RESEARCH ARTICLE

A Clinicopathological Study of Encephalitis in Children with Special Reference to Japanese Encephalitis

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ABSTRACT

Introduction: Encephalitis refers to inflammation of Parenchymal brain tissue. Acute encephalitis begins abruptly (hours to days) whereas chronic encephalitis is insidious in onset, occurring over weeks to months.[1]
Objective: To document etiological, clinical and laboratory features in cases of Encephalitis in general and Japanese encephalitis in particular.
Methods: Fifty Indian children below 15 years of age, with encephalitic features were investigated. They were treated in Bapuji Child Health Institute and Chigateri Government Hospital, Davangere. Various methods (Hematological, Biochemical, Cytological, Microbiological, Radio imaging) were used. Serologic methods were used for detection of IgG and IgM antibody to Japanese encephalitis virus.
Results: The etiological agents responsible were Japanese encephalitis virus followed by Co-existing infections, Mumps and other viral etiology where specific diagnosis could not be clinched
Conclusion: Japanese encephalitis virus is more frequent viral pathogens of childhood encephalitis in Davangere and surrounding areas.

INTRODUCTION

Encephalitis refers to inflammation of Parenchymal brain tissue. Acute encephalitis begins abruptly (hours to days) whereas chronic encephalitis is insidious in onset, occurring over weeks to months.[1] Rates of epidemic encephalitis have fallen over several decades as a result of both improvements in living conditions and vector control and the advent of vaccines against many of the childhood exanthematous diseases. Mortality from encephalitis has not declined proportionately, because the agents that causes most of the severe cases of encephalitis, such as Herpes simplex virus (HSV) and Japanese encephalitis virus (JEV) still exist in the environment.[2] Identifying the agent responsible for suspected cases of central nervous system (CNS) viral infection poses tremendous diagnostic challenges, and a specific organism is identified in only 30% of cases of suspected viral encephalitis.[3] Globally Japanese encephalitis virus is the most important emerging viral encephalitis.[4] Japanese encephalitis is a mosquito-borne arboviral infection which is the leading cause of viral encephalitis[5] and it is an important public health problem in South-east Asia, and its transmission appears to be increasing in several countries.[6] Japanese encephalitis (JE) is the most important forms of epidemic and sporadic encephalitis in the tropical regions of Asia including Japan, China, Taiwan, Korea, Philippines all of the South-east Asia and India.[7] JE is principally a disease of rural agricultural areas, wherever vector mosquitoes proliferate in close association with pigs, which are the principal vertebrate amplifying hosts.[5] JE is a major cause of childhood mortality and morbidity in countries of South East Asia and Western pacific regions. It is the most important cause of arboviral encephalitis.[8] Lack of specific treatment for this complex disease leads to high rates of mortality and disability. In order to reduce complications, the eruption of the disease has to be controlled.[5] Children are mainly affected, with morbidity rate estimated at 0.30 to 1.5 per 100,000 populations and case fatality rate

has ranged from 10% to 60%. Up to 50% of those who recover may be left with neurological deficits. There is a need to develop simple tests for use at the peripheral level both for diagnosis and for epidemiological surveys. It is a vaccine preventable disease.[9]

Objectives - To document etiological, clinical & laboratory features in cases of Japanese encephalitis.

METHODOLOGY

Source of data: This is a prospective study which included pediatric patients from Bapuji Child Health Institute and Research Centre, Chigateri District Hospital, Women and Children Hospital, Davangere with features of encephalitis admitted to the inpatient department during the period July 2007 to June 2009.

Method of collection of Data: The 50 patients with clinical features of encephalitis were enrolled after obtaining informed / written consent from parents / guardians. A complete clinical examination was carried out and relevant investigations done and documented.

Inclusion criteria: All children 15 years of age and below presenting with signs and symptoms of encephalitis / meningoencephalitis were included in the study.

Exclusion criteria: Children with similar clinical features in whom specific diagnosis other than encephalitis was clinched were excluded from the study

Sample collection

1) With strict aseptic precautions blood samples (about 5ml) collected by Venepuncture and transferred in sample collection bulbs.
   - Plain bulb for serological studies including viral serology for JE.

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2) Lumbar puncture for all suspected encephalitis cases
3) Supportive investigations like CT brain and Chest x-ray findings were noted.

Rapid Test : The Onsite JE IgG/IgM rapid test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG anti-Japanese Encephalitis Virus (JE) and IgM anti JEV in human serum or plasma was used as screening test and as an aid in the diagnosis of infection with Japanese Encephalitis.

RESULTS

The present study of clinical, serological, hematological, biochemical and CSF parameters in study of encephalitis in children was conducted between July 2007 to June 2009 in 50 cases. Age of the children ranged from 4 months to 15 years.

Table 1. Age distribution of viral encephalitis cases

	Number of cases	Percentage (%)
< 1 year	01	02
1 to 3 years	11	22
3 to 6 years	12	24
6 to 9 years	09	18
9 to 12 years	08	16
12 to 15 years	09	18
Total	50	100

Majority of the patients were in the age group of 3 to 6 years (24%), followed by 11 patients in the age group of 1 to 3 years (22%) and nine cases each in 6 to 9 years and 12 to 15 years (18%) respectively. Mean age was 7.18 yrs in the present study (Table-1).

Table 2. Viral encephalitis cases

Disease	Total number of cases
Japanese encephalitis	15
J.E + Dengue	1
JE + Dengue + Malaria	2
JE + Dengue + Typhoid fever	2
Mumps encephalitis	2
Unknown viral etiology	8

A total of 15 cases were affected by JEV infections. Five cases had serologic evidence of coexisting infections i.e., (JE and Dengue), (JE, Dengue and Malaria) and (JE, Dengue and Typhoid Fever). Two cases were presumed as Mumps encephalitis due to clinical manifestations. Eight cases were presumed to be of unknown viral etiology and specific diagnosis could not be established (Table-2)

Table 3. Sex incidence in encephalitis cases

Sex	Number of cases	Percentage (%)
Male	27	54
Female	23	46
Total	50	100

Out of 50 patients 27 (54%) were males and 23 (46%) were females. M: F ratio was 1.1:1 (Table 3).

Japanese Encephalitis: 15 cases were diagnosed as Japanese encephalitis

Seasonal Variation: Majority of cases were reported from July-September and in the months of December and January.

Fever and headache, altered sensorium, nausea, vomiting and neck rigidity was seen in all cases (100%) followed by convulsions (80%), disorientation (73.3%), hepatomegaly (73.3%), kernig's sign (53.3%) and splenomegaly (53.3%). Three patients developed complications like acute flaccid paralysis; abnormal behavior with two cases went

into coma and renal failure and later succumbed to the disease within a week.

Table 4. Clinical features in JE patients

Symptoms	No. of cases	Percentage
Fever and headache	15	100
Altered sensorium	15	100
Nausea and vomiting	15	100
Convulsions	12	80
Disorientation	11	73.3
Neck rigidity	15	100
Kernig's sign	8	53.3
Hepatomegaly	11	73.3
Splenomegaly	8	53.3

Serological Test

Fifteen cases of JEV were positive for anti JE antibodies. Both IgG and IgM were seen in 8 cases, IgM in 4 cases and IgG in 3 cases. Onsite JE IgG / IgM rapid test from CTK biotech Inc was used.

Table 5. Rapid test for IgG and IgM JEV antibodies

Serological marker Anti JEV antibodies	Number (%)
IgG	3 (20.00)
IgM	4 (26.66)
IgG and IgM	8 (53.33)

Csf Analysis

CSF analysis was done in 14 of the total 15 cases of J.E viral infection. Lumbar puncture was contraindicated in one patient due to raised intracranial pressure.

Table 6. CSF analysis in JE patients

Parameter	Normal values	Range
Total cell count(cells/mm^3)	< 5	6 – 680
Protein mg/dl	20 – 45	38 – 178
Glucose mg/dl	40 – 70	32 – 72
Chloride (mEq/L)	116 – 122	102 – 120

In 11 of these, the pleocytosis was predominantly lymphocytic and in the rest it was polymorphonuclear.

Five Cases With Co-Existing Infections were Observed in this Study

a) JE with Dengue: One case

The patient had clinical features of fever and headache, altered sensorium, convulsions neck rigidity, hepatomegaly and splenomegaly. It showed positivity for anti-dengue IgM antibody as well as anti JE IgM antibodies.

CSF analysis showed total proteins 34 mg %. Only 4 lymphocytes could be counted.

b) JE, Dengue and Malaria: 2 cases.

Both cases had fever and headache, altered sensorium, nausea, vomiting, neck rigidity, hepatomegaly and splenomegaly. Both cases showed serologic positivity with IgM antibodies for dengue and JE virus infection. Rapid strip test showed positivity for plasmodium Vivax malarial infection.

CSF analysis

Total proteins observed was 86 and 36 mg %. Total cell count ranged from 4 -210 cells. Neutrophils observed were 24 % and 0 %. Lymphocytes were 76 % and 100 % respectively.

c) JE / Dengue and Typhoid fever: Two cases

Clinical features: The symptoms includes fever and headache, altered sensorium, Nausea and vomiting, neck rigidity and convulsion with one of the case showing positive kernig's sign, hepatomegaly and splenomegaly.

Serological test: Both cases showed positivity with widal tests for salmonella typhi. Both cases showed IgM antibody positivity for Dengue and JE virus.

CSF analysis of the two cases showed elevated protein levels (62 and 156 mg %). Total count 140 and 410 cells /mm^3 of CSF. Neutrophils were 42 and 22 %. Lymphocytes 58 and 78% respectively. Lymphocytic pleocytosis were observed in both cases.

DISCUSSION

This study comprises of fifty patients admitted to the hospital attached. These patients had clinical features of encephalitis. Fifteen cases (30%) were serologically proved Japanese encephalitis virus (JEV) infections. Five cases had co-existing infections out of which one case with JE/ DEN, Two cases of JE/ DEN/ Malaria and two cases of JE/ DEN/ Typhoid fever. Two cases were clinically proved Mumps encephalitis. Eight presumed cases (16%) of unknown viral etiology where specific etiological diagnosis could not be clinched.

Table 7. Comparison of Age range of viral encephalitis cases

Study	Number of cases	Age
Xu, Yunhe *et al*[10] (1996)	97	7month-13years
Present study (2009)	50	4 month-15years

A study by Xu, Yunhe *et al*[10] at Beijing children hospital had patients (n= 97) aged between 7 month-13 years. In our study the age ranged from 4 month- 15 years (Table-7).

Table 8. Dengue fever / DHF / DSS (Age comparison study)

Study	Number of cases	Age
S.K. Kabra *et al*[11] (1988)	21	6-12
Rasul CH *et al*[22] (2002)	125	10-14
Present study (2009)	20	4 month-14 years

A study conducted by Kabra SK *et al*[11] at All India institute of medical sciences New Delhi, had patients aged between 6-12 years. Another study conducted by Rasul CH *et al*[22] in Bangladesh had patients in the age group of 10-14 years. In the present study twenty patients (40%) were aged between 4 month-14 years. The Mean age was 7.15 years (Table-8).

Seasonal variation of encephalitis cases

Table 9. Comparison of seasonal incidence of encephalitis cases

Author	Months
Xu, Yunhe *et al*[10] (1996)	July – September
Rasul CH *et al* [22] (2002)	July – October
Present study (2009)	July – September / December and January

Two studies by Rasul CH *et al* and Yunhe *et al* recorded maximum cases between July to October. Our study showed two peaks one from July to September and another from December to January. This could be related to agricultural activities in our area during these periods.

Japanese Encephalitis Cases

Japanese encephalitis is known to affect all age groups

Table 10. Japanese Encephalitis cases (Age comparison study)

Study	Number of cases	Age (yrs)	JE positive cases	Percentage
Donald E. Carey *et al*[21] (1966)	52	3-14	51	98.07
C.S. Kamala *et al*[18] (1989)	120	0-12	60	50
AC Phukan *et al*[16] (2004)	173	1-12	109	63
Present study (2009)	50	3-13	15	30

A study conducted by Donald Carey *et al*[21] at CMC Vellore had a total of 51 cases of JE and age ranged from 3-14 years. Kamala *et al*[18] (1989) recorded age incidence of 0-12 years and Phukan *et al*[16] noted age range of 1-12 years. In our study there were 15 cases of JE and were aged between 3-13 years (Table-2). JE was reported in as young as 8 month old-baby as well as in a 106 year old adult.[18] Incidence is lower among younger children (< 3 year old) than in older children.[12] In South India, the disease was reported to affect children below 15 years of age. Increased incidence in children could be partly explained by socio-economic factors, living conditions and inter-relationship with domestic animals.[15] Risk for JE is more among the children due to poor immune status.[5] JE generally is known to affect rural population of low socio-economic status. The disease here predominantly seen in Hindus. Pigs which are identified as amplifier hosts in India are traditionally banned by Muslims and they do not rear them or allow them near their residential areas. [18] In the present study there was slight male predominance. M: F ratio was 1.1:1. Majority of the patients in the present study were from Davangere. This probably only reflects easy access to health care.

The common clinical features which were seen in almost all studies are fever and headache. Study conducted by Prasad *et al* at PGI, Chandigarh showed 90% of the total cases had fever and headache, altered sensorium with convulsions and Neck rigidity. In a study conducted by Kamala *et al.* at Bellary, Karnataka showed that fever (98.65%), followed by headache (28.6%), vomiting 28.6% and with altered sensorium seen in 6.7% of the total cases studied.   A   study by Kumar *et al* at King George Medical College, Lucknow showed that fever (94.5%), headache (20%), altered sensorium (100%), vomiting (54.3%), and convulsions (84.7%) with few cases showed hepatomegaly (5.43%) and splenomegaly (2.17%). The other problems seen are respiratory problems, mask like facies, with 43.4% cases showing generalized hypertonia. Rathi *et al*[14] at BRD medical college, Gorakhpur showed that fever (98.9%), headache (63.8%), vomiting (54.4%), convulsions (83.8%), altered sensorium (78.2%) Neck rigidity (25.5%) with breathing problems (12.9%) and G.I. bleeding in (13.1%) of the total cases studied.

In the present study, fever and headache (100%) was seen in all cases, while in the studies by Rathi *et al*. Chaudhuri *et al.* and Kamala *et al.* 98% of cases had these features. Altered sensorium was seen in 98% of our cases, while Prasad *et al.* and Rathi *et al.* recorded it 90% and 78.2% of cases respectively. Vomiting was seen in 84% of our cases as compared to around 54% in Kumar *et al.* and Rathi *et al.* studies. Convulsions as a predominant clinical feature were seen in different studies. It was seen in 58 % of our cases. Disorientation / coma were seen in almost all cases studied by Kumar *et al.* and Chaudhuri *et al*. It was around 52% in our study. Lower incidence of convulsions and altered sensorium in our study could be due to quick access to health care intervention and treatment Studies conducted by Prasad *et al* and Chaudhuri *et a.l* showed neck rigidity was 90% and 92% respectively. Our study had 96% of cases with neck rigidity (Table-11).

Table 11. Analysis of clinical features for JE cases

Authors	Fever	Head ache	Vomiting	Convulsion	Altered sensorium	Disorientation / Coma	Meningeal signs		Hepatomegaly	Splenomegaly	Skin Rash	Other features
							Kernig's sign	Neck rigidity				
Prasad et al[24] (1982)	90%	90%	0	90	90	0	0	90	0	0	0	Irregular pupils
Kamala et al[18] (1989)	98.65	28.6	28.6	73.3	96	0	0	37.33	0	0	0	Respiratory (18.6%) Problems Speech disturbance (32%)
Kumar R et al [20] (1990)	94.5	20.6	54.3	84.7	100	0	0	13%	5.43	2.17	5.4	Respiratory problem (46.7%) Mask like facies Rash = 5 (5.4%)
Chaudhri et al[15] (1992)	98	96	0	98	0	98	96	96	0	0	0	Pupillary changes (86%)
Rathi et al[14] (1993)	98.9	63.8	54.4	83.8	78.2	61	61	25.5	0	0	0	Breathing = 12.9 GI bleed = 13%
Present study : (2009)	100	100	84	58	98	52	28%	96	76	56	12	Papilledema = 2% Rash = 6 (12%)

Serological study for JE

According to Rathi et al[14] study, out of 53 patients, 27 cases (51%) were IgM positive in serum. IgG positivity was seen in 498 cases (74.3%) of the total 670 patients. Another study by Kamala et al[18] out of 120 cases where sera was tested, 60 cases (50%) were reactive for JE virus. In a study by Prasad SR et al[17]IgM class of antibodies to JE virus was demonstrated in serum of 5 cases (26.3%) of the 19 encephalitis cases. In our study Both IgG and IgM were positive in 8 cases (53.3%) IgM in 4 cases (26.6%) and IgG in 3 cases (20%). JEV-specific IgM can be detected in CSF and or Serum in approximately 75% of patients within 4-7 days after the onset of illness and nearly all patients are positive7 days after onset of disease.[13]

Table 12. Comparison study of CSF analysis in JE patients

Authors	Proteins > 40 mg/dl	Sugar (40-70 mg %)	n (%)
Kumar et al[20] (1990) n = 85	28.2	N	24(28.3)
Prasad et al[17] (1982) n = 19	85.7	N	7(36.8)
Rathi et al[14] (1993) n = 670	70.8	N	498(74.3)
Present study (2009) n = 15	86.6	N	14(93.3)

Study conducted by Kumar et al[20] showed that CSF protein was raised above 40 mg/dl in 24 cases (28.3%) with maximum protein level was 120 mg/dl and mean 49 mg/dl. CSF sugar was within normal limits in all patients. CSF was examined in 85 of the 92 patients. Pleocytosis in the CSF was found in 28 (32.3%) patients, the cell count ranged from 30-360/mm^3. In 15 of these the pleocytosis was predominantly polymorphonuclear and in 13 predominantly lymphocytic. Study by Prasad et al[17] where CSF was analyzed in 7 of the 19 patients. Five showed lymphocytic pleocytosis (20-40 cell/mm^3). Proteins were moderately raised in 6 patients (46-75 mg/dl). Sugar levels were within normal limits. In our study CSF protein was raised above 40 mg/dl in 13 cases (92.85%) with maximum protein level was 178 mg/dl. CSF sugar was within normal limits. CSF was examined in 14 of the 15 patients. Pleocytosis in the CSF was found in almost all the patients. The cell count ranged from 6-680cells/mm^3. In 11 of these the pleocytosis was predominantly lymphocytic and in the rest polymorphonuclear.

Co existing infections

Out of 50 cases, 5 cases had coexisting infections in our study. They were

a) JEV with Dengue – one case
b) JEV, Dengue and Malaria – two cases
c) JEV, Dengue and Typhoid Fever – two cases.

JEV with Dengue

The clinical features observed in our study were similar in both JEV and Dengue cases. These cases occurred during the post monsoon season. They were serologically positive for IgM antidengue antibodies and IgM anti JE antibodies. A study conducted by Rathi et al[18] had serologically positive reaction for JE, dengue and west wile virus in 5.8% of the cases. This is due to the cross reactivity seen in flaviviruses infections. CSF showed normal protein levels and total cell count had four lymphocytes.

JEV, Dengue and Malaria – Two cases

The clinical features observed in our study had fever and headache, with altered sensorium, vomiting hepatomegaly and splenomegaly. Both cases were serologically positive for IgM antibodies for dengue and JEV. Rapid strip test (Malaria antigen test) showed positivity for plasmodiumVivax malarial infection. Our area is endemic for mosquito borne diseases; hence Malaria, JEV and Dengue virus infection can co-exist.

CSF analysis showed proteins were elevated in single case the total cell count was 4 – 210 cells/mm^3, predominantly lymphocytes.

JE, Dengue and Typhoid fever - 2 cases

These two cases had similar clinical presentation. Both the cases were positive for Widal test by slide agglutination method. The titres were 1:160 (TO) and 1:320 (TH) for salmonella typhi. They were also positive for IgM antibodies for Dengue and JEV.

CSF analysis showed increased in protein levels and total cell count was 140 cells/mm^3. There was lymphocytic pleocytosis in both the cases.

Coexisting and cross reacting infection

In the study conducted by Nuegoonpipat *et al*[23] 2008 at National Institute of Thailand, twenty four (9%) of 258 samples were scored as containing IgM antibody against JE. Levels of JE-IgM were lower than those of dengue IgM in all the serum samples. Serum samples from serologically confirmed JE cases showed IgM in all cases. 13% of them also showed false positivity for dengue – IgM. These results are consistent with an understanding that even though IgM responses are generally specific for Dengue and JE infections, false positivity can be detected. In serum samples from confirmed Dengue patients, about 10% were JE- IgM positive. Another study conducted by Makino *et al*. (1994) too reported that antibody responses were cross reactive among flaviviruses. Martin *et al*. (2002), also reported cross reactive IgM response in flavivirus encephalitis cases.[23] In the present study cross reactive IgM antibodies were detected in three cases out of fifty encephalitic cases. The presence of these antibodies response needs to be considered in areas where Dengue and JE viruses co-circulate. It is recommended that Dengue and JE IgM capture ELISA be performed in parallel in those areas.

Conclusion

Encephalitis and other CNS complications in many viral infections are now well recognized and delay in diagnosis can prove fatal. Dengue and Japanese encephalitis viruses which were initially observed as rare sporadic or seasonal outbreaks have now slowly assuming endemic proportions all over the country. Though these can be explained on various population, occupational, agricultural, environmental factors, urbanization, and rapid transport. The role of preventive public health measures in halting the spread of this vector borne disease cannot be trivialized. Breaking of the vicious circle of disease spread old saying "prevention is the better than cure" should be the rule and aim should be to prevent contracting of infections. Facilities for early diagnosis should be made available. Encephalitis is virtually medical emergency often requiring intensive care and ventilation support. Since encephalitides are unpredictable in their presentation, etiological diagnosis based on clinical presentation is not only difficult but at times it is impossible. Morbidity and mortality associated with these medical emergencies are a testimony to this fact. Etiological diagnosis will go long way in proper treatment, management and prevention of the complication in these diseases.

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