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ORIGINAL RESEARCH

Epidemiological studies on hospital based incidence of Japanese encephalitis in the Jharkhand state of India

Authors:
Noman Alam Md[1], Sahu NP[1] and Sultan Ahmad Md[2].

Institution:
1. Rajendra Institute of Medical Science (RIMS), Ranchi, Jharkhand, India

2. S N (PG) College, Azamgarh, Uttar Pradesh, India

Corresponding author:
Sultan Ahmad Md.

Email Id:
sultansnz@yahoo.com

ABSTRACT:

Study was conducted in Rajendra Institute of Medical Science (RIMS), Ranchi, Jharkhand, during June 2012 to September 2013. The objective of the study was to know the hospital based incidence of Japanese Encephalitis (JE) and to study the age, sex and seasonal pattern of infection. 219 cases were analyzed by the Department of Microbiology, RIMS, Ranchi with clinical diagnosis. These samples were experimentally tested to confirm Japanese encephalitis by IgM Antibody Capture Enzyme Linked Immunosorbent Assay (MAC ELISA). Out of 219 cases, diagnosis was confirmed in 53 cases (24.20%) with male to female ratio of 0.89:1. All were below 15 yrs of age. Most of the cases were children. Clinically, fever (100%), altered sensorium (69.80%) headache (54.71%), neck rigidity (39.62%), Kernig's sign (28.30%), convulsion (43.39%) and vomiting (35.80%) were the major findings observed. Majority of cases were from rural areas. The hospital based incidence of JE was found to be significant in the area of study. Effective measures should be taken to minimize disease transmission.

Keywords:
Japanese Encephalitis, hospital based incidence, Sensorium, Jharkhand, Kernig's sign, Epidemic, seasonal pattern, pediatrics

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INTRODUCTION

An important cause of admission, mortality and permanent neurological sequel in the hospital is viral encephalitis. Hundreds of known arthropod borne viruses (Arbo viruses) causes human diseases and among them the commonest virus is Japanese Encephalitis Virus (JEV) that causes childhood viral encephalitis worldwide.

In India, annual incidence of Japanese encephalitis ranged between 1714 and 6594 (NVBDCP). Epidemics are reported from many parts of Assam, Bihar, Haryana, Tamil Nadu, Uttar Pradesh, West Bengal, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Orissa and union territory of Goa and Pondicherry (Kabilan *et al.*, 2004) and among them Uttar Pradesh and Assam is the major affected state. In Jharkhand, first case was reported in 2010.

Japanese encephalitis virus, a small enveloped plus stranded RNA virus is an arthropod borne Arbo virus, belonging to the family Flaviviridae and genus Flavivirus. Japanese Encephalitis Virus (JEV) causes inflammation of the brain, which may lead to permanent brain damage, and has a high mortality rate. In India, it was first recognized in 1955 when virus was isolated from *Culex vishnui* from Vellore during the outbreak of encephalitis in Tamil Nadu (Namachivayam and Umayal, 1982).

Billions of people worldwide are living in JE endemic area and it was estimated that JEV is responsible to cause 45000 cases of disease and 10,000 deaths per year (Solomon and Winter, 2004; Van den Hurk *et al.,* 2009). The majority of cases (about 85%) occur among children who were less than 15 years of age.

The transmission of JEV occurs through a zoonotic cycle where mosquito acts as an intermediate and replicative vector, whereas pigs and ardeid birds were amplifying hosts. Humans are infected due to biting of an infected mosquito and are dead end hosts. There is no proof of human to human transmission, possibly due to the presence of transient viraemia. Pigs play a major role in the transmission cycle with respect to human infection since they live in vicinity of humans, whereas herons or ardeid birds are important reservoirs. Horses are the only other vertebrate that also develop Central Nervous System (CNS) infection and are considered as dead end hosts, though the amphibians, reptiles and bats can also be infected by JEV. It does not cause encephalitis in pigs and birds and rodents are relatively refractory to infection (MacKenzie, 2005).

MATERIALS AND METHODS

1. Place

The present study has been carried out in the Department of Microbiology, Rajendra Institute of Medical Sciences (RIMS), Ranchi, during the period of MD course. A total of 219 cases were included in this study and the studied cases belong to patient reach in the RIMS, Ranchi and sample reach from the different districts of Jharkhand. Selection of cases was done on the basis of clinical feature of encephalitis attending in the RIMS, Ranchi.

2. Selection of persons

There are certain criteria followed while selecting a person to include in the study. They were

a. The person must be with the symptoms of encephalitis.

b. The person should have fever, headache, coryza or flue like illness during febrile and acute presentation of symptom of encephalitis like headache, nausea, diarrhea, vomiting, myalgia, altered behavior, convulsions, coma and other neurological problems like ocular palsies, hemiplegia, quadriplegia, dystonia, choreoathetosis and coarse tremors, etc.

Keeping the above points in mind, sample was collected and transported to the Department of Microbiology, RIMS, Ranchi, for the IgM detection through ELISA against Japanese encephalitis. The collection, transport and storage of specimens were done according to the standard procedures followed at National Institute of Virology (NIV), Pune (WHO, 1980).

Blood specimen collection

1. Cerebrospinal fluid (CSF)

Cerebro Spinal Fluid (CSF) specimen was collected in sterile screw bottles under all aseptic precaution by trained persons. The containers were properly labeled and transported at earliest to the microbiology laboratory. All attempts were made to collect CSF sample for the confirmation of diagnosis as per the Guidelines for surveillance of acute encephalitis syndrome, government of India, 2006.

1.1Process of CSF Collection

Cerebrospinal fluid flows through subarachnoid space and bathes and protects the brain and spinal cord. Cerebrospinal fluid specimens were collected through lumber puncture by medical expert. The spinal needle is inserted between 3rd and 4th lumbar vertebrae under aseptic condition. Once the needle was properly positioned in the subarachnoid space, pressure was measured and 2-3 ml of CSF is collected in the empty blood collecting vial and stored at +4°C. Small amount of CSF was used for physical, cytological, biochemical, and microscopic examination and the remaining CSF was stored aseptically for serology and viral culture examination.

1.2 Procedure for preparation of different compounds for test

IgM Antibody Capture ELISA (MAC-ELISA) Kit was used supplied by InBios International Inc. (USA). Procedure was followed as recommended by InBios International Inc. (USA).

Monoclonal Antibodies (MAbs) were raised against JE virus and were used to map topographically the epitopes on the envelope protein (Parida *et al.,* 2005). Two separate clusters of epitopes were revealed. It was observed that due to the Haemagglutination Inhibition (HI), Neutralization (NT) reactions that causes positive protection and Antibody Dependent Plaque Enhancement (ADPE) assays with the MAbs, there were five functional domains viz; A, B, C, D, and E were identified (Cecilia *et al*., 1988). It was observed that the cross reactive domain for Haemagglutination Inhibition A (HI'A') was very different for flavivirus. It was also observed that the JE virus specific domain for HI'B' was similar or continuous with the domains that was represented by non HI JE-virus specific MAbs 'C' and flavivirus cross-reactive MAbs'D'. Domain 'E' was expressed by two different MAbs that reacted with both i,e, JE virus as well as uninfected cell nucleus. On the basis of specificity, following conclusions were made:

1. Two different types of domain for antigen i.e. 'A' and 'B' were associated with HI.
2. 'A' domain is also associated with ADPE.
3. HI and NT were dissociated function in the *in vitro* and *in vivo*.
4. All MAbs that react with epitopes in the 'B' domain had HI and NT protective activity unable to show ADPE.

So, for the development of synthetic testing kit 'B' is considered most suitable (Cecilia *et al.*, 1988)

Negative controls

Heat inactivated serum. The JE detect negative control will aid in monitoring the integrity of the kit as well. It was stored at 2-8°C until ready to use for up to 7days. The vial was quick spin briefly before use to collect the content at the bottom.

Positive controls

Heat inactivated serum. The JE detect IgM positive control was added in monitoring the integrity of the kit as well. It was also stored at 2-8°C until ready to use for up to 7days. The vial was quick spin briefly before use to collect the content at the bottom as recommended by supplier.

Test methodology

Monoclonal antibodies (MAbs) were raised against an Indian strain of JE virus and were used to map topographically the epitopes on the envelope protein (Parida *et al.,* 2005). Sample dilution buffer was used to dilute specimen and antibodies. It is necessary to block unbound sites on the solid phase in order to minimize non

-specific reaction. Serums were diluted at 1:100 and CSF at 1:10 with sample dilution buffer and the coated wells were washed thrice with washing buffer. 50 µl of diluted samples was transferred to the appropriate wells and 50µl was reconstituted and added in positive and negative control wells respectively. Test procedures were as followed by standard methodology as shown below by flow diagram

Anti-IgM Antibodies Bound To Solid Phase

↓

Standards and Samples Added

↓

IgM* Anti-IgM Complexes Formed

↓

Unbound Sample Proteins Removed

↓

Anti-IgM-HRP Conjugate Added

↓

Anti-IgM-HRP* IgM* Anti-IgM Complexes Formed

↓

Unbound Anti-IgM-HRP Removed

↓

Chromogenic Substrate Added

↓

Determine Bound Enzyme Activity

0.1 mg/ml TMB substrate was dissolved in 10 ml citrate acetate buffer and further diluted with 25 µl diluted (30%) H_2O_2 and 100 µl TMB stock was prepared. TMB was used for ELISA detection. Reaction between the substrate and immobilized Horse Radish Peroxidase (HRP) conjugated secondary antibodies in the ELISA wells produced a blue colored solution. After reaching the desired color intensity, the reaction was stopped by adding acidic stop solution (1N H_2SO_4) which changed the solution color from blue to yellow. The reactants were allowed to remain stable for one hour and then the plate was analyzed on a microplate reader at 450 nm. Optical densities were recorded as it was known that optical density of the samples is directly proportional to the amount of JE virus specific IgM antibodies in the sample. The positive and one negative control. were mainly for validation of kit.

Expected value are given below

Positive: OD value ≥ 0.5; Negative: OD value≤ 0.18

Interpretation of the result

If OD value of the sample tested exceeds OD of negative control by a factor 5 (Sample OD ≥ Negative OD × 5), the sample was considered as "positive".

Limitations

Diagnosis of JE infection was not based on the results of this test alone but in conjunction with physician's clinical impression. Moreover, epidemiological data and travel history to epidemic area was also considered before making the diagnosis. IgM appears in circulations 3-5 days post onset. Therefore, date of collection of sample after onset of disease also influences the interpretation of the results.

Statistical Analysis

Student's one tailed 't'test was used and the level of significance was tested at <0.05 probability from standard statistical tables (Fisher and Yates, 1963).

RESULTS

In this study, out of 219 cases tested from the different districts of Jharkhand, Ranchi has maximum number of 71suspected cases out of which, 16 cases were found positive for JEV. Latehar and Bokaro have 11.32% positive cases each. Lohardaga, Palamu, and Ramgarh have 5.67% positive cases each and Dumka, Koderma and Saraikela have 1.88% positive cases each. No JE positive cases were found in Jamshedpur, Giridih, Godda, Jamtara, Khunti, Pakur, Sahibganj, Simdega and Chaibasa (Table 4) and for JE MAC ELISA, 53 were positive for JE (24.20%) as shown in Table 1. Out of 109 CSF sample tested, 35 (66.03%) were positive for JE and out of 110 serum sample tested, 18 (33.96%) were positive for JE (Table 3). Age group distribution showed more number of cases between 3-8 yrs of age (66.02%) as shown in Figure

Table 1. Incidence of Japanese Encephalitis during June 2012 to September 2013 in the Jharkhand state

Total No. of suspected cases	JE Positive cases	Percentage
219	53	24.20%

1. Out of 53 positive cases, percentage among male was 47.16% and among female was 52.83% i.e. the ratio was 0.89:1(Table 2). Clinically, it was observed that all patients with JE had a history of fever (100%), headache (54.71%), vomiting (35.8%), altered sensorium (69.8%), convulsion (43.39%), neck rigidity (39.62) and kernigs sign (28.30%) (Table 5). Our findings indicate, statistically significant outbreak of JE in the survey area as calculated 't' values were 6.839 in comparison to tabulated value (2.069, df-23).

Table 2. Gender wise distribution of cases during June 2012 to September 2013 in the Jharkhand state

Sl. No.	Gender	Total No. of suspected cases	JE Positive cases	Percentage
1.	Male	104	25	47.16%
2.	Female	115	28	52.83%
	Total	**219**	**53**	**99.99%**

DISCUSSION

The monsoon season followed by an increase in mosquito breeding due to water logging, leads to JEV spread in farm animals and results in human encephalitis (Mani *et al.*, 1991) in many parts of India as well as in other Asian countries (Erlanger *et al.*, 2009; Hoke *et al.*, 1988). Our observations also support this assumption and it was noticed that sudden increase in the number of patients in the hospitals are found during monsoon months (Figure 2).

Table 3. Ratio of different JE positive samples during June 2012 to September 2013 in Jharkhand

Sl. No	Specimen	Total specimen	JE Positive	Percentage
1.	Serum	110	18	33.96%
2.	C.S.F.	109	35	66.03%
	Total	**119**	**53**	**99.99%**

Culex tritaeniorhynchus, C. gelidus and *C. pseudovishnui* are found to be very efficient vectors for JEV transmission (Solomon, 2004; 2006). Incubation period varies from 5-15 days. Due to sub clinical nature of infection, symptomatic disease ranges from one out of fifty to one out of thousands of human infections (Tsai, 2000). The onset of illness can be abrupt, acute, sub-acute or gradual. Progression of disease can be divided into three stages (i) Prodromal stage - characterized by high fever, headache, malaise, nausea and vomiting (ii) Encephalitic stage - diagnosed by altered sensorium, neck stiffness, tremor, muscular rigidity and speech impairment and (iii) Late stage - characterized by persistent sign of CNS injury such as mental impairment, increased deep tendon reflexes, epilepsy and other behavioral abnormalities (Misra and Kalita, 2010). Case fatality rates for JEV range from 0-30% (Burke *et al.*, 1987). In a study, chronic progressive encephalitis and relapses due to perseverance of JEV in the CNS has been reported in a few patients (Pradhan *et al.,* 2001; Ravi *et al.,* 1993) . Due to shortcomes with other method, IgM ELISA test is the only appropriate method for the provided samples that are collected 3 to 5 days after infection. For example, after the first 9-10 days of illness, the presence of anti JEV IgM in CSF has sensitivity and specificity of >95% (Burke *et al.,* 1985).

Out of 219 clinically suspected JE cases, our study showed 24.20% serologically confirmed JE case during June 2012 to September 2013 in Jharkhand. The disease was fond in children, as observed by earlier worker elsewhere (Rashmi, 1999). This might be due to high exposure to infected mosquito bites with lower immunity. Female preponderance observed in this study might be due to poor caring, illiteracy, low socioeconomic status which leads to higher exposure of female patient to mosquito vectors.

The clinical feature observed in this study was

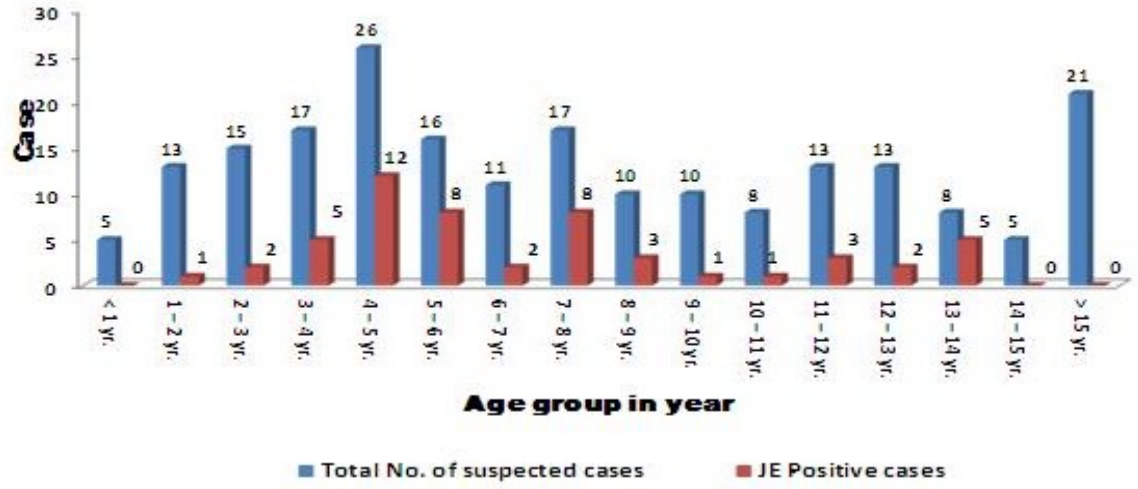

Figure 1 Age wise distribution of JE positive cases in the Jharkhand state of India

not uniform which might be due to the influence of host immune status, viral load and length of time between onset of disease and medical intervention. In our study, JE positivity was more in CSF when compared to serum specimen which signifies the increase in number of cases of CNS infection with JE virus.

Most of the positive cases have occurred from July to September as shown in Figure 2, which corresponds to the monsoon or post monsoon season. It was reported in a previous study that the increased rainfall during monsoon has been shown to be followed by an increase in mosquito, leading to sero conversion in farm animals and later human encephalitis (Mani *et al.,* 1991).

CONCLUSION

The hospital based incidence of JE was found to be significant in the area of study at 5% probability. JE is commonest form of periodic and pandemic encephalitis in the south Asian region due to breeding of mosquito and should be checked before considering the other viral

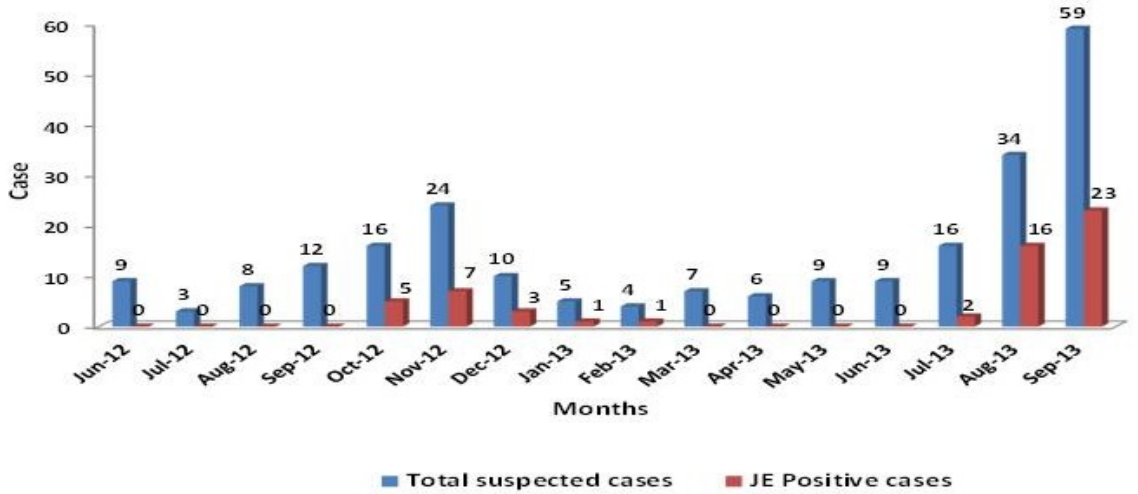

Figure 2 Month wise distribution of JE positive cases in Jharkhand, India

Table 4. District wise distribution of JV positive cases in Jharkhand.

S.N	Districts	Total suspected cases	JV Positive cases	Percent
1.	Bokaro	10	06	11.32
2.	Chatra	05	02	3.77
3.	Deoghar	02	00	00
4.	Dumka	02	01	1.88
5.	Dhanbad	05	02	3.77
6.	Jamshedpur	09	00	00
7.	Giridih	08	00	00
8.	Gumla	06	01	1.88
9.	Garhwa	16	02	3.77
10.	Godda	00	00	00
11.	Hazaribagh	26	06	11.32
12.	Jamtara	01	00	00
13.	Kodarma	01	01	1.88
14.	Khunti	01	00	00
15.	Lohardaga	07	03	5.67
16.	Latehar	17	06	11.32
17.	Palamu	18	03	5.67
18.	Pakur	00	00	00
19.	Ranchi	71	16	30.19
20.	Ramgarh	08	03	5.67
21.	Sahebganj	00	00	00
22.	Simdega	01	00	00
23.	Saraikela	05	01	1.88
24.	Chaibasa	00	00	00
	Total	219	53	99.99

infections, and effective measure should be taken to minimize disease transmission.

Table 5. Analysis of Various symptoms in JE positive cases.

Sl. No.	Clinical feature	JE Positive cases	Percentage
1.	Fever	53	100%
2.	Headache	29	54.71%
3.	Vomiting	19	35.8%
4.	Altered sensorium	37	69.8%
5.	Convulsion	23	43.39%
6.	Neck rigidity	21	39.62%
7.	Kernig's sign	15	28.30%

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