**Abstract**

**Introduction:** Japanese encephalitis (JE) continues to be endemic in West Bengal. The two districts of Bankura and Purulia have consistently reported cases to be occurring both sporadically as well as in the form of small outbreaks.

**Aim of Study:** To find out the diagnostic efficacy of serum immunoglobulin M (IgM) assay in JE patients within 5 days (early) and after 9 days of onset of symptoms.

**Materials and Methods:** The study was done from February 2014 to January 2015. Cerebrospinal fluid (CSF) and serum samples were collected on day 4 from patients admitted with acute encephalitis syndrome in pediatric and medicine wards. Another serum sample was collected on the 10th day of fever. The collected samples were analyzed for JE-specific IgM antibody by IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA).

**Results:** During this period, 276 suspected patients were tested of which 31 (11%) were reactive in CSF. Out of them only 20 (64.5%) were reactive in 4th day serum sample, but 26 (83.8%) were reactive in 10th day serum sample. The positive predictive value of both 4th and 10th day serum samples were 100%, but their negative predictive values were 95% and 98%, respectively. The sensitivity of 4th day serum sample was 64.5% whereas sensitivity of 10th day serum sample was 84%.

**Conclusion:** A single serum sample collection in the early stage of disease missed almost one-third of cases. In many rural hospitals where CSF collection facility is not available, collection of paired sera is a good alternative for diagnosis of JE and also has immense epidemiological value.

**Key words:** Flavivirus, Immunoglobulin M antibody capture enzyme-linked immunosorbent assay, Japanese encephalitis

**INTRODUCTION**

A case of acute encephalitis syndrome (AES) is defined as a person of any age, at any time of year with the acute onset of fever not more than 5-7 days and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures)." Japanese encephalitis (JE) is presently a major cause of “AES.” JE is leading cause of encephalitis in Asia, Asia Pacific, and Western Pacific countries. Now, an approximate 68,000 cases of JE occur globally each year with 20,000 deaths and nearly 15,000 disabled. The vast majority of cases (about 85%) occur among children <15 years and nearly 10% cases among those over 60 years. Mortality rates in locales with intensive care capabilities are 5-10%. In less-developed areas, mortality rates may exceed 35%. JE is reported from different parts of India. The disease is endemic in 18 states — Assam, Bihar, Haryana, Uttar Pradesh, Karnataka, West Bengal, and Tamil Nadu contribute about 80% of cases and death. In India, about 300 million people are at risk. In West Bengal, JE has been reported consistently from Bankura and Purulia.
districts where it occurs both sporadically as well as in the form of small outbreaks.

JE is a viral disease caused by an enveloped single-stranded positive-sense RNA virus of the genus flavivirus family flaviviridae.\textsuperscript{3,4} It is a mosquito-borne disease transmitted by culicine mosquitoes, most notably by *Culex tritaeniorhynchus* and *Culex vishnui* and occasionally caused by *Culex gelidus, Culex fuscocephala, Culex annulus,* and *Culex annulirostris.* It is a zoonotic disease, i.e. infecting mainly animals and incidentally man. It is transmitted mainly by pig to mosquito to pig cycle or arided bird (herons, egrets) to mosquito to arided bird cycle. Pigs are amplifiers of the virus. Birds are the reservoir of JE virus but are asymptomatic.

**Aim of Study**
This study aims to find out the diagnostic efficacy of serum immunoglobulin M (IgM) assay in JE patients within 5 days (early) and after 9 days of onset of symptoms.

**MATERIALS AND METHODS**
The study was done from February 2014 to January 2015 after obtaining ethical clearance from the institutional ethics committee. As isolation of JE virus from clinical specimens is difficult due to low level of viremia and rapid development of neutralizing antibodies against it,\textsuperscript{5} the confirmation of a suspected case of JE requires the detection of JEV specific IgM by IgM capture ELISA in clinical samples. This assay distinguishes between JE and Dengue virus, which are serologically cross-reactive.\textsuperscript{6} Cerebrospinal fluid (CSF) and serum samples were collected on day 4 from patients admitted with AES by lumbar puncture and venepuncture respectively from patients admitted to Paediatric and Medicine wards. Another serum sample was collected on the 10\textsuperscript{th} day of fever. The collected samples were analyzed for JE-specific IgM antibody by IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) using kits from NIV (Pune). Optical density value was taken by ELISA reader. A positive test in CSF sample was taken to be gold-standard for our study.

**RESULTS**
During this period, 276 suspected patients were tested of which 31 (11%) patients were reactive in CSF tested.

Among the 31 reactive patients, 15 (48%) were female and 16 (52%) were male (Figure 1).

Of 31 patients, 13 (42%) were in the pediatric age group (0-12 years). Out of the 18 adults (>12 years) 16 patients (52%) were <60 years of age while rest 2 patients (6%) were >60 years (Figure 2).

The percentage of IgM positive JE was found to be high during the months of September and October. Among 29 cases, 11 cases occurred in September (38%), and 9 cases occurred in October (31%) (Figure 3).

Out of 31 patients, only 20 (64.5%) patients were reactive in 4\textsuperscript{th} day serum sample. Out of 31 patients, 26 (83.8%) patients were reactive in 10\textsuperscript{th} day serum sample (Figure 4).

The positive predictive value of both 4\textsuperscript{th} day and 10\textsuperscript{th} day serum sample were 100% but negative predictive value of 4\textsuperscript{th} day and 10\textsuperscript{th} day serum sample were 95% and 98%, respectively (Figure 5).

**DISCUSSION AND CONCLUSION**
Between February 1, 2014, and January 31, 2015, 276 patients with clinical features of AES were tested for JE by IgM MAC-ELISA at Bankura Sammilani Medical College and Hospital.
IgM positivity was 16 (52%) in males and 15 (48%) in females. Hence, there is no gender predilection which is similar to other studies.7,8

In this study, the sensitivity of 4th day serum sample was 64.5% whereas sensitivity of 10th day serum sample was 84%. In a study from 1987 to 1989 by Chow et al. showed that positivity rate was 65.7% in serum sample collected within the first week.9 Hence, there is a marked increase in sensitivity of second serum sample. A single serum sample collection in the early stage of disease missed almost one-third of cases. In many rural hospitals where CSF collection facility is not available, collection of paired sera is a good alternative for diagnosis of JE. Furthermore, paired serum sample has immense epidemiological value if we collect sera on 4th day and 10th day of onset of fever. Many cases which are undiagnosed at early stage of disease by single serum ELISA can be detected by paired serum sample. Hence, paired serum sample has immense epidemiological value if we collect sera on 4th day or 10th day on onset of fever.

REFERENCES


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