International Journal of Research in Medical Sciences
*Verma RK et al. Int J Res Med Sci. 2015 Nov;3(11):3217-3223*
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20151166

**Research Article**

# Comparative evaluation of antigen detection ELISA and reverse transcriptase PCR in acute stage of Japanese encephalitis prevalent in endemic areas of North-Eastern part of Uttar Pradesh, India

**Rajesh Kumar Verma[1]*, Dharmendra Prasad Singh[1], Ramakant Yadav[2], Ramakant Rawat[3]**

[1]Department of Microbiology, Uttar Pradesh Rural Institute of Medical Sciences & Research, Saifai, Etawah-206130, Uttar Pradesh, India
[2]Department of Neurology, Uttar Pradesh Rural Institute of Medical Sciences & Research, Saifai, Etawah-206130, Uttar Pradesh, India
[3]Department of Medicine, Uttar Pradesh Rural Institute of Medical Sciences & Research, Saifai, Etawah-206130, Uttar Pradesh, India

**Received:** 09 September 2015
**Accepted:** 06 October 2015

***Correspondence:**
Dr. Rajesh Kumar Verma,
E-mail: rshverma@gmail.com



## ABSTRACT

**Background:** Objective of current study was to compare and evaluate different diagnostic tests to establish a reliable and less time consuming diagnostic test for prompt diagnosis of acute Japanese encephalitis cases mainly amongst children and young adults from North-Eastern part of Uttar Pradesh.
**Methods:** A total of 100 subjects, including 50 suspected cases and 50 diseased controls were investigated to establish the diagnosis of JE in acute encephalitis patients. All CSF samples were subjected to MAC-ELISA, virus cultivation and RT-PCR.
**Results:** Out of 50 cases, 6 showed negative results by all the three tests. 50% (22 out of 44), 47.5% (21 out of 44), and 66% (23 out of 44) were found positive by MAC-ELISA, Virus cultivation and RT-PCR respectively. The rate of detection by Virus cultivation and RT-PCR was higher in case of MAC-ELISA negative cases (57.1% and 78.5%) than that of MAC-ELISA positive cases (22.7% and 27.2%) respectively. Mean age of the patients was 12.5 years, which ranged from 1-24 years with male to female ratio of 3:1.
**Conclusions:** The RT-PCR was found most reliable, sensitive and specific method amongst the three chosen methods for detection of JEV in suspected encephalitis patients.

**Keywords:** Antigen detection ELISA, Japanese encephalitis, MAC-ELISA, RT-PCR

## INTRODUCTION

Japanese Encephalitis (JE), an endemic, non-contagious, mosquito borne, acute viral encephalitis, is a global health problem and about 50000 cases and 10000 deaths are recognized annually throughout Asia.[1-3] It is one of the major causes of acute viral encephalitis with high mortality and morbidity and is the most common form of epidemic viral encephalitis.[4-7]

It is endemic in southern and northern parts of India and has continuous irregular outbreaks.[8] The first epidemic of JE occurred in during later half of 1955 in the North Arcot district of Tamil Nadu.[9] Since 1973, outbreaks of JE have occurred in eastern, northeastern and northern states.[8] In UP, after a lapse of score of years first outbreak occurred in 1978 in the northeastern districts.[10] JE was first time isolated in Lucknow in 1978 in KGMC from the brain tissue of a fatal case from Gorakhpur district.[3] A study from Lucknow[11] suggested that disease is endemic

in northeastern districts of U.P., Gorakhpur, Deoria, Azamgarh, Basti, Gonda, Bahraich, Balia, Faizabad,[10] Lakhimpur Khiri.[12] In Lucknow peak incidence is in months after the monsoon i.e. October and November, when mosquito population is at its peak.[13] In India it was found in several studies that JEV infection is common amongst children below 10 years of age due to absence of immunity or low immunity against the virus in them.[14-16]

JEV contains structural and nonstructural proteins. There are seven nonstructural proteins - NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.[17,18] These proteins are essential for viral replication. NS1, NS3 and NS5, the three major non-structural proteins, are found in cells infected with flavivirus. Nonstructural protein 3 (NS3) is a multifunctional protein of 619 amino acid residues.

JE is a serious and fatal condition; it requires prompt and reliable diagnosis to treat the patients without delay. The diagnosis of JE infection is based on four basic types of assays: serologic, viral isolation, molecular, and immunocytochemistry. The most important limitation of serological methods is the cross reactivity of JEV with other closely related viruses like Dengue and West Nile.[19] The definitive method to confirm clinical diagnosis of JE is the isolation of virus. The techniques like monoclonal antibody capture ELISA, IgM antibody capture ELISA and the latest molecular technique based methods like nucleic acid hybridization and amplification have their own drawbacks like they require expertise, expensive reagents and instruments, hence they cannot be used in routine practice, especially in areas where JE is prevalent. Therefore, a combined approach is required which works best for that laboratory.[20] This study was undertaken to evaluate the methods like, virus isolation and RT-PCR, standardized in our laboratory and was employed specifically in the samples negative for JE specific IgM capture ELISA and to determine their correlation in the diagnostics of JE.

## METHODS

The study was conducted in the Virology Section of Department of Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. Which was carried out from November 2001 to July 2003 and samples were collected during September and October 2002 outbreak. A total 100 subjects were investigated. The study group comprised of 50 clinically suspected cases and 50 controls that did not have signs and symptoms of encephalitis but they had come to hospital for some other reasons, were enrolled in this study as controls. The patients were from northeastern regions of Uttar Pradesh, Gorakhpur and its adjoining areas. Suspected patients had acute encephalitis like illness i.e. acute onset, altered sensorium, with/without fever, and other neurological signs and symptoms suggestive of encephalitis. Pyogenic meningitis was excluded by CSF microscopy (wherever possible).[21,22] For case selection, exclusion criteria taken by Kumar et al.,[13] was followed. Informed consent from patients or parents was taken and guidelines for human experimentation were strictly followed during study. 1-2 ml of CSF was collected from each patient by lumbar puncture, under strict asepsis, in a sterile container and was brought to the laboratory, which works as a surveillance center for Japanese Encephalitis in Uttar Pradesh. All samples were stored at -20$^0$C till subjected to various tests. To establish the diagnosis of JE in acute encephalitis patients three chosen methods were IgM antibody capture ELISA (MAC-ELISA), virus cultivation and Reverse Transcriptase Polymerase Chain Reaction (RT- PCR).

MAC-ELISA used was developed by Department of Virology, AFRIMS, Bangkok, 1982-84 and was performed as per their protocol. The CSF samples collected from the suspected patients were tested by MAC-ELISA for JE. CSF was diluted to 1:10 with PBS (Phosphate Buffered Saline), 50 µl of diluted test CSF and controls were loaded in the plates, and incubated overnight at 4°C, plates were washed with PBS-T (PBS with Tween 20) for five times and dried by tapping. Antigen was diluted in 20% acetone extracted normal human sera in 10 ml PBS (enough for two plates), antigen diluted to 1:100 and 100 µl was loaded to each well, plates incubated for 2 hours at room temperature, washed 6 times with PBS-T and dried by tapping. Antiflavivirus IgG-HRP (1:300) was diluted in 20 % NHS containing 0.5% Bovine Serum Albumin, incubated at 37°C for 1 hour, plates washed 6 time with PBS-T and dried by tapping. TMB was reconstituted to 1X concentration, 100 µl of substrate was added to each well, incubated for 30 minutes at room temperature in dark, reaction was stopped by adding 50 µl of 1M $H_2SO_4$. Results were read by ELISA reader at 450 nm wavelength. More than 40 ELISA units were taken as positive.

Virus cultivation was done by inoculating CSF samples in C6/36 cells (mosquito cell lines) to detect the virus by Antigen detection ELISA. After maintaining 5 days, the supernatant of the culture fluid was used to detect E and prM antigen of the virus. Antigen capture ELISA was performed on supernatant of culture fluid in the lab as per Technical Manual of Arboviral Study, Department of Virology, Institute of Tropical Medicine, Nagasaki University, Ngasaki, Japan. Coated plates were brought to room temperature and coating solution was decanted, plates were washed 5 times with PBS-T and dried by tapping several times on the paper towel, 5% fetal calf serum in distilled water was prepared to block the plate to avoid any non-specific binding, 100 µl of blocking was dispensed in each well, plate incubated at 37°C for 1hour, plate was then again washed 5 times with PBS-T and tapping done. Antigen of known concentration (1:400 ELISA Units) standard purified and supplied by Department of Virology, Institute of Tropical Medicine, Nagasaki University, Japan, was used as positive control and serially diluted from 1:400, 1:200, 1:100, 1:50, 1:25, 1:12.5, 1:6.25, and 1:3.125, loaded 100 µl into each well,

uninoculated cell culture supernatant was used as the negative control and JE srain JaOArS982 as the positive control, test sample- CSF were inoculated in cell culture, C6/36, grown at 28°C for 5 days and supernatant was used as antigen for detection, plate was incubated at 37°C for 1 hour, washed five times with PBS-T. Conjugate (1:300) 3.3 µl/1000 µl of blockage was prepared and dispensed 100 µl into each well, plate incubated again at 37°C for 1 hour, washed again 5 times with PBS-T as usual. 50 µl of reconstituted substrate was added into each well and incubated in dark for 20 minutes. Reaction was stopped after 20 minutes with 1M $H_2SO_4$.

Results were read by ELISA reader at 450 nm wavelength. Optical densities of the samples corresponding to optical density of antigen control of 25 ELISA units were presumed to be positive for virus.

RT-PCR. Total RNA was extracted from 100 µl of virus control or samples i.e. CSF by Accuprep viral RNA extraction Kit, (Bioneer Corporation, Korea), as per their protocol. The RNA pellet was suspended in Diethyl pyrocarbonate treated water (DEPC). The RNA was reverse transcribed and amplified by using PCR with reverse transcriptase [Rous-Associated Virus-2 (RAV-2), (Amersham, Arlington Heights, IL) and AmpliTaq, 5 Units/µl, (Perkin Elmer, Norwalk, CT) with the JEV specific primer pairs (Invitrogen) selected from the conserved sequences in the NS3 region of the genome (5'-GCC-TAT-ACG-GCA-ATG-GAG-TT-3' and other 5'-CTG-TTC-GGT-GAC-ATC-AGT-CT-3'). The RT-PCR product, which is 381 base pairs in size, is then subjected to the second round of PCR utilizing an internal set of primers (5'-CTT-GGC-GAT-GGC-TCA-TAC-3' and 5'-TGT-TCT-TAG-GCG-CTG-CTG-3'). 2% agarose gel in 1X TBE buffer containing 0.5 µl/ml ethidium bromide. 2 µl of 10 X gel loading buffer with 18 µl of nested PCR product and loaded in the gel. For DNA size marker, Vth marker (Rosche) mixed with 8 µl of DEPC treated water and 1 µl of 10 X loading buffer. Gel was photographed using gel documentation system. Every assay was accompanied by positive extract controls (JE strain JaOArS982) and negative extracts control: 100 µl of Leibovitz L-15 medium (diluent for JEV stock). A positive specimen was defined as the detection of DNA band of 192 base pairs in the size of on the ethidium bromide stained gel corresponding to DNA amplified from the JEV genome in the positive extract controls. Assays were valid only if the positive controls were positive and all negative controls were clearly negative.

## RESULTS

A total of 50 cases, between 1-24 years of age, clinically suspected to be of Japanese encephalitis and 50 diseased controls were included in the study. Most of the cases belonged to region of Gorakhpur and its adjoining areas. 2 ml of CSF was collected during the peak season of outbreak i.e. September/October 2002. Mean age of the patient was 12.5 years, which ranged from 1-24 years with male to female ratio of 3:2 in cases; approximately the same in controls (Figure 1). A total of 50 CSF samples diluted 1:10 were subjected to MAC-ELISA supplied by AFRIMS Bangkok, Thailand. 22 (50%) were positive and 6 were negative by all three tests. 21 (41.5%) and 29 (66%) CSF samples were positive by Virus cultivation and RT-PCR respectively (Table 1). Out of 44 samples 21 (47.5%) were found to be positive by Virus cultivation but most of the samples grew antigen between 25-50 EU after a passage of 5 days in C6/36 cells (Figure 2). Those samples whose optical density was higher or corresponding to 25 EU of antigen was taken as for the virus positivity after first passage of the sample for 5 days. Most of the JE wild strains grew poorly in cell lines because of low circulation of viral numbers and rapid development of neutralizing IgG antibodies. Out of 44 samples 29 (66%) were positive by RT-PCR using a nested primer from NS3 region of JE Virus genome, which yielded 192 bp fragments (Figure 3). Out of the total 50 cases, 22 (44%) were positive by MAC-ELISA and of these 5 (22.7%) were positive by virus cultivation, 6 (27.2%) were positive by RT-PCR while in those of 28 MAC-ELISA negative cases, we could detect 16 (57.1%) positive by virus cultivation and 22 (78.5%) positive by RT-PCR (Table 2). On comparison of all three tests the maximum number of case could be detected by virus cultivation or RT-PCR. Each test has its own importance depending on the stage of the disease therefore no single test can 100% predict the disease until and unless two tests are used simultaneously. Here we found virus cultivation with RT-PCR could be combined for those cases where MAC-ELISA was negative. On statistical comparison of virus cultivation against RT-PCR by 1x1 table the sensitivity and specificity was found to be 72.41% and 100% respectively whereas positive and negative predictive value was 100% and 72.41% respectively. The diagnostic accuracy was found 84%. When RT-PCR was compared with virus cultivation the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy was found to be between 20-40%. When we compared other tests they did not yield higher sensitivity and specificity.

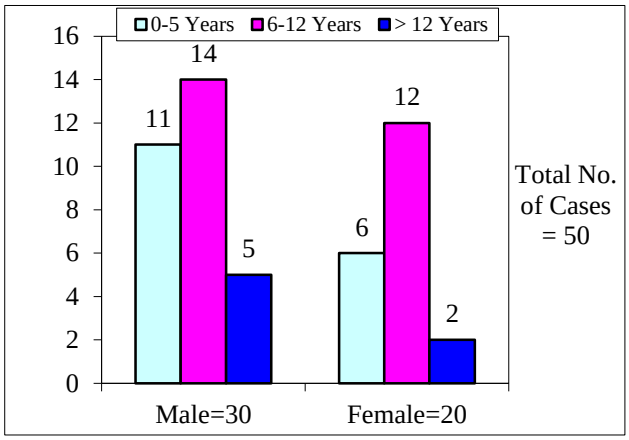


**Figure 1: Age and sex distribution of JE suspected cases.**

**Table 1: Distribution of cases of JE diagnosed by any of the three tests.**

| Total No. of positive cases =44 | MAC-ELISA | Virus cultivation | RT-PCR positive cases |
|---|---|---|---|
| No. of cases Positive | 22 | 21 | 29 |
| Percent of total cases | 50 | 47.5 | 66 |

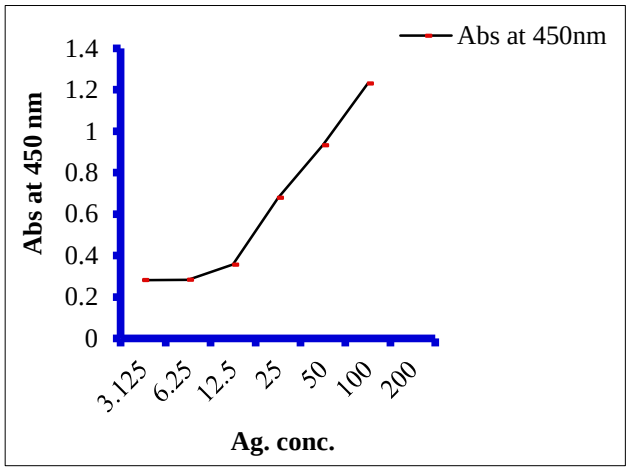


**Figure 2: Curve showing cut off value of antigen detection ELISA.**

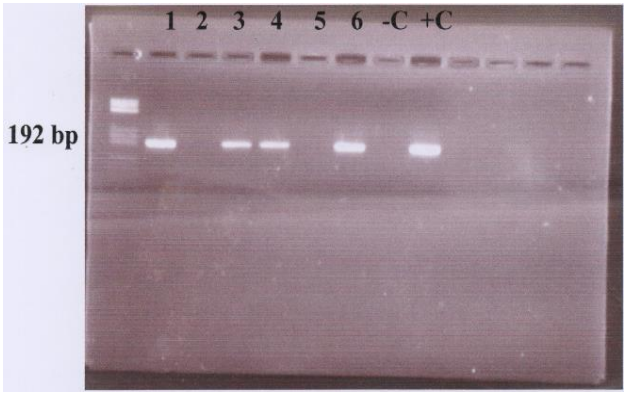


**Figure 3: Amplified bands in gel.**

+C - Positive control; –C - Negative control; 1-6 - Test samples; M - DNA molecular weight marker (V marker - Rosche)

**Table 2: Details of the cases diagnosed by Ag ELISA and RT-PCR in MAC-ELISA positive and negative cases.**

| Total No. of cases = 50 | Virus cultivation | RT-PCR positive |
|---|---|---|
| MAC-ELISA Positive=22 | 5 (22.7) | 6 (27.2) |
| MAC-ELISA Negative=28 | 16 (57.1) | 22 (78.5) |

Figures in parenthesis show percentage

## DISCUSSION

In this study we comparatively evaluated MAC- ELISA, virus culture and RT-PCR for early and rapid diagnosis of acute Japanese Encephalitis. A total of 50 patients and same number of diseased controls living in the northeastern parts of Uttar Pradesh, Gorakhpur, Uttar Pradesh, India and its adjoining areas were included in the study. In our study mean age of the patient was 12.5 years, which ranged from 1 to 24 years with male to female ratio of 3:2 in cases and approximately the same in controls. Both extremes of age and male sex have higher predilection for the disease. This is supported by other studies where JE is common amongst children.[14-16]

We preferred CSF samples over serum samples because normal CSF contains almost no IgM since this immunoglobulin does not cross blood brain barrier but in acute viral encephalitis this level is elevated[23] due to increased intrathecal synthesis of virus specific immunoglobulin from choroid plexus and CSF leucocytes. The specific activity (antigen binding per mole) of IgM in CSF is typically greater than that of the serum obtained simultaneously during acute Japanese Encephalitis. Thus for diagnosis of viral encephalitis by MAC-ELISA, CSF offers both superior sensitivity and specificity over that of serum.

In our study, 22 out of 50 samples (44%) were found to be positive for IgM by MAC-ELISA. All diseased controls were found to be negative for IgM antibody in CSF. Our percentage of the detection limit was low because we did not have exact information about the collection of the sample from the date of onset of the disease. Sensitivity and specificity of MAC-ELISA is greater than 95% after 7$^{th}$ day and samples collected earlier than 7 days may be negative hence collection of CSF and serum is recommended on or after 7$^{th}$ day.[24] We deliberately took more number of MAC-ELISA negative samples so as to prove the utility of virus cultivation and RT-PCR.

In the present study a total of 50 samples were inoculated in C6/36 cells (mosquito cell lines) to detect the virus by cell culture technique. After maintaining 5 days, the supernatant of the culture fluid was used to detect E and prM antigen of the virus. 21 samples out of 50 grew the virus in cell line up to 25 EU on single passage. Mosquito cell lines C6/36 and AP-61 are sensitive systems for viral replication but infection remains silent in C6/36 cells. Hence, inoculated cultures were examined for viral antigen by antigen detection ELISA. Our samples probably collected during first week of illness when virus titer was high and antibody concentration was low. This fact is supported by the virus isolation mainly in the MAC-ELISA negative samples where 16 out of 28 were positive for the presence of virus. Cultivation of the virus from the CSF is said to be associated with grave prognosis and Mathur et al.[25] and Burke et al. observed fatal outcome in all those patients whose CSF specimens

yielded JE virus.[26] But Ravi et al. did not find a correlation between fatal outcome and the virus isolation from CSF.[27] They explained that their failure to isolate virus from fatal cases might have been due to inactivation of virus.[28] We did not know the outcome of the patients, as we did not have the information about their survival. A study during the 1983 epidemic of JE in northern Thailand showed that none of CSF samples of nonfatal cases yielded virus when inoculated in LSTM-AP-61 cell monolayers of Aedes pseudoscutellaris,[29] however this did not happen in fatal cases where one third of the CSF samples were positive for the presence of virus. Subsequently in the next week and no sample was positive for antigen in the third week.[30] This signifies that virus yield is highest during the acute phase of the illness and the samples positive for Ag ELISA in our study could have been of this phase of the disease.

We selected the most conserved region, non-structural protein (NS3), of the viral genome with outer primers 5050 and 5431, and nested primers 5264 and 55073 that yielded 192 base pairs fragment in RT-PCR. Because of high degree of sensitivity and specificity, the RT-PCR method has potential application for routine diagnosis of flaviviral infections.[31] The CSF samples of the cases from the areas, where JE and Dengue are both endemic, may show cross reactivity at IgM level in MAC-ELISA so in such cases PCR assay is more useful. Cross-reactions do not occur among different flavivirus with the use of internal primers in the nested PCR. The designed primers are generally longer than 20 bp. Nested PCR directly detects viral RNA in the clinical sample early which provide an advanced and reliable method for an early and rapid diagnosis of virus infection. This is very meaningful for the prevention and control of such diseases, and is a matter for further study.[32]

In the present study we have evaluated three different diagnostic tests against each other and have tried to establish utility of RT-PCR especially in MAC-ELISA negative cases. The cases which not showed positive result with any of the three tests undertaken in the study was considered true negative. 6 cases out of the total 50 were considered true negative. We suspect these cases to have encephalitis due to the some other etiology. 22 out of remaining 44 cases (50%) were positive by MAC-ELISA. When these cases were subjected to virus cultivation, only 5 (22.7%) cases were positive out of 22 MAC-ELISA positive samples, whereas we could detect 16 (57%) cases out of total 28 MAC-ELISA negative samples. The lower detection rate by virus cultivation in MAC-ELISA positive cases could have been because of difficulty to grow the virus in C6/36 cells and presence of neutralizing antibodies. The higher rate of detection of positive cases by virus cultivation, esp. in those cases that were negative by MAC-ELISA, could be because these samples might have been collected within first seven days of the illness and also their outcome was not known i.e. these might be the fatal cases. As stated earlier that virus isolation rate is good in fatal cases due to high viral load. Diagnostic usefulness of our assay is demonstrated by the analysis of human CSF sample containing JE virus i.e. 29/50 (58%). And other advantage of PCR is speed with accuracy; entire procedure can be completed in 48 hours and multiple samples can be tested simultaneously with minimal technical effort. The RT-PCR was able to detect 29 (66 %) out of total 44 positive cases, by using a set of nested primers from NS3 region of JE virus genome yielding a 192 bp fragment. It detected 6 and 22 cases out of total 22 and 28 MAC-ELISA positive and negative samples respectively. The lower rate of detection in MAC-ELISA cases could have been due to low circulating viral numbers and neutralizing antibodies. Whereas higher yield of positive result in MAC-ELISA negative cases could be due to the fact that these samples might have been collected in acute phase of the illness when the viral load is high and there are scanty level of neutralizing antibodies. PCR assay is limited in its usefulness because only 29 samples out of the total 50 could be amplified. If the samples were transported properly maintained in cold chain, in liquid nitrogen, from the site of outbreak to our lab the results would have been different. This shows that PCR was more sensitive in detecting positive case in those cases, which were negative by MAC-ELISA. Statistical evaluation of virus cultivation against RT-PCR shows the sensitivity and specificity ranging between 70 to 100% and diagnostic accuracy was more than 80% whereas the positive and negative predictive value was 100 and 72.41% respectively. Conversely when RT-PCR was evaluated against virus cultivation the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy was between 20-40%. The main objective was to evaluate virus cultivation against the RT-PCR and we found RT-PCR was a better technique than virus cultivation because of its sensitivity, specificity, rapidity and accuracy. Virus isolation is a confirmatory method of identification and detection of JEV but takes around 7 days, which makes it a barrier in early diagnosis acute encephalitis cases. Whereas, the RT-PCR needs expertise and sophisticated equipments for field evaluation after onset of the disease in a large population at risk. For the purpose of epidemiological surveillance, Japanese encephalitis can be diagnosed by detection of antibodies in CSF and at the most RT-PCR should be used in conjunction to MAC-ELISA for rapid and accurate diagnosis however results must be available to the physician rapidly and early in the illness if improved clinical management is to be achieved.

## CONCLUSION

Japanese encephalitis is a fatal condition of children and constantly associated with post encephalitis neurological sequalae. So, it requires early diagnosis and treatment. There are several diagnostic tools available for the diagnosis of the disease, such as serologic MAC-ELISA, virus isolation and molecular techniques like RT-PCR. Virus isolation is definitive method of detection and identification of JEV but time taking and cumbersome

procedure limits its utility in prompt diagnosis of the disease. MAC-ELISA is rapid, sensitive and specific serologic test but positivity of the test depends on appearance of IgM specific antibody in CSF which takes at least 7 days after onset of disease. It may be useful for epidemiological surveillance. RT-PCR is rapid, highly sensitive and specific molecular method. It is very useful technique for detection of JEV particularly for CSF samples collected in early stage of disease. This technique will be very helpful in prevention of serious neurological sequalae by early diagnosis and treatment. This technique needs expertise and sophisticated equipments for field evaluation after onset of the disease in a large population at risk. Hence, RT-PCR should be used in conjunction to serologic test MAC-ELISA for rapid and early diagnosis.

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: The study was approved by the institutional ethics committee*

## REFERENCES

1. Okuno T. An epidemiological review of Japanese encephalitis. World Health Stats Rep. 1978;31:120.
2. Umenai T, Krzysko R, Bektimirov TA, Assaad FA. Japanese encephalitis: current worldwide status. Bull World Health Organ. 1985;63(4):625.
3. Medappa N. Japanese encephalitis in India. ICMR Bull. 1980;10:29.
4. Shope R. Medical significance of togaviruses: an overview of diseases in man and in domestic and wild vertebrate animals. In: Schlesinger RW, eds. The Togaviruses. 1st ed. New York: Academic Press; 1980: 47-82.
5. Monath TP. Pathobiology of flaviviruses. In: Schlesinger S, Schlesinger M, eds. The Togaviruses and Flaviviruses. 2nd ed. New York: Plenum Press; 1986.
6. Sabchareon A, Yoksan S. Japanese encephalitis. Ann Trop Pediatr. 1998;18:67.
7. Hoke CH, Nisalak A, Sangawhipa N, Jatanasen S, Laorakapongse T, Innis BL, et al. Protection against Japanese encephalitis by inactivated vaccines. N Engl J Med, 1988;319:608.
8. Rodrigues JJ, Shaikh BH, Shendarkar SP, Ramdasi SG. Laboratory confirmed cases of Japanese encephalitis in Maharastra state. Indian J Med Res. 1980;72:471- 4.
9. Work TK, Shah KV. Serological diagnosis of Japanese B type of encephalitis in North Arcot district of Madras state, India with epidemiological notes. Indian J Med Sci. 1956;10:582.
10. Narasimham MVVL, Krishna Rao C, Bendle MS, Yadava RL, Johri YC, Pandey RS. Epidemiological investigations on Japanese encephalitis outbreak in U.P. during 1988. J Comm Dis. 1988;20:263.
11. Kumar R, Mathur A, Kumar A, Sharma S, Saksena PN, Chaturvedi UC. Japanese encephalitis - an important cause of acute childhood encephalopathy in Lucknow, India. Postgrad Med J. 1988;64:18.
12. Mathur A, Tandon HO, Mathur KR, Sarkari NB, Singh UK, Chaturvedi UC. Japanese encephalitis virus infection during pregnancy. Indian J Med Res. 1985;81:9.
13. Kumar R, Mathur A, Kumar A, Sharma S, Chakraborty S, Chaturvedi UC. Clinical features and prognostic indicators of Japanese encephalitis in children in Lucknow (India). Indian J Med Res. 1990;91:321.
14. Dhillon GP, Raina VK. Epidemilogy of Japanese encephalitis in context with Indian scenario. J Indian Med Assoc. 2008;106(10):660-3.
15. Chatterjee S, Chattopadhyay D, Bhattacharya MK, Mukherjee B. Serosurveillance for Japanese encephalitis in children in several districts of West Bengal, India. Acta Paediatr. 2004;93:390-3.
16. Kabilan L, Vrati S, Rameshd S, Srinivasan S, Appaiahgari MB, Arunachalam N, et al. Japanese encephalitis virus (JEV) is an important cause of encephalitis among children in Cuddalore district, Tamil Nadu, India. J Clin Virol. 2004;31:153-9.
17. Chambers TJ, Hahn CS, Galler R, Rice CM. Flavivirus genome organization, expression and replication. Annu Rev Microbiol. 1990;44:649-88.
18. Smiyoshi H, Mori C, Fuke I, Morita K, Kuhara S, Kondou J, et al. Complete nucleotide sequence of the Japanese encephalitis virus genome RNA. Virology. 1987;161:497-10.
19. Mackenzie JS, Barrett ADT, Deubel V. Japanese encephalitis and West Nile viruses. Heidelberg, Germany: Springer; 2002: 12, 19, 36, 40.
20. Calisher CH, Beaty BJ. Laboratory diagnosis of viral infections. In: Lennette EH, eds. 2nd ed. New York: Marcel Dekker, Inc.; 1992.
21. Kumar R, Mathur A, Singh KB, Sitholey P, Prasad M, Shukla R, et al. Clinical sequelae of Japanese encephalitis in children. Indian J Med Res. 1993;97:9.
22. Bharadwaj M, Khanna N, Mathur A, Chaturvedi UC. Effect of macrophage derived factor on hypoferraemia induced by Japanese encephalitis virus in mice. Clin Exp Immunol. 1991;83:215.
23. Ehrenkranz NJ, Zemel ES, Bernstein C, Slater K. Immunoglobulin M in the cerebrospinal fluid of patients with arbovirus encephalitis and other infections of the central nervous system. Neurology. 1974;24:978-80.
24. Solomon T, Thao LTT, Dung NM, Kneen R, Hung NT, Nisalak A, et al. Rapid diagnosis of Japanese encephalitis by using an IgM dot enzyme immunoassay. J Clin Microbiol. 1998;36(7):2030.
25. Mathur A, Kumar A, Sharma S, Kulshreshtha R, Kumar R, Chaturvedi UC. Rapid diagnosis of Japanese encephalitis by immunofluorescent examination of cerebrospinal fluid. Indian J Med Res. 1990;91:1.
26. Burke DS, Lorsomrudee W, Leake CJ, Hoke CH, Nisalak A, Chongswasdi V, et al. Fatal outcome in

Japanese encephalitis. Am J Trop Med Hyg. 1985;34:1203.
27. Ravi V, Vanajakshi S, Gowda A, Chandramuki A. Laboratory diagnosis of Japanese encephalitis using monoclonal antibodies and correlation of findings with outcome. J Med Virol. 1989b;29:221.
28. Gajanana A, Samuel P. Philip, Thenmozhi V, Ranendran R. An appraisal of some recent diagnostic assays for Japanese encephalitis. Southeast Asian J Trop Med Public Health. 1996;27:673.
29. Leake CJ, Burke DS, Nisalak A, Hoke CH. Isolation of Japanese encephalitis virus from clinical specimens using a continuous mosquito cell line. Am J Trop Med Hyg. 1986;35:1045-50.
30. Desai A, Ravi V, Guru SC, Slankar SK, Kaliaperumal VG, Chandramuki A, et al. Detection of autoantibodies to neural antigens in the CSF of Japanese encephalitis patients and correlation of findings with the outcome. J Neurol Sci. 1994;122:109.
31. Puri EA. Henchal, J. Burans, K. R. Porter, W. Nelson, D. M. Watts, C. G. Hayes. A rapid method for detection and identification of flaviviruses by polymerase chain reaction and nucleic acid hybridization. Arch Virol. 1994;134:29-37.
32. Fang Meiyu, Chen Huosheng, Chen Cuihua, Tian Xiaodong, Jiang Lianhua, Peng Yifei, et al. Detection of flaviviruses by reverse transcriptase polymerase chain reaction with the universal primer set. Microbiol Immunol. 1997;41:209-13.

**Cite this article as:** Verma RK, Singh DP, Yadav R, Rawat R. Comparative evaluation of antigen detection ELISA and reverse transcriptase PCR in acute stage of Japanese encephalitis prevalent in endemic areas of North-Eastern part of Uttar Pradesh, India. Int J Res Med Sci 2015;3:3217-23.