care providers and participants were blinded of the study drug. A second sealed envelope was kept with inpatient notes in case a physician urgently needed to know which drug a patient had received.

I took a detailed history and did daily clinical and neurological examinations until death or discharge. Information was recorded on standardised proforma (Appendix O). Treatment side effects were monitored daily from the first day of commencing treatment (the first day of hospital admission) until death or

discharge (on average on eighth day), these included headache, abdominal pain, chest tightness, nausea, vomiting, rash, fever, pallor, local site reaction, diarrhoea, rise in blood urea, rise in blood creatinine, change in blood pressure and urinary output and classified according to the WHO standard protocol for drug side effects. An adverse event was observed as any event, side effect, or other untoward medical occurrence (including dosing errors), that was present during treatment with a study drug. Laboratory adverse events were abnormal values obtained on laboratory tests during the acute and follow-up period. A Serious Adverse Event (SAE) was any adverse event occurring at any drug dose that resulted death, life-threatening event (at immediate risk of death), required hospitalisation or prolongation of existing hospitalisation or resulted in a significant disability. The Data Safety and Monitoring Committee were promptly notified of any serious adverse event, deaths, or life-threatening problems that were regarded as caused by or associated with the administration of the study drug. Occurrence of any adverse events (those that were considered non-serious) during the study was documented in the patient's medical records and on the case record forms. The summary of the study schedule is shown in Appendix P.

Patients were assessed at discharge and 3-6 month follow-up for disability in a range of activities using the LOS (109) (Appendices K & L) and a standardised neurological assessment (Appendix Q). Children who did not return for follow-up were reminded by letter or telephone as available. If they were unable to return to the hospital they were examined at home either by me or my research assistant.

2.6 General Case Definitions

Clinically, a case of AES was defined based on WHO recommended case definition (1). AES case definition has been validated previously in 380 patients (including 149 children below 15 years of age) for diagnosing JEV infected patients in similar setting. It was found to have sensitivity of 100%, specificity of 16%, positive predictive value (PPV) of 6% and negative predictive value (NPV) 100% in adults. In children, sensitivity was 65%, specificity 39%, PPV 48% and NPV 56% (1). JE tests, as part of JE surveillance programme, were conducted on these identified cases during the study period. The AES cases, based on the results of their microbiological and serological tests, were classified as AES of suspected viral aetiology (Confirmed JE, Non-JE and JE Status unknown) and AES of non-viral aetiology (AES-bacterial or parasitic aetiology). The clinical features within each class were examined. The definition for AES was based on WHO recommended case definition:

2.6.1 AES: Acute onset of fever or recent history of fever (of less than 14 days), with change in mental status (including confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures). Other early clinical findings could include an increase in irritability, somnolence or abnormal behaviour greater than that seen with usual febrile illness (1).

2.6.2 AES of suspected viral aetiology or viral encephalitis (VE): was defined by fulfilling the definition for AES (above) and having a discharge diagnosis of suspected viral encephalitis or menigo-encephalitis, supported by

a CSF cell count <1000 cells/mm^3 with a lymphocyte predominance and no positive identification of non-viral pathogens (e.g. bacteria or parasites) in the CSF or blood (37, 38).

2.6.3 Confirmed JE: AES of suspected viral aetiology or VE, which is shown to have IgM antibodies (≥ 40 units) specific to JE virus in a single (CSF and/ or serum) sample (or a rise in titres among paired samples) as detected by IgM-capture ELISA.

2.6.4 Non-JE: A suspected viral case which is shown to have an absence of IgM antibodies specific to JE virus based on a negative test for a single sample collected after the ninth day of illness or no change in titres in paired samples collected at least seven days apart.

2.6.5 JE Status unknown: A suspected viral case which was either not tested for anti-JE IgM antibodies or had samples tested that were collected too early in illness course to confidently rule out JE (as defined above).

2.6.6 AES of unknown viral aetiology: A suspected viral case which was not confirmed as JE; this group included both of the categories described above, i.e. Non-JE, and JE Status unknown.

2.6.7 JE with concomitant infection: A case of confirmed JE, with evidence of bacterial meningitis, typhoid encephalopathy or cerebral malaria.

2.6.8 AES of non-viral aetiology:

2.6.8.1 AES with bacterial meningitis: Case of AES with a CSF cell count > 1000 cells/mm3, a pleocytosis with a polymorph predominance and raised CSF protein (>0.45g/L) and CSF/ plasma glucose <40%, and/or Positive Gram stain from CSF and /or bacterial culture from CSF or antibody titre positive against specific bacteria in serum and /or CSF by PASTOREX MENINGITIS KIT (1, 10, 110).

2.6.8.2 AES of typhoid encephalopathy: Case of AES positive blood culture for Salmonella typhi and/or positive Widal test (10, 37).

2.6.8.3 AES of Cerebral malaria: Children of AES with asexual Plasmodium falciparum parasites in peripheral blood (10).

2.6.8.4 AES of tuberculous meningitis: An AES case having CSF cell count 10-1000 cells (lymphocyte predominance) and CSF/ Plasma glucose ratio <40%, CSF protein 50- 500 mg/dl or Acid-Fast Bacilli detected in CSF modified Ziehl-Neelsen stain or culture.

2.6.8.5 AES of Hepatic encephalopathy: Children with AES with normal CSF finding with abnormal liver enzyme levels (10).

2.6.8.6 AES of acute poisoning: Children of AES with evidence of poisoning on clinical history and examination and laboratory evidence.

2.6.9 Simple febrile seizure: A child aged 6 months to 5 years with fever and a single generalised convulsion lasting less than 5 minutes followed by full recovery of consciousness within 60 minutes of convulsion (1).

2.6.10 Good outcome: Being alive with no impairment of consciousness (GCS 15/15) or neurological sequelae (75).

2.6.11 Bad outcome: Death or incomplete recovery (with altered sensorium or neurological sequelae at discharge) (75). Bad outcome represents patient death or incomplete neurological recovery (with altered sensorium or neurological sequelae at discharge). All children with neurological sequelae were regarded has having a ‘bad outcome’ because JE patients experiencing ‘mild’ sequelae, such as memory impairment or change in personality, have suffered significant limitations in social participation, such as stopping school attendance, causing huge adverse affects to the family (21, 28). In addition, Nepali families of AES patients with moderate or severe sequelae are known to suffer a huge economic burden of US$ 1151 (10 times their median monthly income) (28). I used "bad outcome" because it was absolute term where as "poor outcome" or "worse outcome" were relative.

2.6.12 Liverpool Outcome Score (LOS): This is a simple validated tool for assessing functional impairment in children following JE. It was developed in the University of Liverpool with expert inputs from paediatric neurologists, paediatricians, neurologists, occupational therapists, psychologists and score developers. It was piloted in India and Malaysia in patients of confirmed JE or suspected JEV infections (Appendix R). It is now being used to assess disability of JE in Bangladesh, Indonesia, Cambodia, Vietnam and the Lao People’s Democratic Republic (109).

The score is determined by asking 10 simple questions to patients' care giver and observing 5 activities. While answering 10 questions for the LOS used at the time of discharge, the caregiver compares the child to how they were before admission (Appendix K). For the LOS used at follow up, the

caregiver compares the child with other children of a similar age in the vicinity or the child's sibling when they were at that age (Appendix L). The LOS assesses speech, communication, feeding, ability to be left alone, behaviour, recognition, school or working, seizures, dressing, bladder/bowel control, hearing, sitting, standing, walking and upper limb dexterity. Final LOS is the lowest score for any of the 15 functional domains assessed. A LOS score of 2, 3, 4 or 5 corresponds to an outcome classification of severe, moderate, mild or no functional impairment respectively. A LOS score of 1was given, if the child died; 2, if there were severe sequelae causing impairment of function likely to make the child dependent on others; 3, if there were moderate sequelae only mildly affecting function, probably compatible with independent living, 4, if there were minor sequelae with mild effects on function or personality change or on medication; 5, if there was complete recovery which included normal examination.The lower the score the more severe the impairment. Also a total score is assigned by adding scores for each question. The total score can range from 33-75 points (109).

There are 125 ethnic groups speaking 123 different languages in Nepal.
The written format of some of the languages are more formal and different from spoken language. Therefore, it was not felt appropriate to translate the written questionnaire into local languages. Instead, I and my search assistants administered it verbally in Nepali, which is spoken by around 45% of the population.

2.6.13 Neurological sequelae: Presence of one or more of the following at discharge; impaired consciousness, weakness (monoparesis hemiparesis,

quadriparesis), focal or generalised abnormal limb tone (hypertonia, hypotonia), focal or generalised abnormal limb reflexes (hyperreflexia, hyporeflexia), diagnosis of new onset or recurrent seizures or new or recurrent extra pyramidal movement disorders (75).

Specific case definitions are described in related chapters.

2.7 Sample size

2.7.1 First retrospective study (chapter 3): A sample size calculation was performed to estimate the number of participants needed to detect a significant difference in the proportion of participants with 'bad outcome' (suffered death or neurological sequelae) at hospital discharge among JE compared to non-JE patients. The sample size calculation was performed in OpenEpi, Version 3, using the 'sample size for unmatched case control studies'. Based on a previous publication from Malaysia (21) and supported by our similar experience in Nepal, the expected proportion of participants to have a bad outcome was 60% in JE and 40% in Non-JE patients. Setting the confidence level at 95% and power at 80% and ratio of cases: controls (or JE: Non-JE) at 1:1, the sample size was estimated as 96 participants per arm (192 in total). Based on my previous study (37), I expected to recruit 200 patients over 2 years from two hospitals. 200 participants were estimated to provide sufficient study numbers to examine whether there was a significant difference in the proportion of patients with 'bad outcome' among JE compared to Non-JE patients.

2.7.2 Second study (chapter 4): A sample size calculation was performed to estimate the number of participants needed to detect a significant difference in patients' weight at hospital admission among JE compared to Non-JE patients.

Again the sample size calculation was performed in OpenEpi, Version 3, this time, using the 'sample size for comparing two means'. The mean weight and standard deviation for patients with JE (cases) was 16.2 ± 6.5 Kg and non-JE (controls) 20.5 ± 8.7 Kg respectively based upon data from my previous study (chapter 3) (75). Setting the confidence level at 95%, power at 80% and ratio between the groups at 1:1; the sample size estimated 102 participants in total (51 in each group).

2.7.3 Third study (chapter 5): The sample size calculation was undertaken to assess the number of participants needed to detect a significant difference in plasma urea among participants with a bad outcome as compared to those with a good outcome. Raised plasma urea was used as proxy marker for poor hydration status. The following parameters were based upon data from my earlier study (chapter 3). The sample size calculation was performed in OpenEpi, Version 3, again using the 'sample size for comparing two means'. The mean plasma urea and standard deviation for AES patients with good and bad outcome were 7.9 ± 10.1 mmol/l and 15.8±13.9 mmol/l, respectively; expected proportion of AES patients with good outcome / bad outcome as 60/40 (or 1.5). Setting the confidence level at 95%, power at 80%, the sample size estimated 83 participants in total (50 and 33 with a good and bad outcome respectively).

2.7.4 Fourth study (chapter 6): The aim was to examine feasibility, by studying approximately 20 patients across two sites. I also wanted to determine adverse effects of IVIG or compare anti-JEV antibody titres following administration of IVIG and placebo. I could not find any previous human

clinical trials of IVIG among JE patients or toxicity studies of IVIG in animals with JE to guide sample size. However, there were data on change in anti-JEV titre associated with improved outcome in mice given immune splenocytes. Mice given immune splenocytes generated a mean anti-JEV titre of 400, whereas mice given naïve splenocytes exhibited a mean anti-JEV titre of 40. Based on these data, using a significance of 0.95 and a power of 0.8, mean titres of 400 and 40 and a standard deviation of 400 and 40 for anti-JEV antibody titres in IVIG exposed and non-exposed respectively, 10 subjects were required in each arm. Considering10-20% loss to follow up, I aimed to recruit 22-24 patients.

2.8 Data analysis

Broadly, all the acquired data for all AES patients was initially validated, coded and entered in SPSS Statistics software version 17.0 (IBM-SPSS, New York,) for analysis. Percentage, Proportions and Contingency tables were used for description of the data. Parameters were analysed for normally distributed data using the Student's t-test, non-normally distributed data using Wilcoxon-Mann-Whitney U test and differences between proportions by Fischer's Exact test or Chi square test with Yates correction as necessary. Further detailed analysis is mentioned in related chapters.

2.9 Ethical Consideration

Ethical approval had been sought before commencing the work. All four parts of this thesis were approved by the Ethical Committees of Liverpool School of Tropical Medicine, the Nepal Health Research Council, KCH and BPKIHS.

The purpose, design, risks, benefits, voluntary participation and confidentiality of data were explained to the patients and their parents by a member of the research team. Informed written consent was taken from parents or guardians of all participants of prospective study, including treatment trial, before inclusion in the study. The number of blood and LP tests was the same as recommended by the WHO for diagnosis of JE and were needed by the treating clinicians. In the fourth part of the study consent was taken twice. First consent was taken to recruit participants in the screening process and conducting a LP. A second consent was taken after reviewing results of the LP. If there was high clinical suspicion of the participants having JE, they were then included into the IVIG treatment trial; and blood and CSF samples and photos/ videos were taken for the study. A data safety monitoring committee was constructed to review serious adverse events and to take appropriate action if necessary.

The patient's relevant clinical information was recorded using a data collection sheet which was given a unique study number. Study data was transcribed from the case-record form to the study database. The study number was used to access the data for all future data analyses. All information obtained was kept confidential. The completed proforma were stored in a designated locker of locked research office at each hospital. Databases were stored in a password protected laptop. The database was itself password protected. Data was backed up on a password encrypted portable hard disk (HD-PS12OU2-WH). The password was only accessible to the student and supervisor.

Chapter 3: Clinical and prognostic features among children with Acute Encephalitis Syndrome in Nepal; A retrospective study

Abstract

Background: Acute encephalitis syndrome (AES) is commonly seen among hospitalised Nepali children. Japanese Encephalitis (JE) accounts for approximately one-quarter of cases. Although poor prognostic features for JE have been identified, and guide management, relatively little is reported on the remaining three-quarters of AES cases.

Methods: Children with AES (n=225) were identified through admission records from two hospitals in Kathmandu between 2006 and 2008. Patients without available lumbar puncture results (n=40) or with bacterial or plasmodium infection (n=40) were analysed separately. The remaining AES patients with suspected viral aetiologies were classified, based on positive IgM antibody in the serum or cerebral spinal fluid, as JE (n=42) or AES of unknown viral aetiology (n=103). This latter group was sub-classified into Non-JE (n=44) or JE status unknown (n=59). Bad outcome was defined as "death or neurological sequelae at discharge".

Results: AES patients of suspected viral aetiology more frequently had a bad outcome than those with bacterial or plasmodium infection (31% versus 13%; P=0.039). JE patients more frequently had a bad outcome than those with AES

of unknown viral aetiology (48% versus 24%; P=0.01). Bad outcome was independently associated in both JE and suspected viral aetiology groups with a longer duration of fever pre-admission (P=0.007; P=0.002 respectively) and greater impairment of consciousness (P=0.02; P<0.001). A higher proportion of JE patients presented with a focal neurological deficit compared to patients of unknown viral aetiology (13/40 versus 11/103; P=0.005). JE patients weighed less (P=0.03) and exhibited a higher respiratory rate (P=0.003) compared to Non-JE patients. Lower weight in JE patient could be because of dehydration or malnutrition. However, considering presence of indirect markers of dehydration in them and that those children of JE and AES who arrived early to the hospital did well without receiving treatment of malnutrition, meant dehydration was more likely.

Conclusions: Nepali children with AES of suspected viral aetiology or with JE frequently suffered a bad outcome. Despite no specific treatment, patients who experienced a shorter duration of fever before hospital admission more frequently recovered completely. Prompt referral may allow AES patients to receive potentially life-saving supportive management. Previous studies have indicated supportive management, such as fluid provision, is associated with better outcome in JE. The lower weight and higher respiratory rate among JE patients may reflect multiple clinical complications, including dehydration. The findings suggest a more systematic investigation of the influence of supportive management on outcome in AES is warranted. Whether underlying malnutrition exposed the children to the risk of symptomatic JE or resulted in adverse outcome also needs further evaluation.

3.1 Introduction

Acute encephalitis syndrome (AES) is a constellation of clinical signs and /or symptoms, i.e. acute fever, with an acute change in mental status and/or new onset of seizures (1). These clinical signs suggest the patient has acute inflammation of the brain and are used by clinicians to identify patients with acute encephalitis. Viruses are regarded as the most important cause of the AES worldwide. However, the syndrome can be associated with a range of pathogens, including acute bacterial or parasitic infection. Where population based studies have been undertaken, the incidence ranges between 3.5 and 7.4 cases per 100,000 patient-years (111). Acute encephalitis can be associated with severe complications, including impaired consciousness, seizures, limb paresis or death (10).

In Asia, the major identified cause of acute encephalitis is Japanese Encephalitis (JE) virus. JE affects over 50,000 people annually, leading to 8-30% mortality and 50-60% disability, with children bearing the brunt of the disease burden (36-38, 112). JE is associated with considerable mortality and morbidity among Nepali children specially in endemic areas (37). Consequently, the Ministry of Health and Population of Nepal, supported by the office of the Programme for Immunisation Preventable Diseases, World Health Organisation (WHO-IPD), has integrated JE surveillance with Acute Flaccid Paralysis, Neonatal Tetanus and Measles in its National surveillance network since 2004 (85). Over 23,000 cases of AES and 2500 cases of JE have been reported by the WHO surveillance network since 2004 (personal communication: Mr Tika Sedai, Data Manager, WHO-IPD, Kathmandu, Nepal).

In Nepal, like many countries throughout Asia, test results for JE are often not available until weeks after the patient presents to the health care centre, because they are performed in a centralised government facility. Consequently, health care workers attempt to distinguish JE from other causes of AES based on the patient's clinical features, so that they can focus attention on known complications, such as seizures, and avoid unnecessary treatments, such as antibiotics. However, this approach can be inaccurate, leading to sub-optimal or inappropriate management. There have been several publications relating admission clinical parameters to outcome among JE cases, and the identification of poor prognostic indicators has helped focus attention on treatable complications of infection (24, 52, 78). However, relatively little work has been done identifying prognostic features among the Non-JE AES patients. Therefore it was important to investigate the diagnostic and prognostic features that distinguished JE from other causes of AES. I then decided to conduct a retrospective descriptive study at two major hospitals catering sick children in Kathmandu, Nepal, to examine the aetiology of patients with AES of suspected viral aetiology or viral encephalitis relating to outcome which would help identify which particular aetiology had a bad or a good outcome in this broad group of AES patients. It was also important to study suspected viral encephalitis because it had no definite treatment and had bad outcome as compared to other treatable aetiology of AES such as bacterial meningitis, tubercular meningitis, cerebral malaria and typhoid encephalopathy which were studied separately.

3.2.1 Aim

The aim of this study is to assess the clinical features, laboratory parameters and factors contributing to bad outcome of acute encephalitis syndrome in Nepali children.

3.2.2 Objectives

1. To identify all children that meet World Health Organisation acute encephalitis syndrome case definition in the two hospitals in Nepal.
2. To describe the clinical features, laboratory parameters and immediate outcome of confirmed cases of Japanese encephalitis as compared to confirmed cases of non-Japanese encephalitis and those of unknown causes.
3. To describe the clinical features and laboratory parameters which predict Japanese encephalitis from other causes of acute encephalitis syndrome.
4. To describe the clinical features and laboratory parameters which predict bad outcome of acute encephalitis syndrome and Japanese encephalitis.
5. To obtain baseline data for future prospective studies.

3.3 Methods

The hospital records of all children, aged 1-14 years, presenting either to Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal and Patan Hospital, Lalitpur, Nepal, from January 2006 to January 2008 were screened for a history consistent with AES.

Kanti Children's Hospital is a busy tertiary level referral centre. It has 300 beds and provides health care services to 300-400 children per day. Patan Hospital is a general hospital situated at the southern end of Kathmandu. It has 450 beds and provides health services to around 1,000 people per day, predominantly adults.

I examined the hospital notes, with the help of staff employed in record section, within the respective hospitals, as described in detail in chapter 2. Relevant clinical features and laboratory parameters present at admission were recorded in a standardised proforma (Appendix F). Each proforma was designated a unique study number. The study data were transcribed from the proforma to the study database. The study number was used to access the data for all future data analyses.

The study was approved by the Institutional Review Committee of Kanti Children's Hospital and Patan Hospital, Kathmandu, ethical committee of the Nepal Health Research Council, Kathmandu and the Ethical Review Committee of the Liverpool School of Tropical Medicine, Liverpool, UK.

3.3.1 AES and JE case definitions

Case definition followed was as described in Chapter 2. Patients that self-discharged or were referred to another hospital prior to discharge were excluded from outcome analysis

3.3.2 JE diagnostic test

JE virus exposure was tested by MAC-ELISA (IgM antibody capture-Enzyme Linked Immunosorbent Assay). The ELISA plates were supplied by the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand (113). ELISA measurements were undertaken following the protocol supplied by AFRIMS. Diluted patient sera (1:100) or CSF (1:10) were added to the plates. Absorbance of experimental, positive and negative control samples were measured in duplicate in 96-well plates using a micro-titre plate reader (Huma Reader Single Plus, Human GmbH, Wiesbaden, Germany). Single experimental patient samples with a mean absorbance ≥ 40 units (following subtraction of the absorbance for the negative controls) were considered positive. External quality assurance was undertaken by AFRIMS (113).

3.3.3 Statistical methods

The acquired data for all AES patients was initially validated, coded and entered in SPSS Statistics software version 17.0 (IBM-SPSS, New York) for analysis. Differences between clinical groups were compared using Student independent samples t-tests (for Normally distributed data), Mann-Whitney U-tests (for non-Normally distributed data) and Fisher Exact tests (for categorical data / proportions). All clinical feature variables (with the exception of all duration and numbers of episodes variables apart from duration of fever) were entered into a forward stepwise multiple logistic regression model to identify variables independently associated with bad outcome or JE positive status; multiple imputation methods (10 iterations) were used to overcome problems of missing observations and collinearity statistics were

examined to ensure independence of the predictor variables. The median age and numbers of AES cases were mapped using ArcGIS version 9.3 (Esri Ltd., California) software to show their distribution in the different districts across Nepal in order to identify any spatial patterns in infection dynamics. Statistical significance was set at the conventional 5% level for all analyses.

3.4 Results

3.4.1 Baseline characteristics

Of the total of 17,929 children admitted in the two hospitals (KCH, 13,397 and PH, 4,532) between January 1st, 2006 and January 1st, 2008, 225 (KCH, 208 and PH, 17) children of AES were considered for the study. Forty (18%) of these patients did not have any lumbar puncture (LP) results available in their notes. These patients were analysed separately.

Of the remaining 185 AES patients, 40 (22%) were diagnosed with either bacterial (n=39) or *Plasmodium falciparum* infection (n=1). Eight of these patients also had elevated anti-JE virus titres (≥40 units) on serum IgM testing during acute illness. Since bacterial co-infection can change clinical features and influence patient outcome, these patients were analysed separately (Fig. 3.1).

One hundred and forty five AES patients (145/185;78%) were considered to have AES of suspected viral aetiology. Forty-two of these patients (23%) were confirmed as JE. Among the other 103 patients, 44 tested negative to JE

using samples collected after the ninth day of their illness and therefore were classified as Non-JE. For the remaining 59 patients JE status was unknown (Fig 3.1).

The majority of JE positive patients, 41/50 (82%), were identified by serum testing positive for anti-JEV IgM antibodies, with 31/41 (79%) identified by a single serum sample. The other serum samples tested positive in paired samples. The remaining 9 patients were diagnosed by testing positive for anti-JEV IgM antibodies in the CSF.

The majority of the non-viral AES patients were diagnosed with suspected bacterial meningitis based on a raised cell count with a polymorph predominance and raised protein in the CSF. The commonest identified cause of infection was gram stain positive bacteria in CSF.

Patients presented to Kanti Children's (n=208) or Patan hospital (n=17) from a wide range of outlying districts from Kathmandu including the hill and mountain districts. There were no marked differences in geographic distribution for the number of cases or age at presentation among the different sub-groups of suspected viral AES patients (Fig. 3.2). Route of presentation to hospital was documented in 175 AES patients. Self-referral was the commonest route of presentation, reported by 96/175 (55%) of AES patients.

3.4.2 Patient outcome

Outcome at discharge was recorded for 183/185 patients where LP results were available and 38/40 patients without LP results. Among the patients without LP results, seventeen (45%) had a bad outcome; 10 (26%) died and a further 7 (18%) had neurological sequelae at discharge.

Among the AES patients with non-viral infection, 38/40 (95%) had an outcome at discharge recorded. Five (13%) had a bad outcome; 2 died and a further 3 had neurological sequelae at discharge. All 8 patients with bacterial and JE co-infection exhibited complete recovery at discharge. The one patient with *Plasmodium falciparum* infection recovered completely.

Among the AES patients with suspected viral infection, 45/145 (31%) had a bad outcome; 8 died and 37 had neurological sequelae at discharge. Among the sub-set of confirmed JE patients 20/42 (48%) had a bad outcome; 4 died and 16 had neurological sequelae (Table 3.1).

A significantly higher proportion of patients without LP results died compared to those where LP results were available (10/38 [26%] versus 10/183 [5%]; $P<0.001$). Similarly, the proportion of patients with bad outcome were higher among patients without LP results (17/38 [45%] versus 51/183 [28%]; $P= 0.053$). Exclusion of the patients without LP results may have influenced outcome within the AES groups. To help address this issue, an additional analysis was undertaken, whereby patients without LP results were classified into AES categories based on their hospital discharge diagnosis supported

by their JE serological results. The patients contributed to all AES groups. Each AES category exhibited a higher rate of bad outcome. There were no significant changes in the proportion of patients with bad outcome between groups (Table 3.1).

A significantly higher proportion of patients with AES of suspected viral aetiology had a bad outcome compared to AES patients with a non-viral infection (45/145 [31%] versus 5/38 [13%]; P=0.039). A significantly higher proportion of JE patients exhibited a bad outcome compared to AES patients of unknown viral aetiology (20/42 [48%] versus 25/103 [24%]; P=0.01). A similar trend was observed when JE patients were compared to Non-JE patients, with a higher proportion of JE patients exhibiting a bad outcome (20/42 [48%] versus 12/44 [27%]); P=0.07; Table 3.1).

3.4.3 Prognostic features associated with bad outcome at discharge

Multiple parameters were associated with bad outcome at discharge for both AES cases of suspected viral aetiology (n=145) and confirmed JE (n=42) (Table 3.2). In both groups, bad outcome was associated with a longer duration of fever prior to admission, a lower Glasgow coma score, a focal neurological deficit, older patient age and higher weight at admission.

To identify whether these features were independently associated with bad outcome, these variables were entered into a forward stepwise logistic regression model separately for each patient group. For the larger group, all AES of suspected viral aetiology, fever duration (P=0.002), GCS (P<0.001),

and focal neurological deficit (P=0.001) were retained in the model for outcome. In JE, fever duration (p=0.007) and GCS (p=0.020) were again independently associated with bad outcome (but not focal neurological deficit) together with age (P=0.011).

To assess whether length of time from onset of illness to hospital admission was a prognostic marker for bad outcome, I used length of reported fever prior to admission as a proxy marker. I selected a threshold of 7 days of fever prior to admission (Fig 3.3). I found fever duration over 7 days was associated with a bad outcome. Fever duration was reported in 142/145 (98%) of patients with AES of suspected viral aetiology. Thirty-two patients reported fever duration of more than 7 days prior to admission. Of these, 19 had bad outcome and 13 had good outcome. The remaining 110 patients reported a fever duration under 7 days, of which 23 had bad outcome and 87 had good outcome (19/32 [59%] versus 23/110 [20%]; P< 0.001).This indicates that AES patients who present with a prolonged fever prior to hospital admission have an increased risk of having a bad outcome at discharge (Relative Risk = 2.8 [95% confidence interval: 1.6 – 4.4]).

Among the JE patients, a reported fever duration of more than 7 days prior to admission was again linked to a significantly higher rate of bad outcome compared to those who presented with a shorter duration of fever (9/11 [82%] versus 11/31[36%]; P=0.013). Among patients with AES of non-viral aetiology, prolonged duration of fever prior to admission was also linked to a higher rate of bad outcome (2/6 [33%] versus 3/22 [14%]; P=0.28).

Of the treatments given to AES patients of suspected viral aetiology, mannitol and phenytoin were more frequently prescribed among those with a bad outcome compared to those with a good outcome; 27/61 (44%) versus 19/119 (16%), P<0.001 for mannitol; 23/61 (38%) versus 12/119 (10%), P<0.001for phenytoin, in bad and good outcome patients respectively.

3.4.4 Clinical features that distinguish between confirmed JE and AES of suspected non-JE viral aetiology

Confirmed JE cases were compared to AES cases of unknown viral aetiology (Non-JE and JE status unknown). As the latter group also contained patients that did not have a confirmed negative test for JE, a further analysis was undertaken comparing JE against Non-JE cases.

The rates of many clinical features and laboratory parameters were similar on admission between confirmed JE, confirmed Non-JE and JE-status unknown (Tables 3.3 and 3.4). Importantly, the rates of many recorded neurological features were comparable between the patient groups. There were no significant differences in prevalence of an altered sensorium at admission, depth or duration of coma, frequency, duration or type of seizures between patient groups. However, there was a significantly higher prevalence of patients presenting with focal neurological deficits at admission among confirmed JE patients compared to AES cases of unknown viral aetiology (13/40 versus 11/103; P=0.005; Relative Risk = 3.0 [95% confidence interval: 1.4-6.7]). Among AES patients of suspected viral aetiology, presence of a focal neurological deficit at admission had a positive predictive value of 32%

(sensitivity 54%) for JE. Absence of a focal neurological deficit at admission had a negative predictive value of 89% (specificity 77%) for the patient not having JE.

JE patients presented with a lower body weight (15.25 versus 20 Kg; P=0.031), and a higher respiratory rate (30 versus 28 breaths per minute; P=0.003) compared to confirmed non-JE patients. With the caveat that serum urea, creatinine and electrolytes were measured in only 25 AES patients of suspected viral aetiology, there was a trend for higher serum urea (15 versus 8.9 mmol/L; P=0.27), sodium (137 versus 133 mmol/L; P=0.09) and potassium (4.3 versus 3.9 mmol/L; P=0.21) among JE compared to Non-JE patients (Table 3.4). These parameters again showed a non-significant trend for higher median values among JE patients compared to AES patients of unknown viral aetiology (Table 3.4).

Both weight and respiratory rate, which were significantly different among JE patients compared to Non-JE patients, are in part dependent on patient age. To further dissect the influence of these parameters, age, weight and respiratory rate were entered into a stepwise logistic regression model. Only raised respiratory rate (P=0.011) was retained as independently correlated.

Admission patterns also differed between JE and Non-JE patients. JE patients demonstrated a clear peak in hospital admissions rates in the months immediately following the rainy seasons each year (August and September in 2006-2007). Non-JE patients didn't demonstrate any clear seasonal variation in hospital admission rates (Fig. 3.4).

3.5 Discussion

This study demonstrates that patients with AES of suspected viral aetiology, either where JE was confirmed or where viral aetiology remained unknown, were significantly more likely to have a bad outcome compared to AES patients with bacterial or malaria infection.

Appropriately, JE surveillance is a health priority in Nepal. However, public health and clinical teams should be aware that patients with AES of unknown viral aetiology also have a high risk of morbidity and mortality. Furthermore, since there are up to 3 times more Nepali children with AES of unknown viral aetiology than proven JE, a bad outcome among the former group impacts on a larger number of children Therefore, identification and optimising management of patients with AES should also be a priority.

The lower frequency of bad outcome among the AES patients with bacterial or malaria infection is likely to reflect the availability and effective use of antibiotics and anti-malaria treatment to reduce morbidity among these patients. Non of the patients of bacterial or malaria infection failed to receive antibiotics or anti-malarial respectively.

AES patients without LP results exhibited a significantly higher rate of death. The finding is likely to reflect lumbar punctures being undertaken less frequently on children who were critically ill (Table 3.1). The finding highlights that restricting analyses to patients where LP results are available can lead to an underestimation of the frequency of death and bad outcome linked to AES.

Interestingly, the study has identified that the number of days of fever (reflecting number of days of illness) the patient experienced prior to hospital admission is a prognostic indicator of bad outcome in both patients with AES of unknown viral aetiology and JE. When analysis was applied to AES patients of bacterial aetiology no significant association was identified. Antibiotic use in the community may reduce fever duration prior to hospital admission in patients with bacterial infection and may confound the association between fever duration and bad outcome. In contrast, antibiotics would have limited impact on fever duration during viral infection.

Shorter duration of illness (or fever) prior to admission has previously been associated with good outcome among children for many diseases. What is striking about my finding is that there is no specific treatment for JE or AES of suspected viral aetiology, yet attending hospital earlier in the illness course appears to be of benefit. The findings would suggest that hospital admission and the supportive management received there improves outcome.

Patients with impaired consciousness are often unable to drink themselves. Consequently, dehydration and metabolic acidosis may complicate AES (114). Dehydration and acidosis would fit the significantly lower body weight and higher respiratory rate observed among JE compared to Non-JE patients. A previous prospective survey of JE management identified that fluid supplementation was associated with a positive influence on outcome (93). Although appropriate fluid provision is a delicate balance when managing brain injury (89, 115), one possible explanation of the positive influence of hospital

admission may be that patients receive fluid support in hospital during the illness.

Given the commonest reported mode of presentation was self-referral, the focus on hastening patient attendance would lie with improving patient awareness of the features of AES and encouraging families to attend hospital promptly. Further research is needed to understand the factors that underlie families' health-seeking behaviours when a child is ill.

Low weight in JE patients could also be that they were malnourished. Severe acute malnutrition resulting from a relatively short duration of nutritional deficiency often complicated by concurrent infections has a high mortality rate (116, 117). Previous studies have also reported bacteraemia in 16% - 22% of patients of malnutrition (118, 119). This is probably because they have limited ability to cope up environmental and infective stresses making them vulnerable to infections. Therefore, underlying malnutrition could have also been a risk factor for symptomatic JEV infection in these children. JE patients with malnutrition may also have additional complications of malnutrition such as hypothermia, hypoglycemia, hyponatremia, hypokalemia, anaemia or hypoalbuminemia leading to adverse outcome. However, children who were brought early to the hospital and administered supportive management, without administering separate treatment for malnutrition, in the present study, resulted in good outcome meant that they were probably dehydrated rather than malnourished. However, whether underlying malnutrition increased risk of symptomatic JE or caused bad outcome in these children needs to be further

studied. I also did not have information on socioeconomic status (SES) on the patients which could have been helpful, pitfall of retrospective study.
As shown previously, a low Glasgow coma scale (GCS) and / or a focal neurological deficit at hospital admission were independent clinical markers of bad outcome among children with AES (120). JE patients frequently exhibit raised intra-cranial pressure, brain herniation syndromes (20,52) and focal brain lesions on neuro-imaging studies (121), explaining the preponderance of focal neurological deficits in this group.

In line with previous reports, mannitol was prescribed significantly more frequently among AES patients who exhibited a bad outcome (93). Interestingly, a randomised clinical trial of mannitol among children with raised ICP secondary to cerebral malaria (another cause of non-traumatic brain injury) did not identify any beneficial effect (122). A similar study would help clarify whether mannitol is a useful supportive treatment in AES (123).

JE patients exhibited an increased respiratory rate compared to Non-JE patients. Furthermore, JE patients who exhibited a high respiratory rate were associated with a good outcome, while those with a lower respiratory rate were associated with a bad outcome. This pattern can be observed during evolution of many complications, including metabolic acidosis, pneumonia, acute flaccid paralysis (involving the inter-costal muscles) or brain damage, where there may be an initial compensatory rise in respiratory rate followed by a fall when the body decompensates.

Tachypnea is also a feature of the severe brain injury syndrome - paroxysmal autonomic instability with dystonia (PAID)(124). Patients with this brain injury syndrome exhibit intermittent agitation, diaphoresis, hyperthermia, hypertension, tachycardia, tachypnea, and extensor posturing. All of these signs overlap with features reported in both JE and AES. PAID may exist among AES patients.

As a descriptive analysis this study cannot distinguish cause from consequence. Consequently although several clinical features and interventions appear linked with outcome, this study is unable to determine whether these parameters are causal. Similarly, as a retrospective study it is limited by the breadth and quality of information available in the hospital notes. More detailed information on acid-base status would help discriminate between respiratory and metabolic causes of tachypnea. Similarly, more systematic measurement of urea and electrolytes and additional indicators of fluid balance would help assess the influence of fluid support on outcome. Based on their discharge diagnosis, the AES patients without LP contributed to all AES groups. However, a prospective study with a more systematic investigation of pathogen aetiology is warranted to confirm these findings.

In conclusion, Nepali children with AES of suspected viral aetiology, either where JE is confirmed or where the aetiology remains unknown, exhibit a high rate of death and morbidity. One of the more striking findings from the study was the association between long duration of fever prior to admission and bad outcome. If patients with AES of suspected viral aetiology, including

those with confirmed JE, attend hospital early they are more likely to make a full recovery. Despite no specific treatment for JE or for patients with AES of suspected viral aetiology, the current management in Nepal can limit the development of neurological sequelae. The findings imply that family members, primary and community health care workers should be aware of AES and seek early referral for appropriate and potentially life-saving, supportive management.

Despite many comparable neurological features between JE patients and patients with AES of unknown viral aetiology, significantly more JE patients exhibited a bad outcome. This, in part, may reflect the higher proportion of JE patients that presented with a focal neurological deficit at hospital admission. Further research is needed to understand the factors that underlie bad outcome in AES and JE, including a more systematic investigation of the influence of supportive measures.

3.6 Limitation of study

Since this was a retrospective study, the information present in the notes was limited by the level of training, knowledge and interest of the treating clinician. Therefore, important clinical features for AES and JE patients may have not been recorded. This may have influenced the clinical and laboratory parameters identified to predict JE from other causes of AES. Many patients were excluded from the analysis because no LP was performed. Importantly, there were significantly more number of deaths in those without an LP, as compared to those who had a LP. Again, this could have led to underestimation of the actual

number of patients who suffered a bad outcome; this may have influenced the laboratory parameters, particularly CSF parameters, which predicted bad outcome.

I used a case definition of AES according to WHO surveillance standards. However, this has not been validated in Nepali children. Further, assessment of consciousness and seizure are often difficult in children. Rarely, a bulging anterior fontanel may be the only presentation of encephalitis in young children. Therefore, there is a need to validate clinical features of AES as a screening tool to identify children with suspected viral encephalitis which could have led to exclusion of some of the children of suspected viral encephalitis from this study.

Although, lower weight and indirect markers of JE as compared to non-JE could have been because of dehydration, whether malnutrition is the cause of symptomatic JE or bad come is still speculative. Additional information on the social economic status (SES) of the patients could have also been helpful.

3.7 Summary

On analysing two year's records of two major hospitals of Kathmandu, I found Nepali children with AES of suspected viral aetiology whether JE or of unknown viral aetiology suffered high death rates and severe neurological sequelae. Bad outcome was significantly associated with older age and higher weight in patients of both AES and JE. Surprisingly, despite many comparable clinical features between patients of JE and AES of unknown viral aetiology, JE

patients had significantly more focal neurological deficits and exhibited worse outcome as compared to AES of unknown viral aetiology. JE patients also had lower body weight, higher respiratory rate, a trend for a higher serum urea and potassium levels as compared to patients of non-JE, which could be because of dehydration. Patients with impaired consciousness and focal neurological deficit may not have been able to eat or drink sufficiently which could cause dehydration or metabolic acidosis. Therefore, preventing longer duration of fever by hastening them to attend hospitals to receive supportive management including fluids early could improve outcome. However, lower body weight could also be because of underlying malnutrition in these children. Malnutrition could be a risk factor or complicate management AES patients causing adverse outcome. Since both the condition causes loss of weight, it is important to accurately measure weight and find out it's relationship with outcome.

Understandably in children, weight increases with increase in age. WFA allows you to compare body weight relative to the child’s age and gender at a given time and accurately compare whether the weight measured at the time of admission was low, high or appropriate for that age and sex. WFA can be distributed in different categories based on Z score for a particular gender in children such as +3, +2 +1, 0, -1, -2, -3 WFA Z scores. WFA Z scores below -1 Z score can be taken to be a marker of acute loss of weight in children (91). Therefore, Z scores of 0, +1, +2 and +3 can be considered not to be of low WFA and Z scores -1, -2 and -3 as low WFA. Accurate assessment of different WFA Z score categories together with clinical and laboratory features could be of further value to help differentiate between malnutrition and dehydration.

Community health workers in Nepal are trained to measure accurate weight through public health programmes. Even nurses are trained to measure weight of the attending sick children in order to plan treatment. Although, weight-for-height and body mass index (BMI) maybe better methods to separate malnutrition from dehydration, lack of resources and abnormal posturing and change in body tone make it difficult and inaccurate in patients with AES. Since this was a retrospective study, many necessary clinical data, including accurate weight of all children, were lacking or questionable in some of the cases because it was recorded by different people with different intention and qualification. Therefore, I decided to conduct a more systematic prospective study to assess the association of immediate and long term outcome of AES children with different WFA Z score categories on admission. If successive decrease in lower WFA Z scores were associated with death or neurological sequelae, then educating the parents and health workers to measure weight to help identify high risk patients for immediate referral could save lives. This study has been described in my next chapter.

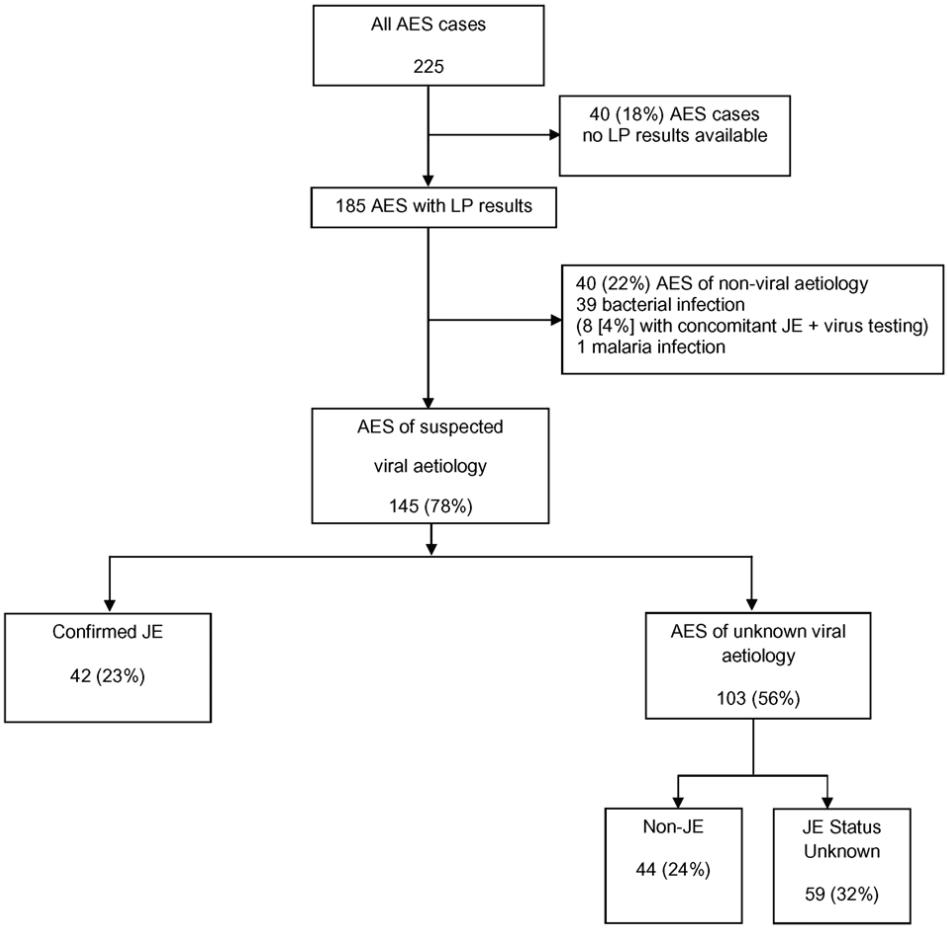


Figure 3.1: Flow diagram of Acute Encephalitis Syndrome patients based on aetiological classification.

225 children with AES were identified. To analyse AES of different aetiologies only patients where LP results were available were taken forward (n =185). Patients with non-viral aetiologies were analysed separately (n = 40). The remaining AES patients were classified as JE (n = 42) or AES of unknown viral aetiology (n = 103) based on presence or absence of high anti-JE virus immunoglobulin titres. AES of unknown viral aetiology was further sub-classified into Non-JE (n = 44) and JE Status Unknown (n = 59) based on presence or absence of low or negative anti-JE virus immunoglobulin titres.

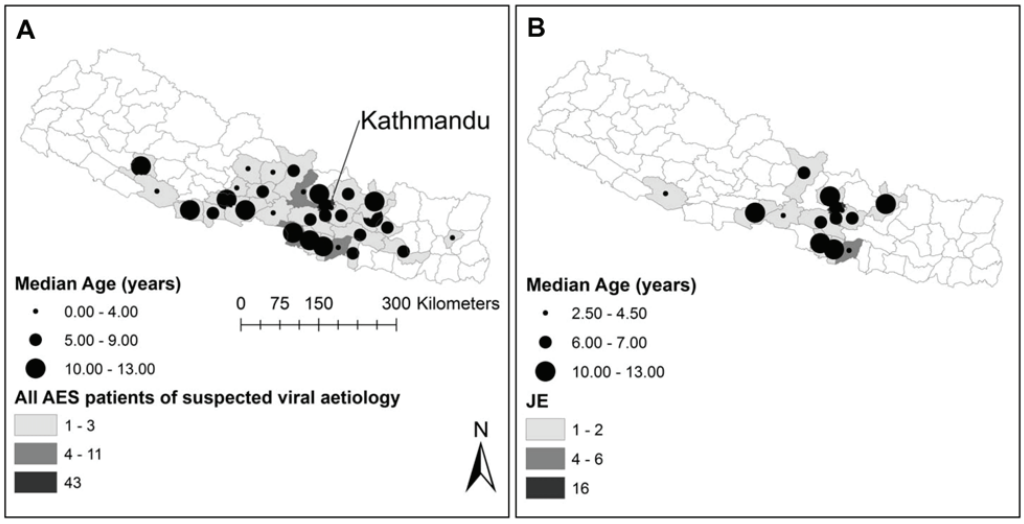


Figure 3.2: Map of residence district for Acute Encephalitis Syndrome patients of suspected viral aetiology.

Panel A, All AES patients of suspected viral aetiology; Panel B, JE patients; Increasing depth of shading within a district indicates a higher number of AES patients were admitted from this district. Unshaded districts indicate no patients were admitted from this district. Increasing circle diameter within a district indicates AES patients of an older (median) age were admitted. Kathmandu is labelled on the map.

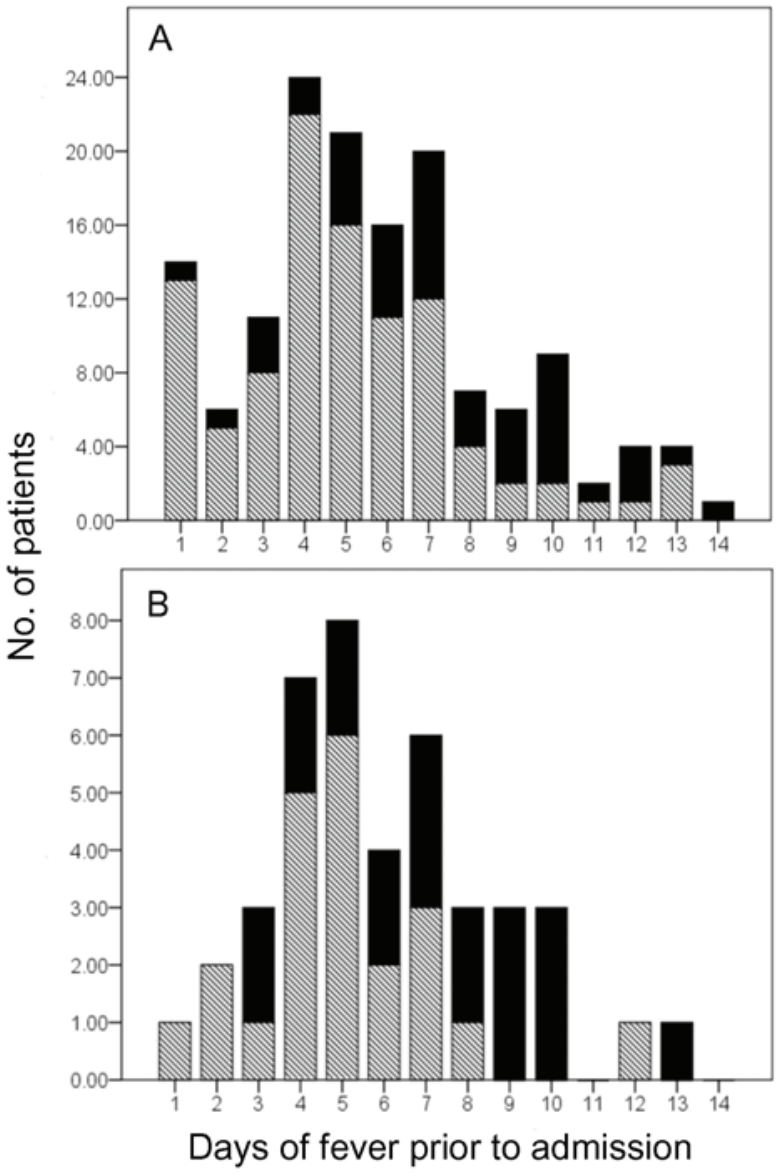


Figure 3.3: Fever duration prior to admission organised by outcome among AES patients of suspected viral aetiology.

Panel A, All AES patients of suspected viral aetiology; Panel B, JE patients

X-axis; fever duration (1-14 days)

Y-axis; Number of patients that presented to hospital at each day of fever duration (1-24)

Solid bar shading; number of patients who exhibited a bad outcome at discharge

Hatched bar shading; number of patients who exhibited a good outcome at discharge

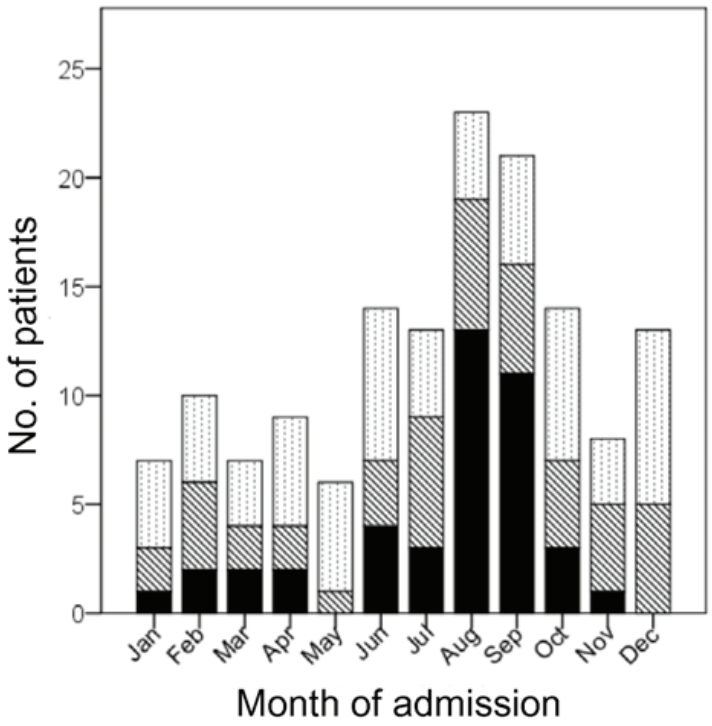


Figure 3.4: Monthly admission numbers for Acute Encephalitis Syndrome (AES) patients of suspected viral aetiology.

X-axis; month of admission

Yaxis; Number of patients admitted to hospital each month

Solid bar shading; number of JE patients

Hatched bar shading; number of Non-JE patients

Dotted bar shading; number of patients where JE Status unknown

Table 3.1: Patient outcome for six aetiological categories of Acute Encephalitis Syndrome.

Outcome	**Suspected viral aetiology**															**Non-viral aetiology**		
	AES of suspected viral aetiology			**Confirmed JE**			**AES of unknown viral aetiology**			**Non-JE**			**JE Status Unknown**			**Bacterial** (n=37) **P.f.** (n=1)		
Lp status	**+Lp**	-Lp	Both	**+Lp**	-Lp	Both	**+Lp**	-Lp	Both	**+Lp**	-Lp	Both	**+Lp**	-Lp	Both	**+Lp**	-Lp	Both
Patient No.	**145**	35	180	**42**	8	50	**103**	27	130	**44**	12	56	**59**	15	74	**38**	3	41
Good	100(69)	19(54)	119(63)	22(52)	3(37.5)	25(50)	78(76)	16(60)	94(72)	22(73)	8(66)	40(71)	46(78)	8(53)	54(73)	33(87)	2(66)	35(85)
Bad	45(31)[*]	16(46)	61(37)[*]	20(48)[§‡]	5(62.5)	25(50)[§‡¶]	25(24)[‡]	11(40)	36(28)[‡]	12(27)	4(33)	16(29)[¶]	13(22)	7(47)	20(27)	5(13)[*§]	1(33)	6(15)[*§]
Neurol. Sequelae	37(25)	7(20)	44(24)	16(38)	3(37.5)	19(38)	21(20)	4(15)	25(19)	12(27)	1(8)	13(23)	9(15)	3(20)	12(16)	3(8)	0(0)	3(7)
Died	8(5)	9(26)	17(9)	4(9.5)	2(25)	6(12)	4(4)	7(26)	11(8)	0(0)	3(25)	3(6)	4(7)	4(27)	8(11)	2(5)	1(33)	3(7)

Outcome at hospital discharge is presented for 2 main aetiological categories of **Acute Encephalitis Syndrome (**AES); suspected viral and non-viral. Based on JE serology, AES of suspected viral aetiology was split into Confirmed JE and Unknown viral aetiology. The latter group was sub-categorised into Non-JE and JE status unknown. AES of non-viral aetiology included Bacterial and Plasmodium falciparum (P.f.) infection. Within each category, patients were split into 3 groups based on availability of LP results; +Lp, those with LP results; -Lp, those without; Both, combining both +Lp and -Lp patients. Patients without LP results were assigned to an AES group based on their discharge diagnosis and JE serological results. Patients with a bad outcome were sub-classified into those with neurological sequelae at discharge or those that died. Number with (%) is presented. Significance was tested via Fisher's Exact test.

[*] Significant difference between suspected viral and non-viral patients (P=0.039 and P=0.022 respectively for +Lp and Both groups).

[§] Significant difference between JE and non-viral patients (P=0.001 and P<0.001 respectively for +Lp and Both groups).

[‡] Significant difference between JE and Unknown viral patients (P=0.01and P=0.008 respectively for +Lp and Both).

[¶] Significant difference between JE and Non-JE patients in the Both group (P=0.029).

A similar trend was observed for outcome between JE and Non-JE patients in the +Lp group (P=0.074).

A higher frequency of death was consistently observed among -Lp patients compared to +Lp patients in each AES category.

Table 3.2: Clinical features for confirmed JE and AES patients of suspected viral aetiology reported by outcome

Clinical Features at admission	Confirmed JE (n=42)			AES suspected viral aetiology (n=145)		
	Bad	Good	p-value	Bad	Good	p-value
No. patients	20	22		45	100	
Age (years)	10 (1-14) [20]	4.5 (1-13) [22]	0.004	8 (1-14) [45]	6 (1-14) [97]	0.005
Number of males	5 (25) [20]	11 (50) [22]	0.121	13 (29) [45]	30 (31) [98]	1.000
Days of fever prior to admission*	7 (3-13) [20]	5 (1-12) [22]	0.010	7 (1-14) [45]	5 (1-13) [100]	<0.001
Altered sensorium	17 (85) [20]	13 (59) [22]	0.091	39 (89) [44]	55 (56) [98]	<0.001
Duration of altered sensorium (days)†	2 (1-5) [13]	2 (1-3) [10]	0.839	2 (1-7) [30]	1 (1-4) [43]	0.009
Modified Glasgow coma score*	11 (5-15) [20]	14 (7-15) [22]	0.001	11 (3-15) [44]	14 (4-15) [98]	<0.001
Focal neurological deficit	9 (50) [18]	4 (18) [22]	0.046	17 (40) [43]	7 (7) [100]	<0.001
Any seizure prior to admission	10 (77) [13]	15 (75) [20]	1.000	24 (77) [31]	67 (83) [81]	0.591
Episodes of seizure prior to admission‡	2 (1-12) [9]	2 (1-4) [14]	0.723	2 (1-12) [19]	2 (1-7) [48]	0.034
Neck stiffness	14 (82) [17]	9 (45) [20]	0.040	29 (71) [41]	49 (54) [91]	0.086
Vomiting	10 (71) [14]	13 (87) [15]	0.390	29 (81) [36]	47 (86) [55]	0.573
Days of vomiting prior to admissionΔ	3 (2-10) [8]	3 (1-7) [9]	0.400	3.5 (1-12) [26]	2 (1-8) [37]	0.019
Weight (kgs)	19.5 (9-30) [20]	13 (1-26) [22]	0.017	19 (4-35) [45]	15 (1-70) [90]	0.040
Axillary temperature (˚C)	38.1 (36.7-40.0) [20]	37.8 (36.7-39.4) [20]	0.170	37.8 (36.1-40.0) [44]	37.8 (36.1-40.0) [86]	0.256
Pulse rate (beats per min.)	101 (52-150) [20]	104 (76-130) [22]	0.905	106 (52-160) [44]	110 (64-170) [96]	0.972
Respiratory rate (breaths per min.)	28 (20-50) [20]	36 (20-80) [22]	0.064	28 (3-60) [45]	30 (16-90) [92]	0.158
Death prior to discharge	4 (20) [20]	0 (0) [22]		8 (18) [45]	0 (0) [100]	
Neurological sequelae at discharge	16 (80) [20]	0 (0) [22]	---	37 (82) [45]	0 (0) [100]	---
Treatment						
Phenytoin	6 (30) [20]	0 (0) [22]	0.007	18 (40) [45]	8 (8) [100]	<0.001
Phenobarbitone	5 (25) [20]	3 (14) [22]	0.445	10 (22) [45]	10 (10) [100]	0.067
Dexamethasone	6 (30) [20]	4 (18) [22]	0.477	12 (27) [45]	22 (22) [100]	0.533
Mannitol	6 (30) [20]	2 (9) [22]	0.123	19 (42) [45]	15 (15) [100]	0.001

Median (range) [number of patients] or Number (%) [number of patients]. Significance of difference between groups by Fisher's Exact test or Mann-Whiney U test. †: patients with altered sensorium only ‡: patients with one or more seizures before admission only Δ: patients with vomiting prior to admission only

* Parmeters identified as independently associated with bad outcome in both AES groups

Table 3.3: Clinical features at admission for five categories of Acute Encephalitis Syndrome patients

Clinical features	Confimed JE	AES unknown viral aetiology (n=103)		AES bacterial aetiology (n=39)	
		Non-JE	JE Status Unknown	Bact. Inf.	Bact. and JEV+
No. patients (% AES patients (n=184†))	42(23)	44(24)	59(32)	31(17)	8(4)
Age (years)	7(1-14)[42]	8(1-14)[44]	4.5(1-13)[56]	4.5(1-12)[31]	3.5(1-10)[8]
Number of males	16(38)[42]	16(36)[44]	16(27)[59]	11(35)[31]	8 [100][8]
Days of fever prior to admission	5.5(1-13)[42]	6(2-14)[44]	5(1-12)[59]	5(1-13)[30]	5 (1-16)[7]
Altered sensorium	30(71)[42]	29(69)[42]	35(60)[58]	13(43)[31]	5 [63][8]
Duration of altered sensorium (days)	2(1-5)[23]	2(1-7)[24]	1(0-5)[27]	3(1-4)[9]	1(1-1.1)[3]
Modified Glasgow coma score	12(5-15)[42]	14(4-15)[44]	14(3-15)[59]	15(4-15)[30]	12(7-15)[8]
Focal neurological deficit	13(32)[40]	7(16)[44]	4(7)[59]	2(12.5)[16]	1(33)[3]
Any seizure prior to admission	25(76)[33]	25(78)[32]	42(89)[47]	21(75)[28]	4(50)[8]
Generalised seizure prior to admission	22(96)[23]	22(92)[24]	35(92)[38]	21(95)[22]	3(38)[8]
Focal seizure prior to admission	1(4)[23]	0(0)[24]	1(3)[38]	0(0)[22]	0(0)[8]
Episodes of seizure prior to admission	2(2-12)[23]	1(1-7)[17]	2(1-9)[27]	1.5(1-7)[18]	1.2 (1-1.5)[2]
Est. duration of longest seizure (mins.)	6(3-60)[16]	6(3-60)[16]	5(4-60)[12]	5(1-5)[3]	10(10-10.2)[2]
Neck stiffness	23(62)[37]	27(66)[41]	28(52)[54]	18(58)[31]	5(63)[8]
Vomiting	23(79)[29]	24(83)[29]	29(88)[33]	18(78)[23]	3(38)[8]
Days of vomiting prior to admission	3(1-10)[18]	4(1-11)[21]	2(1-12)[25]	3.5(1-10)[12]	1.2(1-2)[5]
Weight (Kg)	15.25(1-30)[42]	20(9-70)[38]*	14(1-60)[55]	15(1-60)[29]	11(1-25)[8]
Axillary temperature (˚C)	37.8(36.7-40)[40]	37.2(36.1-40)[38]	37.8(36.1-40)[52]	37.8(36.7-41.1)[29]	38.9(37.8-39.4)[7]
Pulse rate (beats per min.)	101(52-150)[42]	100(69-160)[41]	110[78-170)[57]	100(70-160)[28]	120(72-130)[7]
Systolic BP (mmHg)	96(50-120)[17]	93(78-130)[20]	100(9-110)[14]	100(80-140)[11]	100(90-110)[3]
Dystolic BP (mmHg)	60(20-90)[17]	60(10-100)[20]	60(0-100)[15]	70(50-110)[11]	70(60-80)[3]
Respiratory rate (breaths per min.)	30(20-80)[42]	28(16-60)[40]**	30(3-90)[55]	34(20-50)[30]	34(24-40)[7]

Median (range) [number of patients] or Number (%) [number of patients]. Significance of difference between groups by Fisher's Exact test or Mann-Whiney U test. †Single patient with *Plasmodium* infection not presented.

Significant difference between JE and Non-JE patients; P=0.031*; P=0.003**

Table 3.4: Laboratory parameters at admission for five categories of Acute Encephalitis Syndrome pateints

Laboratory parameters	Confimed JE	AES unknown viral aetiology (n=103)		AES bacterial aetiology (n=39)	
		Non-JE	JE Status Unknown	Bact.	Bact. and JEV+
No. patients	42	44	59	31	8
Blood					
Hemoglobin (g/L)	112 (80-190) [37]	116 (80-140) [37]	112(60-200)[55]	116(30-460)[24]	112(81-120)[7]
Total leukocyte count ($x10^9$/L)	10.9 (2.46-33)[41]	9.6 (1.8-25.8)[40]	12.5(4.0-9.8)[56]	9.5(1.48-110)[27]	12 (1.96- 190)[7]
Polymorphs (proportion)	0.73 (0.08-0.92) [41]	0.72 (0.3-0.96) [39]	0.75(0.16-0.93)[56]	0.77(0.50-0.92)[27]	0.82(0.63-0.95)[7]
Lymphocytes (proportion)	0.21 (0.08-0.82) [41]	26(0.04-0.7)[39]	0.24(0.06-0.84)[54]	0.21(0.08-0.4)[27]	0.18(0.05-0.34)[7]
Blood sugar (mmol/L)	5.1 (2.2-12.1) [13]	5.1(2.2-10.3)[20]	4.4(3.3-7.5)[26]	6.5(3.7-13.3)[4]	7.8(6.4-8.2)[5]
Urea (mmol/L)†	15 (6.8-17.1) [5]	8.9(1.4-18.9)[10]	10.3(0.7-20.3)[10]	Nr	9.6(5.3-21.4)[3]
Creatinine (µmol/L)	44.2(8.8-88)[3]	53.4(8.8-71.6)[11]	53.0(8.8-79.6)[13]	Nr	64.5(35- 80)[3]
Sodium (mmol/L)	137(127-160)[12]	133 (126-146) [17]	133(109-149)[20]	139(129-148)[9]	137 (134- 139)[2]
Potassium (mmol/L)	4.3(3-5)[12]	3.9(3-5)[17]	3.95(2-5)[20]	3.8(3.5-4.9)[9]	3.8 (3.6- 3.9)[2]
Cerebrospinal fluid					
Total leukocyte count ($x10^9$/L)	0.043(0-0.6)[42]	0.041(0-0.83)[44]	0.035(0-0.54)[59]	0.14(0-1.4)[31]*	0.07 (0-0.4)[8]
Polymorphs (proportion)	0.1(0-1)[41]	0.3(0-1)[42]	0.26(0-1)[57]	0.7(0-1)[30]*	0.8(0-0.9)[8]
Lymphocytes (proportion)	0.5(0-1)[41]	0.38(0-1)[42]	0.6(0-1)[57]	0.3(0-1)[30]	0.15(0-1)[8]
Protein (g/L)	0.4(0-1.2)[39]	0.4(0-1.5)[40]	0.4(0.1-1.5)[53]	0.65(0.2-1.5)[30]*	0.1(0.04-5.8)[8]
Sugar (mmol/L)	3.3(1.4-5.7)[41]	3.3(0.9-5.6)[43]	3.3(1.7-6.1)[53]	2.1(1.1-3.8)[30]*	2.1(0.7- 3.3)[8]

Median (range) [number of patients] or Number (%) [number of patients]. Significance of difference between groups by Fisher's Exact test or Mann-Whiney U test. *Significant ($p<0.01$) difference between patients within AES of suspected viral aetiology and AES of bacterial aetiology; nr, not recorded; †normal range for urea (newborn to 16 years): 1.1-6.4 mmol/L (125).

Chapter 4: Weighing a child may help predict outcome in Acute Encephalitic Syndrome

Abstract

Background: Acute encephalitis syndrome (AES) describes a collection of symptoms and signs which help clinicians diagnose acute viral encephalitis (VE). Japanese encephalitis (JE) is a major cause of AES in Nepal. Previously I have shown in a retrospective study that JE patients had lower body weight, higher respiratory rate, a trend for a higher serum urea and potassium levels as compared to non-JE patients. JE patients also had significantly more focal neurological deficits and worse outcome as compared to AES of unknown viral aetiology. In this study, I wanted to find out the association between low weight for age (WFA) on admission and immediate and long term outcomes of children with AES and JE.

Methods: All children, 1-14 years, with fever, altered sensorium and/or seizure attending Kanti children's Hospital (KCH) and BP Koirala Institute of Health Sciences (BPKIHS) in Nepal were recruited. Infection with JE virus was confirmed by MAC-ELISA. Outcome was measured using Liverpool Outcome Score at discharge and follow up to 2 years. Detailed information on socio economic status (SES) could not be obtained and related in the study as the study was conducted in two public hospitals of Nepal catering to a diverse group of patients of different SES.

Results: Of total 152 cases of VE, 39 (25%) were JE, 71 (47%) non-JE and 42 (28%) JE-status unknown. Among children with VE, those with lower WFA were significantly younger, had prolonged illness, more episodes and longer seizure duration, more focal neurological deficit, and higher serum urea level. Among JE patients those with low WFA had significantly longer illness, and prolonged convulsion duration and compared to normal or higher WFA. Moderate to severe sequelae were more common in low WFA group of both VE and JE at discharge and follow up. Complete recovery was significantly more at 1 year follow up in normal or higher WFA group JE patients.

Conclusion: There was significantly progressive increase in residual neurological sequelae as the WFA Z score decreased in both AES and JE patients. Low WFA at admission in children with AES was associated with significantly more sequelae at discharge and persistence of sequelae even at 2 years of follow up. Further work on the relationship between low WFA and fluid and acid base status and malnutrition is recommended.

4.1 Introduction

Acute encephalitis syndrome (AES) describes a collection of symptoms and signs consisting of acute onset of fever with either altered level of consciousness or seizure or both (1). Most patients with the AES have acute viral encephalitis as the underlying cause, but other encephalopathies may also present with the same syndrome, including acute bacterial or parasitic central nervous system infections, and non-infectious encephalopathies (10).

Japanese encephalitis (JE) is a major cause of AES in Nepal. Since 2004, there has been national surveillance for JE by the government of Nepal supported by the World Health Organization (WHO) (126). Because of the large disease burden, the SA-14-14-2 live attenuated JE vaccine was used in a mass vaccination campaign in areas of high JE transmission in Nepal from 2006; from 2009 these areas were also covered by the introduction of JE vaccination into the routine national immunisation schedule. Although JE has been curtailed to some extent by this measure, the burden of AES still remains high. Therefore, a huge number of children with AES still arrive at tertiary care hospitals and health institutions in the country mainly during the summer.

The total weight of a person is summation of different body constituents made up of muscle, fat, water and bone. Generally, body weight indicates nutritional status of the person. Importantly, 60-80% of human body weight is water; less so in adults and more in children. Normally in children, weight increases with increase in age depending on the gender. Weight can be distributed in different categories such as +3, +2 +1, 0, -1, -2, -3 WFA Z scores for a particular gender. WFA Z scores below -1 can be considered a marker of acute loss of weight in children (91). Therefore, Z scores of 0, +1, +2 and +3 can be considered not to be of low and WFA and Z scores -1, -2 and -3 as low WFA (91). Assessment of clinical and laboratory features of patients of different WFA Z score could help in differentiating dehydration from malnutrition.

Prolonged duration of illness has been reported to be a predictor of bad outcome in AES and JE, previously (37, 75). Other indicators of bad outcome

are presence of focal neurological deficit, lower level of consciousness, and restriction of fluid and sodium during treatment (93). Even though there is no specific treatment for most of the patients of AES and JE, in those who arrived early at the hospital in the course of illness, and received supportive care, particularly intravenous fluids, had a better chance of survival and complete recovery (75). Patients with prolonged illness and disability may have difficulty feeding themselves causing malnutrition or dehydration; and lighter in weight. There was also a possibility that those lighter in weight could have been malnourished and vulnerable to JEV infection.

In the retrospective study (chapter 3), when examining all AES cases (both JE and Non-JE patients), higher weight and older age were associated with 'bad outcome'. However, when comparing patients of non-JE with JE, the latter were lighter and younger (75). A higher proportion of JE patients experienced 'bad outcome'. I could not identify a consistent association between weight and bad outcome, because age was a confounder within the AES and JE groups. Therefore, there was a need to investigate whether JE patients had lower weight-for-age (WFA) as compared to non-JE patients (by relating actual weight to expected weight based on population reference values). Lower WFA among the JE patients would suggest these patients were more dehydrated or malnourished. This may help identify potential mechanisms leading to the observed 'bad outcome' among the JE cases.

Loss of weight in children during any illness increases risk of morbidity and mortality (127). Most of the health personnel in developing countries including

Nepal have been trained to measure accurate weight of children through various nutritional related public health programmes. If adverse outcome is found in patients of AES or JE with low WFA, then educating the parents and health workers, to measure WFA of children could be beneficial. WFA may be used as a screening tool to identify high risk patients for immediate referral and explaining the prognosis.

Measurement of the height of the patients and investigating the association of weight-for height and body mass index (BMI) with outcome of AES and JE patients would have been another alternative. Although in the UK, nurses routinely measure height and weight of the attending patients as a part of their hospital practices, it is not the same in Nepal. In most parts of Nepal, nurses are constantly encouraged to measure weight of the attending sick children because it is needed in the treatment to accurately calculate the dose of drugs, amount of intravenous fluids and monitoring fluid status. Most of the emergency departments do not have a measuring tape or a stadiometer to measure height of patients, which could have also discouraged them in measuring height. Even when there is one, abnormal posturing and change in body tone of patients of AES, make it difficult to measure their height. Therefore, I decided to conduct a more systematic prospective crossectional study to find out the association of WFA on admission with outcome of children with AES and JE.

4.2.1 Aim

The aim of this study is to assess the clinical features, laboratory parameters and outcome of acute encephalitis syndrome in Nepali children of different weight-for-age categories.

4.2.2 Objectives

1. To validate the clinical features and laboratory parameters which predict bad outcome of acute encephalitis syndrome and Japanese encephalitis.
2. To validate the clinical features and laboratory parameters which predict Japanese encephalitis from other causes of acute encephalitis syndrome in children.
3. To describe the association of clinical features, laboratory parameters, immediate and long term outcome of children of acute encephalitis syndrome and Japanese encephalitis with different weight-for-age Z score categories on admission.

4.3 Methods

4.3.1 ***Setting:*** The study was conducted in Kanti children's Hospital (KCH) and BP Koirala Institute of Health Sciences (BPKIHS) in Nepal. KCH is the only tertiary care referral public hospital with paediatric subspecialties located in Kathmandu valley (altitude, 4400 feet above sea level, population 2916680, under 14 year population of 887991). Most of the patients of JE attending this hospital are residents of the Southern low land called "Terai" (128, 129). It also receives reffered patients from all over the country. BPKIHS, is a large tertiary care teaching Hospital located in a small South-Eastern town of Dharan in "Terai" and primarily caters to the Eastern region of the country (altitude 200- 29028 feet above sea level, population 6149651, under 14 year population 1697574).

4.3.2 ***Case definition:*** A total of 216 children were recruited from April 2009 to November 2010 in Kanti Children's Hospital, Kathmandu and BPKIHS, Dharan in Nepal. The case definitions were as described in Chapter 2.

4.3.2.1 Weight-for age Z scores: The level of growth (weight) retardation in children can be assessed by the how a child's individual WFA relates to the NCHS/WHO reference child population [http://www.who.int/nutgrowthdb/about/introduction/en/index5.html (as assessed on 3rd March 2016)](92). Z scores equate to standard deviations (SD) around the reference median. In a population with a normal WFA distribution, you would expect 15.9%, 2.3%, or 0.13% of children to have a weight for age Z score of -1, -2 or-3 respectively [http://www.who.int/childgrowth/standards/Growth_standard.pdf (as assessed on 22 February, 2016)]. Historically the WHO define a child with a WFA Z score of less than -2 or -3 as indicating moderate or severe under-nutrition [http://www.who.int/nutgrowthdb/about/introduction/en/index5.html (as assessed on 3rd March 2016)]. Recent studies have shown child mortality risks are not only higher at Z scores of -2 or less, but are also increased (albeit to a lesser extent) at -1 Z scores (91). Low WFA indicates under-nutrition, but in an acute setting it may also indicate poor fluid intake (or dehydration). In this study, I stratified subjects by their WFA Z score and examined outcome among these groups. Since there was no single standard baseline WFA growth chart for Nepali children, WFA was defined according to WHO definition for children below 5 years of age (Appendices H & I) and "Development of the Nepalese Growth Standard for the School-aged Children" for children above 5 years of age (Appendix J).

4.3.2.1.1 High WFA: *Z score* +1 (Z score between +1 and +2)

4.3.2.1.2 Average WFA: *Z score 0* (Z score between +1 and -1)

4.3.2.1.3 Mild low WFA: *Z score -1* (Z score between -1 and -2)

4.3.2.1.4 Moderate low WFA: *Z score* -2 (Z score between -2 and -3)

4.3.2.1.5 Severe low WFA: *Z score -3* (Z score below than -3)

4.3.3 *Sample size:* The sample size estimated was 102 participants in total (51 in each group) as previously shown in chapter 3 (General Methods).

4.3.4 *Recruitment:* All children aged 1- 14 years with AES admitted to KCH and BPKIHS were seen by a member of the investigating team. Blood was sent for complete blood count (CBC), urea, electrolytes, liver function tests (LFTS), glucose, and viral serology as part of routine testing for AES patients. When an LP was performed, opening pressure was recorded and cerebrospinal fluid sent for cell count and differential, glucose, protein, microscopy and culture, and JE serology. Following LP, patients whose LP results were suggestive of AES of suspected viral aetiology (or suspected viral encephalitis) (38), were approached for enrollment in the study. Other investigations were performed at the discretion of the treating physician. A check-list was used to confirm that the inclusion criteria were met. Following this patients who gave consent were recruited into the study. A detailed history was taken from the accompanying parent or guardian by a member of the study team. Clinical examination, including full neurological examination was then peformed. All the information were recorded in specially prepared Proforma (Appendix G). Weight was measured at the time of admission using a digital weighing scale, which was quality controlled every morning using standard weights. The details of the recruitment process was as described in chapter 3 (General Methods).

4.3.5 ***JE serology:*** Infection with JE virus was confirmed by MAC-ELISA (IgM antibody capture-Enzyme Linked Immunosorbent Assay) in serum and/or CSF in NPHL, Teku, Kathmandu or BPKIHS, Dharan as described in Chapter 3 (58, 113, 114).

4.3.6 ***Management:*** The patients were managed according to the hospital protocol for management of AES. All children with suspected bacterial meningitis were given empirical intravenous (IV) crystalline Penicillin or Ceftrioxone. Patients who presented with seizures or were witnessed to have a seizure were immediately given intravenous (iv) midazolam or diazepam and later intravenous phenytoin or sodium valproate. For uncontrolled seizure, intravenous midazolam infusion and mechanical ventilation was provided in intensive care. Patients who had signs of raised intracranial pressure were managed with elevation of head, intravenous 20% mannitol infusion and/or intubation and hyperventilation. Patients who were in shock received volume expanders and inotropic support with intravenous dopamine or dobutamine infusion. All the recruited patients were monitored daily for change in Glasgow coma score (GCS), respiratory rate, pulse rate, blood pressure, temperature, maintenance fluids and use of mannitol and anti-convulsant.

4.3.7 ***Outcome assessment:*** All patients with AES who were suspected of viral encephalitis including JE were reassessed at the time of discharge by a member of the investigating team. During assessment parents were interviewed and patients examined clinically with an emphasis on the neurological system also using the Liverpool Outcome Score (LOS) (109). They were then prospectively

followed-up again between 6- 24 months and were inquired on recovery, deterioration or death based on LOS. Those who did not follow-up were interviewed by telephone (Appendice K and L).

4.3.8 ***Ethics:*** The study was approved by the Institutional Review Committee of Kanti Children's Hospital and BP Koirala Institute of Health Sciences and Ethical Committee of Nepal Health Research Council.

4.3.9 ***Statistical analysis:*** All patients were analysed, including subgroup analysis for JEV positive patients. Normally distributed data was compared using Student's t-test; data that were not normally distributed was compared by the Mann-Whitney U test. Differences between proportions were tested using the chi-square test with Yates' correction or Fisher's exact test. Comparison of more than two groups of normally distributed continuous variables were done using ANOVA and continuous but skewed variables were compared using Krushkar- Wallis test. $P < 0.05$ was taken to be statistically significant.

4.4 Results

A total of 217 cases of AES were recruited in the study from the two hospitals. Thirty eight (12.8%) presented with fever and seizures (no altered sensorium), 62 (20.8%) fever with altered sensorium (no seizures) and 117 (39.3%) had fever, altered sensorium and seizures. The median (range) duration of illness was 14 (3-44) days and median duration of hospital stay was 8 (1-14) days. Overall, 146 (49%) patients had a bad outcome, 50 (16.8%) good outcome and 71 (23.8%) left against medical advice (LAMA) without informing the

hospital staff so the final outcome could not be assessed. Common reasons of LAMA were inability to pay the hospital charges after improvement (because all medical treatments are out-of-pocket in Nepal) and unavailability of beds in the paediatric intensive care unit (PICU). In such situations, they left before formal paper work could have been completed either to home or PICUs of other hospitals. Since these patients could not be followed up, this could have had some effect in the final analysis of the patients. Amongst those who had a bad outcome, there were 28 deaths and 118 had residual neurological sequelae.

First, I validated prospectively the predictors of bad outcome in patients of AES and JE; and predictors of JE from patients of AES, which were reported in my previous retrospective study (75). I found GCS ≤ 12, fever duration ≥7 days and presence of focal neurological deficit to be predictors of bad outcome in AES patients and GCS ≤ 12 as predictor of bad outcome in JE. I also found presence of focal neurological deficit at the time of admission in patients of AES to predict JE. Presence of GCS ≤12 had a positive predictive value of 82% (sensitivity 68%) for bad outcome in AES patients and positive predictive value of 91% (sensitivity 86%) for bad outcome in JE. Absence of GCS ≤12 at admission had a negative predictive value of 36% (specificity 56%) in AES and negative predictive value of 55% (specificity 67%) in JE. I could not validate the presence of focal neurological deficit at the time of admission in AES patients as a predictor of a diagnosis of JE probably because of the small number JE patients (Table 4.1).

28 (13%) out of 217 AES cases were of non-viral aetiology and 37 (17%) cases did not have admission weight recorded appropriately because it was done

by untrained attending doctors who informed late to the research assistant. I therefore excluded these from the analysis. Of the total of 152 cases of VE recruited (Fig 4.1), 39(25%) were confirmed JE, 71(47%) confirmed non- JE and 42 (28%) of unknown JE status. 67 (44%) cases had low weight-for-age (WFA) and 85 (56%) did not have low WFA. According to WFA on admission in patients of VE, normal WFA in 76 (50%) and mild low WFA in 36 (24%) were the most common weight group (Figure 4.1). Similarly, in JE, 16 (41%) of normal WFA and 12 (31%) mild low WFA were also the most common weight group. Overall, in VE, a total of 61/107 (57%) cases discharged were followed up at one year [median (range), 342 (15- 631) days] and 51/107(48%) cases 2 years [median (range), 637 (570- 750) days] after discharge. Also, in JE, 17/34 (50%) cases were followed up at one year [median (range); 321 (15-390) days] and 12/34 (35%) cases at 2 years [median (range); 641 (605- 701) days] after discharge. The WFA distribution of the cases of VE and JE were as shown in figure 4.1.

Children with VE who had a lower WFA were significantly younger, had a prolonged illness, more episodes of seizures, longer seizure duration, more focal neurological deficit, higher serum urea level and neurological sequelae (Table 4.2). There was a non-significant association between low WFA and death (p- 0.08). JE children with lower WFA also had significantly prolonged illness, longer duration of convulsion and bad outcome especially neurological sequelae (Table 4.3).

A total of 19 children with VE died and 91 had residual neurological sequelae at the time of discharge. Moderate sequelae was significantly more in lower

WFA group (-1 or less WFA Z score) and severe sequelae was also more in that group than normal or higher WFA group at the time of discharge (Table 4.4). Complete recovery was observed to be more in normal and higher WFA group. Even up-to 2 years of follow up, more children of lower WFA had residual sequelae and less recovered completely than normal to high WFA group. Similarly in JE, a total of 4 children died and 28 had sequelae (Table 4.5). Severe and moderate sequelae were more common in the lower WFA group at the time of discharge and follow up up-to 2 years. Complete recovery was significantly more in the normal and high WFA group at the time of discharge. It was also higher in the group of children who were followed up for up-to 2 years.

On assessing disability by LOS at the time of discharge, significantly more children with lower WFA had epilepsy or seizures, cognition and motor defect such as recognition, feeding and walking difficulties (Table 4.6). Significantly more JE children with lower WFA also had motor abnormalities such as difficulty in feeding and dressing (Table 4.7).

The recovery profile relating to the neurological sequelae for both VE and JE patients was observed over two years. In VE, those who had moderate (-2) and severe (-3) WFA Z scores had more residual neurological sequelae at the time of discharge as compared to those who had normal (0) and higher (+1) WFA Z scores. Also, the residual sequelae were observed to persist in more than half the cases at 2 years follow up in the severe low WFA group as compared to those with normal or high WFA group (Table 4.8). The lower the LOS on assessment, the more severe the disability. The median LOS at the time of

discharge was lower in moderate and severe low WFA groups which continued to remain low even at 1 year and 2 year follow up as compared to normal and high WFA group. Although there was more residual neurological sequelae at the time of discharge in both moderate and severe low WFA group, there was no improvement in residual sequelae in 67% and 40% of cases at 1 year respectively which persisted in all of the cases at 2 years follow up. Similarly in JE, residual neurological sequelae at the time of discharge were seen more in moderate and severe low WFA group as compared to those who had normal WFA and higher WFA (Table 4.9). The median LOS at the time of discharge was also lower in moderate and severe low WFA group, and remained low at 1 year and 2 year follow up as compared to normal and higher WFA group.

4.5 Discussion

AES is mostly caused by viruses which lack definite treatment. Identifying risk factors and correctable supportive management options would help in explaining the prognosis to anxious patients and parents, prevent death, and decrease disability which could be a source of lifelong misery. Previously prolonged illness, prolonged fever, prolonged duration of altered sensorium, Glasgow coma score (GCS) below 8, multiple convulsions, presence of focal neurological deficit, abnormal breathing, decerebrate posturing and hyponatremia at the time of admission have all been described to be predictors of bad outcome in AES and JE (10, 37). Even a combination of poor perfusion, GCS ≤ 8 and ≥ 2 witnessed seizures have been described to predict bad outcome when more than one risk factors was present in the same patient (21). However, the robustness and generalisability of these findings to a range

of setting was uncertain (21, 37, 77, 78). Therefore I decided to validate my previous findings prospectively in this study.

Amongst the clinical features shown in Table 4.1 which were predictors of bad outcome in AES and JE in my previous retrospective study discussed in chapter 3, I validated prospectively GCS ≤ 12, duration of fever ≥7 days, presence of focal neurological deficits at admission to be robust predictors of bad outcome in AES patients and GCS ≤ 12 also as predictor of bad outcome in JE in this study conducted at two different setting in Nepal. Presence of GCS ≤12 had a positive predictive value of 82% (sensitivity 68%) for bad outcome in AES patients and positive predictive value of 91% (sensitivity 86%) for bad outcome in JE. Absence of GCS ≤12 at admission had a negative predictive value of 36% (specificity 56%) in AES and negative predictive value of 55% (specificity 67%) in JE. However, presence of focal neurological deficits in AES which was found to predict JE in my previous study could not be validated in the present study. This unusual finding may have been because of less number of confirmed JE cases in this study as compared to my previous study. Further re-analysis by boot strap method or reanalyzing the predictors from the first two-thirds of patients and applying this to the latter one-third may have validated my previous finding.

Identifying hydration and nutrition status at admission may be important in the management of patients of AES. On the one hand, because of the fear of cerebral oedema, the traditional approach is to fluid restrict patients, to prevent deterioration of illness (89, 90); on the other hand, when such children

have prolonged illness (fever), poor GCS and focal neurological deficits as mentioned above, they are unwell for many days, vomit frequently, are unable to drink fluid and so may be dehydrated. In these situation, restricting fluids could make dehydration even worse and result in metabolic acidosis and bad outcome. Therefore, assessment of hydration and nutritional status and correction of deficits maybe the key to successful management. One approach is to weigh every child at the time of admission. However, weight is also contributed to by muscle, fat and bone besides water. In general, weight is an indicator of nutritional status. It also increases with increasing age, and differs according to gender. Therefore, WFA reflects body weight of a child relative to age and gender at a given time. Historically the WHO define a child with a WFA Z score of less than -2 or -3 as indicating moderate or severe under-nutrition [http://www.who.int/nutgrowthdb/about/introduction/en/index5.html (as assessed on 3rd March 2016)]. Recent studies have shown child mortality risks are not only higher at Z scores of -2 or less, but are also increased (albeit to a lesser extent) at -1 Z scores (91).

In a population with a normally distributed WFA, you would expect just under 16% of subjects to have a WFA Z score -1 or less. My study shows that almost half of all the cases of AES of suspected viral aetiology and confirmed cases of JE had a WFA Z score of -1 or less at the time of admission. My study also shows that those with WFA Z score of -2 or -3 increasingly tended to suffer a bad outcome, particularly residual neurological sequelae. Prolonged duration of fever, significantly prolonged illness, presence of focal neurological deficits and high serum urea in the lower WFA group in my study suggest these

children could have been dehydrated, because all of these are likely associated with poor fluid intake. However, they could also have been malnourished. Additional signs that could help to separate dehydration from malnutrition are weight-for-height, mid upper arm circumference (MUAC) and skin fold thickness. These would have been normal in dehydration but they would have been below normal in malnutrition. Obvious signs of dehydration (Appendix E) may also be absent in malnourished children. MUAC is being used increasingly to asses malnutrition status through various nutritional programmes in Nepal and most of the health workers would have been trained on it. Weight-for-height measurement, although more accurate to asses malnutrition, would be difficult to assess in unconscious child and skin fold thickness would be new and requiring expensive tool to assess. Therefore, in a low WFA patient of AES or JE, MUAC and other supportive laboratory findings could help differentiate dehydration from malnourishment. Further study with more accurate assessment of signs of dehydration and systematic measurement of serum urea, electrolytes, fluid balance, markers of acid-base balance and MUAC in children of low WFA would help differentiate dehydration from malnutrition in these children.

There were also significantly more episodes of seizures and longer total duration of seizures in the lower WFA group of AES and JE in my study. Repeated seizures and raised intracranial pressures are associated with bad outcome in JE (20). However, I found it to be more common in lower WFA group. Like several nervous system infections, seizures complicate viral encephalitis and JE (20). Cerebral oedema, cortical necrosis and neuronal

injury may cause seizures in encephalitis. In Addition, small haemorrhages and thrombus formation in the brain have been speculated to cause seizures in JE (53). Repeated or uncontrolled seizures are also associated with hypoxemia, hypoglycemia, hyperlactataemia or metabolic acidosis which needs effective management along with the treatment of nervous system infection. Even brief periods of seizure has been associated with raised ICP.

Every year, severe acute malnutrition affects 20 million children below 5 years of age causing around 2 million preventable deaths. The case fatality rate in developing countries is higher with rates as high as 20- 60% (130, 131). In 2011, 2.3 million children in the world died because of lack of nourishment. Hence, malnutrition is one of the important and urgent issues in global development. In nutritional language, low weight-for-age is described as underweight. 58 million children (1 in every 3) in South Asia are under weight which is even more than the entire combined population of England and Wales. According to Demographic and Health Survey of Nepal conducted in 2011, the malnutrition status of children below 5 years of age has been described as moderate to severe wasting in 11%, stunting in 41% and underweight in 29%. Even in the present study, around half of the AES and JE patients were underweight. However, malnutrition is not just because of poor food intake but a combination of frequent illness, poor quality of food, poor feeding practices, poor water and sanitation and inadequate health services in this part the world. There is a significant burden of AES and JE in children living in the South-Asian subcontinent. Therefore malnutrition needs consideration when managing children with encephalitis in these countries where it is very

common. Hypoglycemia and hyponatremia are common complications of malnutrition which are more likely to cause seizure and result in bad outcome in AES children.

Dehydration is classified as weight loss as a marker of fluid deficits (132, 133). WHO defines dehydration as percentage loss of body weight; no signs of dehydration (3-5%), some signs of dehydration (5-9%), and severe dehydration (≥10%) (133). Other symptoms and signs of dehydration are lethargy or increased thirst, decreased urinary output, weak pulse, cool extremities, sunken eyes, absence of tears, sunken fontanel, prolonged capillary refill and poor skin turgor; these could help clinicians to differentiate it from other clinical conditions. Hyponatremia, hypernatremia, hypoglycemia, bacterial toxins, cerebral venous thrombosis are all complications of dehydration which could cause seizures resulting in a poor outcome in children with AES. Moreover, malnutrition, infection and dehydration are inter-related and linked by a vicious cycle. However, management of even dehydration in malnourished children requires administration of ReSoMal which is a half strength oral rehydration solution over a prolonged duration because of the effect of malnutrition in different organs of the body. According to "Nepal Demographic and Health Survey conducted in 2011, 11% of children are malnourished of which 3% are severely malnourished. My findings of almost half of the children with AES and JE of low WFA and significantly associated with bad outcome highlight the need to screen and treat underlying malnutrition and administer effective and appropriate fluid therapy in patients with AES in order to improve outcome. Anticipating and managing repeated seizures in both the conditions could also help improve outcome.

This study also highlights the high burden of disability following AES and JE which could be relevant to all areas of the world where the disease burden is high (28). Importantly I found the functional impairment at the time of discharge was significantly higher in those of lower WFA group as compared to those who had appropriate or higher WFA in both AES and JE. Specifically, feeding difficulties, cognition problems, limb movement difficulties, incontinence and seizures were significantly more common impairments at discharge in the lower WFA group among those with AES; additionally poor feeding, expressive language and dressing difficulties were significantly more common at discharge in the lower WFA group among JE patients. The risk of seizures up to 5 years after CNS infections has previously been reported as almost 7%. Early seizures may occur in 10% of encephalitis patients, and even after 20 years, the risk of unprovoked seizures has been observed to be as high as 22% (53). The identification of children with uncontrolled seizures highlights the need to closely follow up VE related seizures. In both AES and JE children, the disability was more commonly of moderate to severe degree for the lower WFA children. This was the case at the time of discharge and also at 2 years follow up. Five deaths (including 3 JE patients) occurred after hospital discharge, suggesting that outcome at discharge is a poor reflection of the eventual outcome (21, 74). Importantly, disability increased in children of lowest WFA from 20% at 1 year to 33% at 2 year follow up. Although some patients deteriorated after follow up, many others improved (21). Thus it is important for treatment trials to measure both short term and long term outcomes in order to find out the actual efficacy of a drug.

The recovery profile of AES and JE has been less studied. Some have studied long term outcome up-to two years from discharge but have failed to record discharge status in-order to assess change in problems over time to describe recovery profile of disability (16, 73). The discharge outcomes in children with JE are seen to change in long term follow up (10). Complete recovery is possible in follow up from severe motor sequelae at the time of discharge (10, 21, 74). In my study, I found that in both AES and JE patients, the disability seemed to have improved in the 2 year follow up period in children of normal WFA (134). However, it seemed to persist or even worsen in those with lower WFA. Presently in Nepal, children with motor impairment can receive input from the physiotherapist. However, further professional support from speech and language, psychosocial or occupational therapist would likely have been more beneficial.

In conclusion, prolonged illness was a robust predictor of bad outcome in both AES and JE patients. There was a significant increase in the association of bad outcome as residual neurological sequelae in AES and JE patients with decrease in WFA based on their Z score and persistence of sequelae even at 2 years follow up. Although there is clinical evidence to suggest that in these patients the low weight is due to dehydration, there is also the possibility that it could be under nutrition. Hence, to explore further the cause of low WFA, I planned more detailed study, using a structured and standardised proforma that included specific questions on patient hydration status, fluid balance, acid base status, serum electrolytes (as a marker of renal function), and measurement of weight and changes in weight during hospital stay. This forms the subject of my next chapter.

4.6 Limitation of study

A total of 37 (17%) of patients did not have their weight accurately measured using a digital weighing scale. These participants were excluded which could have affected the study results. Since height, discharge weight (therefore, change in weight), total intake of fluids and output, markers of dehydration such as blood lactate, pH, capillary refill time, temperature and oxygen saturation were not measured, it was difficult to confidently separate dehydration from malnutrition in these patients. Since height was also not measured, it was difficult describe whether it was acute weight loss (dehydration) or chronic weight loss (malnutrition). Moreover, WFA reflects composite measure of both acute and chronic under nutrition and cannot distinguish between the two (135).

Since height, discharge weight (or change in weight), total intake of fluids and output, markers of dehydration such as blood lactate, ph, capillary refill time, temperature and oxygen saturation were not measured, it was difficult to confidently differentiate dehydration from malnutrition in these patients. Since height was also not measured, it was difficult describe whether it was acute or chronic weight loss (malnutrition).

I have used two reference standards to assess weight-for-age (WFA) within the study population. This was undertaken because there is no single set of national reference values for weight across all childhood ages in Nepal. The first standard, was the international standard developed by WHO for children below 5 years of age. The second was a local national standard for use among

children above 5 years of age in Nepal. Use of an WHO standard for younger children did have potential disadvantages, since this standard is based on a reference population from the United States. Consequently, some of the children below 5 years of age may have been incorrectly labelled as below a certain WFA Z score (based on WHO standards rather than national standards). Measurement of mid upper arm circumference (MUAC), weight for height, or body mass index (BMI) may have helped differentiate acute from chronic weight loss. Since these measures were not recorded, it is still unknown whether patients were dehydrated or malnourished.

The study was conducted in two public hospitals across a diverse group of patients. Information on social and economic status (SES) may have helped tease apart dehydration form malnutrition among the cases, with the latter being more likely in the lower SES group of patients (136- 138). Similarly, I could have asked the parents about the dietary habits of their children prior to the illness. Again, this information could have further helped differentiate the cause of low WFA. Information on how much and type of food (e.g. using a pictorial food diary) the children ate prior to illness could have provided background information on the child's calorific intake and nutritional status (139, 140).

4.7 Summary

Prospectively, I first validated the clinical and laboratory features identified in my initial retrospective study as predictors of bad outcome in AES and JE. Low GCS and longer duration of fever and presence of focal neurological deficit at the time of admission were validated to be robust predictors of bad

outcome in AES patients. All of these conditions can cause dehydration. Dehydration is expressed as percentage weight loss. JE patients were of lower weight. A higher proportion of JE patients exhibited bad outcome as compared to non-JE patients in my retrospective study. Interestingly in children weight is linked to age. I used Z score to measure weight for age. However, since there was no single standard baseline WFA growth chart for Nepali children, I used WHO child growth standard for children below 5 years of age and the Nepalese growth standard for the school age children developed by Ministry of Health for children above 5 years of age. When outcome was correlated with different admission WFA groups of AES and JE patients formed by Z score value, I found significantly increase in residual neurological sequelae as the WFA Z score decreased in AES patients. I also found significant increase in bad outcome as the WFA Z score decreased in JE patients. There was also persistence of more sequelae in lower WFA group of AES patients even at 2 years follow up in both AES and JE patients. However, weight below -1WFA Z score could be either because of dehydration or malnutrition and sometimes the two conditions were indistinguishable at the time of presentation to the hospital. Malnourished children have anaemia and depressed immunity making them vulnerable to systemic infection resulting in bad outcome. In encephalitis, loss of consciousness, focal neurological deficit, uncontrolled seizures and fluid restrictions by clinicians can cause dehydration and metabolic acidosis causing bad outcome.

Measurement of weight and height for patients with impaired consciousness was not routinely undertaken in my hospital setting. Therefore, I opted to

undertake a more systematic measurement of serum urea, electrolytes, fluid balance, markers of acid-base balance and MUAC in children of different WFA group based on Z score, to help differentiate dehydration from malnutrition. I then studied the relationship of fluid and acid base status with low admission weight and changes in weight during hospital and their relationship to outcome. This has been described in my next chapter.

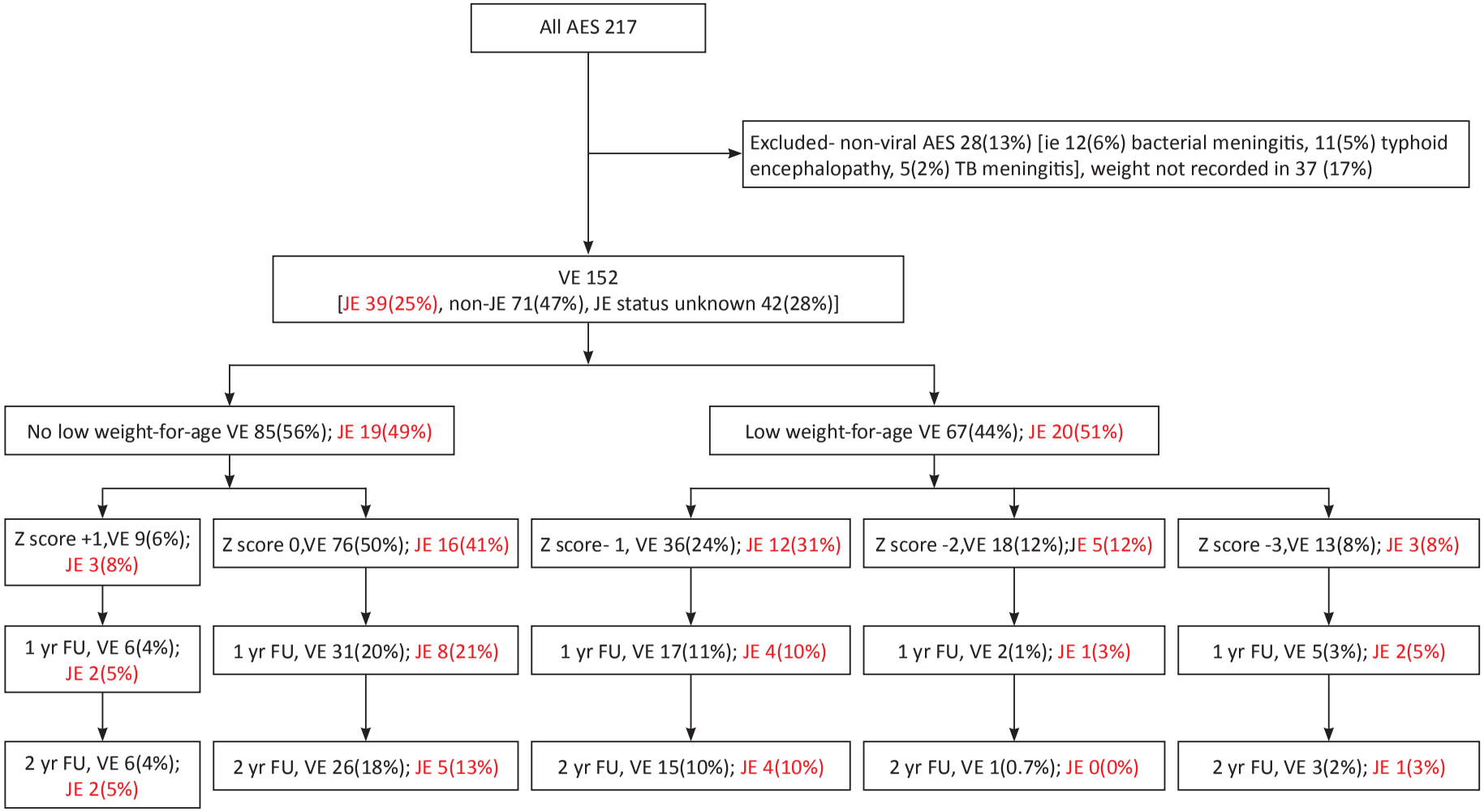


Figure 4.1: Study Profile. Numbers in red font represents number of patients infected with JE. FU, follow up

Table 4.1: Validation of predictors of bad outcome of AES and JE and predictors of JE from AES as derived from previous retrospective study

Features on admission	Present [No (%)]	Absent [No (%)]	*P*-value	OR (95% CI)	RR (95% CI)	Sensitivity	Specificity	PPV	NPV
Bad outcome in AES									
Glasgow coma score (≤ 12)	88 (82.2)	19 (17.8)	0.0059	2.65 (1.2- 5.7)	2.05 (1.2- 3.4)	67.7	55.8	82.2	36.4
Duration of fever (≥7 days)	55 (85.9)	9 (14.1)	0.0069	0.33 (0.1- 0.8)	0.43 (0.2- 0.8)	54.2	21.9	67	14
Focal neurological deficit	90 (86.5)	14 (13.5)	0.000005	0.19 (0.08- 0.4)	0.3 (0.2-0.5)	27.4	33.3	54.8	13.5
Bad outcome in JE									
Glasgow coma score (≤ 12)	31 (91.2)	3 (8.8)	0.003	12.4 (1.8- 77.3)	6.18 (1.9- 20.7)	86.1	66.7	91.2	54.6
Duration of fever (≥7 days)	11 (84.6)	2 (15.4)	0.54	0.73 (0.08- 5.1)	0.8 (0.2- 3.3)	68.6	25	80	15.4
Age (>5 years)	20 (71)	8 (29)	0.12	0.12 (0.67-151)	0.4 (0.6-26.3)				
Diagnosis of JE from all AES									
Focal neurological deficit	33 (75)	11 (25)	0.05	2.09 (0.9- 4.8)	1.8 (0.97- 3.4)	75	41	25.6	85.9

Table 4.2: Clinical features of VE

Clinical features	Z score + 1 (n=9)	Z score 0 (n= 76)	Z score – 1 (n=36)	Z score -2 (n=18)	Z score -3 (n=13)	P- value
Age	9 (4-12)	8 (1-14)	7 (1-13)	6 (2-14)	4 (2-5)	0.01
Weight	28 (17-45)	20 (8-40)	16 (7.5-33)	13 (9-30)	9 (4-12)	< 0.0001
Glasgow coma scale	13 (4-15)	11 (3-15)	11.5 (3-15)	9.5 (5-14)	11 (3-15)	0.63
Duration of fever	4 (1-10)	6 (1-14)	6 (2-14)	6 (2-14)	10 (1-13)	0.11
Headache	5/8 (63)	39/73 (53)	18/34 (53)	6/18 (33)	1/12 (8)	0.026
Duration of headache	4 (1-5)	6 (1-13)	5 (1-13)	7 (0-12)	2 (0-4)	0.27
Vomiting	5/9 (56)	42/73 (58)	21/36(58)	7/18 (39)	3/13 (23)	0.13
Duration of vomiting	2 (1-7)	1 (1-12)	2 (1-7)	3 (0-4)	3.5 (1-6)	0.6
Drowsiness	5/6 (83)	30/57 (53)	18/27 (67)	12/13 (92)	6/11 (55)	0.064
Duration of drowsiness	1 (1-3)	2 (1-10)	2.5 (1-8)	3.5 (1-13)	5.5 (1-11)	0.51
Convulsion	6/9 (67)	55/76 (72)	21/36 (58)	15/18 (83)	10/13 (77)	0.35
episodes of convulsion	1 (1-2)	6 (1-18)	3 (1-12)	6 (1-60)	6 (1-9)	0.008
Duration of each episode of convulsion (minute)	4 (2-15)	4 (1-32)	3 (1-15)	4 (2-15)	6.5 (2-90)	0.88
Total duration of convulsion (minute)	5.5 (2-30)	20 (3-180)	13.5 (3-90)	28.5 (8-180)	24 (6-90)	0.012
Temperature (Farenheight)	101 (98-104)	100 (96-105)	99.5 (97-104)	100.5 (96-104)	100 (98-103)	0.57
Respiratory rate (per minute)	30 (26-56)	30 (12-80)	30 (18-52)	29 (20-49)	30 (24-56)	0.75
Pulse (per minute)	100 (96-152)	104 (58-180)	100 (60- 180)	100 (70- 150)	106 (80- 170)	0.34
Systolic blood pressure (mm Hg)	100 (70-130)	100 (70- 170)	95 (79- 130)	90 (80-160)	90 (80-120)	0.99
Diastolic blood pressure (mm Hg)	60 (50-80)	60 (40-110)	60 (40-90)	60 (50-105)	68 (40-94)	0.94
Focal neurological deficit	2/9 (22)	43/74 (58)	24/34 (71)	13/17 (76)	11/13(85)	0.017
Total duration of illness (days)	9 (5-14)	14 (4-42)	15 (6-28)	16 (7-44)	14 (6-26)	0.034
Hb (gm%)	11.7 (9.7-14.7)	12 (7.8-14.9)	11.2 (8.7-13.5)	11.8 (6.5-13.5)	11.7 (8.7-14.7)	0.58
Random blood sugar (mg/dl)	109 (61-127)	96 (48-219)	91.5 (37-170)	105 (68-161)	85.5 (50-100)	0.044
Urea	21 (13-28)	25 (6.8-100)	24 (12-75)	34 (19-97)	28 (18-92)	0.014
Creatinine	0.8 (0.3-1)	0.7 (0.2-3.4)	0.7 (0.4-5.4)	0.65 (0.4-2.2)	0.7 (0.4-1.8)	0.93
Sodium	135 (129-146)	136(120-156)	136(126-146)	138 (129-146)	138(128-160)	0.69
Pottasium	4.1 (3.4-4.7)	4.3 (2.4-7)	4.4 (3-5.8)	4.3 (3.7-5.3)	4.5 (4.1-6.1)	0.32
CSF opening pressure (cm of water)	20.5 (15-25)	14.5 (6-30)	20 (8-28)	18 (17-19)	23 (9-35)	0.25
CSF white blood corpuscles (per mm3)	2.5 (0-100)	5 (0-4900)	15 (0-575)	101 (0-400)	53 (0-990)	0.26
Raised CSF pressure (cm of water)	2/4 (50)	6/32 (19)	4/12 (33)	0/2 (0)	3/4(75)	0.1
JE	3/9 (33)	16/76 (21)	12/36 (33)	5/18 (28)	3/13 (23)	0.68
Non- JE	3/9 (33)	39/76 (51)	17/36 (47)	5/18 (28)	7/13 (54)	0.38
JE status unknown	3/9 (33)	21/76 (28)	7/36 (19)	8/18 (44)	3/13 (23)	0.4
Bad outcome	5/9 (56)	52/68 (76)	28/33 (85)	14/15 (93)	11/12 (92)	0.13
Neurological sequelae	2/6 (33)	41/57 (72)	27/32 (84)	13/14 (93)	8/9 (89)	0.03

Table 4.3: Clinical features of JE

Clinical features	Z score + 1 (n=3)	Z score 0 (n=16)	Z score – 1 (n=12)	Z score -2 (n=5)	Z score -3 (n=3)	P- value
Age	9 (4-11)	9.5 (1-13)	7(1-13)	6 (2-14)	4 (3-5)	0.44
Weight	28 (17-37)	21 (10-38)	15.5 (8-28)	13 (9-28)	10 (9-10)	0.006
Glasgow coma scale	11 (4-14)	8 (3-15)	11 (5-14)	12 (7-14)	10 (6-12)	0.72
Duration of fever	3 (1-5)	6 (4-14)	5.5 (2-8)	5 (3-8)	8 (5-11)	0.16
Headache	2/3 (67)	9/15 (60)	6/11 (55)	1/5 (20)	1/2 (50)	0.61
Duration of headache	3 (1-5)	5 (2-9)	5 (2-9)	NA	4(4-4)	0.66
Vomiting	2/3 (67)	11/15 (73)	5/12 (42)	2/5 (40)	0/3 (0)	0.13
Duration of vomiting	1.5 (1-2)	1(1-12)	1 (1-7)	2.5 (2-3)	NA	0.43
Drowsiness	3/3 (100)	10/14 (71)	7/9 (78)	5/5 (100)	2/3 (67)	0.57
Duration of drowsiness	1 (1-1)	3 (1-7)	3.5(1-8)	3 (1-6)	1 (1-1)	0.18
Convulsion	2/3 (67)	13/16 (81)	6/12 (50)	5/5(100)	2/3 (67)	0.23
episodes of convulsion	1.5 (1-2)	6 (2-7)	1.5 (1-6)	6 (5-18)	3.5 (1-6)	0.05
Duration of each episode of convulsion (minute)	4 (3-5)	10 (3-32)	3 (3-5)	3 (2-3)	90 (90-90)	0.038
Total convulsion duration (minute)	5.5 (5-6)	20 (15-180)	10 (3-18)	18 (10-54)	90 (90-90)	0.01
Temperature (Farenheight)	101 (98-103)	100 (97-104)	99.5 (97-103)	102 (98-104)	98.6 (98-100)	0.2
Respiratory rate (per minute)	30 (26-56)	33.5 (20-80)	32 (24-52)	26 (20-36)	28 (24-30)	0.35
Pulse (per minute)	100 (100-140)	104 (86-180)	100 (60- 180)	120 (72- 150)	88 (80- 106)	0.45
Systolic blood pressure (mm Hg)	115 (100-130)	107 (90- 170)	90 (79- 120)	90 (90-110)	103 (90-116)	0.73
Diastolic blood pressure (mm Hg)	70 (60-80)	60 (40-110)	60 (60-90)	60 (60-70)	69 (68-70)	0.85
Focal neurological deficit	2/3 (67)	13/16 (81)	9/11 (82)	3/5 (60)	3/3 (100)	0.69
Total duration of illness (days)	9 (5-10)	15 (8-38)	15 (10-28)	14 (13-44)	24 (19-26)	0.041
Hb (gm%)	10.6 (9.7-14.7)	12 (10-14.3)	11.2(9.1-13.5)	11.5(10.6-12.2)	10.4 (10.4-10.4)	0.24
Random blood sugar (mg/dl)	99 (61- 127)	111.5 (85-219)	88.5 (60-112)	105(92-118)	90 (77-98)	0.03
Creatinine	0.9 (0.8-1)	0.7 (0.2-1.9)	0.7 (0.4-0.8)	1.2 (0.4-2.2)	0.6 (0.5-0.6)	0.15
Sodium	144 (132-146)	133 (122-141)	136 (130-146)	137 (130-139)	131(128-134)	0.37
Pottasium	4.4 (3.4-4.7)	4.3 (3-6)	4.2 (3.4-4.8)	4.2 (3.9-5.2)	4.3 (4.1-4.4)	0.95
CSF opening pressure (cm of water)	22 (19-25)	16 (9-30)	19 (8-22)	17 (17-17)	9 (9-9)	0.4
CSF white blood corpuscles (per mm3)	80 (0-100)	30 (0-285)	7 (0-150)	44.5(9-50)	25 (0-50)	0.84
Raised CSF pressure (cm water)	2/3 (67)	3/10 (30)	1/6 (17)	0/1 (0)	0/1 (0)	0.5
Bad outcome	1/3 (33)	12/16 (75)	11/11 (100)	5/5 (100)	3/3 (100)	0.034
Neurological sequelae	0/2 (0)	9/13 (69)	11/11 (100)	5/5 (100)	3/3 (100)	0.005

Table 4.4: Outcome of children with VE at the time of discharge, after one and two years follow up

WFA	+1 Z score	0 Z score	-1 Z score	-2 Z score	-3 Z score	*p*- value
At discharge						
Outcome (LOS)	**n= 9**	**n= 76**	**n= 36**	**n= 18**	**n= 13**	
1- Death	3 (33%)	11 (15%)	1 (3%)	1 (6%)	3 (23%)	0.06
2- Severe sequelae	1 (11%)	20 (26%)	7 (19%)	7 (39%)	4 (31%)	0.5
3- Moderate sequelae	0 (0%)	10 (13%)	12 (33%)	4 (22%)	0 (0%)	0.01
4- Mild sequelae	1 (11%)	6 (8%)	6 (17%)	1 (6%)	0 (0%)	0.4
5- Complete recovery	4 (44%)	16 (21%)	4 (11%)	2 (11%)	2 (15%)	0.2
LAMA	0 (0%)	13 (17%)	6 (17%)	3 (17%)	4 (31%)	0.5
At 1 year follow-up						
Outcome (LOS)	**n= 6**	**n= 31**	**n= 17**	**n= 2**	**n= 5**	
1- Death	0 (0%)	3 (10%)	1 (6%)	1 (50%)	0 (0%)	0.2
2- Severe sequelae	0 (0%)	2 (7%)	0 (0%)	0 (0%)	1 (20%)	0.4
3- Moderate sequelae	0 (0%)	2 (7%)	2 (12%)	0 (0%)	1 (20%)	0.7
4- Mild sequelae	0 (0%)	3 (10%)	0 (0%)	0 (0%)	1 (20%)	0.4
5- Complete recovery	6 (100%)	21 (68%)	14 (82%)	1 (50%)	2 (40%)	0.2
At 2 years followup						
Outcome (LOS)	**n= 6**	**n= 26**	**n= 15**	**n= 1**	**n= 3**	
1- Death	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
2- Severe sequelae	0 (0%)	1 (4%)	0 (0%)	0 (0%)	1 (33%)	0.1
3- Moderate sequelae	0 (0%)	1 (4%)	1 (7%)	0 (0%)	1 (33%)	0.3
4- Mild sequelae	0 (0%)	3 (12%)	1 (7%)	0 (0%)	0 (0%)	0.8
5- Complete recovery	6 (100%)	21 (81%)	13 (87%)	1 (100%)	1 (33%)	0.2

Table 4.5: Outcome of children with JE at the time of discharge, after one and two years follow up

WFA	+1 Z score	0 Z score	-1 Z score	-2 Z score	-3 Z score	*p*- value
At discharge						
Outcome (LOS)	**n= 3**	**n= 16**	**n= 12**	**n= 5**	**n= 3**	
1- Death	1 (33%)	3 (19%)	0 (0%)	0 (0%)	0 (0%)	0.3
2- Severe sequelae	0 (0%)	9 (56%)	4 (33%)	3 (60%)	3 (100%)	0.1
3- Moderate sequelae	0 (0%)	0 (0%)	4 (33%)	2 (40%)	0 (0%)	0.05
4- Mild sequelae	0 (0%)	0 (0%)	3 (25%)	0 (0%)	0 (0%)	0.1
5- Complete recovery	2 (67%)	4 (25%)	0 (0%)	0 (0%)	0 (0%)	0.03
LAMA	0 (0%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)	-
At 1 year follow-up						
Outcome (LOS)	**n= 2**	**n= 8**	**n= 4**	**n= 1**	**n= 2**	
1- Death	0 (0%)	2 (25%)	0 (0%)	1 (100%)	0 (0%)	0.2
2- Severe sequelae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
3- Moderate sequelae	0 (0%)	1 (12%)	0 (0%)	0 (0%)	1 (50%)	0.4
4- Mild sequelae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0.09
5- Complete recovery	2 (100%)	5 (63%)	4 (100%)	0 (0%)	0 (0%)	0.07
At 2 years follow- up						
Outcome (LOS)	**n= 2**	**n= 5**	**n= 4**	**n= 0**	**n= 1**	
1- Death	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
2- Severe sequelae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
3- Moderate sequelae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	-
4- Mild sequelae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
5- Complete recovery	2 (100%)	5 (100%)	4 (100%)	0 (0%)	0 (0%)	-

Table 4.6: Disability as assessed by LOS at the time of discharge in children with VE

Median LOS at discharge	Z score + 1 (n=9)	Z score 0 (n=76)	Z score -1 (n=36)	Z score -2 (n=18)	Z score -3 (n=13)	P- value
Expressive language problem	5 (5-5)	5 (2-5)	5 (2-5)	3 (2-5)	2 (2-5)	0.07
Need help in feeding	5 (5-5)	5 (2-5)	5 (2-5)	3 (2-5)	5 (3-5)	0.024
Can't be left alone	5 (5-5)	3 (2-5)	5 (2-5)	2.5 (2-5)	2 (2-5)	0.067
Behavioral problem	5 (5-5)	5 (2-5)	5 (2-5)	4 (2-5)	4 (2-5)	0.07
Unable to Recognize relatives	5 (5-5)	5 (2-5)	5 (2-5)	5 (2-5)	5 (3-5)	0.04
Unable to attend school of disability	5 (4-5)	4 (3-5)	4 (3-5)	3.5 (2-5)	4 (3-5)	0.18
On anti- epileptic drug or recent seizures	5 (5-5)	4.5 (4-5)	4.5 (4-5)	4 (3-5)	4 (4-5)	0.006
Help needed for dressing	5 (5-5)	5 (2-5)	5 (2-5)	2 (2-5)	2 (2-5)	0.06
Bladder or bowel incontinence	5 (5-5)	5 (2-5)	5 (2-5)	4 (2-5)	5 (2-5)	0.014
Hearing or receptive language problem	5 (5-5)	5 (4-5)	5 (3-5)	5 (3-5)	5 (5-5)	0.06
Difficulty sitting	5 (5-5)	5 (2-5)	5 (2-5)	3 (2-5)	3 (2-5)	0.097
Difficulty in standing up	5 (5-5)	5 (2-5)	5 (2-5)	2 (2-5)	3 (2-5)	0.053
Difficulty in walking	5 (5-5)	5 (2-5)	5 (2-5)	2 (2-5)	2 (2-5)	0.047
Difficulty putting hands on head	5 (5-5)	5 (3-5)	5 (3-5)	5 (2-5)	5 (3-5)	0.12
Difficulty picking up small objects	5 (5-5)	5 (2-5)	5 (2-5)	5 (2-5)	5 (2-5)	0.42
Minimum LOS	4 (1-5)	3 (1-5)	3 (1-5)	2 (1-5)	2 (1-5)	0.39
Total LOS	74 (1-75)	68 (1-75)	71 (1-75)	57 (1-75)	46 (1-75)	0.29

Table 4.7: Disability as assessed by LOS at the time of discharge in children with JE

Mean disability score at discharge	Z score + 1 (n=3)	Z score 0 (n=16)	Z score – 1 (n=11)	Z score -2 (n=5)	Z score -3 (3)	P- value
Expressive language problem	5 (5-5)	3 (2-5)	5 (2-5)	3 (2-3)	2 (2-2)	0.07
Need help in feeding	5 (5-5)	3 (2-5)	5 (5-5)	3 (2-3)	4 (3-5)	0.01
Can't be left alone	5 (5-5)	2 (2-5)	5 (2-5)	3 (2-5)	2 (2-2)	0.2
Behavioral problem	5 (5-5)	5 (2-5)	5 (4-5)	4 (2-5)	3 (2-4)	0.08
Unable to Recognize relatives	5 (5-5)	5 (2-5)	5 (5-5)	5 (2-5)	4 (3-5)	0.3
Unable to attend school of disability	5 (5-5)	4 (3-5)	4 (4-5)	3 (2-5)	4 (4-4)	0.2
On anti- epileptic drug or recent seizures	5 (5-5)	4.5 (4-5)	5 (4-5)	4 (3-4)	4 (4-4)	0.065
Help needed for dressing	5 (5-5)	2 (2-5)	5 (2-5)	2 (2-5)	2 (2-2)	0.037
Bladder or bowel incontinence	5 (5-5)	5 (2-5)	5 (5-5)	3 (2-5)	3.5 (2-5)	0.057
Hearing or receptive language problem	5 (5-5)	5 (4-5)	5 (4-5)	5 (3-5)	5 (5-5)	0.8
Difficulty sitting	5 (5-5)	3 (2-5)	5 (2-5)	4 (2-5)	2.5 (2-3)	0.28
Difficulty in standing up	5 (5-5)	3 (2-5)	3 (2-5)	2.5 (2-5)	2.5 (2-3)	0.27
Difficulty in walking	5 (5-5)	2.5 (2-5)	5 (2-5)	2 (2-5)	2 (2-2)	0.16
Difficulty putting hands on head	5 (5-5)	5 (3-5)	5 (3-5)	5 (3-5)	4 (3-5)	0.73
Difficulty picking up small objects	5 (5-5)	5 (2-5)	5 (2-5)	5 (2-5)	3.5 (2-5)	0.66
Minimum LOS	5 (1-5)	2 (1-5)	3 (2-4)	2 (2-3)	2 (2-2)	0.44
Total LOS	75 (1-75)	51 (1-75)	71 (36-74)	57 (36-66)	47 (36-49)	0.24

Table 4.8: Recovery profile of children of different WFA group with VE based on percentage abnormal LOS

	Z score +1			Z score 0			Z score -1			Z score -2			Z score -3		
	Discharge (n=9)	**1 yr (n=6)**	**2 yr (n=6)**	**Discharge (n=63)**	**1 yr (n=31)**	**2 yr (n=26)**	**Discharge (n=30)**	**1 yr (n=17)**	**2 yr (n=15)**	**Discharge (n=15)**	**1 yr (n=2)**	**2 yr (n=1)**	**Discharge (n=9)**	**1 yr (n=5)**	**2 yr (n=3)**
Median hospital/follow up duration(range) (days)	6.5 (1-11)	346 (254- 400)	649 (584-740)	8 (2- 32)	336 (15-390	639 (570-722)	9 (1-20)	343 (210-631)	634 (600-750)	9.5 (1-42)	278 (210-345)	601 (601-601)	9 (1-21)	321 (178-382)	708 (641-730)
Speech or communication	0	0	0	44	11	4	38	0	0	70	0	0	60	20	33
Feeding	0	0	0	24	7	4	8	6	0	60	0	0	20	20	33
Leaving alone	0	0	0	54	7	4	38	6	0	70	0	0	20	20	33
Behavior	0	0	0	27	7	4	29	6	0	60	0	0	20	20	33
Recognition	0	0	0	10	0	0	8	0	0	40	0	0	40	0	0
School and working	20	0	0	54	14	12	67	6	7	70	0	0	60	20	33
Epilepsy/ seizures	0	0	0	50	14	12	50	6	7	89	0	0	60	40	33
Dressing	0	0	0	35	11	8	21	6	0	56	0	0	60	20	33
Bladder and bowel control	0	0	0	15	0	0	8	0	0	56	0	0	20	0	0
Hearing	0	0	0	5	4	4	8	0	0	33	0	0	0	20	33
Sitting	0	0	0	28	7	4	21	0	0	56	0	0	60	0	0
Standing up	0	0	0	35	11	8	33	0	0	67	0	0	60	20	33
Walking	0	0	0	38	14	8	29	6	0	67	0	0	60	20	33
Hands on head	0	0	0	15	7	4	17	0	0	44	0	0	40	0	0
Picking up	0	0	0	22	11	4	21	0	0	40	0	0	40	20	33
Median minimum LOS (range)	4 (1-5)	5(5-5)	5(5-5)	3 (1-5)	5 (1-5)	5 (2-5)	3 (1-5)	5 (1-5)	5 (3-5)	2 (1-5)	3 (1-5)	5 (5-5)	2 (1-5)	4 (2-5)	3(2-5)
Median total LOS (range)	74 (1-75)	75(75-75)	75(75-75)	68 (1-75)	75 (1-75)	75 (48-75)	71 (1-75)	75 (1-75)	75 (72-75)	57 (1-75)	38 (1-75)	75(751-75)	46(1-75)	74(56-75)	72(56-75)
Improvement		20	0		37	0		69	6		0	0		60	0
No improvement		-	-		46	100		25	94		67	100		40	100
Deterioration		0			17			6			33			0	

Table 4.9: Recovery profile of children of different WFA group with JE based on percentage abnormal LOS

	Z score +1			Z score 0			Z score -1			Z score -2			Z score -3		
	Discharge (n=3)	**1 yr (n=2)**	**2 yr (n=2)**	**Discharge (n=16)**	**1 yr (n=8)**	**2 yr (n=5)**	**Discharge (n=11)**	**1 yr (n=4)**	**2 yr (n=4)**	**Discharge (n=5)**	**1 yr (n=1)**	**2 yr (n=0)**	**Discharge (n=3)**	**1 yr (n=2)**	**2 yr (n=1)**
Median hospital/follow up duration (range) (days)	6 (4-8)	312 (254-370)	637 (619-654)	11 (4-30)	244 (15-390)	660 (605-701)	9 (3- 20)	34 2(229-367)	623 (607-698)	9(1-42)	210(210-210)	0(0-0)	11 (11-21)	250 (178-321)	641 (641-641)
Speech or communication	0	0	0	69	17	0	44	0	0	100	0	0	100	0	0
Feeding	0	0	0	54	0	0	0	0	0	100	0	0	50	0	0
Leaving alone	0	0	0	69	0	0	44	0	0	80	0	0	100	0	0
Behaviour	0	0	0	46	0	0	22	0	0	80	0	0	100	0	0
Recognition	0	0	0	23	0	0	0	0	0	40	0	0	50	0	0
School and working	0	0	0	71	0	0	78	0	0	80	0	0	100	0	0
Epilepsy/ seizures	0	0	0	50	0	0	44	0	0	75	0	0	100	50	0
Dressing	0	0	0	58	17	0	11	0	0	75	0	0	100	0	0
Bladder and bowel control	0	0	0	33	0	0	0	0	0	50	0	0	50	0	0
Hearing	0	0	0	8	0	0	11	0	0	25	0	0	0	0	0
Sitting	0	0	0	58	0	0	33	0	0	50	0	0	100	0	0
Standing up	0	0	0	67	0	0	66	0	0	75	0	0	100	0	0
Walking	0	0	0	67	17	0	33	0	0	75	0	0	100	0	0
Hands on head	0	0	0	42	0	0	22	0	0	25	0	0	50	0	0
Picking up	0	0	0	46	17	0	33	0	0	20	0	0	50	50	100
Median minimum LOS (range)	5 (1-5)	5(5-5)	5(5-5)	2(1-5)	5(5-5)	5(5-5)	3 (2-4)	5(5-5)	5(5-5)	2(2-3)	1(1-1)	0(0-0)	2 (2-2)	3(3-4)	3(3-3)
Median total LOS (range)	75 (1-75)	75(75-75)	75(75-75)	51(1-75)	75(75-75)	75(75-75)	71 (36-74)	75(75-75)	75(75-75)	57(36-66)	1(1-1)	0(0-0)	47(36-49)	73(72-74)	72(72-72)
Improvement		0	0		25	0		0	0		0	0		100	0
No improvement		100	100		50	100		100	100		0	0		0	100
Deterioration		0			25			0			100			0	

Chapter 5: Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

Abstract

Background: Acute encephalitis syndrome (AES) is a group of symptoms and signs used by World Health Organization (WHO) to screen patients of viral encephalitis (VE). There is no definite treatment for most of the cases of VE. Therefore, role of supportive management including fluid treatment may be crucial to improve outcome. Previously, low admission weight has been associated with bad outcome which could have been due to dehydration or malnutrition. Therefore, I decided to investigate the relationship of fluids and acid-base status with low admission WFA and loss of weight in hospital and correlate these with outcome.

Methods: All children aged 1 month to 14 years with fever and altered sensorium and/or new onset seizures from September 2011 to September 2012 attending Kanti Children's Hospital, Kathmandu, Nepal were recruited. Weight-for-age (WFA) using Z score and serum lactate were assessed at admission and discharge. Total fluid input and output was monitored daily.

Results: Of the 92 patients, 62% had admission low WFA or weight loss during hospital stay (low weight group) and 38% without low WFA or no

weight loss (normal weight group). There was 19 times risk of death and 7 times risk of bad outcome (death or sequelae) in low weight group compared to normal weight group. Bad outcome was significantly associated with less admission WFA, more fluid deficit, and trend for higher admission serum lactate. Death was significantly more in patients of longer duration of illness, low admission WFA, more weight loss during hospital stay, more 5% dextrose and 0.45 normal saline recipients, higher sodium and higher urea at admission.

Conclusion: Optimum & appropriate fluids may be a life-saving treatment option in children with AES. A randomised control trial of different volume & types of fluids is recommended.

5.1 Introduction

Acute encephalitis syndrome (AES) is a group of symptoms consisting of acute onset of fever, acute change in mental status and/or new onset of seizure (1). Viruses are regarded as the most important cause of AES in the world. In Asia, the major identified cause of acute encephalitis is by Japanese Encephalitis virus (JEV). However, it can also be caused by other organisms such as bacteria or parasites.

Over 50,000 children present every year to different hospitals with clinical features suggestive of acute brain infection or AES, in Asia, with over 30% of these patients dying or suffering from significant neurological problems following their acute illness (1). In the vast majority of cases there are no definite treatments, although antibiotic and antimalarial drugs are useful for

some of them. Supportive management with adequate intravenous fluids maybe crucial for their survival along with mitigation of the secondary complications, such as seizures and raised intra-cranial pressure. There are guidelines for provision of fluid among patients with impaired consciousness. The fluids generally used are 0·18% saline with 5% dextrose in children (92). Normal maintenance fluid requirement is equal to insensible, faecal and urinary loss. In practice, maintenance fluid is estimated using Holliday-Segar method for calculating maintenance fluid based on the child's weight. They are often delivered at the rates of 100 ml/kg for the first 10 kg of bodyweight, 50 ml/kg for the next 10 kg, and 20 ml/kg for bodyweight exceeding 20 kg (92, 141).

Some standard pediatric literature caution that hypotonic intravenous fluids given at maintenance rates might be unsafe, especially in hospitals in developing countries where serum sodium concentration often cannot be measured (92). Even fluid bolus provision to children with shock, including those who were encephalopathic, has been linked with poor outcome as compared to normal maintenance fluids (142). Hence, there is a general practice in all children with severe altered sensorium (modified GCS < 8) or having clinical features suggestive of raised intracranial pressure to have their fluid intake restricted to two- thirds of maintenance. However, these guidelines have largely been based on traumatic brain injury (89). There is very limited data available indicating the optimum fluid management for patients with non-traumatic brain injury (93, 143, 144).On the contrary, the World Health Organisation (WHO) recommends full maintenance fluids for the routine treatment of bacterial meningitis with an emphasis on glucose and

not sodium content. This is partly based on concerns about dehydration (145). Excessive fluid restriction has been shown to be independently associated with a poor outcome among traumatic brain injury patients (89). More importantly, fluid and sodium restriction have also been reported to have poor outcome in JE patients (93). My previous study also suggested hospital admission with supportive management, including adequate fluids, improved outcome (75).

Fluid provision, with a need to maintain cerebral perfusion pressure without worsening cerebral oedema, is a delicate balance when managing brain injured children. Hence, without specific treatment with anti-viral drugs, optimum supportive management with adequate fluid and acid base status, which may be contributing to raised intracranial pressure and convulsions causing secondary deterioration of patient's health, beside the primary insult, seem vital. This re-opens the question as to what and how much fluids should be provided in acute infectious illness, and makes the area of brain infection even more vexing (20, 123).

My previous study showed patients with JE were of lower weight and had worse outcome as compared to non-JE patients (chapter 3)(75). My another study also showed that there was significantly progressive increase in residual neurological sequelae as the WFA Z score decreased in both AES and JE patients (chapter 4). Those AES and JE patients who had low WFA at the time of admission had significantly worse outcome including residual neurological sequelae at the time of discharge and even at 2 years follow up. Low WFA could have been either by dehydration or malnutrition. It is important to find

out which one of these is contributing to low WFA, because the management is different, which could affect outcome.

Since more than half of the AES patients attended hospital by self referral in my previous study (chapter 3) (75), a simple marker of malnutrition which was cheap, easily available, user friendly, applicable at community level without much training to the community health workers, seemed necessary. Although measurement of weight-for-height, BMI or blood micronutrients level may have been more appropriate for screening malnutrition from dehydration, measurement of mid upper arm circumference (MUAC) was more suitable in a rural based resource poor setting like Nepal. Measurement of loss of weight after admission, gold standard method for assessment of dehydration, would also help in identifying dehydrated patients. Besides clinical history and examination finding of dehydration, indirect markers of dehydration such as oxygen saturation, change in temperature, capillary refill time, change in blood lactate level, blood urea, serum electrolytes and total fluid balance would also be helpful in separating dehydration from malnutrition.

In this study, I therefore aimed to find out the relationship of fluids and acid-base status with low admission weight and weight loss in the hospital. In the absence of consensus on what is the optimum fluid management for children with AES, I also aimed to conduct a systematic investigation of the relationship between fluid and acid base balance and the outcome of AES patients.

5.2.1 Aim

The aim of this study is to assess relationship between fluid balance, change in weight, acid base status and outcome in children with acute encephalitis syndrome

5.2.2 Objectives

1. To describe the association of low admission weight and weight loss in the hospital, as clinical marker of dehydration, with outcome of patients of acute encephalitis syndrome.
2. To describe the association between changes in weight and blood lactate levels, at admission and discharge, as indicators of hydration and acid base status, and outcome in children with acute encephalitis syndrome.

5.3 Methods

The study was conducted at Kanti Children's Hospital, a tertiary level public paediatric hospital located in Kathmandu. Presence of various sub-speciality services in paediatrics such as surgery, intensive care, radiology, neurology, cardiology, endocrinology, nephrology, dermatology, orthopaedics, otolaryngology, dentistry makes it one of its kind in the country. It also makes it the first choice of referral for sick children from all over the country.

A hospital based prospective cross-sectional study was conducted. All children of AES as defined by WHO surveillance standard, presenting to Emergency Department, Paediatric Ward and Paediatric Intensive Care Unit of Kanti children's hospital, Kathmandu, Nepal, were seen either by me or my research assistant. The research assistant received one week of orientation training about

the research methodology before starting the research activities in order to reduce inter- observer variability of research findings.

A detailed history was then taken from the accompanying parents or guardian. Complete clinical examination including a full neurological examination was then performed. Attention was given to the child's weight, mid upper arm circumference (MUAC), clinical signs of dehydration, capillary refill time, peripheral oxygen saturation and axillary temperature. Weight was measured using a digital weighing scale which was quality controlled every morning using standard weights.

MUAC is the circumference of the left upper arm, measured at the mid-point between the tip of the shoulder and the tip of the elbow (olecranon process of the ulna bone and the acromion process of scapula) (146). For that, initially, straight distance was measured between the acromion process of the scapula at the tip of the left shoulder and the olecranon process of the ulna, through the midline, over the triceps, on the posterior aspect of the left upper arm, with the arm hanging loosely and comfortably at the side. The midpoint was determined by measuring the distance between the two landmarks using a tape calibrated in centimeters. The lateral side of the arm was marked with a visible marker pen and measurement of the circumference of the arm at the mark taken by a non stretchable plastic tape. During measurement it was made sure that the tape was not twisted and was parallel to the marking. Measurements were recorded to the nearest 0.5 mm.

Blood was taken for a full blood count, differential leukocyte count, urea, creatinine, sodium, potassium, glucose, liver function test, bacterial culture, viral serology and serum lactate level. A lumbar puncture was performed and CSF taken for leukocyte count and differential, glucose, protein, microscopy and culture, and detection of antibodies for viral (anti-JE IgM ELISA) and bacterial pathogens [antibody titres against specific bacteria *Streptococcus pneumoniae, Hemophilus influenza* type B, *Niesseria meningitidis* measured via a commercial kit (PASTOREX MENINGITIS, BIO-RAD, France, 2011)].

All children fulfilling inclusion criteria mentioned in chapter 2 were included in the study. Written consent was requested from the parents or accompanying guardians, prior to enrolling the children in the study. Patients were assessed (including weight, and blood lactate and oxygen saturation (Spo2) at admission, at 48 hours after admission and again at discharge by a member of the study team. Total fluid input (oral, nasogastric and intravenous including diluents for drugs) and output (urine, vomit) was monitored daily after admission until discharge. The input and output were assessed from the treatment chart, nurses notes, asking the attendant and researchers observation. Use of other supportive management procedures such as head positioning, sedatives (including anti-epileptic drugs), mannitol and analgesia provision were also recorded. All the information was recorded on a case record form (Appendix M). Further details of the methodology is described in chapter 2 (General Methods).

5.3.1 Case Definition

Case definitions followed were as described in Chapter 2.

5.3.1.1 Weight-for-age Z scores: WFA was defined using a z score as described in chapter 4. In this study, I stratified subjects by their WFA Z score and examined outcome among these groups [high WFA: Z score +1 (Z score between +1 and +2), average WFA: Z score 0 (Z score between +1 and -1), mild low WFA: Z score -1 (Z score between -1 and -2), moderate low WFA: Z score -2 (Z score between -2 and -3), severe low WFA: Z score -3 (Z score more than -3)](91). Although, low WFA means under-nutrition, in acute conditions such as AES, it may also suggest poor fluid intake (or dehydration).

5.3.1.2 Low WFA: WFA z-score <0

5.3.1.3 Without low WFA: WFA z-score ≥ 0.

5.3.1.4 Weight Loss: Children who lost weight during hospital stay between admission and discharge (or death).

5.3.1.5 No Weight Loss: Children who did not lose weight during hospital stay (from admission until discharge or death).

5.3.2 Ethics

The study was approved by the Ethical Committees of the Nepal Health Research Council and the Liverpool School of Tropical Medicine as well as the Institutional Review Committee of Kanti Children's Hospital.

5.3.3 Data analysis

Data was coded and entered in statistical package for social sciences (SPSS) Statistics software version 17.0 (IBM-SPSS, New York, USA) and epi info 6. Percentage, Proportions and Contingency tables will be used for description of the data. All the clinical features, laboratory parameters and outcomes of children with AES with low weight-for-age were compared with normal weight-for-age. Normally distributed data was analysed using the Student's t-test, non-normally distributed data using Mann-Whitney U test and differences between proportions by Fischer's Exact test or Chi square test using Yates correction as necessary. Regarding comparison of three groups, normally distributed data were compared using ANOVA and skewed data were compared using Krushkar- Wallis test. $P < 0.05$ was taken to be statistically significant.

5.4 Results

Out of 143 patients admitted with a diagnosis of AES with MUAC ≥ 11.5 cm, 39 (27%) had bacterial meningitis, 7 (5%) typhoid encephalopathy and 5 (3%) patients could not have their discharge weight recorded and were excluded from the study (Figure 5.1). Of the remaining 92 (64%) AES of suspected viral aetiology, 4 (4 %) were JE, 68 (74%) confirmed non-JE and 20 (22%) were of unknown aetiology including unknown JE status. Low WFA was seen in 50 (55%). Amongst the low WFA group, 30 (33%) were mild (WFA Z score between -1 and -2), 10 (11%) moderate (WFA Z score between -2 and -3) and 10 (11%) were severe (WFA Z score below -3). Of the total, 30 (33%) had weight loss after admission. Overall, 23 (25%) were of low WFA and had further weight loss after admission, 27 (29%) were low WFA and had

no weight loss, 7 (8%) did not have a low WFA and had weight loss during admission and 35 (38%) did not have low WFA and no weight loss after admission.

Considering low weight may be associated with dehydration and weight loss with fluid loss, I grouped the participants in two groups as low WFA and/or weight loss and without low WFA and/or no weight loss. When patients of low WFA and/or weight loss and without low WFA and/or no weight loss were grouped together, 57 (62%) were of low WFA and/or weight loss and 35(38%) without low WFA and/or no weight loss. Bad outcome was seen in 59 (64%) patients and good outcome in 33 (38%) (*P*- 0.0002). Bad outcome was significantly higher in those with a low WFA (*P*- 0.005) but not those with weight loss (*P* - 0.13) during admission. Together in those with both low WFA and/or weight loss, there was 7 times risk of bad outcome as compared to good. (Table 5.1) Regarding death, those with low WFA and/or weight loss were 19 times at greater risk of death (Table 5.1).

The clinical features and laboratory parameters of the two groups are shown in Table 5.2. Children with bad outcomes were significantly younger, shorter with lower mid-upper arm circumference (MUAC). They also had prolonged fever and headache duration, more episodes of seizures, focal neurological deficits and lower levels of consciousness. MUAC and height was even lower in the low WFA and/or weight loss group amongst those who had bad outcome. In those who had a good outcome, the low WFA and/or weight loss group had a significantly longer fever duration than those without low WFA and/or no

weight loss. Serum sodium and potassium were significantly higher in the bad outcome group. There was also tendency of higher urea in those who had bad outcome especially in low WFA and/or weight loss group although it was not significant. Also, children who had bad outcome had received significantly more phenytoin and phenobarbitone as compared to those who had good outcome.

Residual neurological sequelae were measured using the Liverpool outcome score (LOS). In children of low WFA and weight loss group, the mean score on the LOS was significantly lower as compared to those without low WFA and no weight loss group [2.9 ± 1.5 versus 34.25 ± 1.22 (*P*- <0.0001)]. Total LOS was significantly lower in the low WFA and/or weight loss group as compared to those without low WFA and no weight loss [51.59 ± 30.19 versus 69.7 ± 14.78 (*P*- <0.0001)].

The total fluid balance constituents monitored are shown in Table 5.3. Children who had good outcome received significantly more intravenous fluids. Even though WFA Z score was used to categorize weight, these children were older and had higher GCS, they could demand and take significantly more fluids orally. Their oral fluid intake increased significantly by 48 hours in those with good outcome as compared to bad outcome group. Interestingly, there was big disparity between IVF ordered by the doctor, written in nurse's note, attendant's observation and what was actually given on the day of admission and after 48 hours. On both the days actual IVF administered was much less than the IVF ordered by the doctor in the bad outcome group as compared to good outcome group.

The fluid status of the recruited patients is shown in Table 5.4. Those who had a bad outcome had a significantly prolonged illness, weighed less at admission and at discharge. They also had significantly higher fluid requirement, fluid deficit and were restricted from receiving full maintenance IVF. Serum lactates at the time of admission were higher in those who had bad outcomes.

When outcomes were grouped into death, residual neurological sequelae and complete recovery, those who died had a significantly longer duration of illness, a lower GCS, more focal neurological deficits, lower WFA Z score, more loss of weight during admission, higher blood sodium and urea during admission. They also had higher serum lactate on admission and higher rise of serum lactate after 48 hours. This all suggest that these children were sicker and more dehydrated than those who survived (Table 5.5). These children had also received significantly more 0.45% normal saline with 5% Dextrose as maintenance IVF as compared to those who had survived with sequelae and recovered completely. Those who had complete recovery, had significantly less fluid requirement, less reduced fluid intake and less fluid restriction and therefore less fluid deficits with progressively increased oral fluid intake and urine output from admission up-to 48 hours.

5.5 Discussion

In this study I found children of AES presenting to the hospital with low WFA Z score were significantly associated with bad outcomes. The odds increased to 7 times more likelihood of having a bad outcome if the patient has a low WFA and/or weight loss. The odds of dying was 13 times more if the patient had a

low WFA Z score at the time of admission, 10 times more if they had weight loss and 19 times if they had a low WFA Z score and/or weight loss. In these children, weight could be representative of body water. Since the MUAC of all children recruited were ≥11.5 cm, we have considered acute loss of weight to be simple surrogate marker of dehydration (141). Therefore, children with low WFA and/or weight loss were dehydrated.

Children who suffered bad outcome were significantly younger, had prolonged fever and headaches, lower GCS and more focal neurological deficit suggesting they were sicker than good outcome group. In addition, in the bad outcome group, more number of children were found with low WFA Z score on admission, more fluid requirements, more fluid deficits, more number of children with fluid restrictions during admissions, lesser intake of oral fluids, higher serum sodium and urea. All of these findings suggest that they these patients were sicker and more dehydrated than the good outcome group. The state of hypertonic hypovolemia evident in these children also confers the likelihood of dehydration (147). This became more evident when the outcome was further sub-grouped as death, sequelae and complete recovery. Significantly more number of children died who had low WFA Z score, higher admission serum urea and sodium and weight loss after admission. Significantly more weight was lost during admission in these children who died as compared to sequelae and recovery group. Admission serum lactate levels were higher and increased further on the second day of admission in this group as compared to others. Not surprisingly, those who recovered completely, were significantly older, had higher GCS scores, suffered less focal neurological

deficits, required lesser maintenance IVF, suffered lesser fluid deficiencies, were less fluid restricted and had more oral fluid intake than those who died or had sequelae indicating good hydration status.

Dehydration is common in children of AES. It may be because they are very young and depend on others for food and drinks and even if older and independent, they are unable to feed because of altered sensorium and neurological deficits. There is also tendency to restrict total fluids to two-thirds of maintenance fearing cerebral oedema by treating clinicians in these children. Adequate perfusion of all the tissues is essential for them to function normally. Dehydration can cause cerebral hypo-perfusion and ischemic injury to the brain causing anxiety, confusion and stupor. It is known to increase risk of cerebral infarction in subarachnoid haemorrhage (93) causing neurological deficit. Renal hypo-perfusion can cause acute renal failure and anuria, hypo-perfusion of the gastrointestinal tract causes erosive gastritis or ischemic pancreatitis, hypo-perfusion of the liver, ischemic hepatitis. It can also cause coagulation abnormalities and thrombocytopenia. The odds of having bad outcome in children with low WFA and/or weight loss can be explained by demonstrating clinical and laboratory evidence of dehydration in them. A significant number of children who suffered a bad outcome were fluid restricted in our study. Restriction of fluids has also been shown to cause adverse outcomes in both traumatic and non-traumatic brain injury patients in other studies (89, 93). Hydration status may also have a role in drug therapy. In dehydration, the volume of distribution of drug may decrease so that even a standard dose for given age and weight may become toxic and produce adverse effects. In

these conditions, normal doses of aminoglycosides could be nephrotoxic and phenobarbitone highly sedative causing adverse outcome.

Dehydration decreases oxygen delivery to cells which reduces availability of oxygen in the energy cycle and promotes anaerobic metabolism. This causes oxidative fermentation of pyruvate to form lactate, which accumulates to cause metabolic acidosis. Excessive production of pyruvate may also cause lactic acidosis (148). Acidosis further shifts the oxygen-haemoglobin dissociation curve to the right, decreasing haemoglobin's affinity for oxygen, and hence decreasing oxygen delivery. If not corrected, severe acidosis can impair the body's metabolic process, impede neurovascular interaction and even prevent pharmacological action of various drugs. This may cause life threatening arrhythmias, myocardial depression, respiratory muscle fatigue, seizures, shock and multi-organ failure. Elevated serum sodium, serum urea, serum creatinine and serum lactate levels on admission with supportive clinical information in the patients who died showed presence of metabolic acidosis with impending renal failure in our study. The median serum sodium was significantly high in those who suffered bad outcomes as compared to good [bad outcome, 139 (122- 172) versus good outcome, 131 (116- 142) (*P*- 0.001)] and those who died as compared to survivors [death, 145 (139- 172) versus survived, 134 (116- 149) (*P*- <0.0001). Raised serum and CSF lactate levels have been previously been reported in both traumatic and non-traumatic brain injury patients who have complications (20, 95- 97). Therefore, adequate hydration to prevent metabolic acidosis and maintain sufficient cerebral perfusion without causing cerebral oedema or rise in intracranial pressure is the key in the management of children with brain injury.

Total body water in the body is spread over two-thirds of the body as intracellular fluid and one-third as extracellular fluid (ECF). A quarter of ECF is in the intravascular space as plasma and the rest is extravascular (interstitial space). A decrease in plasma water causes an increase in plasma sodium concentration and osmolality which is sensed by nuclei in the hypothalamus which results in increased production of ADH and stimulates the thirst mechanism. ADH causes increased renal free water reabsorption in the collecting tubule in the kidney to restore plasma water so that plasma sodium concentration is brought back to normal. Renin-angiotensin-aldosterone system and atrial natriuretic peptides both involved in thirst mechanism also regulate fluid volume by altering sodium excretion. In the cerebral circulation, endothelial tight junction prevents easy passage of sodium through the blood brain barrier (BBB). With an intact BBB, a fall in osmolality by 5 mmol/L decreases osmotic pressure between capillary lumen and brain interstitial space by 95 mmHg. This results in movement of water instead of sodium from hypo-osmolar area towards hyper-osmolar area. This causes accumulation of water in the brain cells or interstitial space to develop cerebral oedema (92, 149).

A Child's daily requirement of sodium as 3mEq/Kg, potassium 2mEq/Kg and glucose 5g/Kg is estimated based on electrolyte composition of human and cow milk. Normally, an infant weighing 6 Kg administered full hypotonic maintenance fluid as 5% Dextrose with 0.18% saline almost receives all the required elements as 100ml/ Kg /day of water, 3 mmol/Kg of sodium and 3.5 mg/Kg of glucose. This estimation is based on normal physiology of a child. However, in severe infections including AES, hyponatremia develops in 20-

45% patients with increasing sodium requirement (92, 150). Therefore, the use of hypotonic saline in these children can further increase risk of severe hyponatremia (141, 151, 152). In this study, significantly more children who died had received 5% Dextrose with 0.45% saline. Also there was a positive trend for those who received 5 % dextrose (0.9%) normal saline to completely recover. This shows that the maintenance IVF calculated based on the normal sodium requirement of healthy children may not have been suitable for severely ill children of AES who may have a higher sodium requirement. Therefore isotonic fluid such as 5% dextrose (0.9%) normal saline may have been more suitable for these children (152).

In this study, elevated serum sodium on admission was suggestive of metabolic acidosis. However serial measurement of electrolytes, lactate and blood gas analysis during hospital stay and discharge would have confirmed this and guide fluid management. Because of the complications related to the illness and fluid status, no single maintenance fluid is ideal and needs to be individualized based on serial findings of body sodium measurement. I also found children who were taking oral feeds significantly associated with good hydration and outcome. Although it can be argued that those children could have been less sick. However, even the markers of dehydration were suggesting good hydration status in these children. Therefore, close attention is required to IVF until the child becomes conscious and can take oral feeds at liberty because one's own body is the most accurate accessor of salt and water needs.

Seizures are a treatable complications of AES and measures aimed at controlling them improve outcome (20). The most accepted pathogenic

mechanism for development of seizures in encephalitis is that of extensive inflammatory response. The inflammatory peptides, which are produced by neurons, astrocytes, and microglia, are increasingly recognized to promote excitatory neurotransmitter release and initiate depolarization which then causes release of certain proinflammatory cytokines, by means of co-stimulation. This dynamic interplay between cytokines, chemokines, neurotransmitters, and other factors are thought to be the likely cause of seizures (153, 154).

I found significant association between more episodes of seizure and bad outcome. The episodes were more frequent in the dehydrated group than the well hydrated group. Median serum sodium level was also significantly higher in those who were dehydrated at the time of admission (*P*-0.049). In those who had seizures, serum sodium level was significantly higher amongst those who had a bad outcome (*P*-0.006); specially those who died (p- <0.0001). There was also no association of serum glucose with seizures (*P*- 0.6), bad outcome (*P*- 0.5) or death (*P*- 0.6).There is 6.8% risk of having unproved seizure in central nervous system infections (53). Single episodes of seizure occur in around 40% of patients of JE but do not affect outcome. However, repeated seizures and status epilepticus are associated with bad outcome (20). Seizures can occur in AES because of cerebral oedema, neuronal injury and neuronal necrosis as a result of inflammatory changes of the brain (53). In JE, cerebral oedema, haemorrhage and formation of thrombus has been reported to cause cerebral hypoxia and seizure (53). Sometimes co-morbidity with cysticercosis can cause seizures (155). However, I found significant association of repeated

seizures with dehydration and metabolic acidosis. Therefore significantly increased disabilities observed at discharge in the dehydrated group could be the result of cerebral ischemia, acidosis and seizures.

Other causes of seizure in dehydration can be due to hypernatremia, hypoglycaemia, hyperlactataemia because of metabolic acidosis, hyponatremia of SIADH in AES, cerebral venous thrombosis, cavernous sinus thrombosis, fever triggered seizure, febrile seizure or CO2 retention. Seizures triggers a vicious cycle of increased cerebral blood flow, then cerebral oedema, raised ICP, reduction in cerebral blood flow, then exacerbation of hypoxic cerebral metabolism, then further increase in cerebral oedema, raised ICP until brainstem herniation. Seizures also causes increased use of anti-epileptic drugs. There was significant association of bad outcome and death in those who were on phenytoin and phenobarbitone. Anti-epileptic drugs can cause excessive sedation, clouding of consciousness, suppression of neurological signs, inhibition of respiration, choking episodes and aspiration pneumonia.

Some of the patients included could have been malnourished. Since all of the children recruited had MUAC $\geq$ 11.5 cm, we had used MUAC to separate children of malnutrition from dehydration. MUAC is a simple age independent anthropometric index which can be conducted at initial evaluation to assess nutritional status. Weight-for-height would have been better if it was possible in children with altered sensorium. Some of the patients of dehydration could have been malnourished or could have co-morbidity. Administering IVF in these children is often difficult because they could develop congestive cardiac

failure at normal rate and volume of administration due to inability to handle normal fluid volume having limited cardiac reserve. Therefore, malnutrition status could affect outcome adversely.

I used only indirect markers of dehydration in my study. Direct markers such as blood ph by arterial blood gas and serum osmolality would have been more accurate in categorising patients to be suffering of metabolic acidosis from dehydration.

In conclusion, AES patients with dehydration suffer bad outcomes. Fluid is a simple method of treatment. The process of accurately carrying out doctors' advice in the ward rounds should be reviewed and strengthened. Present Holliday-Segar recommendations of hypotonic maintenance IVF is not suitable for hospitalized children with AES. IVF needs to be individualized based on the findings of daily measurement of weight, fluid balance, electrolytes (especially serum sodium level) and clinical parameters. There is no definite treatment for most of the viral encephalitides including JE, but maintaining good hydration status with adequate and appropriate IVF may potentially be a simple, cheap, easily available treatment option which could save lives even in resource scarce settings like Nepal. However, only a randomised control trial of different volume (two- third versus full maintenance) and types (DNS, 0.9% NS, 0.45% NS, 0.18% NS) of fluid would be able to prove that increased and appropriate fluid intake could improve outcome.

5.6 Limitation of study

In this study, low WFA at admission and loss of weight after admission were together considered to be markers of dehydration. Both were significantly associated with death. Those who acutely lost weight during hospital stay are likely to have suffered dehydration. However, patients having low WFA on admission cannot be confirmed to have dehydration. In these patients, some could have been malnourished. Further, subanalysis, to examine the clinical features and laboratory parameters of dehydration in LWFA and the weight loss groups may have been helpful.

Anthropometry, including MUAC, has poor specificity to diagnose malnutrition because changes in body measurements are sensitive to multiple factors including intake of essential nutrients, infection, altitude, stress and genetic background. Therefore, some of the patients I included in the study with MUAC ≥ 11.5 cm could have been malnourished.

MUAC < 11.5 cm is a useful screening tool for assessment of malnutrition in children between 1 and 5 years of age in the community. However, it cannot confirm malnutrition in children below 1 year and above 5 years of age. In addition, MUAC < 11.5 cm was used to screen patients of severe malnutrition. This meant that there was possibility of patients of moderate and mild malnutrition to be included in this study which could have affected outcome. There is a possibility that more patients with protein energy malnutrition (PEM), as defined by WHO, could have been excluded if we had taken MUAC <12.5 cm as a cut off value. This has also highlighted the need to validate MUAC <11.5 cm and < 12.5 cm as a marker of severe and moderate malnutrition in Nepali children.

In patients with LWFA and weight loss during hospitalization, I used indirect markers of dehydration and acidosis such as blood lactate, oxygen saturation, capillary refill time. More direct markers such as blood pH and urinary osmolality may have been more accurate. Comparison of clinical features, examination findings and markers of dehydration in patients of MUAC ≥ 11.5 cm and <11.5 cm could have helped characterize dehydration even more accurately.

Weight for height, BMI, Kanawati index, Dugdale index or blood micronutrients level may have been more accurate markers of malnutrition in a well resourced setting. However, I chose MUAC as marker of malnutrition because it was affordable and easily applicable at community level in my resource poor setting.

I included only patients whose LP results suggested AES of viral aetiology because I wanted to accurately describe the finding in patients of viral encephalitis since there was no treatment in majority of the cases. This caused exclusion of patients who did not undergo LP, which could have been because they were very sick and were at risk of procedure related complications. Exclusion of these patients could have affected the study results.

I monitored patients until discharge. Outcome is more accurately characterised as good or bad until 3 months of illness in patients of JE. Therefore, follow up patients up to 6 months for eventual outcome would have been preferable.

Since JE cases decreased over the years after introduction of JE vaccine, there is a need to validate clinical features of AES as defined by WHO surveillance standard, as a screening tool to diagnose other viral encephalitis beyond JE in Nepali children.

5.7 Summary

As the admission WFA Z score decreased there was a significant increase in bad outcome and neurological sequelae in JE and AES patients in my previous study. Therefore, I measured weight of all children at the time of admission or on initial assessment to find out the hydration status of the patient before coming to the hospital and commencement of fluid therapy; and at the time of discharge or death which would reflect receipt of fluids during hospital stay. I also looked for admission serum lactate level to assess hydration and acidosis before hospital admission; and after 2 days to assess dehydration level after admission and commencement of fluid therapy.

In addition, I assessed markers which seperated dehydration from malnutrition by measurement of serum electrolytes and MUAC (≥ 11.5 cm) at the time of admission. Daily monitoring of overall fluid intake such as oral, naso-gastric, diluents of drugs and IVF were done. Daily monitoring of output such as urine and vomitus were also done. Daily fluid intake and output was recorded from treatment chart for doctors order, from nursing notes for what the nurse administered, from interview of attendant for what they observed and from researchers own observations. Interestingly, there was also a mismatch between what was actually given by the staff and that what the doctor ordered.

Since dehydration can be assessed by weight loss, I considered patients with weight < 0 WFA Z score as low WFA group, weight ≥ 0 WFA Z score as without low WFA group, weight lost during admission as weight loss group and no weight loss during admission as no weight loss group. For analysis, low WFA at admission and weight loss during admission were grouped together as dehydrated group and patients without low WFA at admission and no weight loss during admission as non-dehydrated group.

Bad outcome was significantly associated with lower admission WFA Z score, more fluid deficit, and a trend for a higher admission serum lactate. Death was significantly increased in patients with weight loss during admission, low WFA Z score and with a longer duration of illness. Bad outcome patients were also more frequently prescribed 5% dextrose and 0.45 normal saline and had higher sodium and higher urea at admission. There was a 9 times higher the risk of death in the weight loss group. There was also a 19 times higher the risk of death and 7 times higher risk of bad outcome (death or sequelae) in the LWFA and weight loss combined group compared to other group. As in the UK, hospital oral and intravenous fluids guidelines need to be written for children with impaired consciousness. This could be based on existing Advanced Paediatric Life Support (APLS) guidelines supported by Liverpool Life Support Group with a training being conducted in Nepal, every year.

To investigate this further a randomised controlled trial of different types and volumes of fluids in admitted patients of AES and JE could enable us to see whether the association is casual. Then identifying aetiology of AES and

providing aetiology based treatment and immunisation schedules against the disease would also help in improving outcome among AES patients. Since JE was the most common identified aetiology in AES patients identifying new adjunctive treatments for JE, such as IVIG may also help in reducing morbidity and mortality of AES patients in Nepal. Whilst fluid management is important for all causes of AES, for JEV there is the possibility of an adjunctive treatment with IVIG containing neutralizing anti-JEV antibody. I therefore assessed this with a pilot study, described in the next chapter.

Table 5.1: Outcome of the patients based on initial weight and weight loss after admission

	Bad outcome (n=59)	**Good outcome (n=33)**	**P-value**	**OR (95% CI)**
Low WFA	39 (66%)	11 (33%)	0.005	3.9 (1.5- 10.7)
Weight loss	23 (39%)	7 (21%)	0.13	2.4 (0.8- 7.2)
low WFA and/or weight loss	45 (76%)	12 (36%)	0.00005	7.2 (2.5- 20.9)
	Death (n=13)	**Survived (n=79)**	**P-value**	**OR (95% CI)**
Low WFA	12 (92%)	38 (48%)	0.007	13 (1.6- 279)
Weight loss	10 (77%)	20 (25%)	0.0006	9.8 (2.2- 50.7)
low WFA and/or weight loss	12 (92%)	45 (57%)	0.015	19.1 (1.1- 195)

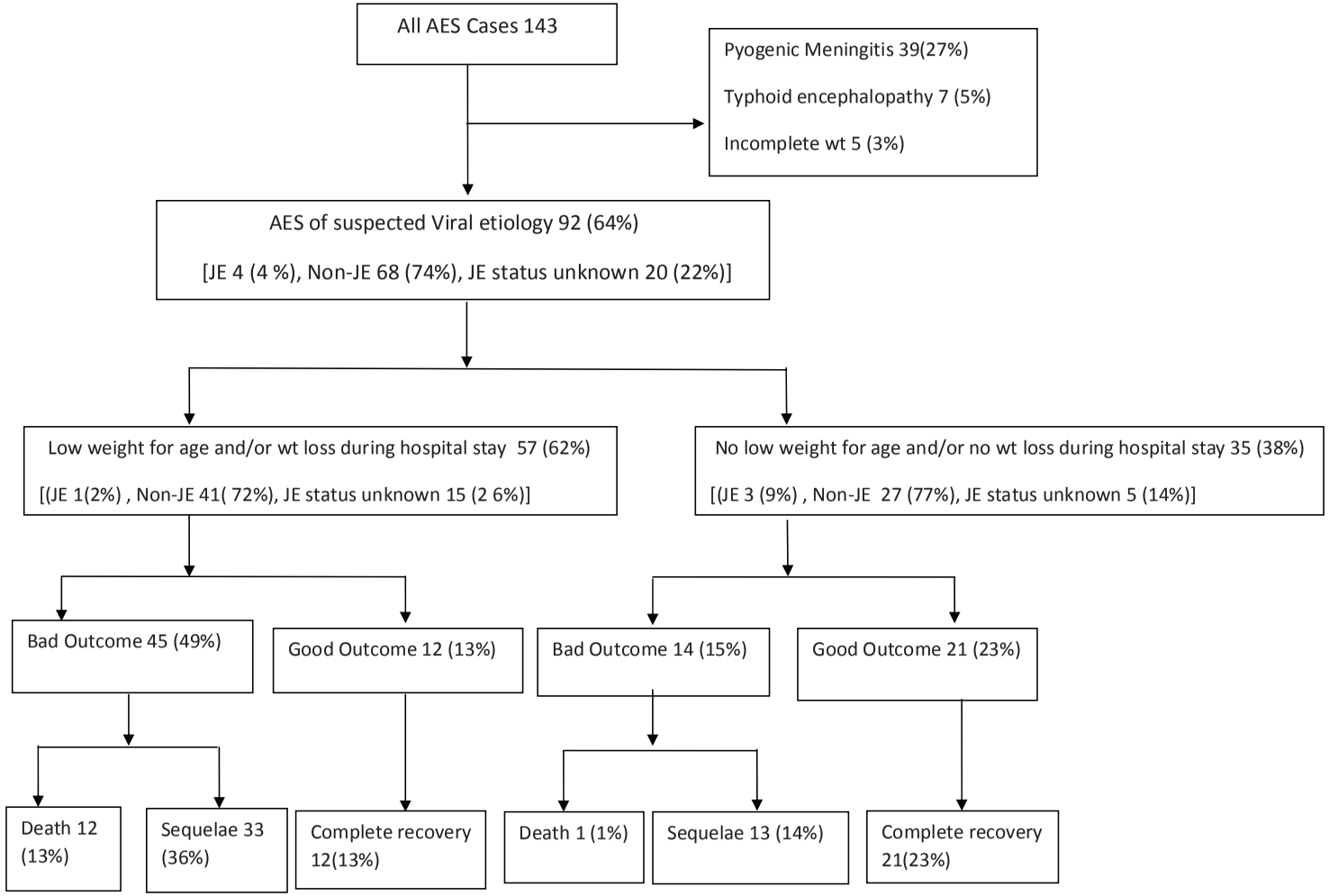


Figure 5.1: Study Profile

Table 5.2: Clinical features and laboratory parameters of the recruited patients at admission

Clinical Feature	Bad outcome				Good outcome				
	Low WFA and/ or weight loss	No Low WFA and/ or no weight loss	Total	*P*-value	Low WFA and/ or weight loss	No Low WFA and/ or no weight loss	Total	*P*-value	*P*-value
	N=45	N=14	n= 59		n= 12	n=21	N= 33		Bad vs good
Age (years)	2.5 (3- 14)	7.5 (2-13)	3 (2- 14)	0.1	7 (4- 13)	8 (2- 14)	7 (2- 14)	0.7	0.035
Male	32 (71)	8 (57)	40 (68)	0.26	7 (58)	11 (52)	18 (55)	0.5	0.15
Fever duration (days)	6 (1- 20)	7.5 (3- 25)	6 (1- 25)	0.11	6 (2- 11)	3 (2- 10)	4 (2- 11)	0.02	0.006
Presence of altered sensorium	42 (93)	13 (93)	45 (76)	1	9 (75)	19 (90)	28 (85)	0.32	0.48
Presence of headache	6 (13)	4 (29)	10 (17)	0.18	5 (42)	6 (29)	11 (33)	0.35	0.06
Headache duration	7.5 (6- 13)	8.5 (5- 11)	7.5 (5- 13)	0.72	4 (3- 11)	2.5 (1- 4)	4 (1- 11)	0.13	0.005
Presence of vomiting	14 (31)	5 (36)	19 (32)	0.49	5 (42)	13 (62)	18 (55)	0.2	0.03
Vomiting duration	1 (1- 5)	1 (1- 10)	1 (1- 10)	0.9	3 (2- 4)	2 (1- 4)	2 (1- 4)	0.21	0.32
Presence of diarrhea	4 (9)	0 (0)	4 (7)	NA	1 (8)	2 (10)	3 (9)	0.7	0.5
Diarrhea duration	1.5 (1- 5)	0 (0- 0)	1.5 (1- 5)	NA	2 (2- 2)	2.5 (1- 4)	2 (1- 4)	1	0.86
Presence of seizure	40 (89)	13 (93)	53 (90)	0.56	9 (75)	16 (76)	25 (76)	0.63	0.069
Presence of GTCS	38 (84)	12 (86)	50 (85)	0.58	8 (67)	15 (71)	23 (70)	0.6	0.5
Seizure episodes	10 (1- 12)	7 (1- 10)	10 (1- 12)	0.66	4 (1- 10)	1.5 (1- 10)	2 (1- 10)	0.28	0.001
Seizure duration	3.5 (1- 600)	3 (1- 10)	3 (1- 600)	0.08	4 (1- 12)	3 (1- 10)	3 (1- 12)	0.3	0.17
MUAC (cm)	14 (12- 19)	15.5 (12- 18)	14.5 (11- 19)	0.04	14.5 (13- 18)	16.5 (13- 24)	15.5 (13- 24)	0.04	0.02
Height (cm)	82 (52- 150)	115.5 (59- 154)	85 (52- 154)	0.04	107.5 (8.5- 13.1)	121.5 (54- 149)	112 (54- 149)	0.31	0.009
GCS	10 (3- 15)	11 (5- 15)	10 (3- 15)	0.38	15 (6- 15)	13 (10- 14)	14 (6- 15)	0.14	<0.001
Presence of FND	24 (53)	8 (57)	32 (54)	0.5	5 (42)	4 (19)	9 (27)	0.16	0.01
Urea (mg/ dl)	34 (22- 86)	32 (20- 60)	33.5 (11- 173)	0.95	31 (11- 66)	29 (18- 80)	29 (11- 80)	0.98	0.23
Creatinine (mg/dl)	0.6 (0.3- 3.5)	0.6 (0.5- 1.5)	0.6 (0.3- 3.5)	0.54	0.6 (0.4- 1.1)	0.6 (0.5- 1.3)	0.6 (0.4- 1.3)	0.5	0.75
Sodium (mmol/L)	139.5 (122- 172)	136 (127- 147)	139 (122- 172)	0.27	131 (119- 135)	131.5 (116- 142)	131 (116- 142)	0.18	0.001
Potassium (mmol/L)	4.4 (2.4- 6.6)	4.6 (3.6- 5.2)	4.4 (2.4- 6.6)	0.9	4.2 (3.5- 4.6)	3.8 (2.3- 6)	3.9 (2.3- 6)	0.8	0.02
Reveived phenytoin	28 (62%)	6 (43%)	34(58%)	0.3	5 (42%)	3 (14%)	8 (24%)	0.1	0.002
Received phenobarbitone	12 (27%)	7 (50%)	19 (32%)	0.1	1 (8%)	0 (0%)	1 (3%)	0.4	0.001

Median (range); number (%); GTCS- generalized tonic clonic seizures; GCS- Glasgow coma scale; FND- focal neurological deficit

Table 5.3: Constituents of fluid balance during hospital stay

	Bad Outcome	**Good outcome**	***P*- value**
	(n= 59)	**(n= 33)**	
Actual IVF given on D0 (ml/day) #	652 (200- 2100)	1090 (390- 2155)	<0.0001
Actual IVF given on D2 (ml/day) ##	570.5 (250- 2260)	958.5 (560- 1650)	0.007
Drug diluents on D0 (ml/day)	100 (14.8- 283)	88 (15- 440)	0.75
Drug diluents at D2 (ml/day)	80 (16- 300)	80 (6- 360)	0.61
Oral fluid on D0 (ml/day)*	0 (0- 1000)	50 (0- 1850)	0.001
Oral fluid at D2 (ml/day)	60 (0- 2000)	900 (0- 2060)	<0.0001
Nasogastric fluid on D0 (ml/day)	0 (0- 240)	0 (0- 0)	0.46
Nasogastric fluid at D2 (ml/day)	0 (0- 260)	0 (0- 600)	0.69
Vomiting on D0 (ml/day)	0 (0- 20)	0 (0- 0)	0.28
Vomiting at D2 (ml/day)	0 (0- 10)	0 (0- 0)	0.43
Urine output on D0 (ml/day)	310 (0- 2150)	500 (80- 1400)	0.2
Urine output at D2 (ml/day)	500 (100- 2325)	650 (150- 1000)	0.36

IVF ordered by doctor(ml/day) D0, bad outcome, 788.5 (25-1728); good outcome, 1040 (223- 2000) (*p*-0.058), IVF in nurse's note (ml/day) D0, bad outcome, 600 (100- 1750); good outcome,1000 (200- 1860) *p*-0.13, IVF on parents audit (ml/day) D0, bad outcome, 555 (150- 2020); good outcome,1000 (445- 1840) (*p*- 0.017)

IVF ordered by doctor(ml/day) D2, bad outcome, 832 (329- 1762); good outcome, 980 (505- 1650) (*p*-0.34), IVF in nurse's note (ml/day) D2, bad outcome, 783.5 (100- 4788); good outcome, 1000 (560- 1650) (*p*-0.09), IVF on parents audit (ml/day) D2, bad outcome, 753.5 (310- 2000); good outcome, 832.5 (505- 1650) (*p*- 0.014)

*Median (range) increase in oral fluid intake from D0 to D2 for bad outcome was 0 (-600-1880) ml/day and good outcome 280 (0-1880) ml/day (*P*- 0.006) Median (range)

Table 5.4: Fluid status of the recruited patients

Clinical Features	Bad Outcome (n=59)	Good outcome (n= 33)	*P*- value
Duration of illness at admission (days)	6 (2- 23)	5 (2- 11)	0.014
Admission weight (Kg) on D0 #	10 (4- 40)	17 (6- 40)	0.008
Discharge or death weight (Kg)	10 (4- 40.5)	17 (6.6- 40)	0.006
Fluids required (ml/kg/day)	100 (55.48- 100)	79.41 (47.5- 100)	0.001
Fluids deficient (ml/kg/day)	20.35 (- 22.2- 68)	0.4 (-30.8- 50)	<0.0001
% difference between fluids required and delivered	26.35 (-33- 90.8)	2.7 (-39.6- 50)	<0.0001
Received IVF 5% Dextrose with 0.45% normal saline	39 (66%)	15 (45%)	0.05
Received IVF Dextrose (0.9%) normal saline	12 (20%)	9 (27%)	0.4
Restriction of IVF to 2/3rd of full maintenance	40 (68%)	15 (45%)	0.04
Serum lactate (mg) on D0 *	2.15 (0.8- 13)	2 (0.8- 3.6)	0.45
Serum lactate (mg) at D2	2.1 (1.2- 8.9)	2.1 (1.1- 7.1)	0.85

A Median (range) change in weight from D0 (admission day) to discharge or death for bad outcome was 0 (- 2.2- 1.5) Kg and good outcome 0 (-1.3- 3) Kg (*P*- 0.3)

*A Median (range) change in serum lactate from D0 (admission day) to D2 (second day of hospitalization) for bad outcome was -0.1 (- 11.4- 7.3) mg and good outcome -0.1 (-1.7- 5.4) mg (*P*- 0.5)

Median (range), number (%); IVF-Intravenous fluids

Table 5.5: Fluid status of the recruited patients by outcome

	Death(n=13)	Sequelae (n=46)	Recovery (n=33)	*P*- value
Duration of illness at admission (days)	7 (4- 12)	6 (2- 23)	5 (2- 11)	0.029
Duration of hospital stay (days)	6 (2- 10)	9 (1- 23)	9 (3- 18)	0.02
Admission weight (Kg)	9 (4.5- 31)	10 (4- 40)	17 (6- 40)	0.028
Change in weight (Kg)¶	-0.5 (-0.8- 0)	0 (-2.2- 1.5)	0 (-1.3- 3)	0.006
Presence of low WFA	12 (92%)	27 (59%)	11 (33%)	0.001
Presence of weight loss	10 (77%)	14 (30%)	7 (21%)	0.001
Admission modified GCS	8 (5- 14)	11 (3- 15)	14 (6- 15)	<0.0001
Presence of FND at admission	9 (69%)	23 (50%)	9 (27%)	0.02
IVF 5% Dextrose with 0.45% normal saline	12 (92%)	27 (59%)	15 (45%)	0.01
IVF Dextrose (0.9%) normal saline	1 (8%)	11 (24%)	9 (27%)	0.35
Never on IVF	0 (0%)	7 (15%)	9 (27%)	0.08
Fluids required (ml/kg/day)	100 (55.5- 100)	100 (56.7- 100)	79.41 (47.5- 100)	0.008
Fluid deficient (ml/kg/day)	16.5 (-8.3- 54.1)	22.5 (- 22.5- 68)	0.4 (-30.8- 50)	<0.0001
Reduced fluid intake (ml/kg/day)*	10 (77%)	32 (70%)	15 (45%)	0.09
% difference between fluids required and delivered	14.6 (- 7- 54.1)	27 (-33- 90.8)	2.7 (-39.6- 50)	0.001
Restriction of IVF to 2/3rd of full maintenance	7 (54%)	33 (72%)	15 (45%)	0.06
Oral fluids (ml/day) D0#	0 (0- 50)	0 (0- 1000)	50 (0- 1850)	0.001
Oral fluids (ml/day) D2#	0 (0- 60)	150 (0- 2000)	900 (0- 2060)	<0.0001
Urine output (ml/day) D0#	355 (30- 500)	250 (0- 2150)	500 (80- 1400)	0.43
Urine output (ml/day) D2#	500 (100- 2350)	461 (120- 1500)	650.4 (150- 1000)	0.65
Serum Na (mmol/L) D0	145 (139- 172)	137 (122- 149)	131 (116- 142)	<0.0001
Serum K (mmol/L) D0	4.1 (2.4- 6.6)	4.4 (3.3- 6.4)	3.9 (2.3- 6)	0.035
Serum urea (mg/dl) D0	45 (20- 173)	29 (11- 78)	29 (11- 80)	0.007
Serum creatinine (mg/dl) D0	0.8 (0.3- 3.5)	0.6 (0.3- 1.7)	0.6 (0.4- 1.3)	0.037
Serum Lactate at D0 (mg) ○	2.6 (1.3- 6.6)	1.8 (0.8- 13)	2 (0.8- 3.6)	0.085
Serum Lactate at D2 (mg)	3.2 (1.6- 4.3)	2.1 (1.2- 8.9)	2.1 (1.1- 7.1)	0.5

¶ The mean ± standard deviation for death was -0.3 ± 0.27, neurological sequelae -0.03 ± 0.5 and complete recovery was -0.003 ± 0.65, death. Also for difference between D2 and D0 for death it was 0.03 ± 1.3. sequelae -0.18 ± 3.4 and recovery 0.72 ± 1.9.

*Reduced total fluid intake relative to expected. Total fluids included IVF, oral and drug diluents. Expected fluids was estimated based on 100 ml/kg for the first 10 kg, 50 ml/kg for next 10 kg, 20 ml/kg for exceeding 20 kg.

Oral fluids include nasogatric fluids. For death, mean ± standard deviation for oral fluids for D0 was 3.8 ± 13.9 ml/day, oral fluids D2- 6.7 ± 20 ml/ day, urine output D0- 326.3 ± 165 ml/day and urine output D2- 752 ± 901.4 ml/day; for neurological sequelae, oral fluids D0 -131.2 ± 270.7 ml/ day, oral fluids D2- 323.6 ± 444.8 ml/ day, urine output D0- 391.3 ± 433.9 ml/ day, urine output on D2- 530.5 ± 350.2; and for complete recovery, oral fluids D0- 431.67 ± 553.8 ml/ day, oral fluids D2- 904.1 ± 632.6 ml/ day, urine output D0- 585.5 ± 446.8 ml/ day, urine output D2- 610.1 ± 268.5 ml/ day.

○ Median (range) change in serum lactate from D0 to D2 for death was -0.3 (-1.1- 1.5) mg, residual -0.1 (-11.4- 7.3) and complete recovery -0.1 (-1.7- 5.4) mg (*P*- 0.7).

Median (range), number (%)

Chapter 6: A preliminary randomised double blind placebo-controlled trial of intravenous immunoglobulin for Japanese encephalitis in Nepal

Abstract

Background: Japanese encephalitis (JE) virus (JEV) is a mosquito-borne flavivirus found across Asia that is closely related to West Nile virus. There is no known antiviral treatment for any flavivirus. Results from *in vitro* studies and animal models suggest intravenous immunoglobulin (IVIG) containing virus-specific neutralizing antibodies may be effective in improving outcome in viral encephalitis. IVIG's anti-inflammatory properties may also be beneficial.

Methods: I performed a pilot randomised double-blind placebo-controlled trial of IVIG containing anti-JEV neutralising antibody (ImmunoRel™, 400mg/kg/day for 5 days) in children with suspected JE at two sites in Nepal. I also examined the effect on serum neutralising antibody titre and cytokine profiles.

Results: 22 children were recruited, 13 of whom had confirmed JE; 11 received IVIG and 11 placebo, with no protocol violations. One child (IVIG group) died during treatment and two (placebo) subsequently died following hospital discharge. Overall, there was no difference in outcome between treatment groups at discharge or follow up. Passive transfer of anti-JEV antibody was seen in JEV negative children. JEV positive children treated with IVIG had

JEV-specific neutralizing antibody titres approximately 16 times higher than those treated with placebo (p=0.2), which was more than could be explained by passive transfer alone. IL-4 and IL-6 were higher in the IVIG group.

Conclusions: A trial of IVIG for JE in Nepal is possible. IVIG may augment the development of neutralising antibodies in JEV positive patients. IVIG appears an appealing option for JE treatment but warrants further study.

Trial registration: ClinicalTrials.gov: NCT01856205

6.1 Introduction

Japanese encephalitis virus (JEV) infection is the most important cause of epidemic encephalitis worldwide (156). JEV is found in Southeast Asia, China, the Pacific Rim and the Asian subcontinent, and its geographical range is expanding (52). JEV is a small single-stranded positive-sense RNA flavivirus, closely related to West Nile virus (WNV) that is transmitted between its natural bird and pig hosts by *Culex tritaeniorhynchus* and other mosquitoes. JEV transmission occurs mainly in rural areas where rice crops are cultivated and where the *Culex* mosquito favours sources of stagnant water in which to breed. People living in rural Asia are exposed to JEV during childhood. A small proportion of those exposed develop a simple febrile illness, with an even smaller proportion developing symptoms of encephalitis. Those who develop JE may present with meningo-encephalitis and/or seizures (20). Around one in every four patients of with neuroinvasive JEV infection die, and half of the survivors have severe neurological sequelae. This imposes a huge

socioeconomic burden in the poor rural settings where JEV is found (157). Although vaccines against JEV have become more widely used in recent years, the animal reservoir cannot be eradicated, so JEV remains a threat. The virus has continued to spread and at present there is no established treatment for JEV, or other related flaviviruses such as WNV.

The pathogenesis of Japanese encephalitis (JE) is believed to involve a combination of viral cytopathology and immunopathology (98- 101); previous attempts to develop treatment have explored both of these. After entering the body through the bite of an infected mosquito, JEV amplifies in the dermal tissues and lymph nodes leading to viraemia. Virions are thought to then bind to the vascular endothelial surface within the CNS, be internalised by endocytosis and transferred across the endothelial cells (52). In the brain, JE is characterised as perivascular inflammation with recruitment of macrophages, neutrophils and lymphocytes (158- 160). The thalamus, basal ganglia, midbrain and anterior horns cells of the spinal cord are particularly affected (11, 161). Viral antigens are predominantly in neurons although microglial cells, astrocytes and vascular endothelial cells are also infected. JEV is thought to cause neuronal cell death in two ways; firstly, by direct neuronal killing (162, 163), whereby viral multiplication within neuronal cells leads to cell death; secondly, by indirect killing, whereby the over activation of microglia, astrocytes and recruited macrophages (164) leads to release of excess proinflammatory cytokines such as interleukin 6 (IL-6), TNF-α, and RANTES (regulated upon activation, normal T cell expressed and secreted), which are

thought to damage neuronal cells, and increase the permeability of the blood brain barrier promoting massive leukocyte migration into the brain and further neuronal cell death (165).

The role of corticosteroids in the treatment of JE was examined in a randomised-placebo controlled trial inThailand. Although dexamethasone caused a reduction in cerebrospinal (CSF) opening pressures and CSF white cell counts, there was no overall benefit in terms of outcome (102). Interferon-α (IFN-α) is produced as part of the innate response to JEV infection, and has antiviral activity against JEV; but a placebo-controlled trial in Vietnam found it did not improve outcome (38). Oral ribavirin also proved to be unhelpful in a controlled trial in India (106). Intravenous immunoglobulin (IVIG) has been postulated as a potential treatment for flavivirus encephalitis caused by JEV and WNV, on account of its antiviral and anti-inflammatory properties. IVIG is postulated to act in two ways; IVIG produced in countries where flaviviruses are endemic contains high titres of specific neutralising antibody, because most of the population have been exposed to the virus, and thus have antibodies (166). In addition IVIG has non-specific anti-inflammatory properties, particularly through the suppression of pro-inflammatory cytokines. However IVIG is more difficult to administer than intravenous IFN-α, intravenous corticosteroids or oral ribavirin (38, 167); it is delivered using a syringe driver, and must be started at a low infusion rate, being increased over time if it is well tolerated. Where-as the previous trial using intravenous agents were conducted in settings with highly developed research infrastructure in Thailand and Vietnam, Nepal has no such establishments.

In this study I looked at symptomatic cases of JE because there was no treatment. IVIG has been postulated to work in in-vitro studies and mouse models in JE. It has also been used in patients of JE on a compassionate basis, but has not been assessed in a clinical trial (165, 168). JE contributes significantly to the total number patients of AES in Nepal. Therefore I was provided with initial fund to look at direct effect of IVIG in patients of JE in Nepal. However, I was not provided with additional grant to take it onto a definite clinical trial. Hence, I conducted a pilot randomised placebo-controlled trial of IVIG treatment in children with suspected JE in Nepal. I also used the opportunity to begin examining changes in immune parameters associated with such treatment.

In the process, regarding safety study, I tested the hypothesis that IVIG could be safely given to children with suspected JE, with no increased risk of serious adverse events compared with placebo. Similarly regarding efficacy, I tested the hypothesis that compared with placebo, treatment with IVIG halves the proportion of JE patients with a bad outcome (death or sequelae) from 66% to 33%.

6.2.1 Aim

To conduct a pilot randomised double blind placebo controlled trial of intravenous immunoglobulin in Japanese encephalitis

6.2.2 Objectives

1. To conduct a pilot randomised double blind placebo controlled trial to assess the safety of intravenous immunoglobulin in Japanese encephalitis in children

2. To conduct a pilot randomised double blind placebo controlled trial to assess the efficacy of intravenous immunoglobulin in Japanese encephalitis in children
3. To explore the effect of intravenous immunoglobulin on immunological markers of efficacy in children with Japanese encephalitis

6.3 Methods

6.3.1 Ethics Statement

The study protocol was approved by the Nepal Health Research Council, and the ethics committees of BPKIHS, Kanti Children's Hospital and the Liverpool School of Tropical Medicine. Informed written consent was obtained for all children from parents or legal guardians. Written consent was also obtained from JEV vaccinated individuals.

6.3.2 IVIG selection

JEV-specific neutralising antibody titre was measured in pharmaceutical grade IVIG obtained from four different suppliers from areas endemic for JEV [Bharat serum, India; Hualan Biological Engineering Inc., China; Sichuan Yuanda Shuyang Pharmaceutical Co., China; Reliance Biopharmaceutical, India] by 50% plaque reduction neutralization titres ($PRNT_{50}$) against wild type JEV (strain P3) infecting a standard culture of Vero cells. IVIG from a low JEV prevalence region (Vigam, Bio Products Laboratory, USA) and a sample of serum from a JEV vaccinated individual were tested as controls. The test was repeated three times. IVIG produced by Reliance Biopharmaceuticals Pvt. Ltd. in India and manufactured in China from blood donors in an endemic area

known to have high levels of JEV seroprevalence. This had the highest mean JEV $PRNT_{50}$, titre and was selected for use in this trial. In order to confirm the JEV-specific neutralising antibody titre as well as characterise background immunity to other flaviviruses known to circulate in Asia, the IVIG was tested by dengue virus (DENV), tick-borne encephalitis virus (using Powassan virus), and WNV IgM and IgG antibody capture ELISA and $PRNT_{90}$ at the US Centers for Disease Control and Prevention (CDC) Division of Vector-Borne Diseases (169-172). The lower limit of quantitation (LLOQ) by PRNT90 was 1:10 serum dilution.

6.3.3 Patients

Patients were recruited from two centres in Nepal: Kanti Children's Hospital in Kathmandu, which is the main pediatric center in Nepal, and the Paediatric Department at BP Koirala Institute of Health Sciences (BPKIHS), a large regional hospital in the town of Dharan in the eastern lowland Terai area of Nepal. The JEV mosquito vector is uncommon in Kathmandu due to the elevation of 1350 metres (4400 feet) and most cases at Kanti Children's Hospital were referred to this centre from the central lowland Terai area where the disease is endemic.

During monsoon of 2009, I approached children between 1 and 14 years of age who were clinically diagnosed AES. AES was diagnosed in children who had a history of fever that lasted less than 14 days, altered consciousness [Glasgow coma score (GCS) <15] with or without a history of seizures (38, 75) as described in chapter 2 (General Methods). After lumber puncture (LP),

children who had CSF findings consistent with viral encephalitis such as white cell count less than 1000 cells/mm^3 with no organisms on Gram stain and a CSF:plasma glucose ratio > 40% (10, 38), and whose parents provided written consent were included in the study. Children who had febrile convulsion were excluded. I also excluded those with a positive blood slide or rapid antigen test for *Plasmodium falciparum* parasites and those referred from a peripheral health center who had clinical and/or laboratory features suggestive of bacterial meningitis where antibiotic treatment had already been commenced. Children with a GCS < 3 (out of 15), who were receiving artificial ventilation without signs of spontaneous respiration, and with absent oculocephalic reflexes were excluded, as prior studies have shown an extremely low chance of meaningful recovery (20).

6.3.4 Procedures

I randomly allocated patients to treatment with IVIG or placebo. Methods of randomisation (38) and recruitment has been described in detail in chapter 2 (General Methods). The randomisation schedule is provided as S1 Table.
I took a detailed history and a member of the study team did daily clinical and neurological examinations until death or discharge as described in chapter 2 (General Methods). I also wanted to examine any difference in anti-JEV antibody titres between children treated and untreated with IVIG. Adverse events were graded using WHO recommendations and reported to an independent data safety monitoring committee. Information was recorded on standardised proforma (Appendix O). Computer tomography scans were performed as indicated at the discretion of the admitting physician. There were

no onsite facilities for magnetic resonance imaging. Patients with prolonged or repeated seizures, respiratory difficulties or severely reduced consciousness, were admitted to a paediatric intensive care unit, which had facilities to intubate and ventilate. Mannitol and dexamethasone were given at the discretion of the admitting physician, and suspected septicaemia was treated with broad-spectrum antibiotics. Patients were assessed at discharge and 3-6 month follow-up for disability in a range of activities using the LOS (109) (Appendix K & L) and a standardised neurological assessment (Appendix Q). Children who did not return for follow-up were reminded by letter or telephone, as available. If they were unable to return to the hospital, they were examined at home. At follow-up, I took particular note of seizures, progress at school, and changes in personality. The trial protocol is available online [http://clinicaltrials.gov/show/NCT01856205 (as accessed on 3rd March 2016)] (Appendices N & P). The CONSORT checklist is also provided as Checklist S1.

6.3.5 Diagnostic and Pathogenetic studies

On admission, patients had routine blood tests (typically; a full blood count and white cell differential, glucose, urea and electrolytes) and a blood film or rapid antigen test for *Plasmodium falciparum*. For diagnostic and pathogenetic studies, I took blood prior to treatment (IVIG or placebo) and 1 hour after the completion of infusion of the 5th (final) dose. If a child required further blood tests for clinical reasons, extra samples were collected. Serum was separated by centrifuge, frozen at -20^{o}C; aliquots were made and one aliquot went to the designated laboratory in Nepal for testing; the other aliquots were transported

on dry ice to the Institute of Infection and Global Health, Liverpool, UK, and subsequently to CDC Division of Vector-Borne Diseases in Fort Collins, Colorado, USA.

On admission, I did a LP with patients in the lateral position, and if the patient was calm the opening pressure was recorded using a CSF manometer. The LP was delayed in patients who were convulsing, or those with clinical signs of raised intracranial pressure (109, 173). CSF samples were examined for cell count and differential, protein, glucose and Gram stain. Further, CSF and serum samples were frozen on site at -20°C, and subsequently transported for further investigations, as above.

Some of the patients had GCS < 3 or on artificial ventilation without signs of spontaneous respiration, who had less chances of meaningful survival. Also, the IVIG looked more viscous in consistency as compared placebo. However, exclusion of those patients and covering the syringes containing the study drug with masking tapes, respectively, helped overcome such biases. Some of the patients were administered the study drug later in the study because of delayed LP. IVIG may have been less effective in these patients as compared to those who were administered in the early period of illness, which is one of the limitations of this study.

To diagnose JE locally, anti-JEV immunoglobulin-M (IgM) antibodies were measured in all serum samples on admission and day 7 of admission, and in all CSF samples by enzyme-linked immunosorbent assay (ELISA) using

the AFRIMS JE MAC IgM ELISA as part of the WHO acute encephalitis syndrome surveillance programme; these samples were measured in batches at the National Public Health Laboratories in Kathmandu, and so the results were not available at the time of randomisation. Classification of the patients as JE negative or positive was based on the results of this initial JEV IgM ELISA testing in Nepal, confirmed at CDC, USA, as below. I selected day 7 post illness onset as the time-point to measure IgM titres because the percentage of cases with raised anti-JEV IgM titres in serum or CSF is typically reported as 100% by then (Figure 6.1) (58, 60).

Confirmatory testing was subsequently performed at the CDC Arboviral Diseases Branch diagnostic laboratory using the testing algorithm described in chapter 2 (General Methods). JEV $PRNT_{50}$ was done on all patient serum at 2 time points: pre-treatment (immediately prior to the first dose) and post-treatment (1hour after the final dose). In the UOL, UK, Interleukin (IL) -6 and IL-4 cytokine measurements were undertaken on aliquots of IVIG and on patients' serum pre-treatment and 1 hour post-treatment which has also been described in chapter 2 (General Methods).

6.3.6 Sample size

I aimed to recruit 22-24 patients. This has been described in chapter 2 (General Methods).

6.3.7 Statistical analysis

Patient clinical features and laboratory parameters were compared between treatment groups (IVIG or placebo). Difference in plaque reduction anti-

JEV antibody neutralizing titres (PRNTs) pre and post treatment were also compared between treatment groups (IVIG or placebo). Similarly, the difference in cytokine abundance pre and post treatment (separately for IL4 and IL6) were compared between treatment groups. Normally distributed data were compared using a Student's t-test. Non-normally distributed data were compared using the Wilcoxon-Mann-Whitney test. Differences between proportions were tested using the Fisher's exact test. To examine whether JE antibody status of the participant prior to treatment was a source of variation in difference in antibody titres or cytokine abundances following treatment, a 2 way ANOVA was undertaken, where treatment (IVIG or placebo) was the column factor and JE status (anti-JEV antibody positive or negative) was the row factor. Prism ver.6.04 (Graph Pad Software, Inc) was used for statistical analyses and graph generation.

6.4 Results

6.4.1 Patients

Between May and August 2009, 22 (96%) children of the 23 screened met the entry criteria; 12 in Kanti Children's Hospital in Kathmandu and 10 in BPKIHS in Dharan. One child was not enrolled in the trial as he was moribund and met criteria for exclusion. Eleven (50%) received IVIG and 11 (50%) placebo (Fig. 6.2). Twenty-two patients were randomised and there were no breaches of randomisation protocol. None of the children were reported to have been vaccinated against JE. 7 (64%) patients in the IVIG group and 6 (55%) in the placebo group were classified as acute JEV infections by JEV/DENV IgM ELISA testing**.** The initial JE diagnostic classification for all patients in Nepal

was confirmed by the testing at the CDC, USA. Seven out of 10 (70%) were positive for JEV in BPKIHS and 6 of 12 (50%) in Kanti Children's Hospital. One child (placebo group) had evidence of a previous flavivirus infection. One patient with an undetermined status after local testing was found to be JEV negative by confirmatory testing at CDC; otherwise there was complete concordance between the test results in Nepal and those at CDC. All CSF tested were negative for JEV RNA by real time RT-PCR.

The baseline characteristics of the two groups were comparable (Table 6.1). The median age was 5 and 7 years for treatment and placebo groups respectively (not significantly different). Most patients came from rural areas, and arrived after approximately 5 days of illness. Median GCS on admission was 8 (range 5-15), and the majority of patents had seizures as part of the presenting syndrome. All concurrently received antibiotics, most commonly cephalosporins. Four patients received dexamethasone alongside study drug; all 4 were in the placebo arm. One child received only 1 dose on the first day after admission, 2 children had 2 doses each on first and second admission days, and 1 child had daily dexamethasone for 5 days (Raw data of patients' characteristics is available as S2 Table).

The adverse and serious adverse events, according to patients' JEV status, and whether they received IVIG or placebo is presented in Table 6.2; there were no significant differences in frequency of adverse events between IVIG and placebo. There were no suspected unexpected serious adverse reactions (SUSARs). Raw adverse events data is available in S3 Table.

6.4.2 Intravenous immunoglobulin

All IVIG preparations produced in JEV endemic regions had anti-JEV $PRNT_{50}$ titres, ranging from 1:320 to 1:640 (Fig. 6.3). Control IVIG from a non-JEV endemic area (Vigam, USA) showed minimal $PRNT_{50}$ titres of 1:10; lower than serum from a JEV vaccinated individual who had a titre of 1:40. ImmunoRel™ IVIG produced by Reliance Biopharmaceutical (India) had the highest anti-JEV $PRNT_{50}$ titre, and was chosen for treatment in this study. This ImmunoRel IVIG showed low $PRNT_{50}$ titres $\leq$1:20 against DENV, WNV and Powassan viruses.

6.4.3 Outcomes

One patient, who received the study drug, died of aspiration pneumonia. He was a 19 month old boy admitted with a 13 day history of high grade fever, repeated generalized tonic clonic seizure, fast breathing and altered sensorium. He had not been immunized against JE. His Glasgow Coma Score 10/15, pulse 140 per minute, respiratory rate 56 breaths per minute, axillary temperature 38.9°C, and he had diffuse wheeze in both sides of the chest. On neurological examination he had a right sided hemiplegia. Investigations revealed a peripheral blood total white cell count of $25.4x10^9$ cells/L (polymorphs 66%, lymphocytes 31%), serum creatinine 0.6 mg/dl, serum sodium 143 mmol/L, serum potassium 4.5 mmol/L. His CSF was clear and colourless with 10 cells/mm^3 (polymorphs 40%, lymphocytes 66%), protein 80mg/dl, glucose 48mg/dl (blood glucose 87 mg/dl). X-Ray chest revealed diffuse infiltration more on the right lung field. He was treated with intravenous ceftrioxone 100mg/kg/day for two days, intravenous midazolam at 0.2mg/kg/dose for two

doses, intravenous phenytoin at 20mg/kg loading dose, followed by 7 mg/kg/day of maintenance dose, intravenous 20% mannitol at 5ml/kg single dose and maintenance intravenous fluids, but died on the fourteenth day of illness. Unblinding after death revealed he had received IVIG (400 mg per kilogram body weight) at 0.01mL/Kg/minute on the first and second day. He was subsequently found to be JEV IgM negative. The investigating member of the Data Safety & Monitoring Committee confirmed that the child died of aspiration pneumonia due to repeated uncontrolled seizures, unrelated to the study drug.

The remaining 21 patients received the full 5 days of treatment. At hospital discharge, the commonest Liverpool outcome score was II (major sequelae) in both IVIG and the placebo groups [7/11 (64%) and 8/11 (73%) patients per group respectively]. When all participants are included at 3-6 month follow-up; 6 out of 11 (45%) exhibited complete recovery (no neurological sequelae) in the IVIG group compared to 2 out of 11 (18%) in the placebo group (Table 6.3). There was no significant difference in the proportion of patients exhibiting complete recovery between the two groups when analysed by intention-to-treat either at hospital discharge or at follow-up [p=1 and 0.36 respectively (Table 6.3)].

6.4.4 Neutralising antibodies

The difference in neutralising antibody titres pre and post treatment were analysed by treatment group (IVIG or placebo) Neutralising antibody titres increased significantly among participants who received IVIG compared to placebo (p=0.038: Fig. 6.4). Separating results by anti-JEV IgM antibody

status, titres showed a greater increase among those who received IVIG compared to placebo in both anti-JEV antibody status groups. The median increase in neutralising antibody titre was greater (16 fold) in anti-JEV IgM positive compared to negative patients (median increase in titre post treatment was 1920 versus 120 for IgM positive and negative patients respectively; S4 Table and Fig. S1). However, the increase in neutralising antibody titres following IVIG treatment was only significant among anti-JEV IgM negative patients (p=0.048 and p=0.24 respectively). Patient anti-JEV IgM status prior to treatment was not a significant source of variation in change in neutralising antibody titre as assessed by 2 way ANOVA (p=0.29; S5 Table).

6.4.5 Cytokines

The difference in cytokine abundance pre and post treatment were also analysed by treatment group (Fig. 6.5). Cytokine abundance for both IL-6 and IL-4 increased among participants who received IVIG compared to placebo. This increase was significant for IL-4 (p=0.043). Separating the IL-4 results by anti-JEV IgM antibody status, IL-4 levels showed a greater increase among those who received IVIG compared to placebo in both anti-JEV antibody status groups. The median increase in IL-4 following IVIG treatment was greater (13 fold) in anti-JEV IgM negative compared to positive patients (median increase in abundance post treatment was 0.65 versus 0.05 pg/ml for IgM negative and positive patients respectively). Neither increase was significant; p=0.06 and p=0.6 for IgM negative and positive patients respectively (S6 Table; S7 Table; Fig. S2). Patient anti-JEV IgM status prior to treatment was a significant source of variation in change in IL-4 abundance (p=0.0018). However, treatment

(IVIG or Placebo) was a more significant source of variation as assessed by 2 way ANOVA (p=0.0005). Patient anti-JEV IgM status prior to treatment was not a significant source of variation in change for IL-6 (S5 Table). A very low concentration of IL-6 was detected in the IVIG (0.4 pg/ml). IL-4 was not detected. Raw data for PRNT levels are available in S8 Table.

6.5 Discussion

JEV, the most important cause of epidemic encephalitis globally, is just one of several important flaviviruses that cause encephalitis. These include tick-borne encephalitis virus, which occurs across northern and eastern Europe, and WNV, which circulates in India, Africa, Europe, and since 1999, the Americas. There is no established treatment for any flavivirus. Although JE vaccine programs are being implemented across Asia, the disease is continuing to spread, and recent estimates put the incidence closer to 70,000 cases annually, rather than the previously estimated 50,000. (174). JE was first recorded in Nepal in 1978 in Rupandehi district of the Western Development Region (42). Thirty four years later there were 29,877 clinically diagnosed cases with 5,589 deaths (personal communication: Dr Santosh Gurung, WHO-IPD, Kathmandu, Nepal). It is currently reported from 65 of total 75 districts. In the early outbreaks, the mortality was up to 60%, but more recently this has improved to approximately 20% (37, 42). Immunisation against JE began in 1999 in 3 districts (83, 84), which had been extended to cover 31 high risk districts by 2011. In addition, the Government vaccinated around 200 000 pigs in the Terai zone in 2001 (42). A recent seroprevalence study in animal hosts in 10 districts in Nepal revealed that 48% of pigs, 27% of ducks and 50% of horses were JEV seropositive

demonstrating a high transmission of JEV in the wild and confirming that it is likely to remain (85). Hence, JE is still a major public health problem in Nepal, and there is a pressing need to develop better treatments.

My pilot study suggests that a multi-centre RCT of IVIG for children with suspected JE is possible in this setting with no protocol violations. The double-blinding procedure, using masking tape to cover syringe contents, has been used in a previous study of IFN-α in JE in a very well resourced research unit in Vietnam, which employs research nurses and technicians (38); I found that it also worked well in this much simpler setting with hospital nurses and technicians. In considering the study, there had been concern that starting an infusion at a low rate, and then increasing over time would be unacceptable to hospital staff if they could not see what drug they were giving; but again this aspect worked well. Although the numbers were small, I did not detect any significant differences in adverse events between the two groups. I did not study the minimum 30 subjects recommended by US FDA Guideline on Safety, Efficacy, and Pharmacokinetic Studies to Support Marketing of Immune Globulin Intravenous (Human) as Replacement Therapy for Primary Humoral Immunodeficiency (2008) (http://www.gmp-compliance.org/guidemgr/files/IGIVIMMUNO.PDF). Nor did I employ a dose escalation designs used in many oncology studies. However I was not studying Primary Humoral Immunodeficiency or cancer, and recommendations from western settings may not always be appropriate for studies in resource-poor settings (175). Three patients died. The one death in the IVIG group was attributed to severe underlying disease rather than the IVIG, and the two deaths which

had occurred by follow-up were both in the placebo group. The proportion of patients exhibiting full recovery (without any sequelae) was similar between the groups at discharge and slightly higher among in the IVIG group at follow. This difference was not significant on intention-to-treat analysis.

In Nepal, IVIG, which has general anti-inflammatory effects, is currently being used to treat a range of paediatric conditions including Guillain-Barré syndrome, idiopathic thrombocytopenic purpura and Kawasaki disease. IVIG from endemic areas is considered a potential treatment for flavivirus encephalitis, including that caused by JEV and WNV, because of the neutralising antibody it contains (167). I found IVIG from a range of manufactures in Asia contained significant neutralising antibody; most had $PRNT_{50}$ titres ≥1:640. Pre-clinical studies suggest that passively transferred antibody may be effective against flavivirus encephalitis (165, 167,168, 176-182).

There is a strong evidence to suggest that JEV, like other neurotropic flaviviruses, may be more susceptible to antibody-mediated, rather than cell-mediated immune responses. For JEV, and other neurotropic flaviviruses, clearance of virus is not dependent on cytolytic T cell activity, in contrast to non-neurotropic viruses (49- 51,179,183, 184). Neurons, as terminally differentiated cells, do not express MHC-1, which would subject them to lysis by CD8 T cells and non-replacement (49). Animal data support the importance of antibody mediated immunity (177, 178). In one study, Konishi *et. al.* immunised mice with plasmid DNAs encoding JEV proteins that induce

neutralising antibody responses or cytotoxic T lymphocyte responses, and then challenged with lethal intraperitoneal doses of virus. They showed that neutralising antibody prevents virus dissemination from the peripheral site to the brain, and that antibody-mediated mechanisms of protection were more efficient than the cytotoxic T cell responses (179). These findings supported earlier work showing that anti-envelope protein antibodies, are the most critical protective component in a JEV challenge model (183), and more recent passive antibody transfer experiments (184). In vitro work in mice shows a protective role for IVIG given prophylactically to prevent the flavivirus causing tick borne encephalitis and a protective effect when used as a treatment. In animal studies in which IVIG containing anti-WNV specific antibody was administered during the viraemic phase but before the virus had entered the CNS, there was a dramatic 100% survival rate (167, 180), and mortality was reduced up to five days after infection (181, 182).

Antibodies and B cells are directly responsible for limiting WNV dissemination in the CNS during early part of infection. Serum viral load has been reported 500-fold increased at day 4 of infection which subsequently has been reported to increase markedly causing huge viral burden in neurons in the CNS at day 6 and rapidly provoking development of fatal encephalitis. Antibodies may reduce viral load available for CNS infection by limiting haematogenous spread through direct neutralisation, complement activation or increased viral uptake by phagocytic cells. Specific IgM produced against the virus during early viral infection may limit dissemination by temporarily containing viraemia and triggering an adaptive IgG response that eliminates

viral infection. However, the amount and site of viral inoculation, the kinetics of viral replication, administration of antibodies, and the absolute amount of natural antibody response, influence the efficacy of natural antibodies in preventing infection (180). More relevant for the treatment of patients presenting to hospital with WN neuroinvasive disease, in which the virus has already entered the CNS, Morrey et al showed that peripheral administration of anti-virus monoclonal antibodies in a mouse model neutralises WNV even after it has entered the brain (185, 186).

As expected, patients had a greater increase in neutralising antibody titres among those treated with IVIG compared to placebo. Interestingly JEV antibody positive children treated with IVIG exhibited higher (approximately 16 times higher) titres of neutralising antibody compared to levels of change following IVIG treatment among JEV antibody negative patients (this change was not significant). The magnitude of this effect appears greater than can be explained by passive transfer of anti-JEV antibody (observed as the increase titres among the JE negative patients). The reason for this is not certain, though it is possible that passively transferred antibody is enhancing the natural production of neutralizing antibody by B cells, perhaps through augmenting the uptake of viral particles by antigen presenting cells. Such a mechanism has been described in a macaque model of HIV, where the administration of low level neutralising monoclonal antibodies to simian HIV led to the rapid development of neutralising antibodies through enhanced B cell responses (187). A relatively modest neutralising antibody titre (i.e. 1:10) has been shown to protect against JEV in animal models, when antibody is administered prior to infection.

However, to my knowledge, no studies have determined what titre is required to limit the evolution of encephalitis once JEV infection is established in humans. My patients all had an antibody titre that was much higher, around 100 fold higher, than this and yet still had encephalitis. This may be because most of the recruited patients presented late to the hospital with a median duration of illness of 10 days. On the basis of mouse models, we know, that the protection caused by passive antibodies is directly related to the amount of antibodies applied and to the time and dose of the infecting virus (24, 40). Although therapeutic effect of virus-specific antibodies is possible by both neutralisation of extracellular virus during the early period of illness and suppression of intracellular viral replication in the late period; it may be that it is most effective if administered during early viraemic phase of the infection (167).

Antibody-dependent enhancement of viral entry into macrophages is also important in secondary DENV infections. In this case non-neutralising antibody from a prior infection with a different DENV serotype is thought to enhance viral entry into macrophages and contribute to increased disease severity (188). In tick-borne encephalitis, passive immunisation with neutralising antibody containing IVIG has been used as prophylaxis in those bitten by infected ticks, before disease develops (189). However, following suspicions that this was actually precipitating disease, through postulated antibody dependent enhancement, this approach was abandoned. My study suggests there were no significant side effects of treatment with IVIG in JE and administration was associated with increased JEV-specific serum neutralising antibody titres.

Neuronal cell death in JE may occur directly, from viral cytopathology, and indirectly via immune mediated mechanisms. This may include over activation of microglia cells (190), which release pro-inflammatory cytokines such as interleukin 6 (IL-6), TNF-α, and RANTES (regulated upon activation, normal T cell expressed and secreted), causing massive migration and infiltration of leukocytes into the brain (191). IL-6, which is produced by neurons, microglia, astrocytes and recruited macrophages in response to viral CNS infection (192-194), causes an increased permeability of the blood brain barrier, which leads to interstitial cerebral oedema, and raised intracranial pressure (195, 196). TNF-α is also produced by astrocytes and macrophages. Its multiple pro-inflammatory properties include upregulation of class I and II MHC expression, upregulation of cellular adhesion molecules, increased permeability of the blood brain barrier (197), and upregulation of inducible nitric oxide synthase (iNOS), leading to the production of nitric oxide (NO) (198). In addition to affecting virally-infected cells, the inflammatory response in the CNS may also damage non-infected cells to cause bystander cell death.

The elevated IL-6 and IL-4 responses observed in my patients support the hypothesis that administration of IVIG modulates the immune response. Accepting cytokine responses are complex, I chose to measure IL-6 and IL-4 because they have been used previously to give a simple reflection of the balance of pro- to anti- inflammatory responses (45). IVIG treatment has previously been linked with both reduced and elevated levels of IL-6 among patients (199-205). Similarly, IVIG treatment has been related to increased IL-4 levels (206,207). Both IL-4 and IL-6 participate in the development of antibody

responses; IL-4 promotes B cell proliferation and isotype switching and IL-6 induces differentiation of B cells into antibody secreting plasma cells (208). The modest increase in IL-4 and IL-6 could be consistent with augmentation of an antibody response; therefore the increase in both antibody and pro-antibody producing cytokines may reflect part of the same process (209). The variation in response among patients with different anti-JEV antibody statuses indicates the immune modulation by IVIG is highly intricate.

Other mechanisms of IVIG induced cytokine production may involve the generation of immune complexes of JEV antigen and IVIG derived antibody. In turn, these complexes may stimulate monocytes to produce IL-6 via Fc-receptor interactions (210). Other immunomodulatory factors (e.g., sCD4, sCD8, sHLA antigen) present in the IVIG could also induce cytokine production (211, 212, 213).

Because previous studies have shown IL6 is sometimes detected in IVIG (205), I checked the levels in my treatment batches and found that although there were low levels of IL6, this was not enough to explain the elevated titre seen in our treated patients.

When JE is due to primary infection (i.e. first flavivirus infection) a quick and effective IgM response occurs in the serum and CSF within few days, and attempts to isolate virus from either sample are unlikely to be successful (52). However, absence of IgM is associated with positive virus isolation from the CSF and a bad outcome (52). When individuals have asymptomatic JEV

infection, antibody probably protects the host by restricting viral replication during the viraemic phase, before the virus crosses the blood brain barrier (52). Antibody may also limit damage during established encephalitis by neutralising extracellular virus and facilitating lysis of infected cells by antibody-dependent cellular cytotoxicity.

A better understanding of immunological changes in JE and the effects of IVIG will be important for the further development of IVIG treatment (45). Since this pilot study showed a randomised double blind placebo controlled trial of IVIG in Nepal was possible, a larger study, with further close monitoring of adverse effects is recommended. The question of efficacy will only be answered with a full phase III randomised placebo-controlled trial.

There may be other wider implications of this study: the number of antibody-mediated encephalitidies being identified is increasing and IVIG is a standard treatment option in these cases (2, 214). In addition, IVIG may have a role in other viral encephalitis as there is evidence that damage caused is over and above that from direct viral infection alone (215, 216). In most series of encephalitis patients the aetiology is unknown in the majority of cases, but is presumed to be a mixture of microbial and immune mediated causes (2, 6); whether IVIG may be a suitable treatment for such patients is unclear. My study provides important pilot data, but further research is needed in this area.

6.6 Limitation of study

The study was restricted to recruiting small of number of patients because of lack of funding to continue to build it into a large clinical trial. A larger study

with patient followed up for a longer period of time would have provided more accurate information about efficacy of IVIG in patients of JE. In this study I looked into only indirect marker of immunological response such as IL-6 and IL-4. A detailed study of T cell and B cell response to treatment of IVIG in JE positive and JE negative patients would have been more informative on immunological markers of efficacy. Another method of investigating efficacy would also have been by looking at immunological mechanism of high antibodies in JE positive patients administered IVIG as compared to placebo. However, I did not have adequate number of patients and funding to explore both of these further. Treatment trial with monoclonal antibodies directed at JE could have been a cheaper alternative to an expensive IVIG trial, in which, probably funding would not have been a major issue.

6.7 Summary

JE is the only known cause of viral encephalitis under the AES surveillance in Nepal. It has a bad outcome and there is no specific treatment. Therefore, identifying treatment for JE would not only help in reducing JE related morbidity and mortality but also improve outcome of AES in Nepal. Intravenous immunoglobulin containing JE virus-specific neutralising antibody is thought to neutralise the virus and suppress damaging inflammation in the brain; but has never been examined in a randomised trial before. Therefore, when I performed a randomised double-blind placebo-controlled trial of intravenous immunoglobulin to assess feasibility, and examine generation of anti-JEV specific antibodies among children admitted with suspected JE, I found children with confirmed JE treated with the intravenous immunoglobulin

had specific neutralising antibody titres16 times higher than those treated with placebo. This was more than could be explained by passive transfer alone, suggesting an immune augmenting effect. Passive transfer of anti-JEV antibody was also seen in JEV negative children. IL-4 and IL-6 were also higher in the intravenous immunoglobulin group. Therefore, such a clinical trial was feasible, intravenous immunoglobulin safe and appeared to augment the development of neutralising antibodies. The anti-inflammatory properties of intravenous immunoglobulin should be useful for a wider use as a treatment for encephalitis. A better understanding of immunological changes in JE and the effects of intravenous immunoglobulin will be important for the further development of intravenous immunoglobulin treatment (45). Since this study showed my approach to be feasible, a larger study, with further close monitoring of adverse effects is recommended. The question of efficacy will only be answered with a full phase III randomised placebo-controlled trial.

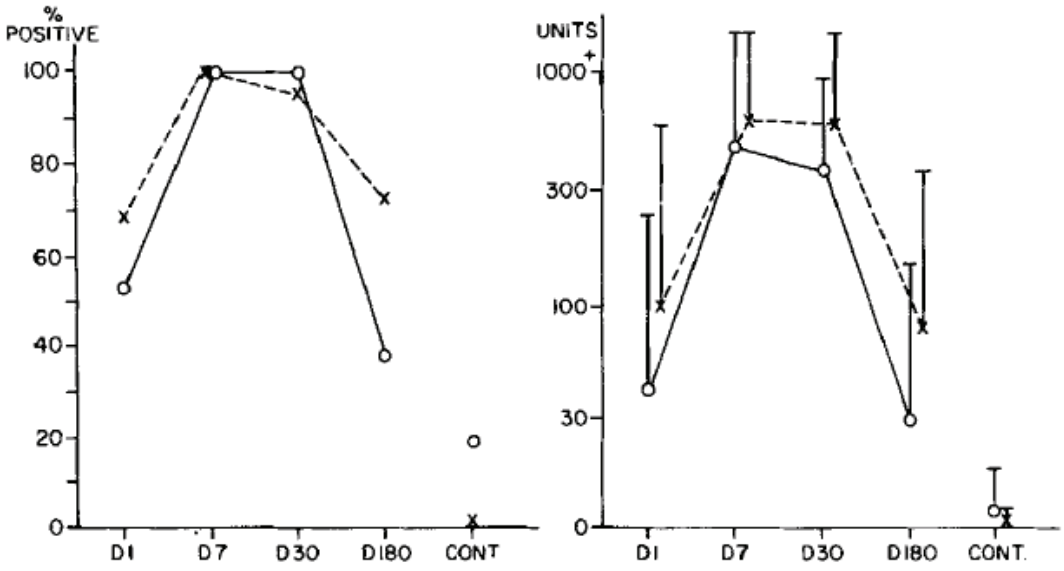


Figure 6.1: Shows kinetics of IgM to JEV by MAC ELISA *(Burke et al, 1982)*

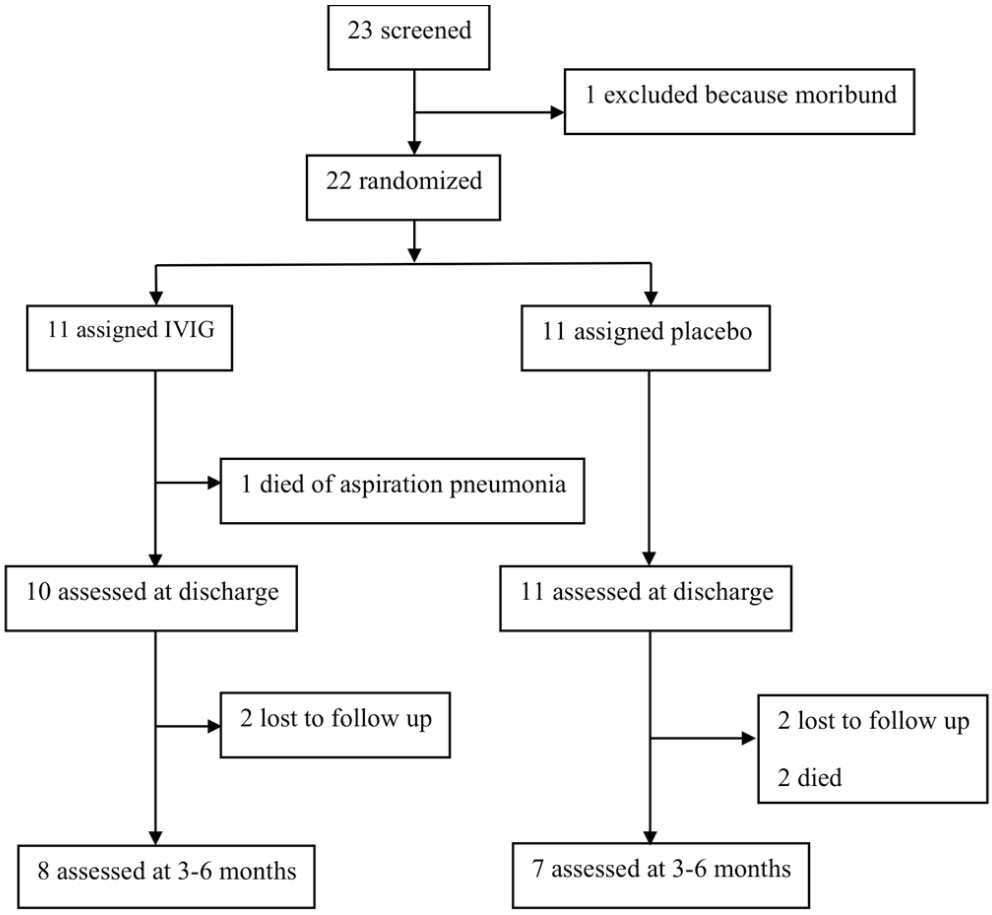


Figure 6.2: Flow diagram of study participants' recruitment and follow-up. All children enrolled, fitting the trial criteria, who were alive at discharge were attempted to be followed-up (n=21). Twenty-one families were successfully contacted. Among these families, two children had died.

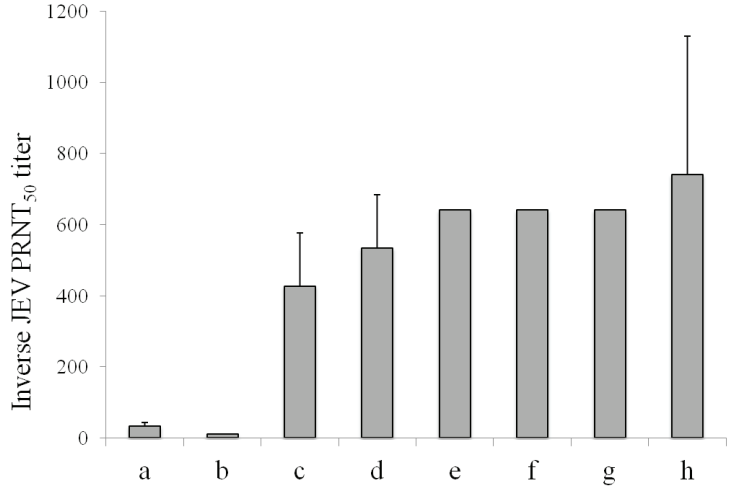


Figure 6.3: Anti-JEV neutralising antibody in commercially available IVIG. Mean and standard deviation of reciprocal 50% plaque reduction neutralisation titres ($PRNT_{50}$) in vero cells using P3 wild type strain of JEV are shown for a: Serum control from JEV vaccinated individual, b: Vigam (USA), c: Bharat (India) batch 1, d: Hualan (China) batch 1, e: Bharat (India) batch 2, f: Hualan (China) batch 2, g: Sichuan (China), h: Reliance (India).

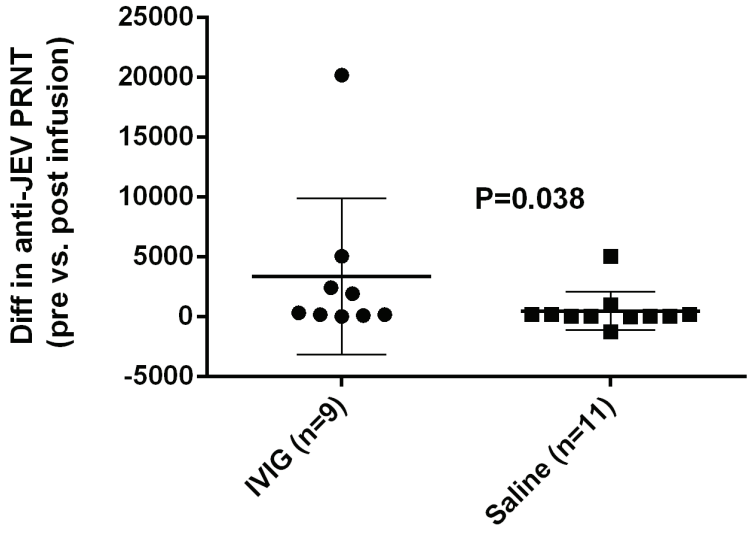


Figure 6.4: Difference in neutralising antibody titres to JEV in children with acute encephalitis syndrome treated with IVIG or placebo.
Median and inter-quartile range of the difference in JEV $PRNT_{50}$ titres pre and post treatment is presented. Patients are grouped according to treatment. Difference in titres was assessed via Wilcoxon-Mann-Whitney test. Note: Two patients who received IVIG were not included in this analysis because of insufficient sample to undertake PRNT measurements.

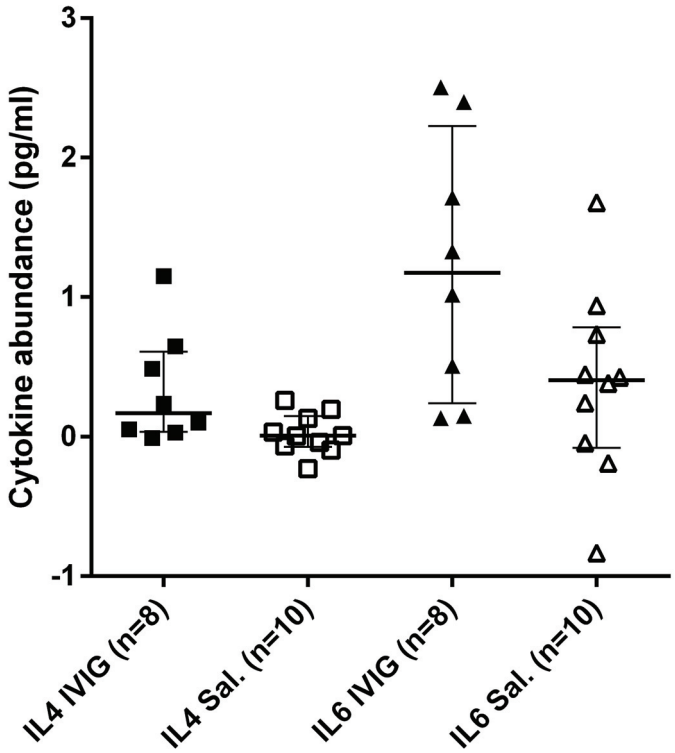


Figure 6.5: Interleukin (IL)-4 and IL-6 abundance in children with acute encephalitis syndrome receiving intravenous immunoglobulin (IVIG) and placebo.

Median and inter-quartile range of change in cytokine abundance (pg/ml) pre and post treatment is presented for IL-4 and IL-6 separately. Cytokine abundance increased for both IL-4 and IL-6. This increase was significant for IL-4 ($p=0.043$ and $p=0.068$ for IL-4 and IL-6 respectively). Difference in abundance was assessed via Wilcoxon-Mann-Whitney test. Note: Four patients (three who received IVIG and one who received saline) were not included in this analysis because of insufficient sample to undertake the ELISA.

Table 6.1: Baseline characteristics of trial participants.

Parameters	No.	IVIG (n=11)	No.	Placebo (n=11)	*P*
Age (years)	11	5 (1-11)	11	7 (1.3-12)	NS.
Male	11	5 (.45)	11	7 (.64)	NS.
Living in rural area	11	11 (1)	11	9 (.82)	NS.
Fever duration (days)	9	5 (2-12)	11	5 (3-10)	NS.
Illness duration (days)	10	5 (4-13)	11	6 (3-13)	NS.
Altered sensorium	11	10 (.91)	11	9 (.81)	NS.
New onset seizures	11	10 (.91)	11	11 (1)	NS.
Glasgow Coma Scale (3-15) on admission	11	8 (5-15)	11	8 (5-15)	NS.
Temperature (°C)	10	38.3 (36.7-40)	10	38.9 (36.7-40)	NS.
Heart rate (beats/minute)	10	95 (68-140)	11	104 (84-130)	NS.
Resp. rate (breaths/minute)	10	33.5 (22-56)	11	28 (22-40)	NS.
Neck stiffness present	11	4 (.36)	11	4 (.36)	NS.
Kernig's sign present	11	1 (.09)	10	2 (.18)	NS.
Abnormal limb tone	8	5 (.63)	9	3 (.3)	NS.
Abnormal posturing	9	2 (.22)	11	0 (0)	NS.
Positive anti-JEV IgM	11	7 (.64)	11	6 (.55)	NS.
Haemoglobin (g/dl)	9	12.1 (9.8-13.8)	10	11.8 (8.2-14.3)	NS.
White cell count (WCC) - $x10^9$/L	9	10.8 (5.2-25.4)	11	13.9 (4.2-18.9)	NS.
Polymorphs (%)	9	66 (30-85)	11	68 (30-90)	NS.
Lymphocytes (%)	9	31 (12-70)	11	30 (10-70)	NS.
Platelets ($x10^9$/L)	7	270 (120-659)	9	200 (88-362)	NS.
Glucose (mg/dl)	11	100 (80-160)	11	85 (66-170)	0.03
Urea (mg/dl)	7	22.8 (15-35)	9	27.0 (18-58)	NS.
Creatinine (mg/dl)	7	0.9 (0.4-2.4)	9	1 (0.5-2.2)	NS.
Cerebrospinal fluid (CSF) WCC -cells/mm^3	11	35 (0-125)	11	30 (0-300)	NS.
CSF polymorphs (%)	11	10 (0-60)	11	10 (0-95)	NS.
CSF lymphocytes (%)	11	70 (0-100)	11	40 (0-100)	NS.
CSF protein (mg/dl)	11	39 (28-100)	10	27 (7-68)	0.01
CSF glucose (mg/dl)	11	70 (48-90)	10	61.5 (45-81)	NS.
CSF/blood glucose ratio	11	62 (50-82)	10	68.5 (46-100)	NS.
Mannitol (given)	11	3 (.27)	11	7 (.64)	NS.
Dexamethasone	9	0 (0)	11	4 (.36)	NS.
Quinine	11	5 (.45)	11	5 (.45)	NS.
Aciclovir	11	1 (.91)	11	2 (.18)	NS.
Chloramphenicol	11	1 (.91)	11	0 (0)	NS.
Cephalosporin	11	10 (.91)	11	10 (.91)	NS.
Phenytoin	11	9 (.82)	11	8 (.73)	NS.
Phenobarbitone	11	3 (.27)	11	2 (.18)	NS.

Data presented as number of patients (proportion) or median (range). No. - number of patients in the clinical group where data for the parameter was available. *P* - P value for Fisher's Exact or Mann Whitney U test between groups.

NS. – not significant.

Table 6.2: Summary of adverse events

Symptoms	IVIG			Placebo			IVIG vs. Placebo	IVIG vs. Placebo
	JE (n=7)	Non-JE (n=4)	Total (n=11)	JE (n=6)	Non-JE (n=5)	Total (n=11)	*P*- value	OR (95% CI)
Fever	2 (.29)	2 (0.5)	4 (.36)	1 (.17)	1 (0.2)	2 (.18)	0.64	2.6 (0.3- 29)
Dyspnoea	1(.14)	2 (0.5)	3 (.27)	1 (.17)	1 (0.2)	2 (.18)	1	1.7 (0.2- 20.1)
Vomiting	0 (0)	0 (0)	0 (0)	1 (.17)	0 (0)	1 (0.9)	1	0 (0- 18.7)
Irritable	1(.14)	1 (.25)	2 (.18)	2 (.33)	0 (0)	2 (.18)	1	1 (0.07- 13.5)
Non-urticarial skin rash	0 (0)	0 (0)	0 (0)	1 (.17)	0 (0)	1 (0.9)	1	0 (0- 18.7)
Hypotension*	1(.14)	0 (0)	1 (0.9)	1 (.17)	0 (0)	1 (0.9)	1	1 (0- 43.7)
Melena*	1(.14)	0 (0)	1 (0.9)	0 (0)	1 (0.2)	1 (0.9)	1	1 (0- 43.7)
Death*	0 (0)	1(.14)	1 (0.9)	0 (0)	0 (0)	0 (0)	1	NA

Data are number of patients (proportion). * Serious adverse events. NA: not applicable

Table 6.3: Outcome for trial participants.

Outcome at discharge	IVIG (n= 11)	Placebo (n= 11)	*P*-value	OR (95% CI)
Median duration of hospital stay (days)	13 (9-21)	12 (6-18)	0.59	-
Median Glasgow coma score	14 (3-15)	14 (7-15)	0.53	-
Number with complete recovery (LOS V)	1 (.09)	1 (.09)	1	1 (0- 43.7)
Number with minor sequelae (LOS IV)	0	1 (.09)	1	0 (0- 18.7)
Number with moderate sequelae (LOS III)	2 (.18)	1 (.09)	1	2.2 (0.1- 74.9)
Number with severe sequelae (LOS II)	7 (.64)	8 (.73)	1	0.7 (0.07- 5.5)
Number that died (LOS I)	1 (.09)	0	1	NC

Outcome at 3-6 months	IVIG (n=11)	Placebo (n=11)	*P*-value	OR (95% CI)
Lost to follow-up	2 (.18)	2 (.18)	1	1 (0.07- 13.5)
Number with complete recovery (LOS V)	5(.45)	2 (.18)	0.36	3.8 (0.4- 41.8)
Number with minor sequelae (LOS IV)	0	3 (.27)	0.21	0 (0- 2.2)
Number with moderate sequelae (LOS III)	1 (.09)	0 (0)	1	NC
Number with severe sequelae (LOS II)	2 (.18)	2 (.18)	1	1 (0.07- 13.5)
Number that died (LOS I)	1 (.09)	2 (.18)	1	0.45 (0.01- 8.4)

Data are number of patients (proportion) or median (range). LOS = Liverpool Outcome Score (LOS): 1 – died; 2 - severe sequelae; 3 - moderate sequelae; 4 - minor sequelae; 5 - full recovery. NC: not calculable

Supporting Information

S1 Table: Randomisation Schedule

Study Site	StudyID	Treatment
BPKIHS	T111	IVIG
BPKIHS	T112	SALINE
BPKIHS	T113	SALINE
BPKIHS	T114	IVIG
BPKIHS	T115	SALINE
BPKIHS	T116	IVIG
BPKIHS	T117	IVIG
BPKIHS	T118	SALINE
BPKIHS	T119	SALINE
BPKIHS	T120	IVIG
KCH	T2	SALINE
KCH	T3	IVIG
KCH	T4	SALINE
KCH	T5	IVIG
KCH	T6	IVIG
KCH	T7	IVIG
KCH	T8	SALINE
KCH	T9	IVIG
KCH	T10	SALINE
KCH	T11	SALINE
KCH	T12	IVIG
KCH	T13	SALINE

S1 Checklist: CONSORT checklist (as published in PLoS ONE)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6,7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	none
Participants	4a	Eligibility criteria for participants	5,6
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6,7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6,7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	none
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	None
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	6
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9,10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9,10

Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, Page 21
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, page 21
Recruitment	14a	Dates defining the periods of recruitment and follow-up	11
	14b	Why the trial ended or was stopped	7
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	22
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	22, 23
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	14, 23
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Fig 3 &4, page 21
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	7
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12,13,14
Other information			
Registration	23	Registration number and name of trial registry	NCT01856205 ClinicalTrials.gov
Protocol	24	Where the full trial protocol can be accessed, if available	attached
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

S2 Table: Participant Characteristics (raw data)

Treatment Group	IVIG	IVIG	IVIG	IVIG	IVIG	IVIG	IVIG	IVIG	IVIG	IVIG	IVIG	SALINE	SALINE	SALINE	SALINE	SALINE	SALINE	SALINE	SALINE	SALINE	SALINE	SALINE
StudyNo	T120	T7	T9	T12	T111	T114	T116	T5	T117	T3	T6	T113	T8	T115	T2	T4	T118	T119	T112	T10	T11	T13
Female (yes=1)	1	0	1	1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	0	1	0
Age (years)	1	7	11	5	5	1.6	7	1.2	8	7	1.7	12	8	4	3	11	6	11	3	9	7	1.3
Living in rural area (yes=1)	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
Duration of illness (days)	4		8	5	5	4	5	4	6	5	13	4	4	5	8	6	6	4	3	10	13	8
Fever duration (days)	4		8	5	5	4		2	6	4	12	4	4	5	8	4	6	4	3	10	10	8
Altered sensorium	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1
New onset seizures	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Glasgow Coma Scale (3-15) on admission	7	8	7	5	12	7	12	15	11	6	10	7	8	8	6	7	12	8	5	14	15	5
Temperature (°C)	39.45	37.78	39.45	37.78	36.67	38.34	38.34	38.34	40.00		38.88	38.56	39.45	38.06		39.45	38.89	38.89	39.45	36.67	37.00	40.00
Resp. rate (breaths/minute)	48	33	48	34	24	26	30	40	22		56	22	28	40	28	34	36	28	30	24	26	32
Pulse (beats/minute)	138	100	90	68	80	84	110	78	130		140	110	104	130	88	100	120	110	100	84	96	120
Neck stiffness present	0	0	0	1	0	0	1	0	1	1	0	0	1	0	0	1	1	0	0	1	0	0
Kernig's sign present	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0
Abnormal posturing	0		0	1	0	0		0	1	0	0	0	0	0	0		0	0	0	0	0	
Abnormal tone	0	1	1	1		0		0	1	0	1	1	0	0	1	1	0	0	0	0	0	
Positive anti-JEV IgM	0	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0	0	0	0
Haemoglobin (g/dl)	9.8	13	10	13.5		11.1		13	11.9	12.1	13.8	11.8	13	14.3		13.5	12.2	11.8	11.2	11.2	10.4	8.2
White cell count (WCC) - $x10^9/L$	19.6	10.8	9.6	9.7		8.2		5.2	22.2	18	25.4	15.6	15.6	15.3	4.2	9.4	7	13.9	18.9	18	11.8	9
Polymorphs (%)	75	63	58	70		77		30	85	61	66	80	90	34	43	66	68	70	81	30	78	31
Lymphocytes (%)	16	37	42	29		16		70	12	39	31	18	10	66	56	34	30	30	14	70	22	66
Platelets ($x10^9/L$)	659	350		270		159		120	326	170		205	180			190	88	328	362	200	210	120
Glucose (mg/dl)	160	100	125	90	98	92	107	80	115	107	87	76	90	69	90	85	105	100	170	66	85	80

Sodium (mmol/l)	135	136	127.6			139		139.3	136	137	143	138	133	131	128	141	130	135	138			138
Potassium (mmol/l)	4.4	4.8	5.1			3.9		4.43	5.2	3	4.5	4.6	4.3	3.3	4.1	6	4.7	4.7	3.6			5.1
Urea (mg/dl)	32	22	23.1	15		35		22.8	20			53	19	20	21	58	30	27	31			18
Creatinine (mg/dl)	1.2	0.6	0.4	0.4		0.9		2.4	1.2		0.6	1.1	0.5	0.8	0.5	1.9	2.2	1	1			0.5
CSF opening pressure (H_2O mm^3)		14	15	22				10					16	18	9	24	17				6	29
Cerebrospinal fluid (CSF) WCC -cells/ µL	70	8	125	0	50	9	0	45	50	35	10	0	285	0	0	35	44	8	20	300	115	30
CSF polymorphs (%)	10	30	35	0	20	0	0	0	0	60	40	0	60	0	0	10	10	95	0	70	40	0
CSF lymphocytes (%)	90	70	65	0	80	100	0	100	100	40	60	0	40	0	0	90	90	5	100	30	60	100
CSF protein (mg/dl)	85	39	40	30	39	28	36	100	30	100	80		30	24	20	30	20	30	19	50	68	7
CSF glucose (mg/dl)	90	76	85	74	58	70	60	50	74	57	48		62	71	60	61	66	81	79	45	50	57
Glucose (mg/dl)	160	100	125	90	98	92	107	80	115	107	100	76	90	69	90	85	105	100	170	66	85	80
CSF/blood glucose ratio	0.56	0.76	0.68	0.82	0.59	0.76	0.56	0.63	0.64	0.53	0.48		0.69	1.03	0.67	0.72	0.63	0.81	0.46	0.68	0.59	0.71
Mannitol (given)	1	0	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1	1	0	0	1
Dexamethasone		0	0	0		0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1
Quinine	1	0	0	0	1	1	1	0	1	0	0	1	0	1	1	0	1	1	0	0	0	0
Aciclovir	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Cephalosporin	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1
Chloramphenicol	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Phenytoin	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	0
Phenobarbitone	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1

S3 Table: Adverse events (raw data)

Study No	T120	T7	T9	T12	T111	T114	T116	T5	T117	T3	T6	T113	T8	T115	T2	T4	T118	T119	T112	T10	T11	T13
Abdominal pain																						
Headache																						
Chest tightness																						
Facial flushing																						
Nausea																						
Vomiting																		1				
Dyspnoea	1				1						1							1	1			
Hot sensation																						
Itching																						
Non. Urt. rash																						
Fever					1		1	1		1								1				1
Pallor																						
Irritable								1	1						1		1					
Hypotension					1												1					
Oliguria / Anuria																						
Hemolysis																						
Melena		1																	1			
Raised Urea																						
Raised Creatinine																						
Death											1											

S4 Table: Change in PRNT titres - pre compared to post treatment.

	IVIG (n=11)			Placebo (n=11)		
	1. Total	**2. JE +ve**	**3. JE -ve**	**4. Total**	**5. JE +ve**	**6. JE -ve**
Number	9	7	2	11	6	5
Minimum	0	0	80	-1280	-1280	-10
25% Percentile	120	160	80	0	-207.5	-5
Median	320	1920	120	0	160	0
75% Percentile	3720	5040	160	160	1980	0
Maximum	20160	20160	160	5040	5040	0
Lower 95% CI	80	0	80	-10	-1280	80
Upper 95% CI	5040	20160	5040	960	5040	160
P val. Grp. 1 vs. 4	0.038					
P val. Grp. 2 vs. 5		0.244				
P val. Grp. 3 vs. 6			0.048			

The table presents change in PRNT titres (pre versus post treatment) among treatment groups. Patients are sub-grouped by their anti-JEV IgM antibody status prior to treatment (JE+ or JE-).

Total - indicates number of patients where PRNT titres were available pre and post treatment.

Confidence intervals (CI) represent estimated 95% limits around the median.

There was a markedly higher increase in PRNT titres following IVIG treatment among anti-JEV antibody positive compared negative patients (16 x higher). This difference was not statistically significant. Negative PRNT values indicate a fall in titres following treatment.

P values calculated by Wilcoxon-Mann-Whitney test.

Note: Two patients, who received IVIG and were JE antibody negative, did not have sufficient sample to undertake PRNT measurement.

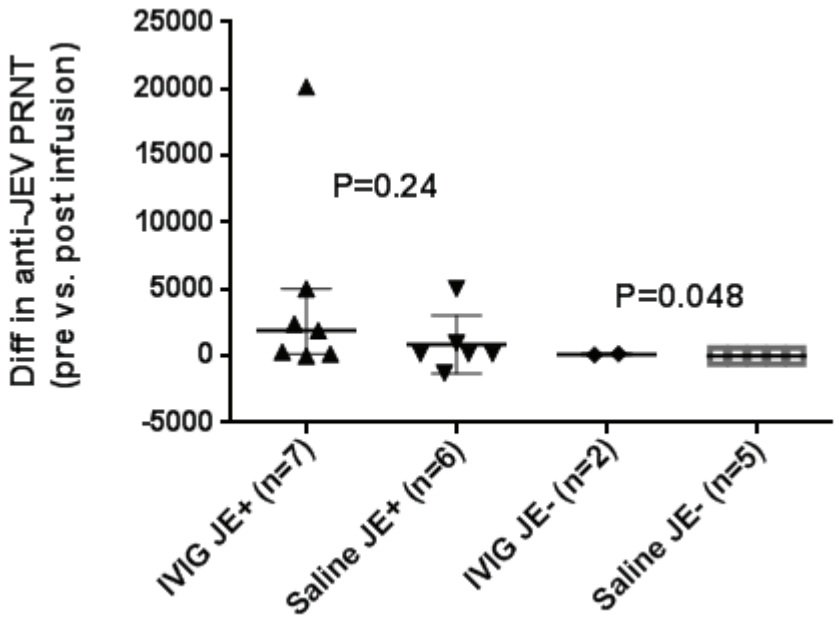


Figure S1: Change in PRNT among treatment participants, sub-grouped by their anti-JEV IgM antibody status

Median and inter-quartile range of the difference in plaque reduction neutralising antibody titres (PRNT) against JEV pre and post treatment is presented as four groups. Patients are sub-grouped by treatment exposure (IVIG or Saline) and anti-JEV IgM antibody status prior to treatment (JE+ or JE-).

Titres showed a greater increase among those who received IVIG compared to placebo in both anti-JEV antibody status groups. However, the increase was only significant among anti-JEV IgM negative patients (p=0.048). Differences between subgroups were assessed via Wilcoxon-Mann-Whitney test.

Note: Two patients who received IVIG and were anti-JEV IgM negative were not included in this analysis because of insufficient sample to undertake PRNT measurements.

S5 Table: ANOVA data based on the linear models for the change in neutralising antibody titres IL-4 and IL-6.

Two-way ANOVA		
Source of Variation	**% of total variation**	**P value**
Interaction for PRNT titres	2.65	0.4843
JE status (Anti-JEV IgM + or -)	6.168	0.2908
Treatment (IVIG or Sal.)	3.057	0.4531
Interaction for IL-4	26.86	0.002
JE status (Anti-JEV IgM + or -)	27.77	0.0018
Treatment (IVIG or Sal.)	38.16	0.0005
Interaction for IL-6	0.01091	0.9667
JE status (Anti-JEV IgM + or -)	0.4206	0.7954
Treatment (IVIG or Sal.)	7.871	0.2715

The table presents source of variation for change in PRNT, IL-4 and IL-6 abundance.

The table shows the interaction between participants' anti-JEV IgM antibody status and Treatment group

The data was calculated via Two-way ANOVA.

S6 Table: Change in cytokine abundance - pre compared to post treatment.

	IL-4		**IL-6**	
	IVIG	**Placebo**	**IVIG**	**Placebo**
Number	8	10	8	10
Minimum	-0.01	-0.23	0.13	-0.83
25% Percentile	0.04	-0.07	0.24	-0.08
Median	0.17	0.01	1.17	0.4
75% Percentile	0.61	0.15	2.23	0.78
Maximum	1.15	0.26	2.51	1.68
Lower 95% CI	-0.007	-0.096	0.133	-0.189
Upper 95% CI	1.151	0.197	2.506	0.938
P val. IVIG vs. placebo	0.04		0.067	

The table presents change in cytokine abundance (pg/ml) pre versus post treatment among treatment groups for IL-4 and IL-6.

Number - indicates number of patients where cytokine abundance measurements were available pre and post treatment. Negative values indicate a fall in abundance following treatment.

Confidence intervals (CI) represent estimated 95% limits around the median.

P values calculated via Wilcoxon-Mann-Whitney test.

Note: Four patients, three who received IVIG and one who received placebo, did not have sufficient sample to undertake cytokine measurements.

S7 Table: Change in IL-4 abundance - pre compared to post treatment, sub-grouped by anti-JEV antibody status

	Total IL-4		**IL-4 JE+**		**IL-4 JE-**	
	IVIG (n=8)	**Sal. (n=10)**	**IVIG (n=5)**	**Sal. (n=6)**	**IVIG (n=3)**	**Sal. (n=4)**
Number	8	10	5	6	3	4
Minimum	-0.01	-0.23	-0.01	-0.23	0.49	-0.07
25% Percentile	0.04	-0.07	0.01	-0.13	0.49	-0.06
Median	0.17	0.01	0.05	0.02	0.65	-0.02
75% Percentile	0.61	0.15	0.17	0.16	1.15	0.15
Maximum	1.15	0.26	0.24	0.26	1.15	0.2
Lower 95% CI	-0.007	-0.096	-0.007	-0.229	0.486	-0.065
Upper 95% CI	1.151	0.197	0.237	0.26	1.151	0.197
P val. IVIG vs. Saline	0.043		0.649		0.057	

The table presents change in IL-4 abundance (pg/ml) pre versus post treatment among treatment groups (IVIG or saline). Patients are sub-grouped by their anti-JEV IgM antibody status prior to treatment (JE + or JE-). Number - indicates number of patients where cytokine abundance measurements were available pre and post treatment.

Confidence intervals (CI) represent estimate of 95% limits around the median.

P values calculated via Wilcoxon-Mann-Whitney test.

The median increase in IL-4 following IVIG treatment was greater (13 fold) in JE- compared to JE+ patients (median increase in abundance was 0.65 versus 0.05 pg/ml respectively).

Note: Four patients, three who received IVIG and one who received saline, did not have sufficient sample to undertake cytokine measurements.

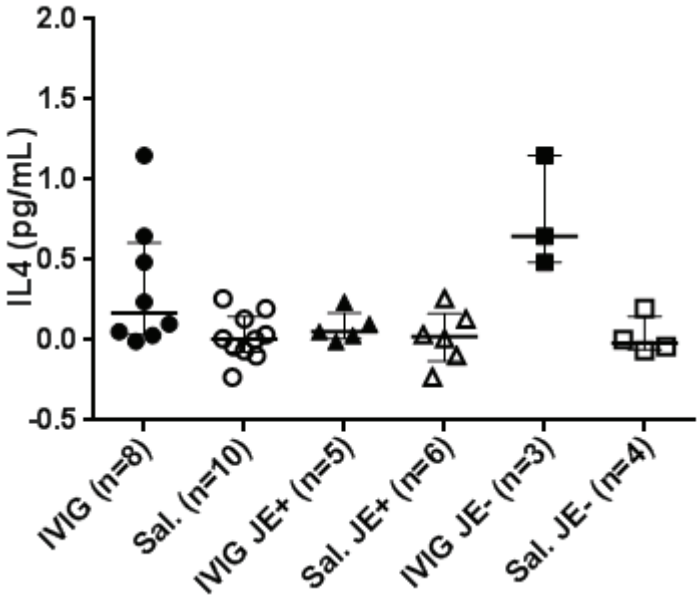


Figure S2: Change in IL-4 abundance among treatment participants, sub-grouped by their anti-JEV IgM antibody status

Median and inter-quartile range of change in IL-4 abundance (pg/ml) pre and post treatment is presented as six groups. Patients are initially grouped according to treatment. Then they are sub-grouped by both treatment exposure (IVIG or Saline) and anti-JEV IgM antibody status prior to treatment (JE+ or JE-). Overall, IL-4 abundance increased significantly among those who received IVIG compared to saline (p=0.043). There was no significant increase in abundances among JE- or JE + sub-groups (p=0.057 and p=0.65 respectively). Differences in abundance were assessed via Wilcoxon-Mann-Whitney test. Note: Four patients (three who received IVIG and one who received saline) were not included in this analysis because of insufficient sample to undertake the ELISA.

S8 Table: PRNT and cytokine levels (raw data)

Treatment	JE status	Pre	Post	Diff
IVIG	JE POSITIVE	80	5120	5040
IVIG	JE POSITIVE	320	20480	20160
IVIG	JE POSITIVE	640	2560	1920
IVIG	JE POSITIVE	160	2560	2400
IVIG	JE POSITIVE	160	320	160
IVIG	JE POSITIVE	320	640	320
IVIG	JE POSITIVE	5120	5120	0
IVIG	NEGATIVE	0	160	160
IVIG	NEGATIVE	0	80	80
IVIG	NEGATIVE	0		na
IVIG	NEGATIVE	0	320	na
SALINE	JE POSITIVE	160	320	160
SALINE	JE POSITIVE	160	320	160
SALINE	JE POSITIVE	10	160	150
SALINE	JE POSITIVE	2560	1280	-1280
SALINE	JE POSITIVE	320	1280	960
SALINE	JE POSITIVE	80	5120	5040
SALINE	NEGATIVE	0	0	0
SALINE	NEGATIVE	0	0	0
SALINE	NEGATIVE	0	0	0
SALINE	NEGATIVE	10	0	-10
SALINE	NEGATIVE	20	20	0

Chapter 7: Final Discussion and Conclusion

AES is associated with high morbidity and mortality affecting all age groups. It can be caused by wide range of organisms such as viruses, bacteria, fungus, parasites or spirochetes. It can also be immune mediated, chemical or toxins induced. Since identifying the pathogen is a laborious, extensive and expensive affair; it has not been systematically studied. In most of the cases specific infectious agent is rarely found (6, 217, 218). Specific pathogens even when identified show considerable geographical and age specific variation. Presently, arbovirus encephalitis is one the most devastating diseases of the nervous system (219). It a disease to fear, not only due to the high mortality rate but also the high rate of residual functional, sensory and cognitive damage. Such a disease can severely impair the socioeconomic status of poor, marginalized and rural communities living in predominantly Asian countries where the disease is particularly prevalent.

Numerically JE is the most important reported cause of AES worldwide. It is estimated, around 3 billion people living in 24 countries mainly of Southeast Asia, China and the Western Pacific Rim are at risk of the disease (http://www.who.int/wer). Since JE surveillance is not well established in many countries and the laboratory confirmation is challenging, the actual prevalence of the virus and the burden of the disease is not known and therefore it will appear as though it's geographical range is ever expanding (52, 220). The recent Public Health Emergency of International Concern caused by the related flavivirus

Zika, shows the ability of these viruses to spread and cause neurological diseases (221). Globally around 68,000 clinical cases and 20,400 deaths are reported every year despite widely available vaccines (175). Involvement of children, high mortality and residual intellectual, behavioural and neurological sequelae in survivors has made it a significant public health problem.

A total of 12.5 million people in Nepal are at direct risk of JE. Laboratory based AES surveillance began in Nepal since 2004. 17,875 cases of AES and 3,067 cases of JE (Table 1.2) have been reported since then. JE surveillance is integral part of AES surveillance and JEV is the only aetiology tested under AES surveillance in Nepal. This may also be the reason why it is the most common identified viral cause of AES in Nepal.

Since there is no specific treatment for most of the AES cases, the clinical management has been largely based on supportive care. Improved insight into pathogen specific clinical outcome, identification of clinical features that predicted outcome, improvement in supportive care, exploring a definitive treatment option for known pathogen such as JEV, could guide strategies for prevention and clinical management to improve disease outcome.

7.1 Clinical features that distinguished confirmed JE from non- JE AES

Many clinical features and laboratory parameters were similar on admission between patients of confirmed JE, confirmed non-JE and JE-status unknown. As discussed in chapter 3. In the past, amongst patients of AES, sudden

onset of fever, altered sensorium, headache, dystonia or movement disorder, opsoclonus or gaze abnormality and residual neuropsychiatric sequelae has been reported to support a diagnosis of JE (222, 223). In my study, JE patients had significantly more focal neurological deficits as compared to patients of unknown viral aetiology (222). Focal neurological deficit was present in 72% of patients at the time of discharge in my study, which was higher than 29%, 33% and 57% reported previously (78, 224,225). However it was similar to 78% reported in India (226). More deficits may have been observed in my study because JE has been disease under surveillance in Nepal since 38 years. This has led to increased awareness of JE and detailed surveillance activities which may recognize subtle neurological signs in JE patients. Deficits reported in past have been abnormal reflexes in 61%, hemiplegia in 42%, papilledema in 22% and cranial nerve palsy in 23% in the past (224). In my study JE patients with focal neurological deficit suffered bad outcome. Additionally, I found it to be more common in JE patients of lower WFA Z score at the time of discharge and follow up.

Absence of focal neurological signs had a very high predictive value which meant that the diagnosis of JE was less likely if there was no focal neurological deficit at the time of admission in patients with AES. This information certainly helps in the diagnosis and management. JE has been reported to be more common in children than adults and focal neurological deficits are a significantly more common clinical feature of JE in children than adults (227- 229). The presence of extrapyramidal features such as dystonia (correlated with thalamic involvement in CT Scan or MRI) has been reported

to be independent predictor of JE amongst patients of AES previously (227). Dystonia is significantly more common in JE in children than adults because of the vulnerability of developing CNS to hypoxic or toxic or metabolic insult (227, 228). Focal neurological deficit can be caused by injury to the nerve, spinal cord or cerebral cortex affecting it's function partially or completely. JE patients frequently develop raised intracranial pressure (20), brain herniation syndromes (20) and focal lesions in the brain (122) which could be the cause of focal neurological deficit. JEV commonly affects the cerebral cortex, cerebellum, thalamus, substantia nigra, brainstem, basal ganglia and the anterior horn cells of the spinal cord. Therefore, different focal neurological deficits reported in JE can be single or a combination of cognitive deficit, mutism, swallowing deficit, gait abnormality, intention tremors, seventh cranial nerve paralysis, cogwheel rigidity, monoparesis, hemiparesis, quadriparesis, diplegia, hyperreflexia, opisthotonus and acute flaccid paralysis of limbs (20, 21, 222, 230). In conclusion, AES patients presenting with single or combination of any of these signs are highly likely to be JE.

7.2 Patient outcome at discharge

Many patients with AES either die or have long term sequelae, especially if they have JE (73). JE patients have also been reported to have more residual sequelae compared to other viral encephalitis (10). Residual neurological sequelae were seen in 38% of JE patients in my first study (chapter 3) and 72% in my second study (chapter 4). This was more than 30% reported previously (52). However, it was comparable to 46% reported in India (225) and 57% in Thailand (224) previously. Sequelae could also have been high because

my studies were conducted in children. Higher rates of sequelae have been reported in children as compared to adults (52, 231). I also found sequelae more common in patients with lower WFA Z score. This difference in outcome between the studies could be due to more children in Nepal with JE having a lower WFA Z score or suffering dehydration than in Thailand. In my study, a significant proportion of AES with suspected viral aetiology also had a bad outcome. Similarly, viral encephalitis patients have previously been reported to have a mortality between 4.6% - 29% and 50% residual sequelae (232). In our setting, all the patients with AES receive antibiotics on admission due to suspicion of bacterial infection, until arrival of investigation reports. Definite treatments are available for bacterial meningitis, cerebral malaria, scrub typhus and typhoid encephalopathy. These are all causes of AES in our setting (37, 233). There are no definite treatments for viral encephalitis secondary to JE, Dengue, Chikungunya and West Nile virus (42, 126,128, 234- 238). A significantly higher proportion of JE patients had bad outcome as compared to non-JE viral infection patients (10). It has been reported that complete recovery is significantly less in children than in adults with JE (227). Children have been reported to have more severe illness as evidenced by deeper coma, higher frequency of seizures, more focal neurological deficit, more extrapyramidal tract involvement such as dystonia and Parkinson like features as compared to adults in previous studies (8, 228). Dystonia usually co-exists with Parkinson like features which could cause severe and protracted illness and a worse outcome.

7.3 Prognostic features suggesting bad outcome at discharge

In both AES and JE patients, older age, higher weight, longer fever duration, lower GCS and presence of focal neurological deficit (227) at the time of admission were associated with bad outcome, as discussed in chapter 3. Fever of long duration has been associated with bad outcome even in a previous JE study (239). Although there is no specific treatment for AES and JE, shorter duration of fever at the time of admission suggested that in patients who had impaired consciousness and focal neurological deficit, were more likely to do better with hospital admission and supportive management. On the contrary, one study has reported longer duration of fever to be associated with complete recovery of patients of JE (10). However, this was a small study conducted in single centre with limited resources. Brain injury has also been reported to be associated with prolonged fever and death (240- 242). Fever has been reported to stimulate formation of heat shock proteins which regulates immune response to protect host cells. Endogenous antipyretics regulate the body's febrile response by suppressing formation of pro-inflammatory cytokines (243). High or prolonged fever may cause inhibition of apoptosis and enhance pro-inflammatory cytokine response (244). The surge of temperature could further damage brain tissue and cause death of patients with brain injury (243). In sepsis, fever has also been reported to decrease tolerance to the rise in metabolic demand, causing hemodynamic instability and hypoxic tissue injury (245). Further cohort studies in hospitalized patients to elucidate the association between fever burden (product of fever duration and intensity) and outcome are needed.

Difficult geographical terrains causing lack of access to roads make the medical referral process unsystematic in Nepal. Most patients are brought to

the hospital by parents on the advice of local paramedics, pharmacists and neighbours. Educating the family members, community leaders, pharmacists, primary and community health workers to attend hospitals promptly for supportive management could be life saving. Thus there is a need for studies investigating the feasibility and benefit of local education to train family members and local health care workers in providing supportive measures among children with AES.

Among the treatments given to AES patients with suspected viral encephalitis, mannitol and phenytoin were significantly associated with bad outcome, in my study. Bad outcome may be explained by mannitol being given to sicker children. In traumatic brain injury, mannitol decreases perivascular oedema and resultant vascular collapse, resulting in enhanced brain oxygenation, without a change in circulation. Therefore, in severe cases of AES, with cerebral oedema and raised ICP, mannitol may have a more important effect on brain oxygenation than on ICP; and therefore a variable effect on outcome (246). Being an osmotic diuretic, it can cause hypokalemic and hypochloremic alkalosis, resulting in hypovolemia and reduction in cerebral perfusion leading to adverse outcome (246). In patients with non-traumatic brain injury, mannitol is reported not to have any beneficial effect on outcome (122, 246, 247).

Seizures are a complication of brain infection and are associated with bad outcome in both AES and JE (3, 20, 217, 222, 248). Again, patients who received phenytoin may have suffered a more severe course of disease and more frequently experienced a bad outcome. Phenytoin is cheap and widely

available for treatment of seizures in Nepal. Intravenous phenytoin at a loading dose of 18- 20 mg/Kg and a maintenance dose of 3- 6 mg/Kg/day in two divided doses is often provided for the treatment of seizures in AES patients (249, 250). Although the oral route is relatively safe and the intravenous route well tolerated, rapid (> 3mg/Kg/min or > 50mg/minute) and sometimes even normal infusion rates of phenytoin can cause cardiac arrhythmia, heart block, severe hypotension or apnoea which could can lead to death (251- 254). Because of toxicity, intravenous phenytoin has been withdrawn from the market and replaced with intravenous fosphenytoin in North America (249). Additionally phenytoin can cause impaired consciousness, suppression of neurological signs or aspiration pneumonia which could also increase the risk of adverse outcome.

7.4 Validation of prognostic features of bad outcome at discharge in AES and JE

CNS infections broadly includes bacterial meningitis, aseptic meningitis and encephalitis. Encephalitis has a global incidence of 3.5- 7.4 per 100,000 person years, suggesting the illness is not uncommon (232, 255). There is a definite treatment for bacterial meningitis. In contrast, the most common aetiology for aseptic meningitis and encephalitis are viruses, with no definite treatment. Although aseptic meningitis is self-limiting, mortality in encephalitis ranges from 4.5% - 29% (5, 232, 256). Half of the survivors have residual neurological sequelae (2). Therefore, identifying risk factors for bad outcome could give clues for improving management. Previously, absence of previous flavivirus infection, prolonged illness, prolonged or high fever, prolonged

altered sensorium, GCS < 8, rapid progression to coma, multiple convulsions, refractory status epilepticus, focal neurological deficit, abnormal breathing, decerebrate posturing, hypotonia, hyporeflexia, poor perfusion, raised ICP, positive Babinski sign, herniation syndrome, albuminuria, hyponatremia, low ani-JE IgM or IgG antibodies in serum or CSF, isolation of JEV from CSF, CSF cells >1000/mm^3 and elevated CSF protein have all been reported to be predictors of bad out come in AES and JE (3, 10, 20- 22, 24, 37, 47, 77, 78, 250, 257). In herpes simplex virus encephalitis, the most common sporadic encephalitis, acute symptomatic seizures, low GCS, delay in aciclovir treatment and older age are predictors of bad outcome (258, 259).

I also validated, prospectively, variables which were predictors of bad outcome in AES and JE, found in my retrospective study (chapter 3), using similar outcome definition, follow up period and clinical setting. As a result of which, I validated that; GCS ≤ 12, duration of fever ≥7 days and a focal neurological deficit at admission to be robust predictors of bad outcome in AES patients with GCS ≤ 12 also as independent predictor of bad outcome in JE. Validity is the extent to which a scale measures what it is intended to measure (260). Therefore, I found that GCS ≤12 in patients of AES or JE could fairly accurately predict a bad outcome. However, absence of GCS ≤12 could not accurately predict absence of bad outcome in neither AES or JE. The decrease in GCS is of the same nature in all patients of AES (specially JE); the difference being only in severity of altered sensorium. In the past, presence of four out of the following six features in AES patients have been regarded as pathognomonic of JE such as altered sensorium, slow speech, mask like face, course ocular tremor, symmetrical neurological paresis and

increased deep tendon reflexes (or abdominal reflex) (77). It has also been reported that JE could be diagnosed with confidence in patients of AES if they had sudden onset of fever, headache and altered sensorium and/or dystonia and various movement disorders and/or opsoclonus and gaze abnormalities with CSF finding of CNS infection of viral aetiology and residual neuropsychiatric problems (222). However, my previous finding of presence of focal neurological deficits among AES patients, could not be validated to predict JE, due to the small number of cases.

7.5 Admission weight for age as a marker of hydration status in AES and JE patients

Dehydration and severe acute malnutrition have been reported to be independent risk factors for mortality in hospitalized children (261). Therefore, identifying and correcting hydration and nutritional status from the time of admission may help improve the management of patients of AES. Both dehydration and malnutrition status predominantly influence weight; best described as WFA in children. Dehydration is assessed by percent change in weight (262). Dehydration can be classified as mild, moderate and severe based on 3-5%, 5- 10% and more than 10% loss of body weight respectively (133). Other clinical features which can help identify dehydration are inability to drink, thirstiness, restlessness, unconsciousness, lethargy, sunken eyes and abnormal skin turgor. Malnutrition is classified as severe or moderate by WHO, if the WFA Z score is less than -3 and -2 respectively. Because of loss of subcutaneous fat, some signs, such as sunken eyes and abnormal skin turgor may also occur in malnutrition and may mislead the treating clinician.

However, prolonged capillary refill time, abnormal respiration and abnormal skin turgor have high positive predictive value for dehydration since there is a possibility of malnutrition being over diagnosed among dehydrated children (262). Well nourished children tend to exhibit higher body temperature compared to malnourished children (263). The recorded body temperature was comparable in all WFA groups. This similarity supports the children with low WFA being dehydrated rather than malnourished. Other laboratory parameters seen in dehydration are; elevated blood urea nitrogen, abnormal serum sodium, elevated serum lactate, decreased serum bicarbonate, low urinary sodium excretion and high urinary osmolality and specific gravity. When I weighed the patients of AES at the time of admission and classified them by WHO's classification of WFA Z score for malnutrition from -3 up-to +1 Z score, almost half of the patients of AES of suspected viral aetiology and JE had a (low) WFA of < -1 Z score (as discussed in chapter 3). Prolonged duration of fever, prolonged illness, presence of focal neurological deficit and high serum urea suggest the low WFA patients could have had restricted oral intake and suffered dehydration compared to normal or high WFA patients. WFA Z score <-2 has also been reported to be independent predictor of death or severe neurological sequelae in patients with suspected viral encephalitis in a similar setting in the past (264). However, I found significantly more patients in WFA Z score < -2 group suffered dehydration (none in WFA Z score > -2), tachycardia and raised serum urea level suggesting death could have been caused by dehydration rather than mal nutrition.

7.6 Use of Liverpool outcome score to assess functional impairment

Previous studies regarding outcome of JE has reported wide variation in neurological impairment (28). The major reason for this uncertainty is the lack of standard method of assessing functional impairment in resource poor settings. Even in resource rich settings tools to assess disability in children are not well developed. Although some tools have been redeveloped for use in resource poor settings, the tools require a lengthy assessment process and the support of a multidisciplinary team (265, 266). Lack of simple tool to measure disability has been identified as one of the reasons for a lack of reliable information on the disease burden of JE.

The Liverpool outcome score (LOS) was used to describe and categorise neurological sequelae. It is a simple score developed to assess disability caused by JE. It can be applied by health care workers in a resource poor setting with minimal training. The tool has been validated for robustness and generalisability (109) (Appendix R).

The scores in individual functional domains and a total score (ranging from 33- 75) from the sum of all individual functional domains is assessed in order to identify severity of functional impairment and plan treatment. More importantly, for the purposes of epidemiological and health economics, it can help classify children with disability into likely to be "dependent" (not capable of independent living) or "independent"(capable of independent living). Children with a minimum LOS of 2 (severe sequelae) were classified

as dependent, while 3- 5 (moderate sequelae, mild sequelae or complete recovery) were classified as independent. During its development, the LOS was validated by comparing the outcome scores with the results of complete clinical examination conducted on the same day. The sensitivity, specificity. positive predictive values and negative predictive values of the LOS to identify children likely to be "dependent" were 100%, 98.4%, 84.2% and 100% respectively in field testing conducted in Malaysia (109). In my study, children who were assessed by LOS in person, also underwent complete clinical examination on the same day (Appendix Q). No clinical findings in neurological examination were detected where corresponding functional problems had not been detected by LOS reconfirming its high sensitivity to detect functional disability. I found LOS simple, easy to administer and user friendly.

In this study, all neurological sequelae were considered as bad outcome because even patients of JE with mild sequelae such as impairment of memory or change in personality has been reported to stop schooling and social participation in past causing huge adverse effect to the family. I used "bad outcome" because it was a absolute term where as "poor outcome" or "worse outcome" were relative terms.

Malawi Development Assessment Tool (MDAT) is a tool developed in Malawi for use by community health workers to assess development outcomes of African (non-western) children under six years of age (267). It is a structured, developmentally detailed and culturally sensitive tool (developed using a local reference population). Assessment is based on 136 items, 34 each in gross motor, fine motor, language and social domains of development. It also has

pictorial representation of many of the items making it understandable to those who use it. It has a sensitivity of 97% and specificity of 82% in assessment of neurodisabilities (267). The tool was not appropriate for use in my study population for several reasons; firstly, the tool is designed for use in children below six years of age; secondly, the assessment is designed to be undertaken by community health workers (not available for my studies); and thirdly, the assessment is reported to take more than 30 minutes to complete in a quiet location (longer time than a routine clinical follow-up consultation would allow).

Denver development assessment-II was developed as a quick and low cost method to screen children for evidence of impaired development for their age. It assesses four areas of child development:gross motor, fine motor, personal-social and language (268). The assessment provides information about the child's performance as compared to performance of children of the same age in all four domains. The result can be interpreted as advances and delays in development. It can be used to demonstrate the child's skills and capabilities. The sensitivity has been reported 83% and specificity 43% in identifying children with development abnormalities (269). Again this tool was not used in my study because it was designed for children from birth to six years of age.

Disability is defined as impairments of structure and/or function which results in limitation of activities and restriction of participation (270). Although large spectrum of behaviour abnormalities and poor school performance have been reported (21), less has been reported on social participation in school, social

function and everyday life. Social activities are affected by communication skills, cognitive and motor function (271). Child and adolescent scale of participation (CASP) composes of 20 questions related with home, community and school. It measures the extent to which children participate in home, school, and community activities compared to children of the same age as reported by parents. It was designed to monitor outcomes of children with traumatic and other acquired brain injuries (272). A good agreement has been reported between CASP score with LOS in patients of AES, meaning high functional ability (high LOS) following AES also had high level of social participation (high CASP) (28). Therefore, CASP would be useful addition to LOS in assessing disability in children.

7.7 Relation of admission weight for age with outcome in AES and JE patients

I found JE patients with lower WFA (Z score of -1,-2 and -3) at admission to have significantly worse outcome at the time of discharge as compared to those with normal or high WFA. There was also trend for AES patients with lower WFA to also have bad outcome. There were significantly more episodes and longer duration of convulsions in low WFA patients, among both AES and JE patients. Patients with confirmed or probable viral encephalitis with a low WFA Z score < -2 have also been associated with death or severe sequelae in a similar setting in the past. The authors attributed the low WFA to undernutrition (264).

According Gomez classification, malnutrition has been defined as first, second and third degree on the basis of 75% - 90%, 60% -74% and < 60% of median

desired body WFA and sex respectively (273). Malnutrition is an important health problem in resource limited countries. WHO states 85% of the world's children live in developing countries, where half of children are malnourished. According to Nepal Demographic and Health Survey (NDHS) 2011, 29 % children below 5 years of age are undernourished (moderate or severe), 41 % stunted and 11 % wasted in Nepal [http://nepal.unfpa.org/sites/asiapacific/files/pub-pdf/GF25.pdf (accessed on February 24, 2016)].

Malnutrition results from an insufficient quantity of calories, protein, carbohydrate, vitamins or minerals in the body. Not enough nutrients is also called undernutrition. Undernutrition is of two types: protein-energy malnutrition (PEM) and dietary deficiencies. Furthermore. there are two severe forms of PEM: marasmus (caused by both protein and calories deficiency) and kwashiorkor (only protein deficiency). Common micronutrient deficiencies observed are of iron, iodine and vitamin A. Other micronutrients which can be deficient includes zinc, copper, magnesium, calcium and vitamins B_6, B_{12}, B_1, B_2, B_3, and C (274).

Malnutrition can be assessed by; seeking a relevant history, clinical signs, biochemical indicators and anthropometry. Inadequate dietary intake can affect functional capacity resulting in adverse health outcomes. Inadequate diet can reduce physical activity and slow growth. Signs of wasting of body parts and alteration of biochemical parameters such as hypoalbuminaemia may then follow. Later activity becomes severely impaired and body wasting becomes marked. Oedema, hair and skin changes also appear.

A detailed dietary history on the amount of calories consumed in a day could help in the diagnosis of malnutrition. Additionally, history of suffering from infectious diseases such as tuberculosis, gastroenteritis, pneumonia, malaria, or measles, which cause increased nutrient requirements, could further help in making the diagnosis (273).

Along with poor weight gain, malnourished children also have poor gain in height and deficits in lean body mass and adipose tissue. The clinical features which suggest malnutrition in children are moon facies, dryness of conjunctiva, Bitot's spots, angular stomatitis, cheilitis, glossitis, spongy bleeding gums, enlarged parotid, sparse and brittle hair, alternating bands of light and normal colour hair (flag sign), alopecia, loose wrinkled skin, koilonychia, wasting of muscles particularly in the buttocks and thighs, hypoalbuminaemia and edema. It is a multi system disorder which may lead to reduced cardiac output causing cardiac failure, reduced gluconeogenesis in the liver causing hypoglycemia, decreased glomerular filtration rate in the kidney causing acidosis and hyponatremia, reduced insulin levels causing glucose intolerance and reduced basal metabolic rate causing hypothermia. Most importantly, there is also diminished cell mediated immunity, IgA synthesis, complement level and host defence's ability to phagocytose making children at risk of sepsis, diarrhoea and dehydration.

Anthropometry has an advantage over clinical and biochemical indicators of malnutrition because the latter tend to be most discriminatory at the extremes of malnutrition. Anthropometry is simple, non-invasive, cheap and easy to

obtain. Anthropometric measurements are important in malnutrition in-order to identify by stunting (low height-for-age) and wasting (low weight-for-height). The disadvantage of low WFA is that it cannot differentiate between stunting (short stature) and wasting (recent weight loss). Therefore low WFA would include both and would be unable to identify children with only recent loss of weight, important in the study. More accurate anthropometric measurements to diagnose malnutrition because of recent weight loss are either weight-for-height or Body Mass Index (even in children above 5 years of age) (273).

After selecting WFA and reference population, I used Z scores (standard deviation scores) to compare the children to the reference population. Although, percentiles and percent-of-median are other classification systems which could be used, Z score is now regarded as the most appropriate descriptor of nutritional status (273). The risk of mortality is related to the anthropometric indicators of nutritional status. Malnutrition has an effect on mortality in patients (273). Therefore, malnutrition can lead to bad outcome in AES or JE. But there is paucity of data on whether malnutrition itself is a risk for AES and JE. In my study it was clear that patients of AES and JE who had low WFA Z score (even if some of them were undernourished before the illness by WHO criteria) may have been dehydrated and this could have been the predominant cause of bad outcome (264).

However, a larger prospective study on a cohort of patients seeking relevant history, clinical signs of malnutrition, blood levels of relevant micronutrients, accurate anthropometric measurements such as weight-for-height, Body Mass

Index and mid-upper arm circumference could have been more helpful in diagnosing malnourished children. Measurements of arterial blood gas, urinary osmolality and specific gravity could help differentiate dehydrated children.

Low WFA could also have been caused by dehydration. The degree of dehydration is indicated by the degree of fluid volume depletion of the patient. This depletion is most objectively measured as a loss of weight from baseline. An estimation of dehydration based on percent loss of body weight is useful when clinical signs are difficult to elicit. This has a sensitivity of 74% for mild (3- 5% of body weight), 33% for moderate (6-9% of body weight), and 70% for severe dehydration (≥10% of bodyweight). Dehydration can also cause an increase in serum lactic acid and a decrease in serum bicarbonate leading to metabolic acidosis. It can lead to a reduction in fractional excretion of sodium, hyponatremia or hypernatremia or cavernous sinus thrombosis causing uncontrolled seizures. It can also cause cerebral hypo-perfusion or infarcts resulting in confusion, stupor or a focal neurological deficit (93).

Repeated seizures have been reported to be associated with bad outcome in JE (20). In my studies, what I found new was that, seizures were more common in low WFA group. Acute symptomatic seizure occurring within 7 days of CNS illness has been reported in 2- 67% of patients (154) following encephalitis. The risk of unprovoked seizure after 7 days of CNS illness is also reported to increase by 16 times than the normal population (and which could occur up-to 20 years) following acute encephalitis illness (258). Often subtle or non-convulsive status epilepticus can be missed in comatose patients. In JE, appearance of seizure is reported to correlate with high CSF opening pressure,

signs of cerebral herniation and death (154). Patients with a witnessed seizure in the hospital were 4 times likely to have bad outcome. Furthermore, those suffering status epilepticus were more likely to have a bad outcome compared to those with self limiting seizures (154).

In order to improve outcome of patients with AES, dehydration should be corrected and seizures controlled appropriately, along with the treatment of infection of the nervous system. However, there is still a possibility that some of the AES patients with low WFA could have been malnourished. Prevention and early management of malnutrition may have a role in preventing occurrence of AES and its complications. Malnutrition can be prevented by initiation of early breast feeding, complementary feeding through nutritional counselling, increasing access to quality food for the poor and marginalized through social protection schemes and safety nets and micronutrients fortification of food. The government of Nepal has been addressing this through its National Nutrition Policy since 2004. Dehydration needs to be corrected following standard guidelines, using resuscitation fluid, replacement fluid and maintenance fluid therapy [http://whqlibdoc.who.int.liverpool.idm.oclc.org/publications/2005/9241593180.pdf (accessed on February 24, 2016)].

The risk factors for developing seizures during CNS infection are not well understood. Risk of seizures appear to relate to the infecting pathogens (such as 6- 67% in JE, 40- 65% in herpes simplex virus encephalitis, 10-20% in La Crosse virus encephalitis and 2.2% in Nipah virus encephalitis), the degree of involvement of cerebral cortex and extent of the inflammatory response (154,

258). Around 60% patients with JE in my first study (chapter 3) and 72% in my second study (chapter 4) had seizures. Repeated seizures are reported to be associated with hypoxia, hypoglycemia, hyperlactataemia or metabolic acidosis. Each of these parameters needs effective management and correction in addition to anti-epileptic drugs. Further studies are required to find out the association between different CNS infections, seizures, anti-epileptic treatment and outcome (154). More research on the outcome and prognosis of post encephalitis seizure is also warranted.

7.8 Relation of admission weight for age with neurological sequelae in AES and JE patients

The range of sequelae observed in my studies were similar to what others have described (3, 16, 21, 27, 73, 74, 76). What I found new was that the residual neurological sequelae at the time of discharge was seen to be significantly higher in those with lower WFA as compared to those who had appropriate or higher WFA in both AES and JE. AES patients of low WFA had significantly more feeding difficulties, cognitive problems, limb movement difficulties, incontinence and seizures at the time of discharge. Whereas, low WFA group of JE patients had significantly more problems of poor feeding, expressive language and dressing difficulties at the time of discharge. In a previous study, when JE patients exhibiting movement disorders, dystonia or parkinsonian features were evaluated by brain imaging, thalamic, basal ganglia and brainstem regions were observed to be hyperintense on T2 and FLAIR and hypointense in T1 weighted images in MRI suggesting regions of hypoperfusion (275). Since Low WFA may have been related to dehydration,

the neurological sequelae in AES and JE could have exacerbated poor cerebral perfusion in the brain. Addition of hypoperfusion injuries to the usual reports of brain pathology, that include diffuse oedema, congestion, infiltration of white blood cells, widespread neuronophagia and necrosis of the brain, meninges and spinal cord in AES and JE patients, could lead to further neurological sequelae in this group of patients. SPECT studies have reported hyperperfusion in acute stage followed by hypoperfusion in subacute and chronic stages of encephalitis (276, 277). In both AES and JE, low WFA patients more frequently exhibited moderate or severe residual neurological sequelae. Similar sequelae were observed among these patients at follow up 2 year post hospital discharge. Some studies report that the degree of cerebral hypoperfusion does not correlate with outcome at 6 months post discharge (275). There is need for a larger cohort study to look into the relationship between dehydration and the occurrence of neurological sequelae.

7.9 Relation of admission weight for age with functional recovery in AES and JE

There are limited studies examining the recovery profile of AES and JE patients. Although some studies have examined outcome up-to two years post hospital discharge; they have not recorded outcome at discharge(or another time-point) making it difficult to describe the patients' recovery profile over time(16, 73). In this study (chapter 4), the patients of AES and JE were followed up at two time-points ; first, at one year and second, at 2 years from discharge. On the whole, there was improvement in patients between 1 and 2 years post discharge, especially in aspects of concern to caregivers, such

as ability to self-feed and be left alone at home. Functional impairment was seen in higher proportion of children with a lower WFA group as compared to those with a normal or higher WFA at discharge and 2 years follow up. Cerebral hypoperfusion is associated with focal neurological deficit in acute vaso-occlusive disease (278). Whether poor recovery in the low WFA group could be because of long term consequence of cerebral hypoperfusion in encephalitis is less understood. The proportion of AES and JE patients who had functional recovery were higher in the higher WFA group as compared to those with a lower WFA. Patients of encephalitis are at risk of developing acute symptomatic seizures within 7 days of CNS infection or unprovoked seizures later in life. The risk of unprovoked seizures is reported to increase by 22 times in those who had developed seizure during acute illness and 10 times in those with no acute seizure as compared to normal population (154). Early seizures in 67% and persistence up-to 20 years in 22% have been reported in encephalitis in the past (53, 154). Acute symptomatic seizures are a predictor of bad outcome. Seizures and status epilepticus are important risk factors for fulminant cerebral oedema in acute encephalitis. This can cause a rise in ICP and brain stem herniation, which could cause sudden death, shock or severe neurological sequelae (279). In my studies, what I found new was that, seizures were present and persisted in higher proportion of AES patients of lower WFA. Seizure improvement has been reported to occur over time following acute encephalitis, such as anti-N-Methyl- D-aspartate receptor (NMDAR) encephalitis (280). Some of the AES patients in my study may have suffered anti-NMDAR or another autoimmune encephalitis. This was not investigated due to lack of resources. I observed uncontrolled seizures in some patients up-

to 2 years after discharge. This finding highlights the need of a cohort study on long term sequelae of encephalitis.

7.10 Relation of admission weight for age with long term outcome which was poor reflection of discharge outcome

Approximately 60-70% of children with encephalitis have neurological sequelae (281). Common sequelae after encephalitis are emotional liability, cognitive deficit, behavioural problems, dystonia, spasticity and epilepsy. Two-thirds of patients of herpes encephalitis develop neurological sequelae at discharge despite treatment with acyclovir (282, 283). Better hospital facilities mean reduction in mortality but an increase in number of patients with sequelae.

There have been reports of patient outcome changing between time of discharge to time of long term follow up (281, 284, 285). JE patients have been reported to have deteriorated functionally at follow up after initial improvement at the time of discharge (21). Some children with encephalitis discharged as "complete recovery" have been later found to have lower intelligence quotient (IQ) scores and a higher prevalence of learning disabilities than general population (281). This could happen in very young children because impairment cognitive function is difficult to assess. Problems with cognitive function could become more evident as the child grows older. Subtle neurological manifestations could also be overlooked because of variations in neurodevelopment among young children (284). Long term low grade neuroinflammation occurring after encephalitis has also been hypothesised to

impair normal function of astrocytes and impair cognitive function in traumatic brain injury patients (286). There are reports on viral infection of CNS in early childhood which have lead to neurodevelopment and psychiatric disorders such as autism and schizophrenia later in life (281, 287). Further studies are needed to find out the relationship between CSF cytokines and neurological sequelae.

There is a pressure on clinicians in low resource settings to discharge patients quickly, because of the huge number of patients attending the hospital during outbreaks of encephalitis and demands from hospital management to reduce hospitalizations days. However, our long term follow up study in JE patients have showed that most changes in outcome occur within 3- 6 months after hospitalization. and not at the time of discharge (21). In this study (chapter 4), I observed 5/61 (8%) additional deaths [including 3/17 (18%) JE patients] among patients after their discharge from the hospital. This observation highlights that outcome at discharge is a poor reflection of the eventual outcome (21, 74). A new finding in my studies were that functional impairments increased in children of low WFA (-3 Z score) from 20% at 1 year to 33% at 2 year follow up (chapter 4). This increase in functional impairment could, in part, also be associated with ongoing seizures in children of low WFA in my study. Both neurological sequelae and seizure occur by similar mechanisms. Cerebral insults can initiate disruption of the blood brain barrier, neuronal reorganization, glial activation and neuronal hyperexcitability amongst others to cause seizure. Seizures can cause prolonged neuronal excitation, inflammation and epileptogenesis in encephalitis (281). Development of epilepsy has strong association with development of sequelae (281, 283, 288, 289). CNS injuries are responsible for 30-49% of seizures

and epilepsy. This increased risk for seizure has been observed in encephalitis patients up-to 20 years post acute illness (283). Seizures could also have directly increased the sequelae in this group of children. In children with low WFA and neurological sequelae, physical and psychological rehabilitation should be initiated in the hospital to help minimize the impact of sequeale.

7.11 LWA and Nepal

LWA can occur because of undernourishment (290). Undernourishment is a physiological impairment created by lack of nutrients in the body. Globally, one in every sixth child is either moderately or severely underweight. According to the NDHS 2011, 3,229,121 children are below 5 years of age in Nepal. Out of these 29% are moderate or severely undernourished. Evidence from animal research shows that malnutrition may augment seizures in known epileptics (291). Malnutrition has also been shown to increase the risk of death in patients with pulmonary tuberculosis (292).

For the first time in 2004, The National Nutrition Policy and Strategy was formed to address the issue of low birth weight, protein energy malnutrition, iodine deficiency disorder, iron deficiency anaemia, vitamin A deficiency disorder, intestinal worm infestation and life style related issues. The country had set targets of reducing undernourished children from 39% in 2010 to 29% in 2015 through the implementation of the Nepal Health Sector Program-2. One of the major policies embraced to reduce the under 5 mortality was to scale up community based newborn care and implement comprehensive nutrition programmes. In 2011, Nepal joined the global "Scale Up Nutrition" campaign to fight against hunger and malnutrition through the Multi-Sector

Nutrition Plan (MSNP) which was initially launched from 6 selected districts. The national multi-sectoral nutrition planning framework can be found as appendix S. Low income countries like Nepal can lose a minimum of 2-3% of their gross domestic product to malnutrition. The World Bank has estimated that an additional $10.3 billion needs to be spent per year to prevent 1 million child deaths and to benefit the 360 million children from 36 countries with the highest burden of malnutrition. The most effective nutritional interventions focus on first 1000 days (as in MSNP) because economic returns of investment then are very high. The 2008 Copenhagen Consensus ranked that providing micronutrients to undernourished children the most cost-effective strategy to improve nutrition and social welfare [http://www.copenhagenconsensus.com/sites/default/files/cc08_results_final_0.pdf (accessed on March 4, 2016)].

Globally, diarrhoea (and dehydration) is estimated in 9.5% of severely undernourished and 3.4% of moderately undernourished children (293). Even in undernourished children with AES, clinical features and laboratory parameters suggests presence of dehydration. In Nepal, where malnutrition is common, dehydration should be suspected when managing children with AES or JE (264). Typical signs of dehydration (Appendix E) (133) may not be obvious in a severely malnourished children. Lack of subcutaneous fat may cause skin turgor to appear poor and eyes sunken in children with marasmus. It may be masked by oedema in kwashiorkor. Presence of irritability or apathy make assessment of mental status difficult in these children. In severe malnutrition, it is difficult to differentiate mild dehydration from severe dehydration, affecting treatment. The only signs which then becomes useful

for assessing hydration status in malnourished children are thirst (mild dehydration), and lethargy, cool extremities, weak radial pulse, reduced urinary output (severe dehydration), and acute weight change. Therefore dehydration can easily be missed during hospital presentation. Modified fluid replacement guidelines are available for the treatment of severe dehydration in undernourished children. Children with severe malnutrition and mild or severe dehydration (not in shock) should be given 5 ml/kg of reduced osmolarity ORS solution (ReSoMal) orally or via naso-gastric tube every 30 minutes for the first 2 hours [ReSoMal contains a lower amount of sodium (45mmol/L) and higher amount of potassium (40 mmol/L) than standard WHO-ORS]. On reassessment, if the child is still dehydrated, 5–10 ml/kg/hour of ReSoMal should be given every alternate hour with F-75 diet, up to a maximum of 10 hours. In children with severe acute malnutrition having signs of shock, who cannot be rehydrated orally or by naso-gastric tube, should be treated with intravenous fluids. Fluids prescribed include; half-strength Darrow's solution with 5% dextrose, Ringer's lactate solution with 5% dextrose or 0.45% saline + 5% dextrose (294). Therefore, rapid correction of dehydration in an undernourished child could cause adverse outcome.

7.12 Low WFA patients of AES or JE could be dehydrated

Low WFA at admission in children with AES and JE is associated with bad outcome at discharge and persistence of sequelae even at 2 years of follow up. Low WFA children can be either dehydrated or undernourished (133, 290). Acute loss of body weight typically reflects loss of body fluid and not body mass. Children are at increased risk of dehydration compared to adults, because

they have relatively a higher surface area to volume ratio with higher insensible water loss, which can easily be accentuated by fever. Very young children and older children with focal neurological deficits, suffering from AES or JE, may not be able to independently access food or effectively communicate their need for fluids as compared to adults. I found patients of low WFA to be younger, have more focal neurological deficit, lower GCS and longer duration of fever as compared to patients with higher WFA in both AES and JE. The WFA Z score was estimated using the WHO reference population.in patients below 5 years of age. The WFA Z score was estimated using a Nepali national reference population in patients above 5 years. The WHO developed their child growth curve reference data based on studies involving children from the United States of America around 1970. The suitability of these curves for international purposes has been challenged more recently (http://www.who.int/nutgrowthdb/about/introduction/en/index3.html). The WHO reference population may not be suitable for comparing Nepali population. Therefore, more children in my study (under 5 years) could have appeared falsely to be of low WFA based on the WHO reference (273). There is a need for a single national reference population to compare the WFA Z score of all age groups of Nepali children. It is also possible that younger children may be dehydrated even by modest deficiency of fluids as compared to older children. This latter suggestion could also explain the prolonged fever and presence of focal neurological deficit observed in younger children. Systematic measurement of serum urea, electrolytes, fluid balance, markers of acid-base balance and MUAC in children of low WFA would help differentiate dehydration from malnutrition in these children.

7.13 Relation of low WFA at admission and/or weight loss during hospital with outcome

I grouped patients with low WFA at admission, and patients exhibiting weight loss during hospital, together during my study. The former patients may have suffered weight loss because of inability to feed. The latter patints may have suffered weight loss secondary to restriction of maintenance fluids by the treating clinicians (fearing cerebral oedema or raised ICP). Of a total 92 AES patients, more than half had a low WFA and/or weight loss. The odds of having a bad outcome was 7 times greater if they were of low WFA and/or had weight loss. Furthermore, the odds of dying were 13 times greater if they had low WFA at the time of admission, 10 times greater if they had weight loss during admission, and 19 times greater if they had low WFA andweight loss. A child's arm contains subcutaneous fat and muscle mass. When there is reduced food intake, lower levels of subcutaneous fat and muscle mass tend to correspond to a decrease in mid upper arm circumference (MUAC). This measurement can be used to diagnose malnutrition in infants and children 6–60 months of age (295, 296). The MUAC of all recruited children between 6- 60 months of age was ≥11.5 cm which meant there was a strong possibility of acute loss of weight being due to dehydration rather than severe malnutrition (141). In 2009, the WHO and United Nation's International Children's Emergency Fund (UNICEF) recommended MUAC cutoff of <11.5 cm as one of three screening criteria for identifying and managing severe acute malnutrition in infants and children 6–60 months of age. Although previously systematic review of the literature showed that children with MUAC measurements <11 cm had significantly elevated risk of mortality (297), WHO and UNICEF recommended a slightly

higher cutoff to increase sensitivity of the measure, while maintaining high specificity. Changing the MUAC cutoff value from 11 cm to 11.5 cm produced a large change in sensitivity (from 16% to 25%) with little loss in specificity in improving the probability of diagnosing severe wasting (298). It also reduced false negative results by 12% (298).The sensitivity and specificity of MUAC < 11.5 cm in identifying severe wasting (weight for age Z score < -3) among children 6 to 59 months was 4.9% - 47.8% and 99.7% - 97.4% respectively (298). MUAC may give false positive result on degree of malnutrition in patients with diseases that cause muscle wasting, such as acquired immune deficiency syndrome (AIDS).

MUAC is a single measurement, independent of age and gender, of children commonly between six months and five years of age. In a time and resource limited setting, it is most useful tool to screen for malnutrition. It is better than other anthropometric measurements in predicting subsequent mortality according to community studies (299, 300). The advantage of MUAC over other anthropometric measurements is that, with color codes of red zone (< 11.5 cm) as severely malnourished, yellow zone (11.5- 12.5 cm) as risk of malnutrition and green zone (> 12.5 cm) as not malnourished, it is not only useful for even illiterate people but also saves users from memorizing and relating the cut-off numbers for each category. It can also be easily taught to minimally trained health workers because it requires a simple equipment; and can be performed even on debilitated individuals. Errors are fewer during measurement of anthropometry by MUAC than Weight-for-Height (297). Since MUAC is less affected than BMI by the localized accumulation of excess fluid in famine, it is being increasingly used in adult undernutrition (301, 302). In

adults, moderate undernutition is considered if MUAC is < 18.5 cm and severe undernutrition < 16 cm.

Another potentially valuable application for MUAC is in screening for obesity. It can provide high accuracy for the assessment of obesity in children and adolescents in resource-poor settings (303). A number of studies have shown that high MUAC has high diagnostic accuracy (sensitivity, specificity and predictive values) for the identification of overweight and obesity (as defined by BMI for age).

7.14 AES patients suffering bad outcome may have been dehydrated

Significantly more AES patients who suffered bad outcome had low WFA on admission. These patients had higher fluid requirements, higher fluid deficits, were more likely to be fluid restricted (to two-thirds of maintenance), had lower intake of oral fluids, higher serum sodium and more likely to have high serum urea and lactate at admission. The findings suggest these patients were not just sicker, but also likely to be dehydrated. Consequently, one explanation for the observed increased risk of bad outcome among children with low WFA and/or weight loss may be due to dehydration.

7.15 Relation of death, sequelae and recovery with hydration status of AES patients

When outcome was further sub-grouped into death, sequelae or complete recovery, I found significantly more children who died had a low WFA, higher serum urea and higher serum sodium at the time of admission. Patients

who died, exhibited more weight loss during admission, than those who had sequelae or who recovered completely. There was also tendency for higher admission serum lactate levels in those who died. Serum lactate tended to further increase on their second day post admission.. Hence, there was evidence of more severe dehydration among those who died, compared to patients who suffered sequelae or who recovered completely. In contrast, patients who recovered completely required significantly less fluid replacement, received less maintenance IV fluid, had more oral fluid intake, and were less fluid restricted.

7.16 Effect of dehydration in body metabolism

Dehydration causes hypo-perfusion of the tissues. Therefore, there is decreased oxygen delivery to cells, reduced availability of oxygen in the energy cycle and promotion of anaerobic metabolism. This leads to oxidative fermentation of pyruvate to form lactate, which accumulates in the blood to cause metabolic acidosis. Akin to sepsis, there may also be excessive production of pyruvate, which again is fermented to lactate to further exacerbate lactic acidosis (148). Presence of acidosis will shift oxygen-haemoglobin dissociation curve to the right, decreasing haemoglobin's affinity for oxygen causing decrease in oxygen delivery. This starts a vicious cycle of poor oxygen delivery- lactic acidosis-poor oxygen delivery. Severe metabolic acidosis can impair the body’s metabolism,impede neurovascular interaction and affect pharmacological action of drugs. In my study, elevated serum sodium, serum urea, serum creatinine and serum lactate on admission, together with supporting clinical information, indicated that patients who died showed signs of metabolic acidosis with impending renal failure.

7.17 Effect of dehydration in different tissues

Adequate tissue perfusion is essential for normal cell function. In the nervous system, dehydration can cause cerebral hypo-perfusion and ischemia, causing confusion and stupor. Dehydration increases the risk of cerebral infarction, in turn, increases the risk of neurological deficit and/or seizures (93). In the kidney, dehydration can cause hypo-perfusion and acute renal failure. It can also cause hypo-perfusion of the gastrointestinal tract, trigger erosive gastritis or ischemic pancreatitis. Hypo-perfusion of the liver can cause ischemic hepatitis. In the blood, it can produce coagulation abnormalities and thrombocytopenia. In the heart, it can cause life threatening arrhythmias or myocardial depression. In the lungs it can cause respiratory muscle fatigue and hypoxia. Most importantly it can cause life threatening shock and multi-organ failure.

7.18 Seizures and low WFA and dehydration

Seizures are a treatable complication of AES. Effective management of seizuers could improve outcome (20). Of the 70 million people living with epilepsy around the world, 60 million live in low or medium income countries; half of whom are children (304). Acute symptomatic seizures occur in around 30% of CNS insults at the time of acute infection. This is also a risk factor of later development of epilepsy when recurrent seizures occur after an epileptogenesis event. The risk of developing epilepsy after CNS infection depends on the aetiology of the infectious agent, severity of brain injury, age, genetic factors and other co-morbidities (304). Between infection and development of epilepsy various changes in the brain occur such as initiation

of immune or inflammatory response, increased permeability of BBB, neuronal hyperexcitability, neuronal loss, gliosis, molecular and structural reorganisation and epigentic reprogramming which could cause occurrence of spontaneous recurrent epileptogenic seizures (304). Although there is paucity of information in humans, in mouse models, IL-1β, TNF- α and IL- 6 trigger an inflammatory response, cause neuronal hyprexcitability, and lower the neuronal seizure threshold, to cause seizures (304). Inflammation can permanently alter expression of glutamate receptor subtypes and K^+/Cl^- co-transporter in the brain which in-turn may trigger immediate or long term pathophysiology. Inflammation can impact on seizure recurrence, cause acute and long term reduction in the seizure threshold, as well as other cognitive co-morbidities (behaviour, learning and memory deficits) observed in adulthood. Inflammation may also contribute to disease progression (305, 306). Epilepsy and persistence of neuroinflammation can occur in the immature brain following just a single episode of acute symptomatic seizure (304). In my study, I found children of low WFA were significantly younger than those with higher WFA. Episodes of seizure and bad outcome were also significantly more frequent in young children. Young children have less mature brain development. This may put them at higher risk of seizures and the pathophysiological changes that can follow.

In my study (chapter 5), I found serum sodium was significantly elevated among the dehydrated patients, particularly in the bad outcome group (bad outcome 140 mmol/L versus good outcome 131 mmol/L); and in those who died (death, 145 mmol/L versus recovered, 131 mmol/L). Hypernatremia can

cause repeated seizures. Repeated seizures or status epilepticus are associated with bad outcome (20). There was a significant association between repeated seizures with dehydration and metabolic acidosis in my study. Acidosis can inhibit voltage gated calcium pumps. These pumps facilitate influx of calcium from the extracellular to intracellular space. Pump failure is linked to hypocalcemia and seizure (307). Acidosis could trigger seizures, which in turn could be the reason for increased neurological sequelae in dehydrated patients. Other causes of seizure in dehydration can be hypoglycaemia, hyperlactataemia, hyponatremia, cerebral venous thrombosis, cavernous sinus thrombosis, fever triggered seizure or CO2 retention. I did not find hypoglycemia as a cause of seizure in these groups.

7.19 Relation of intravenous fluids and AES

Intravenous fluids (IVF) are used in the care of sick and injured children. The IVF commonly used is 5% dextrose with 0.18% normal saline. Maintenance IVF is expected to supply sufficient sodium chloride for the body's metabolism, water to excrete nitrogenous waste by kidneys and glucose to prevent glycogen breakdown (Appendix T).

The Holliday- Seger equation, which estimates amount of kilocalories expended and equates with fluid requirement in millilitre, has been the current standard method of calculating and administering maintenance IVF (141). It has been argued that a sodium concentration of 30 - 50 mmol/L makes fluid hypotonic, and that sodium concentration should be increased to approximately 75 mmol/L (0.45% sodium chloride) to be isotonic (308). The WHO, showing concern for dehydration, recommends full maintenance fluids in children

with bacterial meningitis with an emphasis on providing IV glucose. Full maintenance hypotonic fluid can cause hospital acquired hyponatremia. Such a fluid regime may be more harmful to children in resource poor settings, where serum sodium cannot be regularly monitored. Many researchers have described adverse neurological events, such as seizures, status epilepticus, cerebral oedema, intracranial hypertension and encephalopathy in children with serious infection who have become progressively hyponatremic or who have received prolonged hypotonic fluid (150, 309- 313). Urinary loss of sodium, SIADH and shift of water from intracellular to extracellular space has been estimated to account for hyponatremia, in 29- 45% of children with encephalitis. In the brain, even with an intact BBB, a fall in serum osmolarity favours water accumulation in the interstitium or brain cells to cause cerebral oedema (149). Assuming normal renal function, 0.18% saline at 100 ml/kg/day is estimated to trigger a fall in serum sodium from 135 mmol/L to 131 mmol/L and cause a 5% rise in total body water. Whereas 0.9% saline at 75ml/kg/day is estimated to trigger an increase in serum sodium by 2 mmol/L and an increase in total body water by 1.5% within a day, without any increase in intracellular water (92).

Unrestricted maintenance fluids, in children with impaired renal function, can disrupt the BBB, cause cerebral oedema and other adverse neurological outcomes, depending on the volume of fluid administered (92). Isotonic saline has a pH of 5- 6. Metabolic acidosis can still persist, when saline is used in large volumes for children in shock. In such circumstances, bicarbonate or another buffer may be needed (92). Rapid rehydration by bolus, using ringer lactate or normal saline, in patients with hypernatremia (where sodium

monitoring is not available) or receipt of hypertonic rehydration solution prior to admission, can cause death. Excess mortality from fluid boluses in patients of severe acidosis and severe shock has been reported previously (314). A landmark study conducted in Africa based on a large sample size, multi-national sites, good concealment of treatment, high treatment adherence and patient follow up in children with compensated shock, concluded there was no additional advantage of bolus albumin over saline in resuscitation of sick children (142). The study also found children given boluses of fluid, whether albumin or saline, exhibited an increased rate of death, compared to control group of children who received normal maintenance IVF over 2 days and 4 weeks of treatment (142). However, it has been argued that the sick children they recruited were neither hypovolemic nor septic, as reported, rather they appeared to have malaria or bronchiolitis, in which, fluid boluses were detrimental (142). This difficulty of case diagnosis is seen in Nepal. Pneumonia is frequently diagnosed following WHO criteria. These children may instead have bronchiolitis, and could inadvertently receive fluid boluses because of suspected pneumonia with septicaemia. It can be argued in shock, when the body is compensating with vasoconstrictor response to reduce perfusion to non vital tissues, when resuscitated rapidly with IVF for reversal, maybe deleterious. Also fluid boluses could cause reperfusion injury, cardiac failure, pulmonary oedema or rise in ICP. However, the African study may have erroneously excluded true septic or hypovolemic sick children as gastroenteritis, severe malnutrition or non-infectious shock. Applying their recommendation and denying fluid boluses to children with true hypovolemic shock could cause adverse outcome (142).

Healthy children excrete larger volumes of water compared to sick children. It has been suggested that the quantity of IVF provided should be tailored to disease state. Maintenance fluid should be reduced to less than 75% of normal in patients whose free water clearance is reduced by 50%, to avoid oedema. However, this approach should not be misinterpreted. Otherwise, there is a risk of restricting fluids to the point of dehydration, because of fears that cerebral oedema may adversely affect outcome (315). For example at Kanti Children's Hospital, all children with severe altered sensorium (modified GCS < 8) or having clinical features suggestive of raised ICP have their fluid intake restricted to two- thirds of their maintenance fluid. Adequate IVF also helps in appropriate distribution of drugs to achieve therapeutic concentration Furthermore, dehydrated patients are at risk for water soluble drug toxicity (141).

There is paucity of data on optimum fluid management for patients with non-traumatic brain injury (93, 143, 144). My study aimed to better understand the association between hydration (including fluid supplementation) and outcome in children with AES. I observed significantly more children who received 0.45% saline with 5% Dextrose compared to other IV fluids died. In contrast, children who received 0.9% saline IV tended to exhibit full recovery. I also observed that children who were taking oral feeds more frequently exhibited better outcome. My findings re-opens the question as to what fluid management should be provided in acute infectious illness, and makes the area of brain infection, where clinicians are balancing the support of cerebral perfusion pressure against the risk of cranial or pulmonary oedema, even

more challenging (20, 123). Without any specific anti-viral treatment for AES, optimum supportive management of patients during their acute AES illness is vital. A large randomised controlled trial of hypotonic versus isotonic saline or different volumes (two- third versus full maintenance) of systemic fluid supplements in children with severe infections, stratified for types of infections to find out the differences in frequencies of severe hypernatremia, neurological complications or death may tell us which volume and type of IVF could improve outcome. Until then 0.9% sodium chloride with dextrose tailored for free water clearance maybe the most appropriate for very sick children (92).

7.20 Intravenous immunoglobulin as a treatment of JE

Intravenous immunoglobulin (IVIG) has been postulated as a treatment for flavivirus encephalitis caused by JEV and WNV, on account of its antiviral and anti-inflammatory properties (168). Pre-clinical studies suggested passively transferred antibody could be useful against flavivirus encephalitis (165, 167, 168, 176- 182). Konishi *et al* demonstrated that neutralising antibody prevents virus dissemination from the peripheral site to the brain, and that antibody-mediated mechanisms of protection were more efficient than cytotoxic T cell responses (179). In animal studies in which IVIG containing anti-WNV specific antibody was administered during the viraemic phase but before the virus had entered the CNS, the studies demonstrated a dramatic 100% survival rate (167, 180), or that mortality was reduced up to five days after infection (181, 182). Peripheral administration of anti-virus monoclonal antibodies in a mouse model has been shown to neutralize WNV even after the virus has entered the brain (185, 186).

IVIG in humans has been used on a compassionate basis, but has not been assessed in a randomised trial for JEV or WNV (165, 168). The previous trials assessing adjunctive agents for JE were conducted in Thailand and Vietnam, both highly developed research settings, where-as Nepal has no such establishments. Therefore a preliminary randomised placebo-controlled trial of IVIG was conducted for treatment of children with suspected JE in Nepal.

7.21 Selection of Intravenous immunoglobulin for treatment

IVIG developed using serum from donors living in endemic areas is considered most appropriate for flavivirus encephalitis, including that of JEV and WNV, because of the neutralising antibody it contains (167). I found IVIG from a range of manufactures in Asia contained significantly high neutralising antibody; most had $PRNT_{50}$ titres ≥1:640. ImmunoRel™ IVIG produced by Reliance Biopharmaceutical (India) had the highest anti-JEV $PRNT_{50}$ titre, and was chosen for treatment in my study. However, ImmunoRel IVIG was found to show low $PRNT_{50}$ titres ≤1:20 against DENV, WNV and Powassan viruses.

7.22 Intravenous immunoglobulin treatment and neutralising antibodies

IVIG treated patients had a greater increase in neutralising antibody titres as compared to placebo. What I found new was that, JEV antibody positive children treated with IVIG showed approximately 16 times higher titres of neutralising antibody compared to levels following IVIG treatment among JEV antibody negative patients. The extent of rise appeared greater than can be

explained by passive transfer of anti-JEV antibody. Although the mechanism is unclear,, it is possible that passively transferred antibody is augmenting the natural production of neutralising antibody by B cells, through enhancing the uptake of viral particles by antigen presenting cells (187). A 1:10 neutralising antibody titre has been shown to protect against JEV in animal models, when antibody is administered prior to infection. However, no studies have determined appropriate titre required to limit the evolution of encephalitis once JEV infection is established in humans.

7.23 Intravenous immunoglobulin treatment and cytokines

Neuronal cell death in JE may occur directly, from viral cytopathology, and indirectly via immune mediated mechanisms. This may include over activation of microglia cells (190), which release pro-inflammatory cytokines such as interleukin 6 (IL-6), TNF-α, and RANTES (regulated upon activation, normal T cell expressed and secreted), causing massive migration and infiltration of leukocytes into the brain (191) increasing permeability of the blood brain barrier leading to interstitial cerebral oedema and raised intracranial pressure (195, 196) as discussed in chapter 3. In addition to affecting virally-infected cells, the inflammatory response in the CNS may also damage non-infected cells to cause bystander cell death.

The rise in IL-6 and IL-4 responses in our patients support the hypothesis that administration of IVIG modulates the immune response. IVIG treatment has been associated with both reduced and elevated levels of IL-6 (199- 205),

and increased IL-4 levels (206, 207) in previous studies. IL-6 and IL-4 were chosen because they have previously been used to show balance of pro- to anti- inflammatory responses (45). Both IL-4 and IL-6 are secreted by T cells and participate in the development of antibody responses; IL-4 promotes B cell proliferation and isotype switching and IL-6 induces differentiation of B cells into antibody secreting plasma cells. It may be that the cytokine changes I observed reflect the augmentation of antibody response; therefore the increase in both antibody and pro-antibody producing cytokines may have lead to the same process (209). The differences in response among patients with different anti-JEV antibody statuses indicates immune modulation by IVIG. Other mechanisms of cytokine production may involve patients generating immune complexes against antibodies present in IVIG which could stimulate monocytes to produce IL-6 via Fc-receptor interactions (210). Also, other immunomodulatory factors (e.g., sCD4, sCD8, sHLA antigen) in the IVIG could stimulate cytokine production (211, 212). Clinically, in asymptomatic JEV infection, antibody probably protects the host by restricting viral replication before the virus crosses the blood brain barrier (52). However, in established encephalitis, it may also restrict damage by neutralising extracellular virus and facilitating lysis of infected cells by antibody-dependent cellular cytotoxicity.

7.24 Other implications of intravenous immunoglobulin treatment

In recent years, neurological conditions account for approximately 43% of the IVIG use in clinical practice worldwide (316). IVIG used therapeutically,

supplements the immune system with immunoglobulins collected from healthy donors which broaden the spectrum of immune response and attenuate autoimmune response in recipients (317- 319). Unlike steroids, it modulates immune response without increasing risk of opportunistic infection. IVIG is prepared from pooled serum immunoglobulins collected from 3000- 10,000 blood donors. The variety of donors helps expand the spectrum of the IVIG's antibody activities. IVIG contains mostly IgG1 and IgG2. Other minor components include IgM, IgA, soluble CD4, CD8, HLA molecules, small amount of coagulation factors and cytokines. IVIG acts through multiple immunomodulatory and antigen recognizing pathways involving humoral and cellular immunity. IVIG also acts on various subsets of B cells and T cells, scavenges complement, modulates cytokines and blocks idiotypic antibodies (317, 318, 320). IVIG may also alter gene expression associated with inflammation, fibrosis, and regeneration (321). Typically, the dose for acute treatment with IVIG is 2 g/kg over 2 to 5 days (316). Subcutaneous is more popular than intravenous route because of convenience in administration at home and fewer systemic side effects, (322).

In my study, many patients with AES (i.e. confirmed non-JE) may have been suffering from antibody mediated or autoimmune encephalitis (AIE). AIE cases are commonly missed in resource poor setting like Nepal because of lack of awareness, skilled human resources and laboratory facilities. Antibody mediated encephalitis can be grouped according to the serum antibodies and their specific targets such as a) intracellular antigens (GAD65) b) intranuclear antigens (Hu, Yo and Ma2) c) neuronal membrane antigens

(VGKC), various glutamate receptors (NMDA or AMPA receptors) or the GABAB receptor (304). Recently many cases of autoimmune encephalitis associated with antibodies against the N-methyl D-aspartate (NMDA) receptor called N-methyl-D-aspartate receptor antibody (NMDARAb) encephalitis have been reported. The disorder is likely mediated by antibodies against the NR1 subunit of the NMDA receptor causing state of reversible NMDA receptor hypofunction and characterized by prodromal stage of fever, nausea, vomiting followed after few weeks by rapid or subacute onset behavioural change, neuropsychiatric features, seizures, unresponsive/catatonic state, dyskinesias, and autonomic instability (323- 325). Initially, it was described as a paraneoplastic syndrome associated with ovarian teratoma (323), but it is now clear that many patients don't have tumours, and that men and children are also affected (325). Presence of NMDA receptor antibodies in the serum and/or CSF is diagnostic. Early tumour resection (if present) along with immunotherapy (intravenous and/or oral steroids, IVIG, and/or plasma exchange) and second-line immunotherapy with cyclophosphamide or rituximab is recommended (325), although spontaneous prolonged recoveries have been reported (324). Over 75% of patients exhibit recovery. IVIG is being increasingly used for its treatment.

Another autoimmune encephalitis is Voltage-gated Potassium Channel-complex Antibody-associated Limbic Encephalitis (VGKC-LE), where antibodies are directed against potassium channel complex proteins, such as contactin-associated protein-like 2 (Caspr2) and leucine rich, glioma-inactivated 1 protein (LGI1). The latter proteins are tightly associated with

potassium channels in the brain (326). Clinically, these patients present with memory loss, confusion, behavioural changes and seizures (326). In addition, patients with LGI1 antibodies present with facio-brachial dystonic seizures preceding limbic encephalitis (327). Histopathologically, patients with antibodies specific for LGI1 or caspr2 show inflammation and severe degeneration in the hippocampus. Diagnosis is confirmed by clinical feature and presence of VGKC-antibodies in the serum and CSF. Importantly, antibody lowering treatments like plasma exchange and IVIG improve neurological deficits in these patients, suggesting that antibodies directed against the VGKC complex are responsible for clinical symptoms (326). Treatment may be continued for 1-2 years. Relapse occurs in at least 5% of cases.

Acute Disseminated Encephalomyelitis (ADEM) accounts for around 10% of all known cases of encephalitis. ADEM usually affects children 3- 10 years of age and begins after a childhood rash (exanthema), other viral infections or immunisations. The white matter of the brain is predominantly affected. The initial clinical features are less-specific, and include fever, headache, stiff neck, vomiting and anorexia. Later there is loss of consciousness, confusion, coma, visual deterioration, hemiparesis and seizures. It has a monophasic course from weeks to a month. MRI of the brain typically shows multiple areas of characteristic white matter abnormality. Antibodies and cellular immune responses against specific brain antigens has been reported on investigation. Although intravenous methyl prednisolone is treatment of choice, IVIG and plasma exchange is useful in resistant cases (328). Complete remission can occur in 75% of cases.

IVIG is also indicated for use in various neuropathies, myopathies and disease of neuromuscular junction. It is commonly used in chronic inflammatory demyelinating polyneuropathy (CIDP), a chronic autoimmune neuropathy associated with demyelination of peripheral nerve and abnormal humoral and cellular immunity. The induction dose of IVIG is 2 g/kg, given over 2 to 5 days. In Guillain–Barre syndrome (GBS), remission is reported in up to 53% patients with IVIG (329). Multifocal motor neuropathy (MMN) with conduction blocks is a slowly progressive motor neuropathy which also respond to IVIG in 94% of patients, even though they may require higher dosage of IVIG. IVIG is also administered in IgM associated paraproteinemic neuropathies (330), idiopathic inflammatory myopathies, dermatomyositis, polymyositis, refractory necrotizing myopathy, sporadic inclusion body myositis (331, 332), exacerbation of myasthenia gravis (MG) (333) and Lambert–Eaton myasthenic syndrome (LEMS) (334). IVIG is administered as first line treatment for GBS, MMN and CIDP. It is a second line treatment for severe and worsening MG, dermatomyositis and LEMS (322).

IVIG is a potential adjunctive treatment for JE in the future. However, further knowledge is required. The role of subcutaneous provision of IgG for maintenance therapy, once IV induction has been completed, should be explored in JE patients. The ever growing demand for IVIG, its limited supply and its expensive processing, all influence the cost of immunoglobulin treatment (335). Not all patients benefit from immunomodulatory treatment, therefore surrogate markers, which could predict which patients respond best, needs to be developed. One surrogate marker may be to profile expression

levels of Fcγ receptors. It has been found that impaired expression of inhibitory FcγRIIB on myeloid cells and B lymphocytes in patients with CIDP are up regulated following clinically effective IVIG therapy (336, 337). Making recombinant antibodies for IVIG could also address expense. A variety of critical pathways targeted by IVIG therapy have been explored for replacement by recombinant antibodies, such as reduction of auto-antibody half-lives by recombinant antibodies blocking neonatal Fc receptors, increased activation of Fcγ receptors by forming multimeric IgG Fc preparations that block immune complex binding to them and making IVIG preparations with enhanced levels of anti-inflammatory sialic acid-rich IgG glycovariants. Further research is also required to improve understanding of most appropriate IVIG preparation and most suitable target diseases. Improvement in understanding of IVIG's mechanism of action, especially among neurological illnesses, would help develop a more targeted IVIG action, like native IgG.

7.25 Newer treatments of JE and AES

Around 70,000 cases of JE occur every year throughout the world, despite effective vaccine. This indicates a pressing need for development of an effective antiviral drug. Minocycline decreased neuronal apoptosis, microgial activation, proinflammatory mediators and JEV viral titres in mice models of JEV. A randomised controlled trial of minocycline (initially 5 mg/kg/day followed by 2.5 mg/kg 12 hourly for ≤ 12 years and initially 200 mg followed by 100 mg 12 hourly in older patients) for seven days in AES patients (including JE patients) in India, was not able to demonstrate significant benefit on patient mortality at three months (338). However, when patients that were moribund or dying at admission were excluded from analysis, minocycline

treated patients showed a trend towards better outcome. Although the study was designed to study the effect of minocycline in JE patients, low numbers of JE cases were recruited, undermining the power of the study and making the impact in JE inconclusive (338). A variety of other drugs have also shown activity against JEV infection in animal models, and are potential drugs for human trial in future as shown in Appedix U (339).

The neuro-protective effect of cooling, known to be beneficial in perinatal asphyxia, was explored in a study involving children with acute encephalitis (340). Delayed cooling, 12 hours after the acute insult was found to be deleterious. In contrast, early cooling appeared neuroprotective in children with acute encephalitis and encephalopathy. Plasma exchange had been reported to be effective in the treatment of severe relapses of acute inflammatory CNS demyelinating diseases in past (341). When children of autoimmune-mediated CNS disorders, including encephalitis refractory to standard therapy, were treated with daily therapeutic plasma exchange for 5 sessions (at 80-110 ml/kg of plasma using 4% albumin solution and fresh frozen plasma), efficient removal of the inflammatory particles were achieved and more than half of the children were observed to have a good outcome (342). A randomised trial of Ligustrazini hydrochlorioi (at 4 mg/kg infusion intravenously over 4 hours per day for 7 days), a traditional Chinese medicine extracted from *Ligusticum Wallichii* Franch plant, known to promote recovery of degenerated nerve cells, conducted in China, has also been reported to be safe and effective drug for the treatment of encephalitis (http://www.nepjol.info/index.php/JNPS/article/view/3451).

7.26 Challenges of JE control strategies

There has been an increase in geographic spread and disease incidence for JE, in much of southeast and southern Asia. This spread is probably because of extensions in land irrigation, increases in paddy growing practices and lack of co-ordinated immunisation programmes (36). Therefore, JE control has been challenged in many areas. During JE surveillance, one of the most important practical steps, is to improve on number of patient CSF samples collected after 10 days of illness and enhance provision of appropriate sample transport back to the laboratory. The average CSF sample collection rate in Nepal from 2013 up-to 2015 is 57%. Therefore, to improve JE surveillance, the Nepal National Committee on Immunisation Practices (NCIP), regarding JE surveillance strategy, has recommended to continue JE surveillance despite fall in number of cases of JE amongst AES, collect serum or CSF samples 10 days after onset of symptoms to increase sensitivity of JE test, collect CSF for testing for recipient of JE vaccination, monitor evidence of waning immunity which may require booster dose of vaccine and 6 months follow up of AES and JE cases to determine actual disability. They have also recommended to expand the JE laboratory network to other areas of the country and strengthen capacity at the National Public Health Laboratory (NPHL) in Kathmandu to be able to conduct plaque reduction neutralising tests (PRNTs) for JEV.

In 2011, in Nepal, the percentage of children who received JE vaccine was estimated between 8-73%. This estimate was low compared to target of 87% of all vaccine coverage. This low vaccine coverage may make it difficult to contain JE outbreaks in the future. Furthermore, if the live attenuated

vaccine is not provided to people, because of ongoing concerns around safety in immunocompromised people, HIV patients, and pregnant women, vaccine coverage could decrease further. Even if the percentage of vaccine coverage improved, JE control is challenged by the necessity to sustain a high rate of immunisation indefinitely. Hence, there is a fear that even with current vaccination campaign resulting in 95% reduction of cases, hundreds of cases may still arise every year, and the disease burden remain high. A comprehensive information, education and communication package emphasizing the need for JE vaccine in routine immunisation would also help in maintaining high rates of immunisation status and promote JE control efforts.

Difficulty in maintaining JE control in Nepal has been indicated by reports on JE cases spreading to higher elevation in the Himalayas (128, 220) (Appendices A & B). This spread could be a result of climate change and rise of temperature, A warmer climate maybe more suitable for vector and viral replication, human habitation and population growth, growth in practice of rice cultivation and pig rearing. All these factors create a more suitable environment for JE transmission. Alternatively, the increase in JE cases it may simply be the result of improved case detection, secondary to expansion of effective disease surveillance, diagnostics and public awareness. The true reason is yet to be known. Phylogeographic studies of JEV and its vectors collected from highland and low land areas using Bayesian Evolutionary Analysis Sampling of Trees (BEAST) and comparing seroprevalence of reservoir and amplifier hosts living in highland and lowland areas could provide information on whether the

emergence of JE in the highlands is recent or recently discovered, and improve insight on the extent of transmission (343).

Between 2006 and 2015, there has been a decrease in AES and JE cases by 36% and 53% respectively since the introduction of JE vaccine in 2006. However, there has only been 5% decrease in proportion of JE cases out total AES cases. Through laboratory based surveillance, from 2004 up-to 2015, clinical notes and serum and/or CSF samples have been collected from 17875 patients. JE has been diagnosed in17% of those cases, based on samples tested for JE under the surveillance system. The identification of the aetiology among the remaining 83% of AES cases will be important to reduce the burden of AES in Nepal. There has been reports on presence of Dengue virus (235, 344), Chikungunya virus (234), Chandipura virus (345), Nipah virus (346), Herpes Simplex virus (347), human parvovirus 4 (348), Enteroviruses (347), Influenzae virus (349), Measles virus (350), Mumps virus (351), Varicella Zoster virus (351), West Nile virus (238), HIV (352), rabies (353), Streptococcus pneumoniae (347), Neisseria meningitidis (347), Staphylococcus aureus (347), Haemophilus influenzae (354), Leptospira (233, 355), Orientia tsutsugamushi (233) in Nepal and neighbouring areas which are known to cause AES. Therefore, it is now time to move forward from JE surveillance to screen for the entire spectrum of pathogens associated with AES. One way forward, with limited resources, is to test initially for common known viruses and bacteria in AES patients. Improved insight into the specific viral aetiology and pathogen-specific clinical outcome of acute encephalitis in this region is essential to guide strategies for prevention and clinical management

so that evidence based public health actions can be planned and carried out. The integrated efforts of clinicians, veterinarians and entomologists would be valuable to achieve better JE control.

7.27 Limitation of the study

The first study (chapter 3) was a retrospective study. Information was limited to the knowledge and interest of the treating clinicians which may or may not have been of interest to the researcher- a pitfall of a retrospective study. Lack of important variables in this retrospective analysis could have lead to inability to validate predictors of JE amongst cases of AES in the prospective study.

After the JE vaccination campaign with SA-14-14-2 vaccine from 2009 in Nepal, there was a fall in the diagnosis of JE. Out of 143 patients with AES, only 4 patients were diagnosed with JE in the study. This prevented me from having a large enough sample size to assess the association of fluid management with outcome in children with JE.

Because of the different practice of follow up among of the treating clinicians, geographical location, access to transport and distance from research site, patients of AES and JE were followed up at wide variety of intervals during intended follow up at one and two years after discharge. Therefore, the follow up for one year ranged from 15- 631 days and second year 570- 750 days in suspected viral aetiology of AES and 15- 390 days and 605- 701days in JE respectively. However, there was no significant difference in the follow up duration between different WFA groups in both AES and JE.

There were many patients of AES and JE who were willing to participate in the study. Despite this, a high proportion of patients were lost to follow up. In AES of suspected viral aetiology, 57% of the patients attended an outcome assessment as follow up at one year and 48% at two years post hospital discharge. Among JE patients, 50% attended follow up at one year and 35% at two years. My prospective study had fewer patients attending outcome assessment at follow up compared to other follow up studies [81% in Malaysia (21) and 90% in Indonesia (74)]. Poor follow up of AES patients could have been because of work commitments of parents, difficulty in access to transport or improvement in child's condition. This loss to follow-up may have biased my findings.

Despite routine blood and CSF examination, including culture for bacteria, in all AES patients, no definite aetiology was found in the majority of participants. In previous studies, lack of aetiological diagnoses has been reported in a high proportion of suspected encephalitis patients (2, 6). Limitation of serological diagnosis could have led to false negative for JE if samples were collected too early, and false positives may have occurred due to recent JE vaccination and infection with dengue virus not tested under the surveillance network (356). In this study, the clinical, laboratory features and outcome of confirmed JE negative AES and JE status unknown were similar, suggesting the unknown group were less likely to be undiagnosed JE. Even in those who were diagnosed JE, results of their testing were often only available after discharge from hospital because of limited resources. Given the relative scarcity of positive diagnostic results, it was important to approach and manage all the cases as AES, based on their clinical syndrome.

In my study, I only used indirect markers of dehydration. Direct markers such as blood pH by arterial blood gas and serum osmolality would have been more accurate in distinguishing patients with metabolic acidosis from dehydration. Arterial blood gas measurements were initially attempted in the study. However, lack of consent and inconsistency in arterial blood gas results made me discard this parameter.

Percentage change in weight is used as a measure to assess hydration status. Obvioulsy, any food, drinks and passage of stool or urine shortly before the measurement may alter weight, which may have introduced variability in the measurements.

The duration of intravenous fluid administration was not recorded in my study. Recording the total amount of fluid administered when the child was 'nil by mouth' may have provided further information on the patient's fluid balance.

There can be serological cross-reactivity between flaviviruses causing false positive results. Cross-reactivity between West Nile (WN) and St Louis Encephalitis (SLE) viruses by MAC-ELISA was reported as one reasons why the outbreak of WN virus in New York in 1999 was initially thought to be caused by SLE virus. Later, comparative studies of SLE, WN, and JE viruses by PRNT and recovery of WN virus from human brain and bird tissue were completed confirming WN as the cause of the outbreak (357). Currently, the most accurate serologic method for distinguishing closely related flavivirus infections, is PRNT, performed on paired acute phase and convalescent phase

serum or CSF against a variety of related flaviviruses (357). Even in the present study, there was a possibility of cross-reactivity between JEV and other flaviviruses such as Dengue.

There are many laboratory techniques available to diagnose JE. In the present study, anti-JEV immunoglobulin-M (IgM) antibodies were measured locally using the AFRIMS JE MAC IgM ELISA kit as recommended by WHO. This has been proved to be a reliable method to diagnose JE (61, 65). In the IVIG treatment trial (chapter 6), confirmatory testing was subsequently performed at the Arboviral Diseases Branch diagnostic laboratory at the CDC, USA. The last serum sample collected from each patient was tested by JEV and DENV IgM capture ELISA. Positive or equivocal results were confirmed by JEV and DENV $PRNT_{90}$, with a 4-fold or greater difference in titre interpreted to be virus specific. If the final sample was positive or equivocal for JEV and/or DENV IgM all samples for that case were tested. The CSF from that patient was also tested by JEV and DENV IgM ELISA. In addition, CSF was also tested for the presence of JEV RNA by RT-PCR assays where appropriate. JEV $PRNT_{50}$ was done on all patient serum at the 2 time points. As a result, one patient with an undetermined status after local testing by MAC ELISA in Nepal was found to be JEV negative at CDC; otherwise there was complete concordance between the test results in Nepal and those at CDC. Therefore, although the possibility of cross-reactivity of other flavivirus cannot be completely excluded in those JEV positives, there are many reasons to believe that the JE results were correct, as shown by the results of the confirmatory testing of the IVIG treatment trial.

7.28 Clinical implications of the research

Identifying and validating the clinical features which could predict bad outcome at the time of admission in AES and JE patients, would not only help in explaining the prognosis to the patient and parents, but also help improve patient management in the future.

This study has highlighted the need of a single standard growth chart for Nepali children of all age groups, in order for local health care staff to identify children with abnormal weight. Since low WFA at admission in children with AES and JE is associated with bad outcome at discharge and persistence of sequelae at 2 years follow up, the practice of measuring weight and classifying into different WFA group of all AES patients at initial contact could be useful, particularly if early referral of those of low WFA for supportive management with fluids was shown to reduce immediate and long term morbidity and mortality of these children in the future.

Maintaining good hydration status with adequate and appropriate IVF may potentially be a simple, cheap, easily available treatment option which could save lives even in resource poor settings, like Nepal. Therefore, as in the UK, hospital oral and intravenous fluids guidelines need to be written for children with impaired consciousness. This could be based on existing Advanced Paediatric Life Support (APLS) guidelines, with local training being conducted on an annual basis.

There is a need to develop hospital protocols for hospital management and follow-up of AES patients. Knowledge about uncertainty of definite

outcome at the time of discharge and recovery profile of AES patients could help clinicians counsel the parents for further care at home; also help in identifying an end point in assessment of outcome in treatment trials. Access to a multidisciplinary team of specialists, besides the general paediatrician, such as neurologist, physiotherapist, speech therapist, psychologist, dietician and occupational therapist is required during follow up since many sequelae, including behavioural problems and uncontrolled seizures were seen to persist up-to two years after discharge. IVIG can be safely given to children with suspected JE. IVIG appears an appealing potential option for future treatment of JE that warrants further investigation.

7.29 Implications for future research

Early hospital admission and supportive management improved outcome of AES and JE patients. Since most of the patient were brought to the hospital by self referral, educating the family members to attend hospitals promptly for supportive management could be life saving. Therefore, there is a need for further research on "health seeking behaviour of the families with sick children" to develop resources for appropriate education. Also more systematic investigation on the role of supportive measures on outcome is needed.

The recovery profile of AES patients changed over time post discharge. Seizures can be treated. Exploration of the prevalence of unprovoked seizure in the community following acute AES and its association with eventual outcome needs further study. There is also need to find out the proportion of autoimmune encephalitis cases within AES children in Nepal.

A large number of children with LWFA suffered AES and JE and who also had bad outcome. This raises the question, is LWFA a contributory factor in developing encephalitis following infection with JEV or other viruses? Is greater prevalence of dehydration or undernutrition in children in Asia the reason for the large number of cases of JE and AES?

AES patients who died were more likely to suffer weight loss during hospital admission. Similarly, AES patients who suffered bad outcome were more likely to have LWFA on admission or experience weight loss during admission. When there is no definite treatment for most AES patients, maintaining good hydration status with adequate and appropriate IVF may potentially be a simple treatment option. Therefore, a randomised control trial of different volumes and types of fluid is needed to confirm that increased or maintenance fluid intake could improve outcome.

Since seizure was more common in dehydrated children, the role of interventions such as training of health workers on accurate diagnosis and effective management of seizures through ongoing International Paediatric Epilepsy Training (IPET) and Advanced Paediatric Life Support (APLS) trainings in Nepal, and the role of health education materials such as a video documentary on epilepsy for patients and parents in improving outcome needs to be studied.

In order to find out the cause of changing epidemiology of JE in highlands of Nepal, there is a need to find out if this emergence is recent or recently

discovered. There is also a need to improve insight on the mode and extent of transmission in these areas.

IVIG was safe, and appeared to augment the development of neutralising antibodies. The anti-inflammatory properties of IVIG may have implications for the wider use of IVIG as a treatment for encephalitis which needs further research. More research on immunological changes in JE and the effects of IVIG will be important for the further development of IVIG treatment (45). A double blind randomised placebo controlled trial including large number patients would be required to find out if clinical outcome improves among JE patients treated with IVIG. Newer immunogenic JEV proteins have also been identified and effect of monoclonal antibodies against them as the treatment of JE could be potential research area in the future (358).

Since many participants were of confirmed non-JE viral encephalitis, improved detection of pathogens suspected to cause AES would help to plan rationalised management and treatment strategies. If any vaccine preventable aetiology is identified, it will help plan future National immusization strategies, particularly, what other routine vaccines need to be introduced to reduce prevalence of AES in Nepal.

7.30 Final concluding remarks

AES is a devastating illness, that affects children and adults in many countries, including Nepal. AES is associated with substantial mortality and neurological sequelae in survivors. The range of pathogens causing AES is not well

described. Although many neurotropic viruses are reported, JEV remains the leading identified cause in most of Asia, including Nepal. Since there is no specific treatment for most AES cases, identifying and correcting prognostic features of bad outcome, being aware of the value of supportive management in improving outcome, and identifying novel effective treatments for treating JE, are important future strategies to improve outcome among AES patients. Patients with AES, particularly JE, were often identified to suffer weight loss or be of low weight for age (LWFA) and appeared dehydrated at the time of admission. AES cases who died significantly more frequently experienced weight loss during hospital admission. Similarly, AES cases who suffered bad outcome, more frequently exhibited weight loss or were LWFA at admission. Dehydration can be assessed by identifying symptoms and signs of dehydration and measuring anthropometric indices, even in resource poor settings. Dehydration can be corrected relatively easily through careful fluid supplementation. Training health care workers to identify dehydration and developing a safe rehydration strategy for AES cases could save lives. Encouraging AES patients to attend hospitals more quickly, to enable them to receive supportive management, including adequate and appropriate fluids could also improve outcome.

Seizures were common in LWFA children. Seizures were again associated with bad outcome. Training of health workers on the diagnosis and effective management of seizures could help reduce adverse outcomes among AES patients. Health education on managing seizures could be provided through short documentaries.. The documentaries could advise carers on what to do and

what not to do during an acute seizure at home. The documentaries could be broadcast via Nepali national television channels, to maximise coverage across the country. Public engagement and further public education strategies takling AES in Nepal are required.

My pilot study shows IVIG to be safe to be prescribed in AES children. IVIG augmented the development of neutralising antibodies in JE patients making it an appealing treatment option. My study supports IVIG being taken forward as a phase III trial in JE. IVIG's anti-inflammatory properties could also be useful for treatment of other encephalitis. Therefore, in the future IVIG could reduce JE related morbidity and mortality and potentially also improve overall outcome of AES in Nepal.

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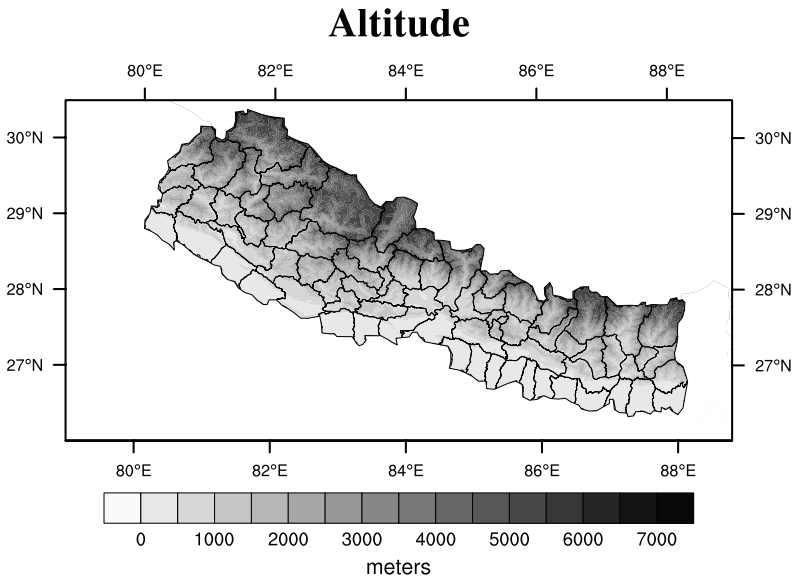
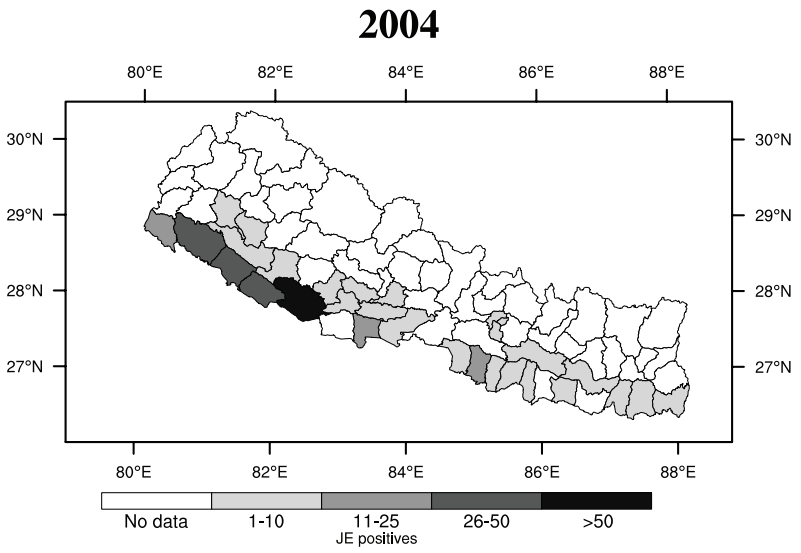
APPENDICES

Appendix A

Figures showing geographical distribution of JE cases from 2004 - 2015 in Nepal





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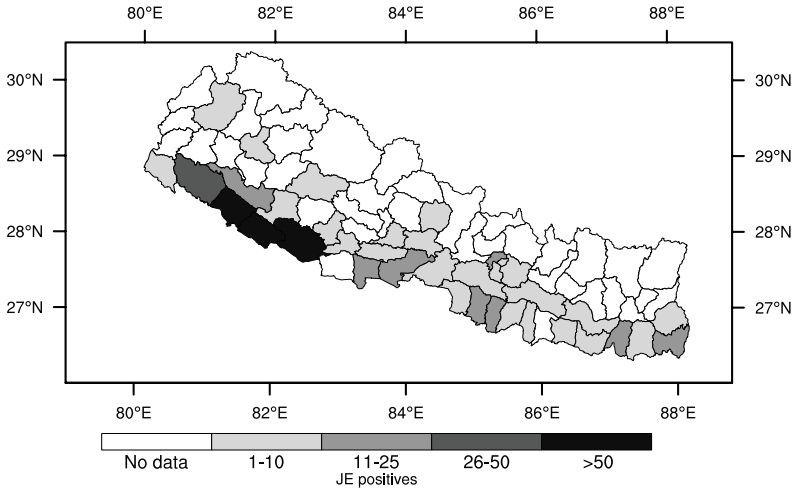
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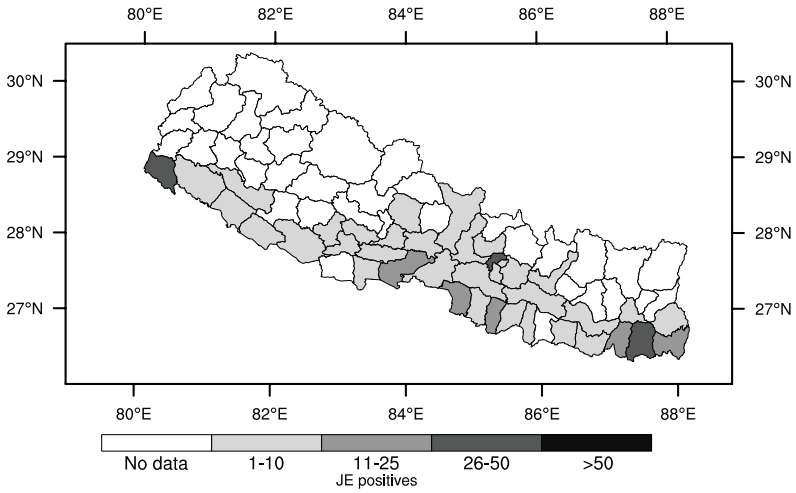
No data
1-10
11-25
26-50
>50
JE positives

2006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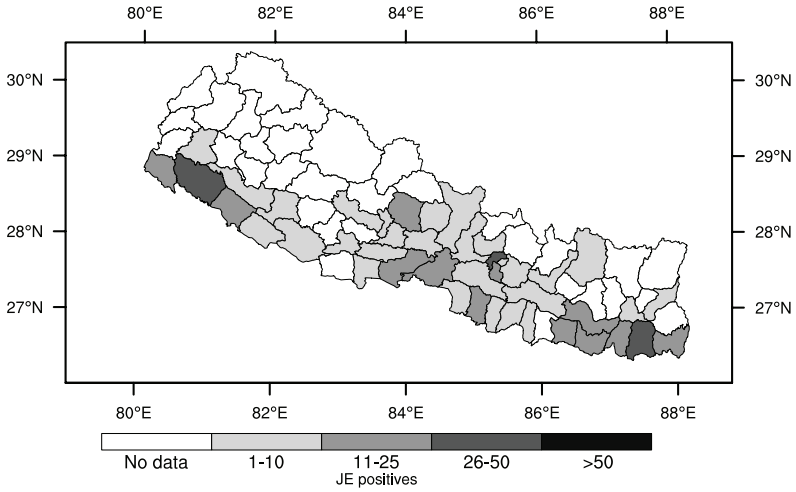
JE positives

2007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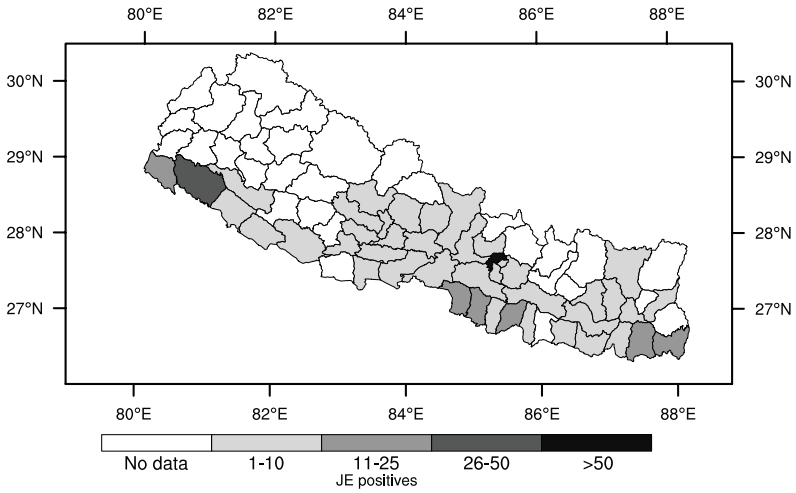
No data
1-10
11-25
26-50
>50
JE positives

2008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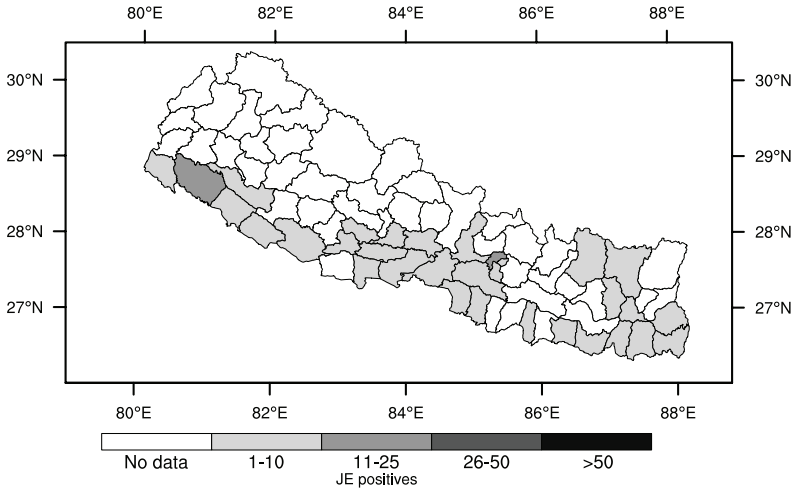
26-50
>50
JE positives

2009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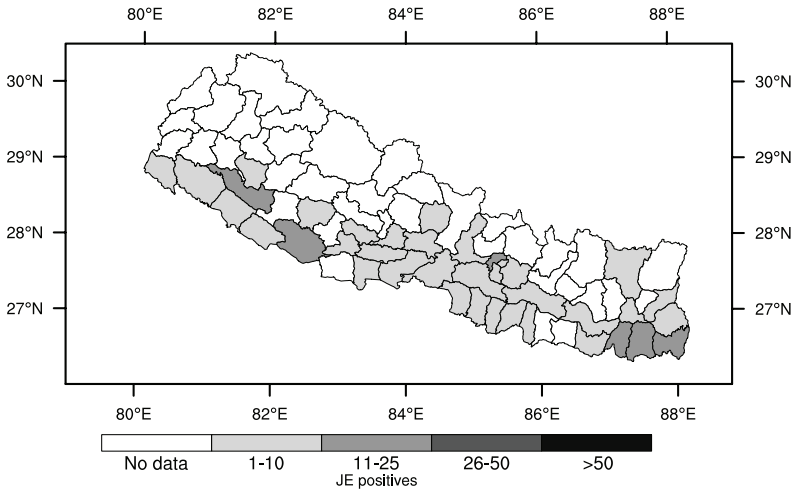
>50
JE positives

2010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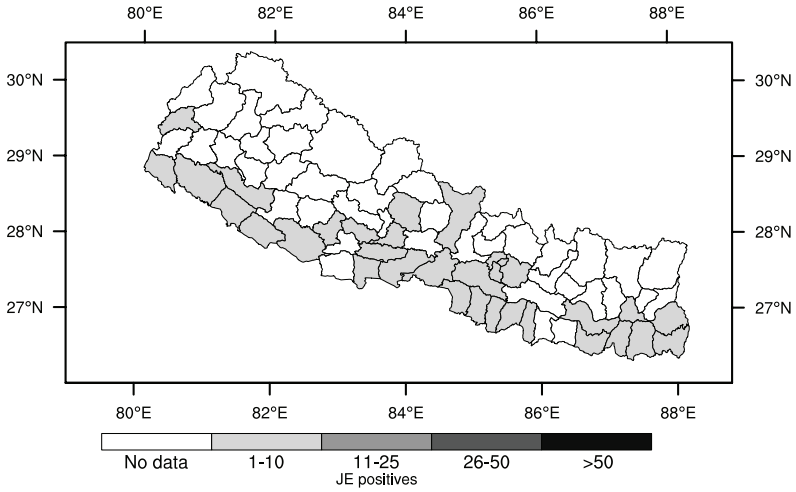
JE positives

2011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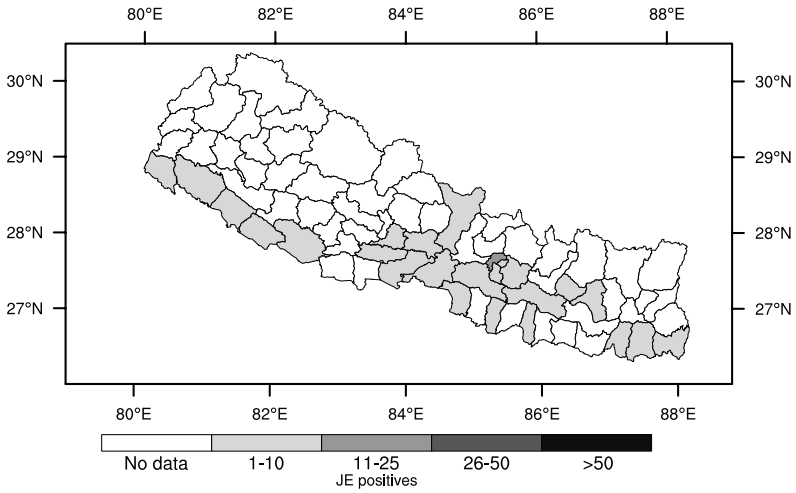
JE positives

2012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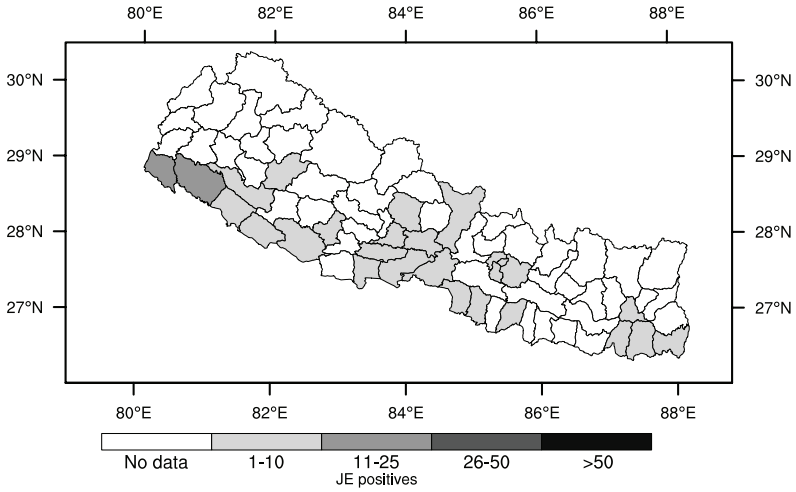
>50
JE positives

2013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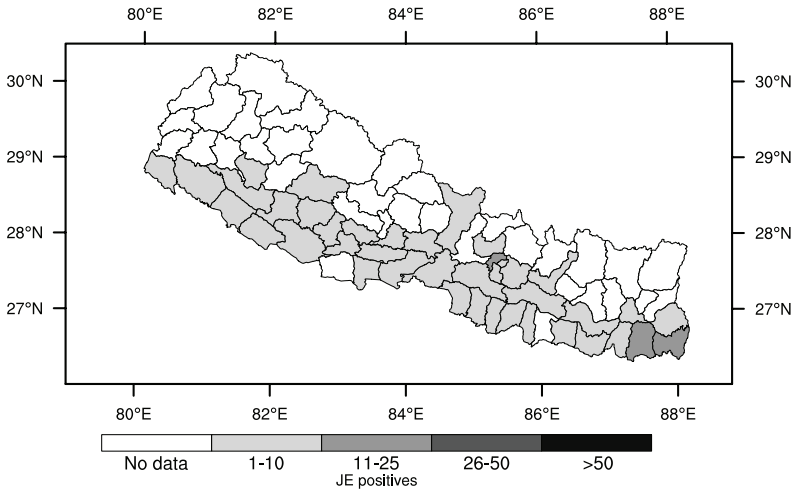
>50
JE positives

2014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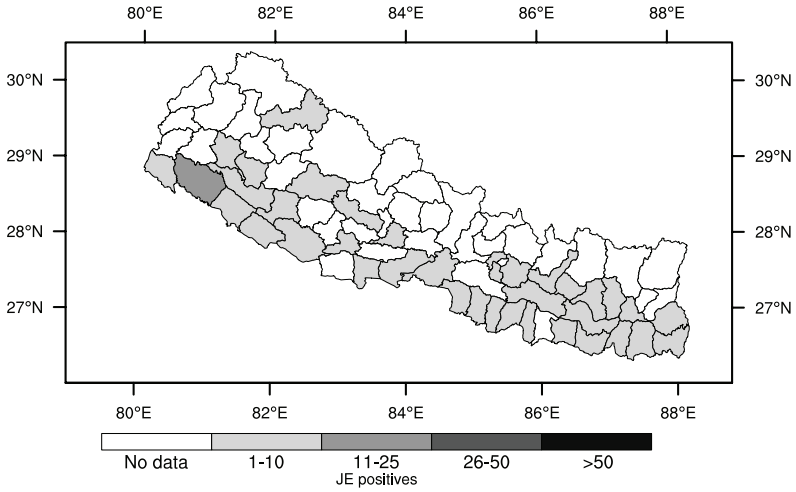
JE positives

2015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26-50
>50
JE positives

Appendix B

Table showing distribution JE cases from 2004- 2015 by districts under the National AES surveillance programme.

• DISTRICT	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
• ACHHAM	1											1
• ARGHAKHANCHI	2	2	2	2	1	3	2				3	1
• BAGLUNG				2	2							1
• BAITADI								1				
• BAJHANG		1										
• BANKE	44	94	10	10	4	2	5	7	3	7	6	2
• BARA	11	17	10	14	11	7	8	2		2	3	5
• BARDIYA	37	105	6	11	2	2	2	2	5	9	6	3
• BHAKTAPUR	3	4	4	9	8	3	1	1	2	1	9	
• BHOJPUR					1	1						2
• CHITWAN		3	3	11	7	9	4	4	1	5	5	4
• DAILEKH	1						2				1	1
• DANG	88	89	9	10	8	2	19	4	2	4	9	3
• DHADING			2	7	3	2	1					
• DHANKUTA			1	3	1	1	3	2		1	4	1
• DHANUSHA	6	8	7	15	6	4	3	8		2	7	7
• DOLKHA		1		1	1						4	
• DOTI				1								
• GORKHA			2	5	7			1	1	1	4	
• GULMI	1		2		3	1	2	1			1	
• ILAM		2	1			2	2	2			1	2
• JAJARKOT										1		
• JHAPA	8	11	11	20	14	5	11	4	3	2	14	4
• KAILALI	47	39	7	33	31	19	4	10	8	18	7	24
• KALIKOT		1										
• KANCHANPUR	13	10	28	20	12	2	4	5	1	14	6	10
• KAPILVASTU	13	32	16	6	3	2	3	2	1	4	1	1
• KASKI			1	15	9			1		1		
• KATHMANDU	9	25	26	44	59	22	23	9	13	5	18	5
• KAVRE		6	4	4	4		1	4	1	4	4	2
• KHOTANG					1				1			1
• LALITPUR	3	7	10	14	3	6	3	2	7	4	5	2
• LAMJUNG		1		1	1		1					

• MAHOTTARI	9	3	2	7	1	3	3	5	1		3	4
• MAKWANPUR		3	4	3	3	4	3	1	1		2	
• MORANG	5	9	29	43	17	7	16	6	5	8	15	8
• MUGU												1
• MYAGDI					1							
• NAWALPARASI	8	16	15	13	9	5	3	9	5	9	7	6
• NUWAKOT			3	4	3						1	
• OKHALDHUNGA									1			1
• PALPA	2	3	3	2	7	4	2	3	1	1	2	
• PANCHTHAR				1	1		1					
• PARBAT				3	4							
• PARSA	3	8	11	9	14	8	3	4	4	8	10	2
• PYUTHAN	3	2	1					1		1	1	
• RAMECHHAP			1	1							4	1
• RAUTAHAT	6	12	12	4	10		3	6	1		6	1
• ROLPA							3				3	
• RUKUM		1									1	2
• RUPANDEHI	16	19	3	5	5	1	3	2		3	5	3
• SALYAN	1	1		4							2	3
• SANKHUWASABHA					1	1	2					
• SAPTARI		2	5	12	3	2	4	1			5	3
• SARLAHI	6	7	5	7	11		5	1		2	6	3
• SINDHULI	3	3	2	5	9		3		2		2	3
• SINDHUPALCHOWK			2	2	6		1			2	3	
• SIRAHA	1	4	5	14	7	2					1	3
• SOLUKHUMBU				1		1						
• SUNSARI	6	18	19	21	4	7	13	9	4	4	7	5
• SURKHET	10	11	2	5	9	1	21	1		1	6	3
• SYANGJA	1	1	1	4	3	1	1	3	2	1	3	1
• TANAHU		1	2	3	4	4	1		3	2	5	
• TERHATHUM			1								3	
• UDAYAPUR	1	2	3	11	5		2	4			5	3

(WHO-IPD, Nepal)

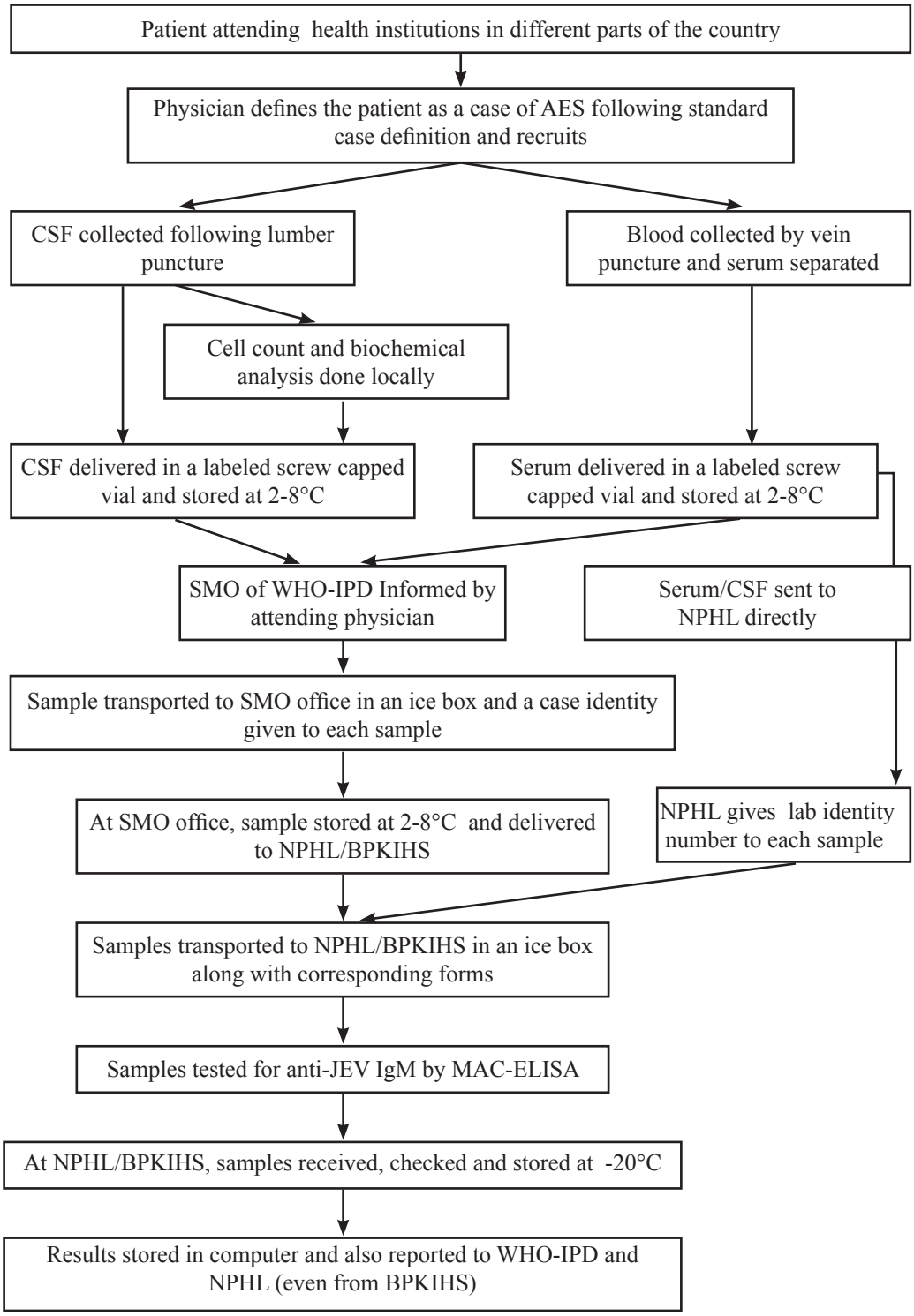
Appendix C

Table showing JE epidemiology in Nepal from 1978- 2012

Year	Number of suspected cases	Deaths	CFR (%)
Syndromic surveillance			
1978	422	119	28.2
1979	182	49	26.9
1980	622	231	37.1
1981	54	16	29.6
1982	843	390	46.3
1983	242	36	14.9
1984	142	45	31.7
1985	629	183	29.1
1986	1615	415	25.7
1987	502	140	27.9
1988	1403	380	27.1
1989	868	227	26.2
1990	365	102	27.9
1991	650	114	22.3
1992	702	127	18.1
1993	446	108	24.2
1994	1836	383	20.9
1995	1246	255	20.4
1996	1450	260	17.9
1997	2953	407	13.8
1998	1161	149	12.3
1999	2924	434	14.8
2000	1729	169	9.8
2001	1888	275	10.0
2002	842	168	20.0
2003	330	69	20.9
Laboratory based surveillance			
2004	1533	370	26
2005	2290	669	53
2006	1471	295	42
2007	1650	442	61
2008	1988	339	39
2009	1515	147	9
2010	1572	197	1
2011	1337	129	0
2012	952	75	0

Appendix D

Figure showing National JE Surveillance Program of Nepal



(Department of Health, Ministry of Health, Nepal)

Appendix E

Table showing assessment of dehydration

Variable	Mild, 3-5%	Moderate, 6- 9%	Severe, ≥ 10%
Blood pressure	Normal	Normal	Normal to reduced
Quality of pulses	Normal	Normal or slightly decreased	Moderately decreased
Heart rate	Normal	Increased	Increased
Skin turgor	Normal	Decreased	Decreased
Fontanelle	Normal	Sunken	Sunken
Mucous membrane	Slightly dry	Dry	Dry
Eyes	Normal	Sunken orbits	Deeply sunken orbits
Extremities	Warm, normal capillary refill	Delayed capillary refill	Cool, mottled
Mental status	Normal	Normal to listless	Normal to lethargic or comatose
Urine output	Slightly decreased	< 1 ml/Kg/h	<< 1 ml/Kg/h
Thirst	Slightly decreased	Moderately increased	Very thirsty or too lethargic to indicate

(Duggan et al, 1992)

Appendix F

Proforma of Retrospective study

Clinical, laboratory and prognostic features among children with Acute Encephalitis Syndrome in Nepal; a retrospective study

History **(WHO CASE ID: VENEPCDR_____)** **Study No: _____**

Initials / Name _____ Hosp IP No. _____ Age___ Sex M ☐ / F ☐ D.O.B._____

Address of home: _____

Corresponding Address _____

Date Admitted to Hospital_____Date Admitted to Study_____ Time_____ Date of discharge_____ Outcome_____

Referred from: District Hospital ☐ / General Practioner ☐ / Primary health centre ☐ / home ☐ / other:_____

Does the child meet WHO AES case definition?

Acute Febrile Illness	N ☐ / Y ☐ Details _____
PLUS Change in mental status	N ☐ / Y ☐ Details _____

(including symptoms such as confusion, disorientation, coma, or inability to talk)
AND/OR

New onset of seizures (excluding simple febrile seizures*)	N ☐ / Y ☐ Details _____

⇒ **Enter AES Documentation study**

Collect samples

Acute CSF	N ☐ / Y ☐ Date _____
Acute Serum	N ☐ / Y ☐ Date_____
Convalescent serum (later)	N ☐ / Y ☐ Date _____

SUMMARY INFORMATION (Complete after patient discharge)

Adm GCS E= ___ M= ____ V=____ Discharge GCS E = ____ M =____ V = _____
Discharged Day __ Date _____ **Discharge Outcome score** I (died) / II (Severe) / III (Mod) / IV (Mild) / V (No) Sequelae
JE status Acute CSF Pos / Neg Acute Serum Pos / Neg Conv Serum Pos / Neg

Clinical, laboratory and prognostic features among children with Acute Encephalitis Syndrome in Nepal; a retrospective study

Study Admission Date:		**Initials:**	**Age**			**M/F**			**Study No:**		
Progress summary	Day of study:	**Day: 0**	**Day: 1**	**Day: 2**	**Day: 3**	**Day: 4**	**Day: 5**	**Day: 6**	**Day: 7**	**Day: 8**	
	Day of illness:										
	Date:										
GCS	Examination time now	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	
Best Eyes	spontaneous and seeing	4	4	4	4	4	4	4	4	4	
	to loud voice	3	3	3	3	3	3	3	3	3	
	to pain	2	2	2	2	2	2	2	2	2	
	not at all (or vacant stare)	1	1	1	1	1	1	1	1	1	
Best Motor	obeys commands	6	6	6	6	6	6	6	6	6	
	localises painful stimulus (knuckles on	5	5	5	5	5	5	5	5	5	
	withdraws (from pressure to nailbed)	4	4	4	4	4	4	4	4	4	
	abnormal flexion posturing to pain	3	3	3	3	3	3	3	3	3	
	extension to pain	2	2	2	2	2	2	2	2	2	
	none	1	1	1	1	1	1	1	1	1	
Best Verbal (2-5y in brackets)	orientated (words of any sort)	5	5	5	5	5	5	5	5	5	
	confused (monosylables)	4	4	4	4	4	4	4	4	4	
	inappropriate words (cries/screams/moans)	3	3	3	3	3	3	3	3	3	
	incomprehensible sounds (grunts)	2	2	2	2	2	2	2	2	2	
	none	1	1	1	1	1	1	1	1	1	
TOTAL											
Improving?	No change/Yes/No										
TICK	Oxygen / Endotracheal intubation / Central line										
	Nasogastric tube										
	Urinary catheter										
	Feeding orally / NG / IV fluid										
General Changes	Pulse rate/Resp rate/RS/CVS/PA/										
Seizures/Spasms/Posturing	Yes/No										
Neuro	Changes in Bulk/Power/Tone/Reflexes										
	Dolls Eye Reflex (normal / abnormal										
	Sit/Stand/Walk with or without assistance										
	Changes in cranial nerves / other focal signs										
DRUG TREATMENT (please list dose & frequency)											
Mannitol											
Phenytoin											
Phenobarb iv / Gardinal											
Diazepam											
Antimicrobials											
*Other Drugs (avoid **Dexa** if possible)*											

Other Changes / Notes / Details:

Clinical, laboratory and prognostic features among children with Acute Encephalitis Syndrome in Nepal; a retrospective study

History **Study No: _____**

Initials/ Name _____ Age_____ Sex M ☐ / F ☐ D.O.B._____

Date Admitted to Hospital_____Date Admitted to Study_____ Time_____

Date admitted to any hospital:_____ Where?_____
Referred from: District Hospital ☐ / General Practioner ☐ / Primary health centre ☐ / home ☐ / other:_____

Handedness: Right ☐ / Left ☐ Ethnicity: _____

Attends school Y ☐ / N ☐ / too young for school ☐ Does family rear pigs? Y ☐ / N ☐

Normal Milestones Y ☐ / N ☐ Describe_____

Do parents think child is normal self today? Y ☐ / N ☐ Details_____

Date of onset of illness _____ Today is day_____ of illness.

Fever	U ☐ / N ☐ / Y ☐ _____day to _____day	**Feeding**	Normal ☐ / reduced ☐ / not at all ☐
If fever	High grade ☐ / low grade ☐	**Drowsy**	U ☐ / N ☐ / Y ☐ _____day to _____day
Rigors/Chills:	U ☐ / N ☐ / Y ☐ _____day to _____day	**Irritable**	U ☐ / N ☐ / Y ☐ _____day to _____day
Headache:	U ☐ / N ☐ / Y ☐ _____day to _____day	**Confused:**	U ☐ / N ☐ / Y ☐ _____day to _____day
Neck pain	U ☐ / N ☐ / Y ☐ _____day to _____day	**Unconscious:**	U ☐ / N ☐ / Y ☐ _____day to _____day
Photophobia	U ☐ / N ☐ / Y ☐ _____day to _____day	**Convulsions**	N ☐ / Y ☐ describe below
Sore throat	U ☐ / N ☐ / Y ☐ _____day to _____day	**Spasms**	N ☐ / Y ☐ _____describe below
Cough	U ☐ / N ☐ / Y ☐ _____day to _____day		
Runny nose	U ☐ / N ☐ / Y ☐ _____day to _____day		
Vomiting:	U ☐ / N ☐ / Y ☐ _____day to _____day		
Diarrhoea	U ☐ / N ☐ / Y ☐ _____day to _____day		
Illness biphasic	U ☐ / N ☐ / Y ☐ decribe: _____		

Rash / Conjuctivitis / Abdo distension / GI Bleed / Jaundice / Others U ☐ / N ☐ / Y ☐ decribe, including days: _____

_____

Confused, N ☐ / Y ☐ describe, giving example(s)_____

Describe convulsions: number of attacks, when occurred, duration, partial, generalised, Status? incontinence? Frothing?

Post-ictal phenomenon, Todd's paresis?woke up after? _____

_____

Simple febrile convulsions only? N ☐ / Y ☐ (details) _____

Facial or Limb paralysis: N ☐ / Y ☐ (where and when, day to max weakness) _____

Preceeding illness in the last 2 months? None ☐ / Yes ☐ (give details)_____

Recent Vaccines No ☐ /Yes ☐

JE Vaccine No ☐/ Yes ☐/ Not Sure ☐ Date_____ Any reaction to JE vaccine No ☐ /Yes ☐

Details _____

Other history (including other symptoms and past Hx of convulsions, unusual features, family Hx):

_____

_____

_____

Treatment given before admission to study: N ☐ / Y ☐ / unknown medication ☐

(if yes tick and give details: what, dose, when, where from):
Aspirin ☐ / Paracetamol ☐ / Over the counter medicine ☐ / Traditional Medicine ☐ / Unknown antibiotic ☐

Chloramphenicol ☐ / Penecillin ☐ / Ceftrioxone ☐ / Midazolam ☐ / Diazepam ☐ / Phenobarb ☐ / Phenytoin ☐ / Haloperidol ☐

Saline ☐ / Dextrose ☐ **Mannitol ☐ / Steroids ☐ details:_____**

Other drugs: No ☐ / Yes ☐ (details) _____

Clinical, laboratory and prognostic features among children with Acute Encephalitis Syndrome in Nepal; a retrospective study

General Examination **Study No._____**

Initials / Name_____ Date:_____ Time:_____

Temp _____degrees F	Resp. rate _____ / min
Pulse_____ / min	Height/ length_____cm (if available)
BP:_____mmHg	Weight _____kg

Neck stiffness N ☐ / Y ☐ Saliva dribbling: N ☐ / Y ☐

Kernig's positive N ☐ / Y ☐ Jaundice: N ☐ / Y ☐

Vacant stare N ☐ / Y ☐ Malnutrition (calculate late N ☐ / Y ☐

N ☐ / Y ☐ Cough: N ☐ / Y ☐

Bulging fontanelle: N ☐ / Y ☐ Coryza: N ☐ / Y ☐

Full bladder: N ☐ / Y ☐ Lymphadenopathy N ☐ / Y ☐ (details) _____

Rash N ☐ / Y ☐: Macular ☐ / papular ☐ /vesicular ☐ /other ☐: where_____

Enanthemas (mucous membrane eruptions) N ☐ / Y ☐ (describe type and location)_____

Oedema N ☐ / Y ☐ (Where?)_____

Any haemorrhagic manifestations: N ☐ / Y ☐ (describe)_____

Heart Sounds: Normal ☐ / Abnormal ☐(specify)_____

Breath sounds: Normal ☐ / Abnormal ☐(specify)_____

Pattern: Regular ☐ / Hypervent ☐ / Cheyne Stokes ☐ / Pauses ☐ / Irregular ☐ / Gasping ☐

ENT: normal / abnormal (describe) _____

Hepatomegaly: No ☐ / Yes ☐ ____ cm in mid clav line Texture: _____ Tender No ☐ / Yes ☐

Ascites: N ☐ / Y ☐ Tense:N ☐ / Y ☐ Splenomegaly: _N ☐ / Y ☐

Abdominal other: N ☐ / Y ☐

Nasogastric tube: N ☐ / Y ☐ Reason for NG tube: aspiration ☐ / feeding ☐

NG aspirate N ☐ / Y ☐ (coffee grnd aspirate? N ☐ / Y ☐ fresh bld ? N ☐ / Y☐)

Urinary Catheter: N ☐ / Y ☐ For: retention ☐ / incontin ☐ / monitoring output ☐

If catheterised current hourly urine output _____ mls / hr

Endotracheal intubation: N ☐ / Y ☐ Froth from E/T tube? None ☐ / White ☐ / Pink ☐

Clinical, laboratory and prognostic features among children with Acute Encephalitis Syndrome in Nepal; a retrospective study

Admission Neurological Examination **Study No:_____**

Initials / Name_____ Date:_____ Time:_____

Cranial nerves

Pupil Size Right: pinpoint ☐ / normal ☐ / dilated ☐

Left: pinpoint ☐ / normal ☐ / dilated ☐

Pupil response Normal ☐ / Abnormal ☐ (describe) _____

Fundi Right: Normal ☐ / Not done ☐ / Abnormal ☐ (describe)_____

Left: Normal ☐ / Not done ☐ / Abnormal ☐ (describe)_____

Spont Eye movements Normal ☐ / Abnormal ☐ (describe) _____

Doll's Eye reflex Normal ☐ / Abnormal ☐ (describe) _____

Response to menace Normal ☐ / Abnormal ☐ (describe) _____

VIIth nerve Normal ☐ / Abnormal ☐ (describe) _____

Swallowing Normal ☐ / Abnormal ☐ IF ABNORMAL or PATIENT UNCONCIOUS:

Gag: Normal ☐ / Abnormal ☐ / Absent ☐

Stridor No ☐ / Yes ☐

Speech Normal ☐ / Abnormal ☐ (describe) _____

Abnormal movements No ☐ / Yes ☐

Tremor ☐ / Lip smacking or chewing ☐ / Teeth grinding ☐ / Agitated ☐ / twitching ☐ / Choreoathetosis ☐ /

Hiccoughs ☐ / Rigidity spasms ☐ (Details) _____

Posture

Head position: Normal ☐ / turned to left ☐ / turned to right ☐

Extensor / Flexor posturing : No ☐ / Yes ☐

Mental State Normal ☐ / Abnormal ☐ (describe) _____

Other Comments _____

Clinical, laboratory and prognostic features among children with Acute Encephalitis Syndrome in Nepal; a retrospective study

Admission Neurological Examination **Study No:_____**

Peripheral nervous system

Able to Sit: Independently ☐ / With help ☐ / Not at all ☐

Able to Stand: Independently ☐ / With help ☐ / Not at all ☐

Any fasciculation: N ☐ / Y ☐ details: _____

Neck tone: Normal ☐ / Increased ☐ / Neck rigid ☐

Flaccid limbs (LMN signs): No ☐ / Yes ☐ Details:_____

Head lag (when pulled to sit): No head lag ☐ / head lag ☐

Limb Tone/Posture [Normal (N) /Flexion (F) / Extension (E) / Gen Increased (I)/ Decreased (D) / Cogwheel (C)

Right Arm: ___ Left Arm: ___

Right Leg ___ Left Leg ___

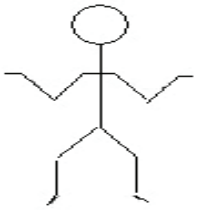
Equine feet (toes pointing downwards): Right: No ☐ / Yes ☐ Left: No ☐ / Yes ☐

Clonus: Right Ankle: No ☐ / Yes ☐ Left Ankle: No ☐ / Yes ☐

Tendon Reflexes, Draw:

Code:

- absent
+ reduced
++ normal
+++ increased



Symetrical/Asymetrical Sym ☐ /Asy ☐

Plantar reflexes:

Right: Flexor (down) ☐ / Extensor (up) ☐ / No movement ☐ / Unsure ☐

Left: Flexor (down) ☐ / Extensor (up) ☐ / No movement ☐ / Unsure ☐

Abdominal reflexes Any present ☐ / none present ☐

Tone Overall: Normal ☐ / Generally Flaccid ☐ / Generally Spastic ☐ / Mixed ☐ / Neither ☐

Power (Grade 1-5, 1= no movement, 2= not against gravity, 3 = against gravity, 4= reduced, 5= full)

Right Arm _____ Left Arm _____

Right Leg_____ Left Leg _____

Hands fall on face Right Hand N ☐ / Y ☐ Left hand N ☐ / Y ☐

Knees flop out Right Knee N ☐ / Y ☐ Left Knee N ☐ / Y ☐

Paresis: Hemiparesis ☐ / Paraparesis ☐ / Quadraparesis ☐ / Monoparesis ☐ /

None ☐ / Unknown ☐ / Moving all 4 limbs ☐

Clinical, laboratory and prognostic features among children with Acute Encephalitis Syndrome in Nepal; a retrospective study

Results **Study No:**

Initials / Name_____ Time:_____ Date:_____ Time:_____

DATE					
	Hb	**Random blood sugar:** _____			
	Total Count				
	Diff Count	Polym %	Lymphs %	Eosin %	Mono %
	Plats				
	RBS (gluc)				
	ESR				

	Malaria screen	neg/pos		Specify (PS report/Ag):
	(Widal if done)			
	(Dengue if done)			

	Blood cultures	neg/pos		Specify:

CSF Results	Date	Opening Pressure/cm water	WCC	% Polys	% Lymps	Protein	CSF Glucose	RBS Glucose	CSF / Plasma Glucose	Other info
1st										
2nd										
3rd										

CSF Gram stain _____ Hib Latex _____ CSF Culture _____

LFT: TB _____ Direct Bilirubin _____ SGOT _____ SGPT _____ Alp Phos _____ PT _____

Renal: Urea_____ Creat _____ Na _____ K _____

Others

Radiology

CXR (if done) Date _____ Result _____

CT head (if done) Date _____ Result _____

MRI head (if done) Date _____ Result _____

Others

JE serology	CSF 1	Date _____	neg / pos	Date _____	neg / pos	CSF 2 (if taken)	Date _____ neg / pos
	Serum 1	Date _____	neg / pos	Date _____	neg / pos	Serum 2	Date _____ neg / pos

OTHER (please fill below):

Appendix G

Proforma of WFA study

Weighing a child may help predict outcome in Acute Encephalitic Syndrome

History **(WHO CASE ID: VENEPCDR_____)** **Study No: _____**

Initials / Name _____ Hosp IP No. _____ Age___ Sex M ☐ / F ☐ D.O.B._____

Address of home: _____

Corresponding Address _____

Date Admitted to Hospital_____Date Admitted to Study_____ Time_____ Date of discharge_____ Outcome_____

Referred from: District Hospital ☐ / General Practioner ☐ / Primary health centre ☐ / home ☐ / other:_____

Does the child meet WHO AES case definition?

Acute Febrile Illness	N ☐ / Y ☐ Details _____
PLUS Change in mental status	N ☐ / Y ☐ Details _____
(including symptoms such as confusion, disorientation, coma, or inability to talk) AND/OR	
New onset of seizures (excluding simple febrile seizures*)	N ☐ / Y ☐ Details _____

Collect samples

Acute CSF	N ☐ / Y ☐ Date _____
Acute Serum	N ☐ / Y ☐ Date_____
Convalescent serum (later)	N ☐ / Y ☐ Date _____

SUMMARY INFORMATION (Complete after patient discharge)

Adm GCS E= ___ M= ____ V=____ Discharge GCS E = ____ M =____ V = _____
Discharged Day __ Date _____ **Discharge Outcome score** I (died) / II (Severe) / III (Mod) / IV (Mild) / V (No) Sequelae
3-6 mo FU Day __ Date _____ **FU Outcome score** I (died) / II (Severe) / III (Mod) / IV (Mild) / V (No) Sequelae
Others FU
JE status Acute CSF Pos / Neg Acute Serum Pos / Neg Conv Serum Pos / Neg

Study Admission Date: **Initials:** **Age** **M/F** **Study No:**

Progress summary	Day of study:	Day: 0	Day: 1	Day: 2	Day: 3	Day: 4	Day: 5	Day: 6	Day: 7	Day: 8
	Day of illness:									
	Date:									
GCS	Examination time now	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:
Best Eyes	spontaneous and seeing	4	4	4	4	4	4	4	4	4
	to loud voice	3	3	3	3	3	3	3	3	3
	to pain	2	2	2	2	2	2	2	2	2
	not at all (or vacant stare)	1	1	1	1	1	1	1	1	1
Best Motor	obeys commands	6	6	6	6	6	6	6	6	6
	localises painful stimulus (knuckles on	5	5	5	5	5	5	5	5	5
	withdraws (from pressure to nailbed)	4	4	4	4	4	4	4	4	4
	abnormal flexion posturing to pain	3	3	3	3	3	3	3	3	3
	extension to pain	2	2	2	2	2	2	2	2	2
	none	1	1	1	1	1	1	1	1	1
Best Verbal (2-5y in brackets)	orientated (words of any sort)	5	5	5	5	5	5	5	5	5
	confused (monosylables)	4	4	4	4	4	4	4	4	4
	inappropriate words (cries/screams/moans)	3	3	3	3	3	3	3	3	3
	incomprehensible sounds (grunts)	2	2	2	2	2	2	2	2	2
	none	1	1	1	1	1	1	1	1	1
TOTAL										
Improving?	No change/Yes/No									
TICK	Oxygen / Endotracheal intubation / Central line									
	Nasogastric tube									
	Urinary catheter									
	Feeding orally / NG / IV fluid									
	General Changes Pulse rate/Resp rate/RS/CVS/PA/									
Seizures/Spasms/Posturing	Yes/No									
Neuro	Changes in Bulk/Power/Tone/Reflexes									
	Dolls Eye Reflex (normal / abnormal									
	Sit/Stand/Walk with or without assistance									
	Changes in cranial nerves / other focal signs									
DRUG TREATMENT (please list dose & frequency)										
Mannitol										
Phenytoin										
Phenobarb iv / Gardinal										
Diazepam										
Antimicrobials										
*Other Drugs (avoid **Dexa** if possible)*										

Other Changes / Notes / Details:

Weighing a child may help predict outcome in Acute Encephalitic Syndrome

History **Study No: _____**

Initials/ Name _____ Age_____ Sex M ☐ / F ☐ D.O.B._____

Date Admitted to Hospital_____Date Admitted to Study_____ Time_____

Date admitted to any hospital:_____ Where?_____
Referred from: District Hospital ☐ / General Practioner ☐ / Primary health centre ☐ / home ☐ / other:_____

Handedness: Right ☐ / Left ☐ Ethnicity: _____

Attends school Y ☐ / N ☐ / too young for school ☐ Does family rear pigs? Y ☐ / N ☐

Normal Milestones Y ☐ / N ☐ Describe_____

Do parents think child is normal self today? Y ☐ / N ☐ Details_____

Date of onset of illness _____ Today is day_____ of illness.

Fever	U ☐ / N ☐ / Y ☐ _____day to _____day	**Feeding**	Normal ☐ / reduced ☐ / not at all ☐
If fever	High grade ☐ / low grade ☐	**Drowsy**	U ☐ / N ☐ / Y ☐ _____day to _____day
Rigors/Chills:	U ☐ / N ☐ / Y ☐ _____day to _____day	**Irritable**	U ☐ / N ☐ / Y ☐ _____day to _____day
Headache:	U ☐ / N ☐ / Y ☐ _____day to _____day	**Confused:**	U ☐ / N ☐ / Y ☐ _____day to _____day
Neck pain	U ☐ / N ☐ / Y ☐ _____day to _____day	**Unconscious:**	U ☐ / N ☐ / Y ☐ _____day to _____day
Photophobia	U ☐ / N ☐ / Y ☐ _____day to _____day	**Convulsions**	N ☐ / Y ☐ describe below
Sore throat	U ☐ / N ☐ / Y ☐ _____day to _____day	**Spasms**	N ☐ / Y ☐ _____describe below
Cough	U ☐ / N ☐ / Y ☐ _____day to _____day		
Runny nose	U ☐ / N ☐ / Y ☐ _____day to _____day		
Vomiting:	U ☐ / N ☐ / Y ☐ _____day to _____day		
Diarrhoea	U ☐ / N ☐ / Y ☐ _____day to _____day		
Illness biphasic	U ☐ / N ☐ / Y ☐ decribe: _____		

Rash / Conjuctivitis / Abdo distension / GI Bleed / Jaundice / Others U ☐ / N ☐ / Y ☐ decribe, including days: _____

_____

Confused, N ☐ / Y ☐ describe, giving example(s)_____

Describe convulsions: number of attacks, when occurred, duration, partial, generalised, Status? incontinence? Frothing?

Post-ictal phenomenon, Todd's paresis?woke up after? _____

_____

Simple febrile convulsions only? N ☐ / Y ☐ (details) _____

Facial or Limb paralysis: N ☐ / Y ☐ (where and when, day to max weakness) _____

Preceeding illness in the last 2 months? None ☐ / Yes ☐ (give details)_____

Recent Vaccines No ☐ /Yes ☐

JE Vaccine No ☐ / Yes ☐ / Not Sure ☐ Date_____ **Any reaction to JE vaccine No ☐ /Yes ☐**

Details _____

Other history (including other symptoms and past Hx of convulsions, unusual features, family Hx):

_____

_____

_____

Treatment given before admission to study: N ☐ / Y ☐ / unknown medication ☐

(if yes tick and give details: what, dose, when, where from):
Aspirin ☐ / Paracetamol ☐ / Over the counter medicine ☐ / Traditional Medicine ☐ / Unknown antibiotic ☐

Chloramphenicol ☐ / Penecillin ☐ / Ceftrioxone ☐ / Midazolam ☐ / Diazepam ☐ / Phenobarb ☐ / Phenytoin ☐ / Haloperidol ☐

Saline ☐ / Dextrose ☐ **Mannitol ☐ / Steroids ☐ details:_____**

Other drugs: No ☐ / Yes ☐ (details) _____

Weighing a child may help predict outcome in Acute Encephalitic Syndrome

General Examination **Study No._____**

Initials / Name_____ Date:_____ Time:_____

Temp_____degrees F	Resp. rate_____/ min
Pulse_____/ min	Height/ length_____cm (if available)
BP:_____mmHg	Weight_____kg

Neck stiffness N ☐ / Y ☐ Saliva dribbling: N ☐ / Y ☐

Kernig's positive N ☐ / Y ☐ Jaundice: N ☐ / Y ☐

Vacant stare N ☐ / Y ☐ Malnutrition (calculate late N ☐ / Y ☐

N ☐ / Y ☐ Cough: N ☐ / Y ☐

Bulging fontanelle: N ☐ / Y ☐ Coryza: N ☐ / Y ☐

Full bladder: N ☐ / Y ☐ Lymphadenopathy N ☐ / Y ☐ (details) _____

Rash N ☐ / Y ☐: Macular ☐ / papular ☐ /vesicular ☐ /other ☐: where_____

Enanthemas (mucous membrane eruptions) N ☐ / Y ☐ (describe type and location)_____

Oedema N ☐ / Y ☐ (Where?)_____

Any haemorrhagic manifestations: N ☐ / Y ☐ (describe)_____

Heart Sounds: Normal ☐ / Abnormal ☐(specify)_____

Breath sounds: Normal ☐ / Abnormal ☐(specify)_____

Pattern: Regular ☐ / Hypervent ☐ / Cheyne Stokes ☐ / Pauses ☐ / Irregular ☐ / Gasping ☐

ENT: normal / abnormal (describe) _____

Hepatomegaly: No ☐ / Yes ☐ ____ cm in mid clav line Texture: _____ Tender No ☐ / Yes ☐

Ascites: N ☐ / Y ☐ Tense:N ☐ / Y ☐ Splenomegaly: _N ☐ / Y ☐

Abdominal other: N ☐ / Y ☐

Nasogastric tube: N ☐ / Y ☐ Reason for NG tube: aspiration ☐ / feeding ☐

NG aspirate N ☐ / Y ☐ (coffee grnd aspirate? N ☐ / Y ☐ fresh bld ? N ☐ / Y☐)

Urinary Catheter: N ☐ / Y ☐ For: retention ☐ / incontin ☐ / monitoring output ☐

If catheterised current hourly urine output _____ mls / hr

Endotracheal intubation: N ☐ / Y ☐ Froth from E/T tube? None ☐ / White ☐ / Pink ☐

Weighing a child may help predict outcome in Acute Encephalitic Syndrome

Admission Neurological Examination **Study No:_____**

Initials / Name_____ Date:_____ Time:_____

Cranial nerves

Pupil Size Right: pinpoint ☐ / normal ☐ / dilated ☐

Left: pinpoint ☐ / normal ☐ / dilated ☐

Pupil response Normal ☐ / Abnormal ☐ (describe) _____

Fundi Right: Normal ☐ / Not done ☐ / Abnormal ☐ (describe)_____

Left: Normal ☐ / Not done ☐ / Abnormal ☐ (describe)_____

Spont Eye movements Normal ☐ / Abnormal ☐ (describe) _____

Doll's Eye reflex Normal ☐ / Abnormal ☐ (describe) _____

Response to menace Normal ☐ / Abnormal ☐ (describe) _____

VIIth nerve Normal ☐ / Abnormal ☐ (describe) _____

Swallowing Normal ☐ / Abnormal ☐ IF ABNORMAL or PATIENT UNCONCIOUS:

Gag: Normal ☐ / Abnormal ☐ / Absent ☐

Stridor No ☐ / Yes ☐

Speech Normal ☐ / Abnormal ☐ (describe) _____

Abnormal movements No ☐ / Yes ☐

Tremor ☐ / Lip smacking or chewing ☐ / Teeth grinding ☐ / Agitated ☐ / twitching ☐ / Choreoathetosis ☐ /

Hiccoughs ☐ / Rigidity spasms ☐ (Details) _____

Posture

Head position: Normal ☐ / turned to left ☐ / turned to right ☐

Extensor / Flexor posturing : No ☐ / Yes ☐

Mental State Normal ☐ / Abnormal ☐ (describe) _____

Other Comments _____

Weighing a child may help predict outcome in Acute Encephalitic Syndrome

Admission Neurological Examination **Study No:_____**

Peripheral nervous system

Able to Sit: Independently ☐ / With help ☐ / Not at all ☐

Able to Stand: Independently ☐ / With help ☐ / Not at all ☐

Any fasciculation: N ☐ / Y ☐ details: _____

Neck tone: Normal ☐ / Increased ☐ / Neck rigid ☐

Flaccid limbs (LMN signs): No ☐ / Yes ☐ Details:_____

Head lag (when pulled to sit): No head lag ☐ / head lag ☐

Limb Tone/Posture [Normal (N) /Flexion (F) / Extension (E) / Gen Increased (I)/ Decreased (D) / Cogwheel (C)

Right Arm: ___ Left Arm: ___

Right Leg ___ Left Leg ___

Equine feet (toes pointing downwards): Right: No ☐ / Yes ☐ Left: No ☐ / Yes ☐

Clonus: Right Ankle: No ☐ / Yes ☐ Left Ankle: No ☐ / Yes ☐

Tendon Reflexes, Draw:

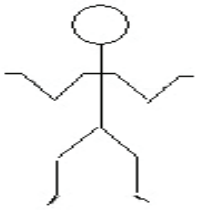
Code:

- absent

+ reduced

++ normal

+++ increased



Symetrical/Asymetrical Sym ☐ /Asy ☐

Plantar reflexes:

Right: Flexor (down) ☐ / Extensor (up) ☐ / No movement ☐ / Unsure ☐

Left: Flexor (down) ☐ / Extensor (up) ☐ / No movement ☐ / Unsure ☐

Abdominal reflexes Any present ☐ / none present ☐

Tone Overall: Normal ☐ / Generally Flaccid ☐ / Generally Spastic ☐ / Mixed ☐ / Neither ☐

Power (Grade 1-5, 1= no movement, 2= not against gravity, 3 = against gravity, 4= reduced, 5= full)

Right Arm _____ Left Arm _____

Right Leg_____ Left Leg _____

Hands fall on face Right Hand N ☐ / Y ☐ Left hand N ☐ / Y ☐

Knees flop out Right Knee N ☐ / Y ☐ Left Knee N ☐ / Y ☐

Paresis: Hemiparesis ☐ / Paraparesis ☐ / Quadraparesis ☐ / Monoparesis ☐ /

None ☐ / Unknown ☐ / Moving all 4 limbs ☐

Weighing a child may help predict outcome in Acute Encephalitic Syndrome

Results **Study No:**

Initials / Name_____ Time:_____ Date:_____ Time:_____

DATE

	Hb	**Random blood sugar:** _____			
	Total Count				
	Diff Count	Polym %	Lymphs %	Eosin %	Mono %
	Plats				
	RBS (gluc)				
	ESR				

	Malaria screen	neg/pos		Specify (PS report/Ag):
	(Widal if done)			
	(Dengue if done)			

	Blood cultures	neg/pos		Specify:

CSF Results	Date	Opening Pressure/cm water	WCC	% Polys	% Lymps	Protein	CSF Glucose	**RBS Glucose**	Plasma Glucose ratio	Other info
1st										
2nd										
3rd										

CSF Gram stain _____ Hib Latex _____ CSF Culture _____

LFT: TB _____ Direct Bilirubin _____ SGOT _____ SGPT _____ Alp Phos _____ PT _____

Renal: Urea_____ Creat _____ Na _____ K _____

Others

Radiology

CXR (if done) Date _____ Result _____

CT head (if done) Date _____ Result _____

MRI head (if done) Date _____ Result _____

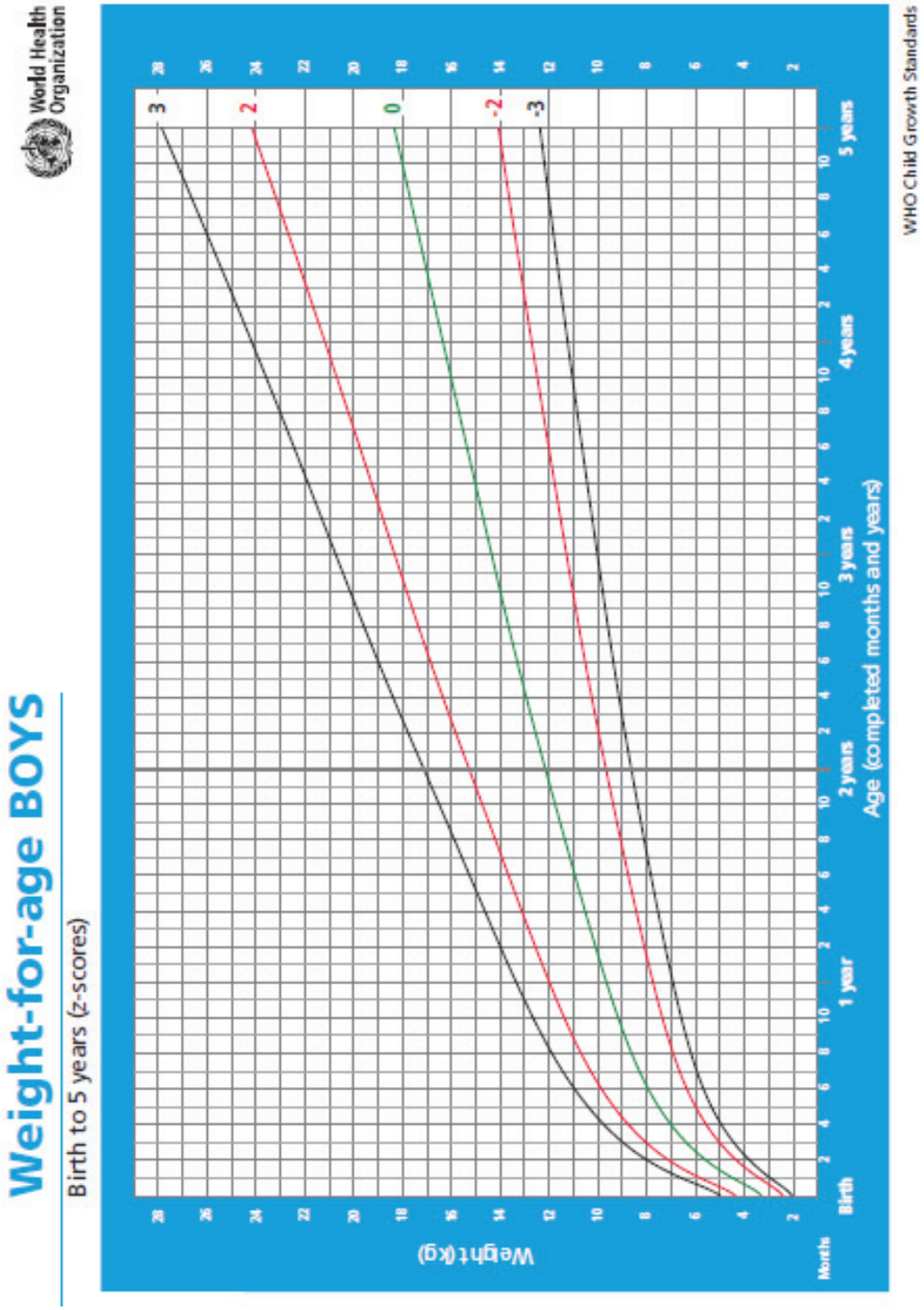
Others

JE serology	CSF 1	Date _____	neg / pos	Date _____	neg / pos	CSF 2 (if taken)	Date _____ neg / pos
	Serum 1	Date _____	neg / pos	Date _____	neg / pos	Serum 2	Date _____ neg / pos

OTHER (please fill below):

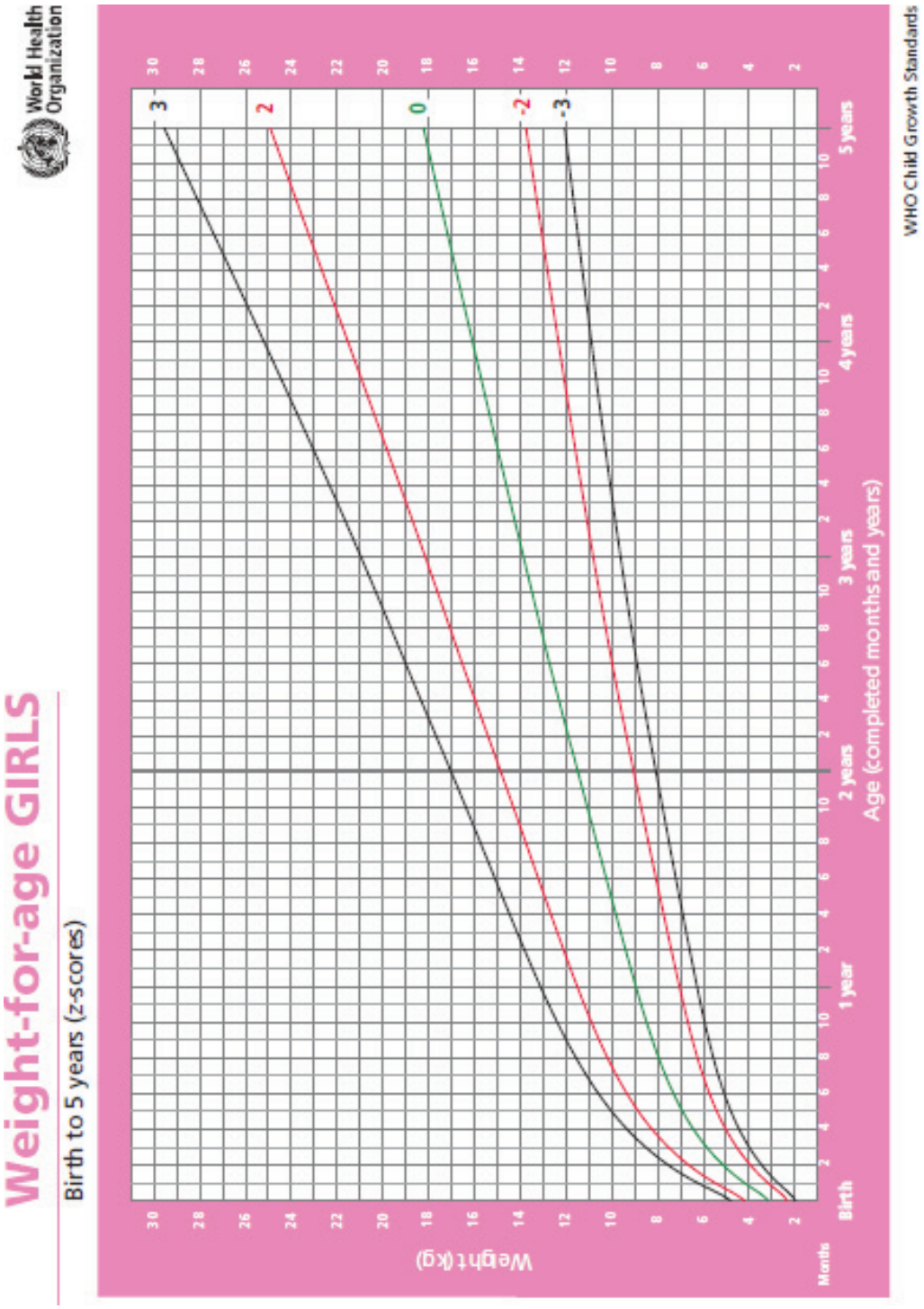
Appendix H

Weight for age in boys



Appendix I

Weight for age in girls



Appendix J

A. Table showing Weight (Kg) for age for male students

Age (years)	M-3SD	M-2SD	M-1SD	Median	M+1SD	M+2SD	M+3SD
5	9.6	12.0	14.4	16.8	19.2	21.6	24.0
6	7.15	10.7	14.4	17.9	21.5	25.1	28.7
7	9.3	12.7	16.1	19.5	22.9	26.3	29.7
8	9.4	13.5	17.6	21.7	25.8	29.9	34.0
9	11.1	15.3	19.5	23.7	27.9	32.1	36.3
10	10.2	15.4	20.6	25.8	31.0	36.2	41.4
11	14.1	19.0	23.9	28.8	33.7	38.6	43.5
12	12.5	19.0	25.5	32.0	38.5	45.0	51.5
13	16.2	23.3	30.4	37.5	44.6	51.7	58.8
14	21.3	28.7	36.1	43.5	50.9	58.3	65.7
15	27.1	34.3	41.5	48.7	55.9	63.1	70.3
16	28.9	36.3	43.7	51.1	58.5	65.9	73.3
17	33.8	39.7	45.6	51.5	57.4	63.3	69.2
18	35.0	41.4	47.8	54.2	60.6	67.0	73.4
19	36.9	43.1	49.3	55.5	61.7	67.9	74.1

[School Health and Nutrition Project, Child Health Division, MOH, 2011 (http://library.nhrc.org.np:8080/nhrc/bitstream/handle/123456789/128/657.pdf?sequence=1)]

B. Table showing Weight (Kg) for age for female students

Age (years)	M-3SD	M-2SD	M-1SD	Median	M+1SD	M+2SD	M+3SD
5	8.8	11.2	13.6	16.0	18.4	20.8	23.2
6	9.7	12.3	14.9	17.5	20.1	22.7	25.3
7	10.2	13.1	16.0	18.9	21.8	24.7	27.6
8	8.3	12.5	16.7	20.9	25.1	29.3	33.5
9	10.9	15.2	19.5	23.8	28.1	32.4	36.7
10	10.3	15.4	20.5	25.6	30.7	35.8	40.9
11	12.0	17.9	23.8	29.7	35.6	41.5	47.4
12	13.4	20.3	27.2	34.1	41.0	47.9	54.8
13	21.2	27.2	33.2	39.2	45.2	51.2	57.2
14	25.8	31.5	37.2	42.9	48.6	54.3	60.0
15	25.2	31.8	38.4	45.0	51.6	58.2	64.8
16	27.4	33.3	39.2	45.1	51.0	56.9	62.8
17	28.8	35.0	41.2	47.4	53.6	59.8	66.0
18	29.7	35.6	41.5	47.2	53.3	59.2	65.1
19	29.4	35.6	41.8	48.0	54.2	60.4	66.6

[School Health and Nutrition Project, Child Health Division, MOH, 2011 (http://library.nhrc.org.np:8080/nhrc/bitstream/handle/123456789/128/657.pdf?sequence=1)]

Appendix K

Liverpool Outcome Score at Discharge

Answer each question. Write the score in the square

Ask the parent or caregiver the following questions:

For some of these questions, you ask the parent or caregiver how the child compares with how they were immediately before the illness (irrespective of length of time in hospital).

1. Speech or communication

Compared with before the illness, is the child's speech or communication:

Score (2- 5) ☐

- The same (5)
- Reduced (3)
- Not speaking or communicating (2)

2. Feeding

The child's feeding is: ☐

- The same as before illness (5)
- Occasionally needs help (3)
- Always needs more help (2)

3. Leaving Alone

Before the illness, could this child be left alone without coming to harm?

- If No score 5 (5)

If Yes, can this child now be left alone?

- Yes (5) ☐

- Yes briefly in familiar environment (3)
- No (2)

4. Behaviour

Compared with before the illness do the caregivers think the child's behaviour is altered?

- No completely normal (5)
- Gets angry easily (4)
- Other behavioural problems (4)
- Severely abnormal (2)

If abnormal give details: _____

5. Recognition

Could the child recognise their family members, other than their main carer, before the illness?

- If No, score 5 (5)

If Yes, can this child now recognise their family members, other than their main carer?

- Yes (5)
- Some (3)
- None (2)

6. School and working

Before the illness, was the child at school or working?

If Yes, do the carers think the child will go back to school or work?

- Yes (5)

- No (3)

If No, do the carers think the child will still able to do the same tasks at home and follow the same routine:

- Yes (5)
- No (3)

7. Epilepsy/ Seizures

Did the child have any seizures during this illness?

- If No, score 5 (5)

If Yes, Is the child still having seizures?

- No seizures and not on anti-epileptic drugs (5)
- No seizures and on anti-epileptic drugs (4)
- Yes still having seizures (3)
- Yes, seizures most days (2)

8. Dressing

Is the child's ability to dress:

- The same as before illness (5)
- Occasionally needs extra help (3)
- Needs more help than before (2)

9. Bladder and Bowel control

Is urinary and faecal continence:

- The same as before the illness (5)
- Needs more help or is incontinent of bowel or bladder (2)

10. Hearing

Does the parent think this child's hearing is:

- Normal (5)
- Reduced in one or both ears (4)
- Cannot hear at all (3)

Observation of the child's abilities

For these questions you observe what the child can do. If you cannot get the child to cooperate, answer these questions based on what the caregiver says.

11. Sitting

Could the child sit before the illness?

- If No, score 5 (5)

If Yes, observe, can this child sit?

- Yes independently (5)
- Needs help (3)
- Not at all (2)

12. Standing up

Could the child get from sitting to standing before the illness?

- If No, score 5 (5)

If Yes, observe, can the child get from sitting to standing?

- Yes, independently (5)
- Needs help (3)
- Not at all (2)

13. Walking

Could the child walk before the illness?

- If No, score 5 (5)

If Yes, observe this child walking 5 metres across room. The child walks:

- Normally (5)
- Abnormally, but independently +/- crutches/stick (3)
- Not able to walk (2)

14. Hands on head

Put both your hands on your head, and ask the child to copy you. Child is:

- Too young (5)
- Normal both hands (5)
- Abnormal one or both hands (4)
- Unable one or both hands (3)

15. Picking Up

Ask child to pick up pea-sized ball of paper or small coin. Child is:

- Too young (5)
- Normal pincer grasp both hands (5)
- Unable one hand (3)
- Abnormal one hand or both hands (3)
- Unable both hands (2)

Outcome Score =Lowest score for any single question (range 2-5):

Total Score = all the individual scores added up (range 33 –75):

(If the child died, the score = 1)

Appendix L

Liverpool Outcome Score at follow up

Answer each question. Write the score in the square

Ask the parent or caregiver the following questions:

For some of these questions, you ask the parent or caregiver how this child compares with other children of a similar age in their locality, e.g., how does this child compare in speaking or walking or talking to other children of the same age in the community.

1. Speech or communication:

Compared with other children the same age in the community, is the child's speech or communication: Score (2- 5)

- The same as other children of this age (5)
- Changed or reduced (3)
- Not speaking or communicating (2)

2. Feeding

The child's feeding is:

- The same as other children (5)
- Occasionally needs help (3)
- Always needs more help (2)

3. Leaving Alone

Before the illness, could a child of this age be left alone without coming to harm?

- If No score 5 (5)

If Yes, can this child be left alone?

- Yes (5)
- Yes briefly in familiar environment (3)
- No (2)

4. Behaviour

Compared with other children of this age, do the caregivers think the child's behaviour is altered?

- No, completely normal (5)
- Gets angry easily (4)
- Other behavioural problems (4)
- Severely abnormal (2)
- If abnormal give details_____

5. Recognition

Can other children of this age recognise their relatives, other than their main carer?

- If No, score 5 (5)

If Yes, can this child recognise their relatives, other than their main carer?

- Yes (5)
- Some (3)
- None (2)

6. School and working

Are other children of the same age at school or working?

If Yes, is the child

- Now back to normal at school or work (5)
- Not doing as well (4)
- Dropped a school grade or no longer attending school or work (3)

If No, is the child:

- Still able to do the same tasks at home and follow the same routine (5)
- Not able to do as well as before (4)
- Not able to do at all (3)

7. Epilepsy/ Seizures

Has the child had any seizures in the last 2 months?

- No seizures and not on anti-epileptic drugs (5)
- No seizures and on anti-epileptic drugs (4)
- Yes has had seizures (3)
- Yes, seizures most days (2)

8. Dressing

Is the child's ability to dress:

- The same as other children the same age (5)
- Occasionally needs extra help (3)
- Always needs more help than other children of the same age (2)

9. Bladder and Bowel control

Is urinary and fecal continence:

- The same as other children the same age (5)
- Needs more help or is incontinent of bowel or bladder (2)

10. Hearing

Does the parent think this child's hearing is:

- Normal (5)
- Reduced in one or both ears (4)
- Cannot hear at all (3)

Observation of the child's abilities

For these questions you observe what the child can do. If you cannot get the child to cooperate, answer these questions based on what the caregiver says.

11. Sitting

Can other children of the same age sit?

- If No, score 5 (5)

If Yes, observe, can this child sit?

Yes independently (5)

- Needs help (3)
- Not at all (2)

12. Standing up

Can other children of this age get from sitting to standing?

- If No, score 5 (5)

If Yes, observe, can the child get from sitting to standing?

- Yes, independently (5)
- Needs help (3)
- Not at all (2)

13. Walking

Can other children of this age walk?

- If No, score 5 (5)

If Yes, observe this child walking 5 metres across room. The child walks:

- Normally (5)
- Abnormally, but independently +/- crutches/stick (3)
- Not able to walk (2)

14. Hands on head

Put both your hands on your head, and ask the child to copy you. Child is:

- Too young (5)
- Normal both hands (5)
- Abnormal one or both hands (4)
- Unable one or both hands (3)

15. Picking Up

Ask child to pick up pea-sized ball of paper or small coin:

- Normal pincer grasp both hands (5)
- Unable one hand (3)
- Abnormal one hand or both hands (3)
- Unable both hands (2)

Outcome Score =Lowest score for any single question (range 2-5):

Total Score = all the individual scores added up (range 33 –75):

(If the child died, the score = 1)

Appendix M

Proforma of fluid study

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

History **(WHO CASE ID: VENEPCDR_____)** **Study No: _____**

Initials / Name _____ Hosp IP No. _____ Age___ Sex M ☐ / F ☐ D.O.B._____

Address of home: _____

Corresponding Address _____

Date Admitted to Hospital_____Date Admitted to Study_____ Time_____ Date of discharge_____ Outcome_____

Referred from: District Hospital ☐ / General Practioner ☐ / Primary health centre ☐ / home ☐ / other:_____

Does the child meet WHO AES case definition?

Acute Febrile Illness	N ☐ / Y ☐ Details _____
PLUS Change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk)	N ☐ / Y ☐ Details _____
AND/OR New onset of seizures (excluding simple febrile seizures*)	N ☐ / Y ☐ Details _____

Collect samples

Acute CSF	N ☐ / Y ☐ Date _____
Acute Serum	N ☐ / Y ☐ Date_____
Convalescent serum (later)	N ☐ / Y ☐ Date _____

SUMMARY INFORMATION (Complete after patient discharge)

Adm GCS E= ___ M= ____ V=____ Discharge GCS E = ____ M =____ V = _____
Discharged Day __ Date _____ **Discharge Outcome score** I (died) / II (Severe) / III (Mod) / IV (Mild) / V (No) Sequelae
3-6 mo FU Day __ Date _____ **FU Outcome score** I (died) / II (Severe) / III (Mod) / IV (Mild) / V (No) Sequelae
Others FU
JE status Acute CSF Pos / Neg Acute Serum Pos / Neg Conv Serum Pos / Neg

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

History **Study No: _____ Hospital IP No_____**

First Initials of Name _____ Age_____ Sex M ☐ / F ☐ D.O.B._____

Date Admitted to Hospital_____Date Admitted to Study_____ Time_____

Date admitted to any hospital:_____ Where?_____
Referred from: District Hospital ☐ / General Practioner ☐ / Primary health centre ☐ / home ☐ / other:_____

Address: District_____, Ward no_____ VDC_____ Mobile no:
Handedness: Right ☐ / Left ☐

Attends school Y ☐ / N ☐ / too young for school ☐

Normal Milestones Y ☐ / N ☐ Describe_____

Animals/birds near the home /school (within 50 metres)?: N ☐/ Y ☐ which? pigs ☐/ chickens ☐/ other_____

Do parents think child is normal self? Y ☐ / N ☐ Details_____

Date of onset of illness _____ Today is day_____ of illness.

Fever	N ☐ / Y ☐ _____day to _____day	**Feeding**	Normal ☐ / reduced ☐ / not at all ☐
If fever	High grade ☐ / low grade ☐	**Vomiting:**	N ☐ / Y ☐ _____day to _____day
Rigors/Chills:	N ☐ / Y ☐ _____day to _____day	**Diarrhoea**	N ☐ / Y ☐ _____day to _____day
Headache:	N ☐ / Y ☐ _____day to _____day	**Abdo distens.**	N ☐ / Y ☐ _____day to _____day
Neck pain	N ☐ / Y ☐ _____day to _____day	**GI bleed**	N ☐ / Y ☐ _____day to _____day
Photophobia	N ☐ / Y ☐ _____day to _____day	**Jaundice**	N ☐ / Y ☐ _____day to _____day
Sore throat	N ☐ / Y ☐ _____day to _____day	**Drowsy**	N ☐ / Y ☐ _____day to _____day
Cough	N ☐ / Y ☐ _____day to _____day	**Irritable**	N ☐ / Y ☐ _____day to _____day
Runny nose	N ☐ / Y ☐ _____day to _____day	**Confused:**	N ☐ / Y ☐ _____day to _____day
Conjunctivitis	N ☐ / Y ☐ _____day to _____day	**Unconscious:**	N ☐ / Y ☐ _____day to _____day
Rash	N ☐ / Y ☐ _____day to _____day	**Convulsions**	N ☐ / Y ☐ fill in convulsion sheet
Illness biphasic	N ☐ / Y ☐	**Spasms**	N ☐ / Y ☐ _____describe below

Confused, N ☐ / Y ☐ describe, giving example(s)_____

Facial or Limb paralysis: N ☐ / Y ☐ (where and when, day to max weakness) _____

if paralysis complete additional examination sheet

Preceeding illness in the last 2 months? None ☐ / Yes ☐ (give details)_____

Recent Vaccines No ☐ /Yes ☐

JE Vaccine No ☐/ Yes ☐/ Not Sure ☐ Date_____ Any reaction to JE vaccine No ☐ /Yes ☐

Details _____

Other history (including other symptoms and past Hx of convulsions, unusual features, family Hx):

_____

_____

_____

_____

Treatment given before admission to study: N ☐ / Y ☐ / unknown medication ☐

(if yes circle and give details: what, dose, when, where from):
Aspirin ☐ / Paracetamol ☐ / Over the counter medicine ☐ / Traditional Medicine ☐ / Unknown antibiotic ☐

Midazolam ☐ / Diazepam ☐ / Phenobarb ☐ / Phenytoin ☐ / Chloramphenicol ☐ / Haloperidol ☐

Saline/ fliuds ☐ / Type of fluids _____ Dextrose ☐ **Mannitol ☐ / Steroids ☐ details:_____**

Other drugs: No ☐ / Yes ☐ (details) _____

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

General Examination **Study No._____** **Hosp IP No_____**

First Initials of Name_____ Date:_____ Time:_____

Temp_____degrees C	Resp. rate_____/ min		
Pulse_____/ min	Height/ length_____cm	(if possible)	
BP:_____mmHg	Weight _____kg	MUAC_____cm	CRT_____sec

Neck stiffness N ☐ / Y ☐ Mouth dribbling: N ☐ / Y ☐

Kernig's positive N ☐ / Y ☐ Jaundice: N ☐ / Y ☐

Fixed stare N ☐ / Y ☐ Malnutrition N ☐ / Y ☐

McEwans sign N ☐ / Y ☐ Cough: N ☐ / Y ☐

Bulging fontanelle: N ☐ / Y ☐ Coryza: N ☐ / Y ☐

Full bladder: N ☐ / Y ☐ Lymphadenopathy N ☐ / Y ☐ (details) _____

Rash N ☐ / Y ☐: Macular ☐ / papular ☐ /vesicular ☐ /other ☐: where_____

Enanthemas (mucous membrane eruptions) N ☐ / Y ☐ (describe type and location)_____

Oedema N ☐ / Y ☐ (Where?)_____

Bleeding from oral mucosa? N ☐ / Y ☐

Other haemorrhagic manifestations: N ☐ / Y ☐ (describe)_____

Signs of dehydration N ☐ / Y ☐ if yes describe _____

Heart Sounds: Normal ☐ / Abnormal ☐(specify)_____

Breath sounds: Normal ☐ / Abnormal ☐(specify)_____

Pattern: Regular ☐ / Hypervent ☐ / Cheyne Stokes ☐ / Pauses ☐ / Ataxic ☐ / Gasping ☐

ENT: normal / abnormal (describe) _____

Hepatomegaly: No ☐ / Yes ☐ ____ cm in mid clav line Texture: _____ Tender No ☐ / Yes ☐

Abdominal other: N ☐ / Y ☐

Abdomen tender other than liver? N ☐ / Y ☐

Ascites: N ☐ / Y ☐ Tense:N ☐ / Y ☐ Splenomegaly: _N ☐ / Y ☐

Nasogastric tube: N ☐ / Y ☐ Reason for NG tube: aspiration ☐ / feeding ☐

NG aspirate N ☐ / Y ☐ (coffee grnd aspirate? N ☐ / Y ☐ fresh bld ? N ☐ / Y☐)

Urinary Catheter: N ☐ / Y ☐ For: retention ☐ / incontin ☐ / monitoring output ☐

If catheterised current hourly urine output _____ mls / hr

Endotracheal intubation: N ☐ / Y ☐ Froth from E/T tube? None ☐ / White ☐ / Pink ☐

ABG Done N ☐ / Y ☐ If yes answer: Prolonged bleeding after ABG N☐ /Y☐

Bleeding for _____ seconds after arterial blood gases

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

Admission Neurological Examination **Study No:_____**

Fill GCS and drugs and history on daily progress sheet

Cranial nerves

Pupil Size — Right: pinpoint ☐ / normal ☐ / dilated ☐
Left: pinpoint ☐ / normal ☐ / dilated ☐

Pupil response — Normal ☐ / Abnormal ☐ (describe) _____

Fundi — Right: Normal ☐ / Not done ☐ / Abnormal ☐ (describe)_____
Left: Normal ☐ / Not done ☐ / Abnormal ☐ (describe)_____

Eye movements — Normal ☐ / Abnormal ☐ (describe) _____

Doll's Eye reflex — Normal ☐ / Abnormal ☐ (describe) _____

Response to menace — Normal ☐ / Abnormal ☐ (describe) _____

VIIth nerve — Normal ☐ / Abnormal ☐ (describe) _____

Swallowing — Normal ☐ / Abnormal ☐ IF ABNORMAL or PATIENT UNCONCIOUS:
Gag: Normal ☐ / Abnormal ☐ / Absent ☐

Stridor — No ☐ / Yes ☐

Speech — Normal ☐ / Abnormal ☐ (describe) _____

Pout reflex: — Absent ☐ / Present ☐

Abnormal movements — No ☐ / Yes ☐

Tremor ☐ / Lip smacking or chewing ☐ / Teeth grinding ☐ / Agitated ☐ / twitching ☐ / Choreoathetosis ☐ /
Hiccoughs ☐ / spasms ☐ (Details) _____

Posture

Head position: — Normal ☐ / turned to left ☐ / turned to right ☐

Decerbrate / decorticate posturing : No ☐ / Yes ☐

Rigidity spasms — No ☐ / Yes ☐ (details)_____

Mental State — Normal ☐ / Abnormal ☐ (describe) _____

Other Comments _____

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

Admission Neurological Examination

Peripheral nervous system **Study No:_____**

Able to Sit: Independently ☐ / With help ☐ / Not at all ☐

Able to Stand: Independently ☐ / With help ☐ / Not at all ☐

Any fasciculation: N ☐ / Y ☐ details: _____

Neck tone: Normal ☐ / Increased ☐ / Neck rigid ☐

Flaccid limbs (LMN signs): No ☐ / Yes ☐ Details:_____

Head lag (when pulled to sit): No head lag ☐ / head lag ☐

Limb Tone [Normal (N) /Flexion (F) / Extension (E) / Gen Increased (I)/ Decreased (D) / Cogwheel (C)

Right Arm: ___ Left Arm: ___

Right Leg ___ Left Leg ___

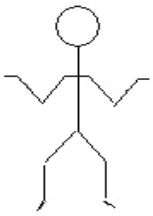
Equine feet (toes pointing downwards): Right: No ☐ / Yes ☐ Left: No ☐ / Yes ☐

Clonus: Right Ankle: No ☐ / Yes ☐ Left Ankle: No ☐ / Yes ☐

Tendon Reflexes, Draw:

Code:

- absent
+ reduced
++ normal
+++ increased



Symetrical/Asymetrical: Sym ☐ /Asy ☐

Plantar reflexes:

Right: Flexor (down) ☐ / Extensor (up) ☐ / No movement ☐ / Unsure ☐

Left: Flexor (down) ☐ / Extensor (up) ☐ / No movement ☐ / Unsure ☐

Abdominal reflexes Any present ☐ / none present ☐

Tone Overall: Normal ☐ / Generally Flaccid ☐ / Generally Spastic ☐ / Mixed ☐ / Neither ☐

Power (Grade 1-5, 1= no movement, 2= not against gravity, 3 = against gravity, 4= reduced, 5= full)

Right Arm _____ Left Arm _____

Right Leg_____ Left Leg _____

Hands fall on face Right Hand N ☐ / Y ☐ Left hand N ☐ / Y ☐

Knees flop out Right Knee N ☐ / Y ☐ Left Knee N ☐ / Y ☐

Paresis: Hemiparesis ☐ / Paraparesis ☐ / Quadraparesis ☐ / Monoparesis ☐ /

None ☐ / Unknown ☐ / Moving all 4 limbs ☐

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

Admission Date: **Initials:** **Age** **M/F** **Study No:**

Progress summary	Day of study:	Day: 0	Day: 1	Day: 2	Day: 3	Day: 4	Day: 5	Day: 6	Day: 7	Day: 8
	Day of illness:									
		Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:
GCS		Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:
Best Motor (2-5y in brackets) Best Verbal	spontaneous and seeing	4	4	4	4	4	4	4	4	4
	to loud voice	3	3	3	3	3	3	3	3	3
	to pain	2	2	2	2	2	2	2	2	2
	not at all (or fixed stare)	1	1	1	1	1	1	1	1	1
	oriented (words of any sort)	5	5	5	5	5	5	5	5	5
	confused (monosylables)	4	4	4	4	4	4	4	4	4
	inappropriate words (cries/screams/moans)	3	3	3	3	3	3	3	3	3
	incomprehensible sounds (grunts)	2	2	2	2	2	2	2	2	2
	none	1	1	1	1	1	1	1	1	1
	obeys commands	6	6	6	6	6	6	6	6	6
	localises painful stimulus (knuckles on	5	5	5	5	5	5	5	5	5
	withdraws (from pressure to nailbed)	4	4	4	4	4	4	4	4	4
	abnormal flexion posturing to pain	3	3	3	3	3	3	3	3	3
	extension to pain	2	2	2	2	2	2	2	2	2
	none	1	1	1	1	1	1	1	1	1
TOTAL										
Improving?	No change/Yes/No									
New complaints										
Seizures/Spasms/Posturing	Yes/No									
	If Yes describe / complete seizure chart									
General: Pulse, RR, Temp, BP, CRT										
	RS/CVS/PA/Skin									
Neuro	MacEwan's/Neck stiffness									
	Focal neurology/Acute flaccid paralysis									
	Cranial Nerves									
	Pupils/Fundi									
	Tone									
	Reflexes									
	deep tendon reflexes									
	plantar reflexes									
	dolls eye reflex (norm/abnorm)									
	Gait									
	Sit/Stand/Walk with or without assistance									

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

Results

DATE **Study No:** **Hosp IP No__**

	Hb				
	Total Count				
	Diff Count	Polym %	Lymphs %	Eosin	Mono %
	Plats				
	RBS (gluc)				
	ESR				

	Malaria screen	neg/pos	Specify (PS report/Ag):

	Blood cultures	neg/pos	Specify:

	JE serology	Sample 1	neg/pos
		Sample 2	neg/pos
		Sample 3	neg/pos

CSF Results	Date .	Pressure /cm water	WCC	% Lymphocytes	Protein .	Glucose (Glu st/ lab)	RBS Glucose	Other info
1st								
2nd								
3rd								

	Urea		Creatinine		T Bil		D Bil		SGPT		SGOT
Date:		Date:		Date:		Date:		Date:		Date:	
	Alk Phos		Na		K						
Date:		Date:		Date:							

Pastorix test				Date
serum	Pos/Neg	Organism_____		
csf	Pos/Neg	Organism_____		

EEG performed Y ☐ / N ☐ Nerve conduction (if flaccid) Y ☐ / N ☐ CT (unless full recovery) Y ☐ / N ☐

OTHER (please fill below):

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

Date _____ Study No:_____

	Date	Date	Date
Variables	**Admission**	**Day 2 (48 hours)**	**Discharge**
IVF intention (Maintenance/ Restricted)			
Actual total fluids (ml/kg/day)			
Actual Total fluids (ml/day)			
Intravenous fluids (doctor) (ml/day)			
Intravenous fluids (nurse) (ml/day)			
Intravenous fluids (attendant) (ml/day)			
Intravenous fluids (observation) (ml/day)			
Type of fluids (DNS/ 1/2 NS/ 1/5 NS/ RL)			
Nasogastric fluids (ml/day)			
Oral fluids (ml/day)			
Diluent for drugs (ml/day)			
Urine output (ml)			
Vomit (ml)			
BP (mm Hg)			
Resp Rate (per min)			
Temperature			
SPO2			
Head position (elevated/flat)			
CRT (sec)			
Weight (Kg)			
Urea		X	
Creatinine		X	
Lactate			
Na			X
K			X

Role of fluid management in the outcome of children with Acute Encephalitis Syndrome

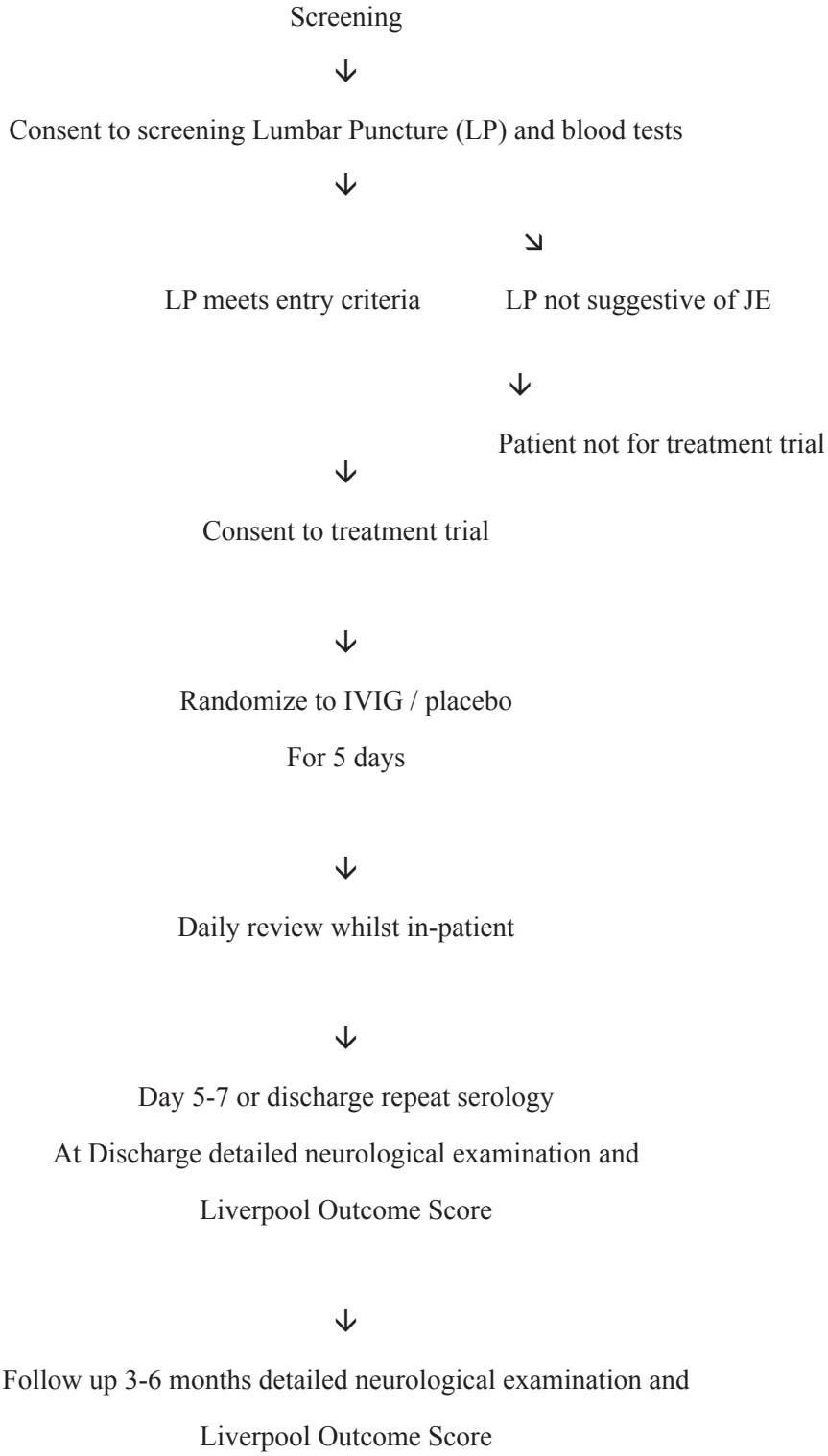
Treatment record

Study No: **Name:** **M/F** **Age**

Day of study:	Day: 0	Day: 1	Day: 2	Day: 3	Day: 4	Day: 5	Day: 6	Day: 7	Day: 8	Day: 9
DRUG TREATMENT (please list dose & frequency)										
Mannitol (inc. dose+diluent fluid(ml/day)										
Phenytoin (inc. dose+diluent fluid(ml/day)										
Phenobarbitone (inc. dose+diluent fluid(ml/day)										
Diazepam (inc. dose+diluent fluid(ml/day)										
Dexamethasone										
Other Drugs										
Ceftriaxone (inc. dose+diluent fluid(ml/day)										
Vancomycin (inc. dose+diluent fluid(ml/day)										
Penicillin (inc. dose+diluent fluid(ml/day)										
Cefotaxime (inc. dose+diluent fluid(ml/day)										
Gentamycin (inc. dose+diluent fluid(ml/day)										
Others										

Appendix N

Flow diagram shows summary of protocol timeline and recruitment process



Appendix O

Proforma of IVIG study

A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for JE in Nepal

History **(WHO CASE ID: VENEPCDR_____)** **Study No: _____**

Initials / Name _____ Hosp IP No. _____ Age___ Sex M ☐ / F ☐ D.O.B._____

Address of home: _____

Corresponding Address _____

Date Admitted to Hospital_____Date Admitted to Study_____ Time_____ Date of discharge_____ Outcome_____

Referred from: District Hospital ☐ / General Practioner ☐ / Primary health centre ☐ / home ☐ / other:_____

Does the child meet WHO AES case definition?

Acute Febrile Illness	N ☐ / Y ☐ Details _____
PLUS Change in mental status	N ☐ / Y ☐ Details _____
(including symptoms such as confusion, disorientation, coma, or inability to talk) AND/OR	
New onset of seizures (excluding simple febrile seizures*)	N ☐ / Y ☐ Details _____

Collect samples

Acute CSF	N ☐ / Y ☐ Date _____
Acute Serum	N ☐ / Y ☐ Date_____
Convalescent serum (later)	N ☐ / Y ☐ Date _____

SUMMARY INFORMATION (Complete after patient discharge)

Adm GCS E= ___ M= ____ V=____ Discharge GCS E = ____ M =____ V = _____
Discharged Day __ Date _____ **Discharge Outcome score** I (died) / II (Severe) / III (Mod) / IV (Mild) / V (No) Sequelae
3-6 mo FU Day __ Date _____ **FU Outcome score** I (died) / II (Severe) / III (Mod) / IV (Mild) / V (No) Sequelae
Others FU
JE status Acute CSF Pos / Neg Acute Serum Pos / Neg Conv Serum Pos / Neg

A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for JE in Nepal

History **Study No: _____**

Initials/ Name _____ Age_____ Sex M ☐ / F ☐ D.O.B._____

Date Admitted to Hospital_____Date Admitted to Study_____ Time_____

Date admitted to any hospital:_____ Where?_____

Referred from: District Hospital ☐ / General Practioner ☐ / Primary health centre ☐ / home ☐ / other:_____

Handedness: Right ☐ / Left ☐ Ethnicity: _____

Attends school Y ☐ / N ☐ / too young for school ☐ Does family rear pigs? Y ☐ / N ☐

Normal Milestones Y ☐ / N ☐ Describe_____

Do parents think child is normal self today? Y ☐ / N ☐ Details_____

Date of onset of illness _____ Today is day_____ of illness.

Fever	U ☐ / N ☐ / Y ☐ _____day to _____day	**Feeding**	Normal ☐ / reduced ☐ / not at all ☐
If fever	High grade ☐ / low grade ☐	**Drowsy**	U ☐ / N ☐ / Y ☐ _____day to _____day
Rigors/Chills:	U ☐ / N ☐ / Y ☐ _____day to _____day	**Irritable**	U ☐ / N ☐ / Y ☐ _____day to _____day
Headache:	U ☐ / N ☐ / Y ☐ _____day to _____day	**Confused:**	U ☐ / N ☐ / Y ☐ _____day to _____day
Neck pain	U ☐ / N ☐ / Y ☐ _____day to _____day	**Unconscious:**	U ☐ / N ☐ / Y ☐ _____day to _____day
Photophobia	U ☐ / N ☐ / Y ☐ _____day to _____day	**Convulsions**	N ☐ / Y ☐ describe below
Sore throat	U ☐ / N ☐ / Y ☐ _____day to _____day	**Spasms**	N ☐ / Y ☐ _____describe below
Cough	U ☐ / N ☐ / Y ☐ _____day to _____day		
Runny nose	U ☐ / N ☐ / Y ☐ _____day to _____day		
Vomiting:	U ☐ / N ☐ / Y ☐ _____day to _____day		
Diarrhoea	U ☐ / N ☐ / Y ☐ _____day to _____day		
Illness biphasic	U ☐ / N ☐ / Y ☐ decribe: _____		

Rash / Conjuctivitis / Abdo distension / GI Bleed / Jaundice / Others U ☐ / N ☐ / Y ☐ decribe, including days: _____

_____

Confused, N ☐ / Y ☐ describe, giving example(s)_____

Describe convulsions: number of attacks, when occurred, duration, partial, generalised, Status? incontinence? Frothing?

Post-ictal phenomenon, Todd's paresis?woke up after? _____

_____

Simple febrile convulsions only? N ☐ / Y ☐ (details) _____

Facial or Limb paralysis: N ☐ / Y ☐ (where and when, day to max weakness) _____

Preceeding illness in the last 2 months? None ☐ / Yes ☐ (give details)_____

Recent Vaccines No ☐ /Yes ☐

JE Vaccine No ☐/ Yes ☐/ Not Sure ☐ Date_____ Any reaction to JE vaccine No ☐ /Yes ☐

Details _____

Other history (including other symptoms and past Hx of convulsions, unusual features, family Hx):

_____

_____

_____

Treatment given before admission to study: N ☐ / Y ☐ / unknown medication ☐

(if yes tick and give details: what, dose, when, where from):

Aspirin ☐ / Paracetamol ☐ / Over the counter medicine ☐ / Traditional Medicine ☐ / Unknown antibiotic ☐

Chloramphenicol ☐ / Penecillin ☐ / Ceftrioxone ☐ / Midazolam ☐ / Diazepam ☐ / Phenobarb ☐ / Phenytoin ☐ / Haloperidol ☐

Saline ☐ / Dextrose ☐ **Mannitol ☐ / Steroids ☐ details:_____**

Other drugs: No ☐ / Yes ☐ (details) _____

A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for JE in Nepal

General Examination **Study No._____**

Initials / Name_____ Date:_____ Time:_____

Temp_____degrees F	Resp. rate_____/ min
Pulse_____/ min	Height/ length_____cm (if available)
BP:_____mmHg	Weight_____kg

Neck stiffness N ☐ / Y ☐

Kernig's positive N ☐ / Y ☐

Vacant stare N ☐ / Y ☐

N ☐ / Y ☐

Bulging fontanelle: N ☐ / Y ☐

Full bladder: N ☐ / Y ☐

Saliva dribbling: N ☐ / Y ☐

Jaundice: N ☐ / Y ☐

Malnutrition (calculate late N ☐ / Y ☐

Cough: N ☐ / Y ☐

Coryza: N ☐ / Y ☐

Lymphadenopathy N ☐ / Y ☐ (details) _____

Rash N ☐ / Y ☐: Macular ☐ / papular ☐ /vesicular ☐ /other ☐: where_____

Enanthemas (mucous membrane eruptions) N ☐ / Y ☐ (describe type and location)_____

Oedema N ☐ / Y ☐ (Where?)_____

Any haemorrhagic manifestations: N ☐ / Y ☐ (describe)_____

Heart Sounds: Normal ☐ / Abnormal ☐(specify)_____

Breath sounds: Normal ☐ / Abnormal ☐(specify)_____

Pattern: Regular ☐ / Hypervent ☐ / Cheyne Stokes ☐ / Pauses ☐ / Irregular ☐ / Gasping ☐

ENT: normal / abnormal (describe) _____

Hepatomegaly: No ☐ / Yes ☐ ____ cm in mid clav line Texture: _____ Tender No ☐ / Yes ☐

Ascites: N ☐ / Y ☐ Tense:N ☐ / Y ☐ Splenomegaly: N ☐ / Y ☐

Abdominal other: N ☐ / Y ☐

Nasogastric tube: N ☐ / Y ☐ Reason for NG tube: aspiration ☐ / feeding ☐

NG aspirate N ☐ / Y ☐ (coffee grnd aspirate? N ☐ / Y ☐ fresh bld ? N ☐ / Y☐)

Urinary Catheter: N ☐ / Y ☐ For: retention ☐ / incontin ☐ / monitoring output ☐

If catheterised current hourly urine output _____ mls / hr

Endotracheal intubation: N ☐ / Y ☐ Froth from E/T tube? None ☐ / White ☐ / Pink ☐

A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for JE in Nepal

Admission Neurological Examination **Study No:**_____

Initials / Name_____ Date:_____ Time:_____

Cranial nerves

Pupil Size	Right: pinpoint ☐ / normal ☐ / dilated ☐
	Left: pinpoint ☐ / normal ☐ / dilated ☐
Pupil response	Normal ☐ / Abnormal ☐ (describe) _____
Fundi	Right: Normal ☐ / Not done ☐ / Abnormal ☐ (describe)_____
	Left: Normal ☐ / Not done ☐ / Abnormal ☐ (describe)_____
Spont Eye movements	Normal ☐ / Abnormal ☐ (describe) _____
Doll's Eye reflex	Normal ☐ / Abnormal ☐ (describe) _____
Response to menace	Normal ☐ / Abnormal ☐ (describe) _____
VIIth nerve	Normal ☐ / Abnormal ☐ (describe) _____
Swallowing	Normal ☐ / Abnormal ☐ IF ABNORMAL or PATIENT UNCONCIOUS: Gag: Normal ☐ / Abnormal ☐ / Absent ☐
Stridor	No ☐ / Yes ☐
Speech	Normal ☐ / Abnormal ☐ (describe) _____

Abnormal movements No ☐ / Yes ☐

Tremor ☐ / Lip smacking or chewing ☐ / Teeth grinding ☐ / Agitated ☐ / twitching ☐ / Choreoathetosis ☐ / Hiccoughs ☐ / Rigidity spasms ☐ (Details) _____

Posture

Head position: Normal ☐ / turned to left ☐ / turned to right ☐

Extensor / Flexor posturing : No ☐ / Yes ☐

Mental State Normal ☐ / Abnormal ☐ (describe) _____

Other Comments _____

A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for JE in Nepal

Admission Neurological Examination **Study No:_____**

Peripheral nervous system

Able to Sit: Independently ☐ / With help ☐ / Not at all ☐

Able to Stand: Independently ☐ / With help ☐ / Not at all ☐

Any fasciculation: N ☐ / Y ☐ details: _____

Neck tone: Normal ☐ / Increased ☐ / Neck rigid ☐

Flaccid limbs (LMN signs): No ☐ / Yes ☐ Details:_____

Head lag (when pulled to sit): No head lag ☐ / head lag ☐

Limb Tone/Posture [Normal (N) /Flexion (F) / Extension (E) / Gen Increased (I)/ Decreased (D) / Cogwheel (C)

Right Arm: ___ Left Arm: ___

Right Leg ___ Left Leg ___

Equine feet (toes pointing downwards): Right: No ☐ / Yes ☐ Left: No ☐ / Yes ☐

Clonus: Right Ankle: No ☐ / Yes ☐ Left Ankle: No ☐ / Yes ☐

Tendon Reflexes, Draw:

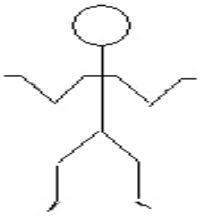
Code:

- absent

+ reduced

++ normal

+++ increased



Symetrical/Asymetrical Sym ☐ /Asy ☐

Plantar reflexes:

Right: Flexor (down) ☐ / Extensor (up) ☐ / No movement ☐ / Unsure ☐

Left: Flexor (down) ☐ / Extensor (up) ☐ / No movement ☐ / Unsure ☐

Abdominal reflexes Any present ☐ / none present ☐

Tone Overall: Normal ☐ / Generally Flaccid ☐ / Generally Spastic ☐ / Mixed ☐ / Neither ☐

Power (Grade 1-5, 1= no movement, 2= not against gravity, 3 = against gravity, 4= reduced, 5= full)

Right Arm _____ Left Arm _____

Right Leg_____ Left Leg _____

Hands fall on face Right Hand N ☐ / Y ☐ Left hand N ☐ / Y ☐

Knees flop out Right Knee N ☐ / Y ☐ Left Knee N ☐ / Y ☐

Paresis: Hemiparesis ☐ / Paraparesis ☐ / Quadraparesis ☐ / Monoparesis ☐ /

None ☐ / Unknown ☐ / Moving all 4 limbs ☐

Study Admission Date: **Initials:** **Age** **M/F** **Study No:**

Progress summary	Day of study:	Day: 0	Day: 1	Day: 2	Day: 3	Day: 4	Day: 5	Day: 6	Day: 7	Day: 8
	Day of illness:									
	Date:									
GCS	Examination time now	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:
Best Eyes	spontaneous and seeing	4	4	4	4	4	4	4	4	4
	to loud voice	3	3	3	3	3	3	3	3	3
	to pain	2	2	2	2	2	2	2	2	2
	not at all (or vacant stare)	1	1	1	1	1	1	1	1	1
Best Motor	obeys commands	6	6	6	6	6	6	6	6	6
	localises painful stimulus (knuckles on	5	5	5	5	5	5	5	5	5
	withdraws (from pressure to nailbed)	4	4	4	4	4	4	4	4	4
	abnormal flexion posturing to pain	3	3	3	3	3	3	3	3	3
	extension to pain	2	2	2	2	2	2	2	2	2
	none	1	1	1	1	1	1	1	1	1
Best Verbal (2-5y in brackets)	orientated (words of any sort)	5	5	5	5	5	5	5	5	5
	confused (monosylables)	4	4	4	4	4	4	4	4	4
	inappropriate words (cries/screams/moans)	3	3	3	3	3	3	3	3	3
	incomprehensible sounds (grunts)	2	2	2	2	2	2	2	2	2
	none	1	1	1	1	1	1	1	1	1
TOTAL										
Improving?	No change/Yes/No									
TICK	Oxygen / Endotracheal intubation / Central line									
	Nasgogastric tube									
	Urinary catheter									
	Feeding orally / NG / IV fluid									
General Changes	Pulse rate/Resp rate/RS/CVS/PA/									
Seizures/Spasms/Posturing	Yes/No									
Neuro	Changes in Bulk/Power/Tone/Reflexes									
	Dolls Eye Reflex (normal / abnormal									
	Sit/Stand/Walk with or without assistance									
	Changes in cranial nerves / other focal signs									
DRUG TREATMENT (please list dose & frequency)										
Study drug										
Mannitol										
Phenytoin										
Phenobarb iv / Gardinal										
Diazepam										
Antimicrobials										
*Other Drugs (avoid **Dexa** if possible)*										

Other Changes / Notes / Details:

A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for JE in Nepal

Adverse events

Initials / Name_____ Time:_____ Study No:
Date:_____

No	**Clinical Features**	**Yes**	**No**
1	Abdominal pain		
2	Headache		
3	Chest tightness		
4	Facial flushing		
5	Nausea		
6	Vomiting		
7	Dyspnoea		
8	Hot sensation		
9	Itching		
10	Non. Urt. rash		
11	Fever		
12	Pallor		
13	Irritable		
14	Hypotension		
15	Oliguria / Anuria		
16	Hemolysis		
17	Melena		
18	Raised Urea		
19	Raised Creatinine		
20	Death		
21	Others		

A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for JE in Nepal

Results **Study No:**

Initials / Name_____ Time:_____ Date:_____ Time:_____

DATE

	Hb	**Random blood sugar:** _____			
	Total Count				
	Diff Count	Polym %	Lymphs %	Eosin %	Mono %
	Plats				
	RBS (gluc)				
	ESR				

	Malaria screen	neg/pos		Specify (PS report/Ag):
	(Widal if done)			
	(Dengue if done)			

	Blood cultures	neg/pos		Specify:

CSF Results	Date	Opening Pressure/cm water	WCC	% Polys	% Lymps	Protein	CSF Glucose	**RBS Glucose**	CSF / Plasma Glucose ratio	Other info
1st										
2nd										
3rd										

CSF Gram stain _____ Hib Latex _____ CSF Culture _____

LFT: TB _____ Direct Bilirubin _____ SGOT _____ SGPT _____ Alp Phos _____ PT _____

Renal: Urea_____ Creat _____ Na _____ K _____

Others

Radiology

CXR (if done) Date _____ Result _____

CT head (if done) Date _____ Result _____

MRI head (if done) Date _____ Result _____

EEG performed Y ☐ / N ☐ Nerve conduction (if flaccid) Y ☐ / N ☐ CT (unless full recovery) Y ☐ / N ☐

RT-PCR

IL-4 IL-6

Others (JEV neutralizing Antibody levels)

JE serology	CSF 1	Date _____	neg / pos	Date _____	neg / pos	CSF 2 (if taken) Date _____ neg / pos
	Serum 1	Date _____	neg / pos	Date _____	neg / pos	Serum 2 Date _____ neg / pos

OTHER (please fill below):

Appendix P

The Table shows summary of study schedule

	Screening	Day 0 Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Daily follow up until discharge	Discharge	Follow Up
		If meets LP/ clinical criteria								3-6 months
Initial assessment	♦									
Full Clinical & Neurological Examination		♦							♦	♦
Brief Clinical examination			♦	♦	♦	♦	♦	♦		
Study Drug IVIG/ Placebo			•	•	•	•	•			
Study drug adverse effect			♦	♦	♦	♦	♦	♦	♦	
Clinical Outcome									♦	♦
Weight		♦								
Glasgow Coma Score (GCS)		♦	♦	♦	♦	♦	♦	♦	♦	♦
Liverpool Outcome Score (LOS)									♦	♦
Negative *P. falciparum*	♦									
Clinical Investigations										
CBC, RFT, glucose, LFTs		♦								
(U&Es and other investigations as clinically indicated)										
Lumbar puncture and opening pressure	♦		♦*					♦*		
CSF cytokines & JE viral PCR	♦		♦*					♦*		
JE serology	♦		♦							
Discharge JE serology										
(Day 5-7 of illness or discharge date if earlier)							♦		♦	
Blood Cytokines	♦		♦						♦	
Serum for measurement of JEV neutralizing Antibody		♦					♦ Pre- & post dose			

* As clinically indicated

Appendix Q

A preliminary randomized double blind placebo-controlled trial of intravenous immunoglobulin for Japanese encephalitis in Nepal

Neurological assessment form

Date _____ Study No:_____

First Initials of child_____ Age_____ Sex: M ☐ / F ☐

Cranial nerves

Vision	Right _____ Left _____
Fundi	Right: Normal ☐ / Abnormal ☐ / Not done ☐ Left: Normal ☐ / Abnormal ☐ / Not done ☐
Diplopia	N ☐ / Y ☐ (describe) _____
Vth nerve	Normal ☐ / Abnormal ☐ (specify) _____
VIIth nerve	Normal ☐ / Abnormal ☐ (specify) _____
Hearing	Normal ☐ / Abnormal ☐ (specify) _____
Tongue movement	Normal ☐ / Abnormal ☐ (specify) _____
Pout reflex:	Absent ☐ / Present ☐

Peripheral nervous system

Neck tone: Normal ☐ / Increased ☐ / Neck rigid ☐

Limb Tone [Normal (N) /Fixed Flexion (F) / Extension (E) / Gen Increased (I)/ Decreased (D) / Cogwheel (C)

(write letter next to limb space)

Right Arm:_____ Left Arm: _____

Right Leg _____ Left Leg _____

Equine feet (toes pointing downwards): Right: No ☐ / Yes ☐ Left: No ☐ / Yes ☐

Clonus: Right Ankle : No ☐ / Yes ☐ Left Ankle : No ☐ / Yes ☐

Tendon Reflexes, Draw:

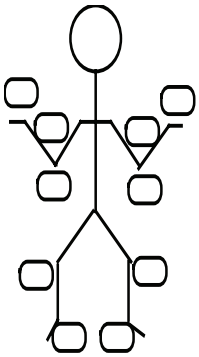
Code:

- absent

+ reduced

++ normal

+++ increased



Plantar
reflexes:

Right: Flexor (down) ☐ / Extensor (Up) ☐ / No movement ☐ / Equivocal (Unsure) ☐

Left: Flexor (down) ☐ / Extensor (Up) ☐ / No movement ☐ / Equivocal (Unsure) ☐

Hemiparesis ☐ / Paraparesis ☐ / Quadraparesis ☐ / Monoparesis ☐ / None ☐ / Unknown ☐

Tone Overall: Normal ☐ / Generally Flaccid ☐ / Generally Spastic ☐ / Mixed ☐ / Neither ☐

Response to Pain: Extensor ☐ / Flexor ☐ / Neither ☐

Abdominal Reflexes: Present ☐ / absent ☐

Sensation: Not tested ☐ / Normal ☐ / Abnormal ☐ (specify, or draw over page)_____

Back at school yet school Y ☐ / N ☐ (why not):_____

Any other Comments / Problems : _____

Appendix R

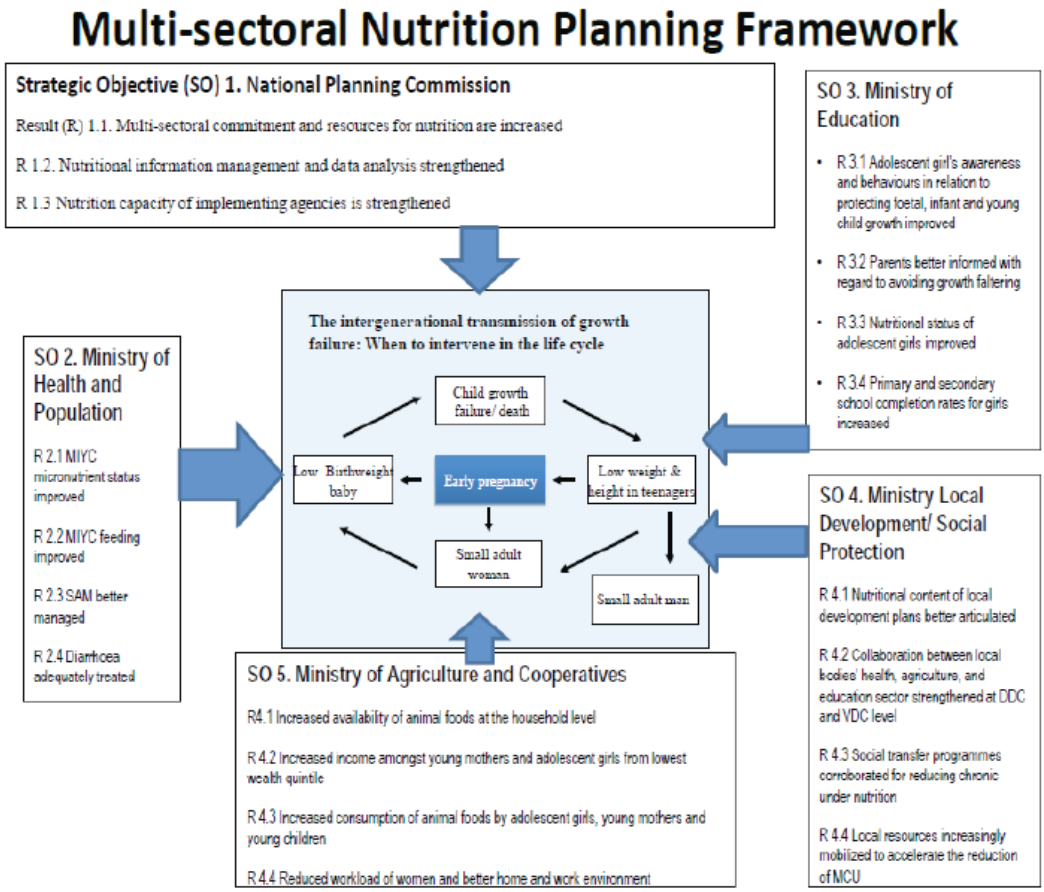
Table showing validation of Liverpool outcome score (109)

	Malaysia Percent (95% CI)	India Percent (95% CI)
Sensitivity	100% (89.1- 100)	100% (91.2- 100)
Specificity	98.4% (96.5- 99.4)	93.8% (90.7- 96)
Positive predictive value	84.2% (68.7- 94)	65.6% (52.3- 77.3)
Negative predictive value	100% (98.6- 100)	100% (98.5- 100)
Intra observer agreement (kappa value)	1.0	0.9 (0.82- 0.99)
Inter observer agreement (kappa value)	0.94 (0.86- 1.0)	0.79 (0.67- 0.91)

(Lewthwaite P. et al, 2010)

Appendix S

Figure showing Multi-sectoral Nutrition Planning Framwork of Nepal



(http://scalingupnutrition.org/wp-content/uploads/2013/03/Nepal_MSNP_2013-2017.pdf)

Appendix T

Table showing different concentration of sodium in commonly used intravenous fluids

Commonly used intravenous fluids	Sodium (mmol/L) content
5% dextrose + 0.9 % normal saline	154
5% dextrose + 0.45 % normal saline	77
5% dextrose + 0.25 % normal saline	34
5% dextrose + 0.18 % normal saline	31
Normal saline (0.9% Nacl)	154
Ringer's lactate	130

(Martin GS, 2016)

Appendix U

Table shows list of anti-JE drugs

Category	Antiviral drugs	Target/Mechanism
Non-specific broad spectrum	Arctigenin	Antioxident
	Fenofibrate	Antioxident
	Curcumin	Antioxident
	Pentoxifylline	Assembly or release
	Nitazoxanide	Early-mid replication cycle
Nucleic acid based	siRNA	C,M,E,NS1, NS3, NS4B, NS5
	PNA	UTR
	Morpholino Oligomer	UTR
Virus replication cycle based	Heparan Sulfate	Receptor binding
	E-binding peptide	Receptor binding
	NSQ	Attachment
	Indirubin	Attachment
	Bovine lactoferrin	Receptor binding
	Griffithsin	Receptor binding
	Recombinant E	Receptor binding
	MCPIP1	RNA replication
	Kaempferol	RNA replication
	SCH 16	Translation