International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

*Original Research Article*

# Serological Evaluation of Early Cases of Japanese Encephalitis by ELISA In Patients at a Tertiary Care Teaching Hospital

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## ABSTRACT

**Introduction:** Japanese encephalitis virus (JEV) is the most common cause of viral encephalitis in the world, causing an estimated 45,000 cases and 10,000 deaths annually. Even with the best laboratory facilities, JEV cannot usually be isolated from clinical specimens, probably because of low circulating viral numbers and the rapid development of neutralizing antibodies. The diagnosis is therefore usually made serologically. Hence, present study was undertaken to serologically evaluate early cases of J. encephalitis by ELISA in patients reporting to Patna medical college; a tertiary care teaching hospital.

**Material and Methods:** The present study was carried among 304 cases, reporting to Patna medical college, Patna and samples reaching from different districts of Bihar were included in the study. Samples were collected and transported to department of microbiology Patna Medical College for the IgM against Japanese encephalitis detection through ELISA.

**Results:** A total 304 serum samples were tested for JE antibody from suspected encephalitis patient of Bihar state area. The persons with acute history of fever, headache, corrhyza or flue like acute illness for last few days prior to collection of samples. Out of 304 selected case 65 (21.38%) showed antibody against JE virus. Through JE viral activity was seen in all age groups yet the activity was more prominent among the children age groups. Both male and female had the virus activity with equal frequency ($p > 0.05$).

**Conclusion:** JE test by ELISA i.e, IgM capture ELISA is highly sensitive and confirmatory and can differentiate, infection type, intensity and presence of JE viral strain, detect specific IgM in CSF or in the blood.

**Keywords:** Epidemics; Bihar; Japanese Encephalitis Virus.

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**Article History:**

**Received:** 03-05-2017, **Revised:** 28-05-2017, **Accepted:** 11-06-2017

| Access this article online | |
|---|---|
| Website: www.ijmrp.com | Quick Response code |
| DOI: 10.21276/ijmrp.2017.3.4.006 | |

## INTRODUCTION

Japanese encephalitis virus (JEV) is the most common cause of viral encephalitis in the world, causing an estimated 45,000 cases and 10,000 deaths annually. Up to 50% of survivors are left with severe neurological sequelae.[1] Japanese encephalitis virus (JEV), a mosquito-borne pathogen of the genus Flavivirus, family Flaviviridae, is the main cause of viral encephalitis in Asia.[2] Affordable vaccines are now becoming available, but implementation programmes are hampered because the epidemiological data and surveillance for JE are poor; this is largely because of lack of standardized diagnostics. JE is endemic in resource-poor areas and in these settings; patients often have a clinical diagnosis only.[3]

Even with the best laboratory facilities, JEV cannot usually be isolated from clinical specimens, probably because of low circulating viral numbers and the rapid development of neutralizing antibodies. The diagnosis is therefore usually made serologically.[1] Hence, present study was undertaken to serologically evaluate early cases of J. encephalitis by ELISA in patients reporting to Patna medical college; a tertiary care teaching hospital.

## MATERIALS AND METHODS

The present study was carried out in the department of Microbiology in Patna Medical College Patna over a period of two years. A total number of 304 cases, reporting to Patna medical college, Patna and samples reaching from different districts of Bihar were included in the study. Cases were selected on the basis of clinical features of encephalitis among patients reporting to Patna Medical College, Patna.

The following criteria were followed while selecting patient to include in the study.

1. The person must be with the symptoms of encephalitis.
2. The person having fever, headache, corrhyza or flu like illness

during febrile and acute presentation of symptoms of encephalitis like headache, nausea, diarrhea, vomiting, myalgia, irritable, altered behavior, convulsions and coma or difficulty of speech and other neurological deficits like ocular palsies, hemiplegia, quadriplegia, and extra pyramidal signs in the form of dystonia, choreoathetosis and coarse tremors.

In view of above, samples were collected and transported to department of microbiology Patna Medical College for the IgM against Japanese encephalitis detection through ELISA.

The collection, transport and storage of specimens were done according to the standard procedures followed at national institute of virology (NIV), Pune (Japanese encephalitis in india, international Document 1980 and WHO manual march 2007).

Blood (serum) or CSF specimen were collected. Blood specimen was collected atleast after 5 days after onset of illness for detection of IgM antibodies. A second convalescent sample was collected 10-14 days after the first sample.

**Test Procedure**

Serum/plasma 1:100 or CSF 1:10 with sample dilution buffer was diluted. The coated /post coated wells were washed thrice with wash buffer. 50 µl of diluted samples were transferred to the appropriate wells; 50µl of reconstituted positive control and negative were added to the respective wells. The plate was kept in a humidified fox (a bread box with a soaked cotton /tissue paper) and was incubated in the plate at 37°C for 1 hour. At the end of incubation, the plate was washed five times with wash buffer. The plate was tapped after last wash on a tissue paper.

50µl of antigen was added to each well. The plate was then again kept in a humidified fox (a bread box with a soaked cotton /tissue paper) and was incubated at 37°C for 1 hours. At the end of incubation, the plate was washed five times with wash buffer. Tap the plate after last wash on a tissue paper. Then 50µl of Hx-B was added to each well and the procedure was repeated. Then 50 µl Avidin –HRP was added to each well and the procedure was repeated. After that, 100 µl of substrate (TMB/H2O2) was added to each well. The plate was incubated in a dark at room temperature till the color was developed. After stopping the reaction with 100µl of 1n $H_2SO_4$,

The absorbance was measured at 450 nm within 10 minutes.

Each kit was supplied with one positive control and one negative control for quality control.

**Expected value are given below**

Positive: OD value ≥ 0.5

Negative: OD value≤ 0.18

**Interpretation of the result:**

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

## RESULTS

Sample (blood and CSF) from 304 symptomatic ill and suspected encephalitis patient were collected. The result of test (ELISA for JE) conducted in 290 serum and 14 CSF samples are depicted in the table 1. Out of 304 samples, 186 were male and 118 were female. Out of 304 serum, 65 showed reactive (>negative OD (optical density) i.e. presence of antibody against JE virus activity.

Age wise distribution of JE virus activity: The evidence of JE viral positivity was detected in all the age groups. However the activity was more prominent in the age group of 0-14 years.

Out of 304 investigated cases, 65 cases were positive and all cases were in age group of 0-14 years, thus JE virus activities are maximum in childhood.

**Table 1: Age wise distribution of JE virus activity**

| Age group | Total investigated | positive | Percentage |
|---|---|---|---|
| **0-4** | 083 | 08 | 9.63 |
| **5-9** | 127 | 37 | 29.13 |
| **10-14** | 082 | 18 | 21.95 |
| **15-19** | 02 | 00 | 00 |
| **Above 20** | 10 | 02 | 20 |

**Table 2: Gender wise distribution of JE virus activity**

| Gender | No of total population | Number of positive | Percentage |
|---|---|---|---|
| **Male** | 186 | 44 | 23.65 |
| **Female** | 118 | 21 | 17.79 |

Sex wise distribution of JE virus activity (table 2): The virus activity among the 304 serum sample of suspected encephalitis patient in relation to gender is shown in table 2. Out of 186 suspected male patients 44 (23.65%) were positive and out of 118 suspected female patients, 21 (17.79 %) were showing JE virus activity. It signifies that both male and female had JE virus activity with equal frequency ($p>0.05$).

Month wise distribution of JE virus activity among the tested serum sample is shown in table 3. It is seen from table 3 that the JE virus activity was not prevalent throughout the year under reference in Bihar. The activity was high during the month of July, August, September, October and November, however JE was found minimum during November to May occasionally a few cases were found in November.

**Table 3: Month wise distribution of JE cases and JE activity in suspected encephalitis patient**

| Month | Total Investigated | Positive Case | Percentage |
|---|---|---|---|
| **JulY.09** | 1 | 1 | 100 |
| **Aug.09** | 22 | 4 | 18.18 |
| **Sept.09** | 4 | 1 | 25 |
| **Oct.09** | 5 | 1 | 20 |
| **Nov.09** | 8 | 1 | 12.5 |
| **Dec.09** | 3 | 0 | 0 |
| **Jan.10** | 0 | 0 | 0 |
| **Feb.10** | 0 | 0 | 0 |
| **Mar.10** | 0 | 0 | 0 |
| **Apri.10** | 1 | 0 | 0 |
| **May.10** | 1 | 0 | 0 |
| **June.10** | 5 | 0 | 0 |
| **Jul.10** | 15 | 0 | 0 |
| **Aug.10** | 6 | 0 | 0 |
| **Sept.10** | 5 | 1 | 20 |
| **Oct.10** | 1 | 0 | 0 |
| **Nov.10** | 2 | 0 | 0 |
| **Dec.10** | 2 | 0 | 0 |
| **Jan.11** | 0 | 0 | 0 |
| **Feb.11** | 1 | 0 | 0 |
| **Mar.11** | 4 | 0 | 0 |
| **Apri.11** | 2 | 0 | 0 |
| **May.11** | 3 | 0 | 0 |
| **June.11** | 18 | 0 | 0 |
| **Jul.11** | 25 | 3 | 12 |
| **Aug.11** | 71 | 18 | 22 |
| **Sept.11** | 99 | 35 | 35 |
| **Total** | 304 | 65 | 21 |

## DISCUSSION

The Japanese encephalitis virus is the largest cause of viral encephalitis in the world today. Although it originally was the cause of summer epidemics in Japan in the 19th century and the early part of the 20th century, the virus has been almost eradicated from that country.[4] The first case in India was reported in the 1950s from the southern state of Tamil Nadu.[5] The first major epidemic occurred in India in West Bengal in 1973.[6]

The present study was conducted among three hundred and four patient having symptoms of encephalitis, out of which 65 showed the antibody titre against JE virus. All these individuals had symptoms related to encephalitis- prodromal or full-blown. They did not know exact history of immunization against Arbovirus. Out of 304 selected case 65 (21.38%) showed antibody against JE virus. Through JE viral activity was seen in all age groups yet the activity was more prominent among the children age groups. Both male and female had the virus activity with equal frequency ($p > 0.05$).

Brahma et al[7] studied 226 AES patients admitted in 6 different units of Medicine ward in GMCH, out of which 76 patients were diagnosed to be JE positive with CSF IgM ESLIA, coming from 17 districts of Assam.

Although JE virus spill over from the animals/birds etc. and causes human infection, yet the pattern of its appearance in different places within the country is quite variable. In north-eastern region, the highest peak was also observed in August and September. In the Bihar region the highest peak is observed particularly in September month which period summer continues. The ratio of unapparent human infection to clinically apparent disease is high for JE. In Japan, it was found to be between 500 to 1000 inapparent infections for every cases of JE. In India, the ratio is probably similar to that in Japan as judged by the high proportion of the population possessing antibodies to JE virus in the endemic areas and relatively low incidence of the disease.[8]

A seroepidemiological study of Japanese encephalitis (JE) in Dimapur, Nagaland by Angami K et al[9] was carried out following an outbreak of the disease between July, 1985 and February, 1986. Altogether 50 persons were affected with 30 (60 per cent) deaths. A total of 311 serum samples comprising 95 humans, 166 animals and 50 birds were tested for the presence of haemagglutination inhibition test. The prevalence of JE antibody was 77.7 per cent in dogs, 52 per cent in cattle, 34 per cent in pigs and 21.1 per cent in goats. Of the five species of birds, flavivirus

and JE antibodies were detected in 21.4 per cent pigeons and 22.2 percent heron egrettes. Neutralisation test established the distinct role of JE virus over other related flavivirus antigens. During that period haemagglutination inhibition test was available the data interpretation on that test. The study showed mostly JE antibody activity found in July and February in our study maximum cases found in august and September. Kumar R et al[10] conducted study in Uttar Pradesh, eighty-six randomly selected children between 6 months and 12 years of age admitted with acute unexplained encephalopathy over a one year period were examined for evidence of Japanese encephalitis. One or more indicators of the infection were present in 36 (41.8%). Viral isolation from brain tissue was possible in 2 of 12 patients and from cerebrospinal fluid in 19 out of 62 patients. Serological evidence of probable Japanese encephalitis was found in 21 out of 36 patients. Japanese encephalitis is an important cause of acute childhood encephalopathy in the Lucknow area, where it is probably endemic. This study showed JE is the common cause of encephalitis in children our study also showed JE is the common cause of encephalitis mainly in children.

The presence of JEV-specific immunoglobulin M (IgM) in the CSF is thought to be diagnostic of JE, as opposed to infection with JEV without encephalitis which results in increased IgM in the sera but not in the CSF.[11]

The presence of infectious virus in the CSF and low levels of JEV specific IgM in both CSF and serum at presentation are associated with a poor outcome.[12]

A recent study of samples from Thai patients by Chanama S et al[13] showed that IgM detection in CSF was more sensitive than serum IgM for the early diagnosis of JEV encephalitis. Using an IgM capture EIA, IgM was found in 60% of serum samples and 90% of CSF samples collected 1–4 days after the onset of illness. All CSF samples were positive by day 7 while 100% of serum samples are not positive until day 13.

Diagnosis of JE infection should not be 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 should also be 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 influence thee interpretation of the results.

## CONCLUSION

JE test by ELISA i.e, IgM capture ELISA is highly sensitive and confirmatory and can differentiate, infection type, intensity and presence of JE viral strain, detect specific IgM in CSF or in the blood.

## REFERENCES

1. Solomon T, Thao LTT, Dung NM, et al. Rapid Diagnosis of Japanese Encephalitis by Using an Immunoglobulin M Dot Enzyme Immunoassay. Journal of Clinical Microbiology. 1998; 36(7):2030-2034.

2. Litzba N, Klade CS, Lederer S, Niedrig M. Evaluation of Serological Diagnostic Test Systems Assessing the Immune Response to Japanese Encephalitis Vaccination. Singh SK, ed. PLoS Neglected Tropical Diseases. 2010; 4(11):e883.

3. Lewthwaite P, Veera Shankar M, Tio PH, Daly J, Last A, Ravikumar R, Desai A, Ravi V, Cardosa JM, Solomon T. Evaluation of two commercially available ELISAs for the diagnosis of Japanese encephalitis applied to field samples. Tropical Medicine & International Health. 2010 Jul 1; 15(7):811-8.

4. Umenai T, Kryzsko R, Bektimorov TA, et al. Japanese encephalitis: current worldwide status. Bull WHO 1985; 63:625–31.

5. Webb JKG, Perriera S. Clinical diagnosis of an arthropod borne type virus encephalitis in children in north Arcot district, Madras state, India. Indian J Med Sci 1956; 10:573–80.

6. Sengupta SN, Sen M, Das PK, et al. Epidemic of Japanese encephalitis in west Bengal: A clinical appraisal of the first 143 cases at Bankura. J Assoc Phys India 1974; 22:463

7. Brahma B, Abdul Barik Ahmed, Tribeni Sarma, Debabrata Goswami, Pankaj Patowary. Clinical and Demographic Study of Japanese Encephalitis Patients Admitted in Gauhati Medical College and Hospital, Guwahati, Assam, India During 2014 Epidemic. Journal of Evidence based Medicine and Healthcare 2015; 2(51):8637-8642.

8. Japanese encephalitis in India, ICMR bulletin 10:29 1980.

9. Angami K, Chakravarty SK, Das MS, Chakraborty MS, Mukherjee KK. Seroepidemiological study of Japanese encephalitis in Dimapur, Nagaland. The Journal of communicable diseases. 1989 Jun; 21(2):87-95.

10. Kumar R, Mathur A, Kumar A, Sharma S, Saksena PN, Chaturvedi UC. Japanese encephalitis--an important cause of acute childhood encephalopathy in Lucknow, India. Postgraduate medical journal. 1988 Jan 1; 64(747):18-22.

11. Burke DS, Nisalak A, Ussery MA, Laorakpongse T, Chantavibul S. Kinetics of IgM and IgG responses to Japanese encephalitis virus in human serum and cerebrospinal fluid. Journal of Infectious Diseases. 1985 Jun 1;151(6):1093-9.

12. Burke DS, Lorsomrudee W, Leake CJ, et al. Fatal outcome Japanese encephalitis. Am J Trop Med Hyg.1985; 34:1203–1210.

13. Chanama S, Sukprasert W, Sa-ngasang A, A-nuegoonpipat A, Sangkitporn S, Kurane I, Anantapreecha S. Detection of Japanese encephalitis (JE) virus-specific IgM in cerebrospinal fluid and serum samples from JE patients. Japanese journal of infectious diseases. 2005 Oct 1; 58(5):294.

**Source of Support:** Nil.

**Conflict of Interest:** None Declared.



**Cite this article as:** Ravi Kumar, Krishna Kumar Mani. Serological Evaluation of Early Cases of Japanese Encephalitis by ELISA In Patients at a Tertiary Care Teaching Hospital. Int J Med Res Prof. 2017; 3(4):27-30. DOI:10.21276/ijmrp.2017.3.4.006