

Japanese Encephalitis Virus: Common Cause of Viral Encephalitis in Paediatric Age Group in Bellary, Karnataka, India

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ABSTRACT

Background: A study was conducted in VIMS hospital at Bellary during the epidemic of August 2004 to July 2005.

Objectives: To know the hospital based incidence of J.E and to study the age and sex pattern of the infection in the paediatric age group.

Materials and Methods: 82 children admitted to the Paediatric ward, VIMS, Bellary with clinical diagnosis of acute viral encephalitis, during the epidemic of August 2004 to July 2005 were studied. After pooling the samples, they were subjected to J.E. MAC ELISA.

Results: Out of 82 patients tested by J.E. MAC ELISA, 19 (24%) were positive for J.E. and 12 (14.63%) were positive for flaviviral infection. CSF was positive in all 19 cases with 100% positivity.

Serum was positive in 10 cases with 15.87% positivity. This indicates that there are significant number of J.E. cases in Bellary emphasising the rampant nature and stressing the measures to be taken to overwhelm this devastating disease.

Conclusion: The hospital based incidence of J.E. was found to be significant in the area of the study. The paediatric age group, between 4 to 6 years was found to be most predisposed to J.E., probably because of low immunity in the age group. Male predominance was seen with male to female ratio of 1.42:1. Illiteracy, low socio economic status and living in unhygienic conditions near rice fields contributed to the high incidence of J.E. in and around Bellary. The serological results clearly establish the etiology. Isolation of JEV from specimens would have strengthened the diagnosis.

Key Words: Japanese encephalitis, MAC ELISA

KEY MESSAGE

- Japanese encephalitis is endemic in Bellary
- MAC ELISA helps in rapid and specific diagnosis of Japanese encephalitis
- Isolation of JEV would have strengthened the diagnosis

INTRODUCTION

Viral encephalitis is an important cause of hospital admissions, mortality and permanent neurological sequelae in children in India. A wide variety of viruses may cause encephalitis. The ones which are of public health importance in India include Japanese encephalitis (J.E.) and Rabies [1]. J.E is one of the important arthropod borne zoonotic diseases of public health importance, causing encephalitis in humans leading to heavy morbidity and mortality in different parts of the world [2].

OBJECTIVES

1. To study the hospital based incidence of Japanese encephalitis in Bellary.
2. To study the pattern of age and sex distribution of Japanese encephalitis in Bellary.

MATERIALS AND METHODS

The study group consisted of 82 patients clinically diagnosed as acute viral encephalitis and admitted to the department of Paediatrics, VIMS, Bellary. The study was conducted over a period

of one year from August 2004 to July 2005. Specimen collection and transport were considered as of paramount importance in the laboratory confirmation. 2-3 ml of CSF was collected by lumbar puncture after ruling out papilledema by the paediatrician. 2-5 ml of blood samples were collected by venipuncture [2]. Both samples were transported to the Microbiology laboratory in vaccine carriers. After receiving the CSF and blood samples along with the duly filled requisition forms from the Paediatric department, serum was separated by centrifugation of the whole blood sample. The CSF and serum samples were transferred to sterile aliquots, labelled with the particulars of the patient and preserved in the refrigerator at 4°C. A register was maintained in which the particulars of the patient were entered. The MAC ELISA for J.E. was performed using a commercial kit, JEV CHEX procured from X-CYTON diagnostics, marketed by NIMHANS, Bangalore, NII, New Delhi and KGMC, London. JEV MAC ELISA is a semi quantitative method for the detection of IgM antibodies to JEV in CSF and serum. MAC assays have one great advantage over conventional indirect assays in that they eliminate competition by IgG molecules. This results in enhanced sensitivity of IgM detection. In JEV infection

the use of MAC ELISA is especially advantageous since the ratio of diseased to in apparent infections is 1:300. As a result, populations living in endemic areas for J.E invariably will have JEV IgG in their serum indicating past exposure to the virus. JEV CHEX uses anti human IgM coated plates to capture IgM antibodies in the clinical samples. The test was performed according to the manufacturer's instructions. All samples and reagents were brought to room temperature. The aluminium pouch, containing the ELISA plate, was cut at the notch above the Zip lock pouch. If only few strips were to be used, what is needed of the plate mould was taken and the rest of the strips were returned to the pouch and the pouch is resealed. A1 well was left blank. 10 micro litre of negative control (NC) was added to B1, C1 and D1. 10 micro litre of positive control (PC) was added to E1 and F1. 10 micro litre of weak positive control (WPC) CSF was added to G1 and H1. 10 microlitre of WPC serum was added to A2 and B2. The samples were diluted, CSF (1 in 10) and serum (1 in 20) with the sample diluent. After dilution the ELISA plates were covered with a lid or sealer. The plate was incubated at 37°C for 1 hour. After 1 hour, the contents were discarded into hypochlorite solution and the plates were washed 5 times with the wash buffer. 100 micro litre of ready to use antigen was added to each well. The plate was incubated at 37°C for 1 hour. The antigen was flicked off the plate. The plate was washed 5 times. 100 micro litre of diluted biotinylated Mab was added to each well. The plate was incubated at 37°C for half an hour. The plate was washed another 5 times. 100 micro litre of diluted Streptavidin-peroxidase conjugate was added to each well. It was left for 15 minutes at room temperature. The plate was washed 5 times. 100 micro litre of diluted substrate was added to each well. It was left for 10 minutes at room temperature. 100 micro litre of stop solution was added to each well to arrest the reaction. The plate was read at 450 nm in an ELISA reader within 60 minutes of completing the reaction. Well A1 was read as blank. 630 nm filter was used as the reference filter.

TEST RESULTS

The test results were calculated using the below formula.

Calculation of Elisa Units

$$\text{Test of OD value} = \frac{\text{Mean OD value of NC} \times 100}{\text{WPC-Mean OD value of NC}}$$

All samples with equal to or greater than 100 EU were considered as reactive for JEV. All samples with 30 to 99 EU were suspected of a recent flaviviral infection, such as dengue and West Nile. All samples with less than 30 ELISA units (EU) were considered as non reactive for JEV. A positive reaction of CSF sample is confirmatory for JEV infection.

RESULT

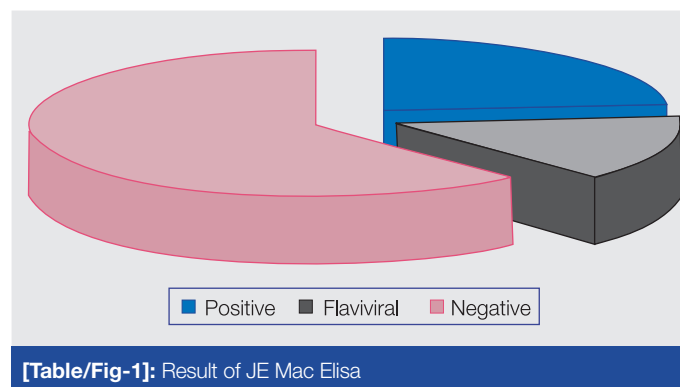
In this study, out of the 82 patients tested by J.E. MAC ELISA, 19 (23.17%) were positive for J.E., 12 (14.63%) were positive for flaviviral infection and 51 (62.20%) were negative for J.E. Out of the 82 CSF samples tested, 19 (23.17%) were positive for J.E., 1 (1.22%) was positive for flaviviral infection and 62 (75.61%) were negative for J.E. Out of the 63 serum samples tested, 10 (15.87%) were positive for J.E., 11 (17.46%) were positive for flaviviral infection and 42 (66.67%) were negative for J.E.

Age distribution among males showed more number of cases between the age group of 7 to 9 years (38.88%), followed by the age

group of 4-6 years (26.31%). Among the females highest number of cases occurred in the age group of 1 to 3 years followed by the age group of 4 to 6 years. Percentage of positive cases among the males was 28.7% and among the females was 15.15%. However difference in the percentage of positive cases did not differ statistically ($p > 0.05$).

DISCUSSION

Encephalitis is an inflammation of the brain, which is a reaction of the body's immune system to infection or invasion. Arthropod borne viral encephalitis is responsible for most of the epidemic viral encephalitis. JEV encephalitis is the most common form of epidemic and sporadic encephalitis in the tropical region of Asia. Basically, J.E. is a zoonotic disease maintaining JEV in nature by bird- mosquito- bird and pig- mosquito- pig cycles. Pigs are the known amplifiers of JEV. Bats can also carry the virus for longer periods of time. Human beings are the only incidental hosts forming a dead end [3]. Kumar Rashmi opines that Japanese encephalitis may affect all age groups but children between 5 to 15 years of age bear the brunt of the disease [1]. Benakappa et al. have stated in their study that the major brunt of the disease is on the paediatric age group. As the epidemic recurs it tends to affect the younger age group more, since the immunity is low in them [2,3,4]. It appears that J.E. may become one of the major public health problems in India considering the quantum of the vulnerable paediatric population, the proportion of JEV infections among the encephalitic children and wide scattering of J.E. prone areas [3]. P Suvarna Devi et al. have reported in their study that the mean age of occurrence of J.E. was 5.6 and 7.5 years, with a male to female ratio of 2.1:1 and 3:1 respectively for encephalitis and J.E [5]. Our study correlates very well with this study. In this study, out of the 82 patients tested by J.E. MAC ELISA, 19 (23.17%) were positive for J.E., 12 (14.63%) were positive for flaviviral infection and 51 (62.20%) were negative for J.E. Out of the 82 CSF samples tested, 19 (23.17%) were positive for J.E., 1 (1.22%) was positive for flaviviral infection and 62 (75.61%) were negative for J.E. Out of the 63 serum samples tested, 10 (15.87%) were positive for J.E., 11 (17.46%) were positive for flaviviral infection and 42 (66.67%) were negative for J.E. The serological results clearly establish the JEV etiology [6]. MAC ELISA has been standardised to detect in serum and CSF IgM class of antibodies in J.E. and dengue. The test is rapid, sensitive and specific. JEV specific IgM antibodies



[Table/Fig-1]: Result of JE Mac Elisa

Sample	Total	Positive	Flaviviral infection	Negative
CSF	82	19 (23.17%)	1 (1.22%)	62 (75.61%)
SERUM	63	10 (15.87%)	11 (17.46%)	42 (66.67%)

[Table/Fig-2]: Specimen wise result of J.E. Mac Elisa

Age group (in years)	Male positive cases	Male total cases	% of positive cases (Males)	Female positive cases	Female total cases	% of positive cases (Females)
1-3	0	1	0	1	2	50
4-6	5	19	26.31	3	17	17.64
7-9	7	18	38.88	0	8	0
10-12	2	11	18.18	1	6	16.67
Total	14	49	28.57	5	33	15.15

[Table/Fig-3]: Age and gender wise distribution of cases

can be distinguished easily and clearly from dengue virus specific IgM antibodies. The diagnosis can be made with a single specimen collected during the acute phase [7]. Flavivirus isolation from patient's tissues and body fluids is difficult and time consuming. Serological findings are sometimes confusing because of high degree of cross reactivity amongst the flaviviruses. A rapid, sensitive and specific detection of one of the markers of virus infections would definitely help in establishing the cause of infection. IgM is one such marker which can be detected in acute phase serum or CSF [7]. MAC ELISA differentiates among JEV and DEN virus IgM antibodies and the diagnosis can be made from a single sample (preferably CSF) collected early during the acute phase [7].

CONCLUSION

The hospital based incidence of J.E. was found to be significant in the area of study. J.E. is the most common form of sporadic and epidemic encephalitis in the tropical regions and should be ruled out first before considering the other viral causes. The pediatric age group, between 7 to 9 years was found to be most predisposed to J.E., probably because of low immunity in the age group. Male

predominance was seen with male to female ratio of 1.48:1. The serological results clearly establish the JEV etiology.

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DECLARATION ON COMPETING INTERESTS:

No competing Interests.

Date of Submission: **Mar 23, 2011**
Date of per review: **Apr 25, 2011**
Date of acceptance: **Apr 29, 2011**
Online first: **May 21, 2011**
Date of Publishing: **Jun 13, 2011**