

## DETECTION OF JAPANESE ENCEPHALITIS VIRUS AND HERPES SIMPLEX VIRUS IN ACUTE ENCEPHALITIS SYNDROME CASES BY ELISA AND PCR IN A TERTIARY CARE HOSPITAL OF EASTERN INDIA

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### ABSTRACT

**Introduction:** Encephalitis is defined as inflammation of the brain parenchyma associated with neurologic dysfunction. AES occurs in explosive epidemics or in a non-epidemic (sporadic) form. Epidemic in India have been attributed to Japanese encephalitis virus (JEV) infection. Herpes simplex virus (HSV) is responsible for most sporadic cases of AES. This study was undertaken to detect JEV and HSV from CSF samples. **Method:** Two to three ml of CSF was collected in a dry sterile container. Samples were divided into 2 vials and kept at -20oC. One vial was used for ELISA test in while the other vial was used for PCR. **Result:** Of 90 cases, 61 were males (68%), 29 were females (32%). Majority belonged to 1 month-5years in 34 (38%) cases followed by 6-10 years in 23 (26%) cases. The commonest symptom was fever in 90 (100%) cases followed by change in mental status in 86 (95.6%). Of 90 samples 2 (2.2%) was positive for JEV in PCR while only 1 (1.1%) was positive in ELISA. A total of 9 (10%) samples were positive for HSV 1&2, of which 8 (8.9%) samples were positive in ELISA assay and 8 (8.9%) were positive in PCR assay. In our study only 2 (2.2%) samples were positive for JEV in PCR while one was negative in ELISA. 9 (10%) samples were tested positive for HSV which signifies the sporadic nature of the virus in this region. **Conclusion:** PCR was found to be more sensitive in detection of JEV, while in HSV detection both ELISA and PCR were equally sensitive.

**Keywords:** Acute encephalitis syndrome, Enzyme linked immunosorbent assay, Herpes simplex virus, Japanese B encephalitis virus, Polymerase chain reaction.

### INTRODUCTION

The term encephalitis corresponds to an inflammation of the brain parenchyma. Viruses are the most common infectious agents associated with acute encephalitis. The cardinal symptoms and signs of acute viral encephalitis are fever, altered level of consciousness, headache, focal neurological deficits, and seizure [1]. According to World health organization (WHO) definition, clinically, a case of acute encephalitis syndrome is defined as a person of any age, at any time of year with the acute onset of fever 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). Other early clinical findings may include an increase in irritability, somnolence or abnormal behavior greater than that seen with usual febrile illness [2]. Annual incidence of acute encephalitis ranges between 3.5-7.5 cases per 1,00,000

person and 16 cases per 100,000 children worldwide [3]. AES was reported from 171 endemic districts in 17 states of India. AES occurs in explosive epidemics or in a non-epidemic (sporadic) form. Epidemic in India have been attributed to Japanese encephalitis virus (JEV) infection and have predominantly affected children. Various novel agents have been reported more recently as cause of AES including Enterovirus, Chandipura virus and Nipah virus [4]. Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein-Barr virus (EBV), Mumps, Measles and Enterovirus are responsible for most of the sporadic cases of acute viral encephalitis [5]. Herpes simplex encephalitis (HSE) is one of the most common and serious sporadic encephalitis. Over 90% of cases are caused by herpes simplex virus type 1 (HSV-1) and the remaining cases were due to herpes simplex virus type 2 (HSV-2) [6]. Diagnosis of AES is done by examination of cerebrospinal fluid, serological testing, CT, MRI, electro-encephalography, and brain biopsy [1]. Recent introduction of reverse transcriptase polymerase chain reaction (RT-PCR) based molecular technique have proved to be rapid and sensitive in detection of JE viral isolates [7-8]. The evaluation of an antibody response against HSV is interpreted with care because of the possible cross reaction with varicella zoster virus (VZV) antibodies during VZV infection of



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the CNS, and of a polyspecific antibody response as found in multiple sclerosis and certain other autoimmune diseases of the CNS. Recent studies have suggested that detection of HSV DNA by PCR increases the sensitivity of viral infection detection compared to antigenic detection or cell culture methods [9]. Keeping in view of the above facts, this prospective study was carried out to assess the incidence of Japanese encephalitis, Herpes simplex encephalitis and to compare between ELISA and PCR for detection of JEV & HSV in acute encephalitis syndrome after obtaining permission from the institutional ethics committee.

## MATERIALS AND METHODS

**Study design:** Prospective

**Ethics approval:** Approved by the institutional ethics committee

**Study period:** The study was done from October 2014 to September 2016

**Study place:** This is a prospective study conducted in SCBMCH, Cuttack in collaboration with RMRC, Bhubaneswar

**Sample size:** 90

**Inclusion criteria:** which comprised of ninety patients admitted to different departments of SCB Medical College & Hospital presenting with fever or history of fever (>38°C), encephalopathy altered level of consciousness persisting for > 24 hours and seizures, lethargy/irritability, change in personality and behaviour.

**Exclusion criteria:** Patients with aseptic meningitis without encephalopathy, patients with non-infectious CNS disorders due to hypoxic, ischemic, vascular, toxic and metabolic causes, patients with CNS disorders lasting less than 24 hours were excluded from the study.

**Sample collection:** Two to three ml of CSF was collected in a dry sterile container from the patients, which were divided into 2 vials and kept at -20°C. One vial was used for ELISA test in our laboratory while the other vial was used for PCR assay which was done at RMRC, Bhubaneswar. Precautions were taken to prevent repeated freezing and thawing of the samples.

### Method:

For ELISA test, HSV (1+2) IgM ELISA kit (Calbiotech, CA, USA) and JEV specific IgM antibodies by ELISA kit (InBios, Seattle, USA) were used. Test was done as per manufacturer's instruction.

For PCR detection of **JEV**, viral RNA was extracted from CSF using QIA amp Viral RNA Minikit (Qiagen GmbH, Germany). The oligonucleotides for nested PCR were selected from the conserved regions of the E protein genes: Outer primer: JEC3:5'-AGG AATCCTGGCTATGCT

TATGCT TTCCTG-3' and JEC1:5'-AGC ATG CAC ATT-GGTCGC-3', Inner primer: JEC4:5'-GTG CAC ATG CCA-TAGGT-3' and JEC2:5'-GTG CATGGAACC ACC ACT-3'. For the second round of PCR, 5 ml PCR-lampicon was taken as the template and the inner set of primers was used, implementing the same protocol. Next, 1.5% (w/v) agarose gel electrophoresis (0.5 mg/ml containing ethidium bromide) was performed in order to visualize the 488-bp amplified product.

Extraction of viral DNA (HSV 1&2) done with QIA amp viral RNA kit as per manufacturer's instruction (QIAGEN Ltd, Crawley, United Kingdom). The oligonucleotides for nested PCR were selected from the conserved regions of the Glycoprotein D genes. Outer primer: ATC CGAACG CAG CCC CGCTG-TCC GG(G/C) GGC AGCAGG GTG CT, Inner primer: -GCG CCGTCA GCG AGG ATAAC- AGCTGTATA (G/C) GG CGA CCGTC. Amplification products were identified by their molecular weights following electrophoresis of 10 ml of the secondary reaction mixture through an ethidium bromide-stained 2% agarose gel and UV light transillumination.

## RESULTS

The season with the highest average total AES case occurrence was monsoon (June, July, August, September, and October) and the minimum occurrence was observed in winter (December, January and February) during the period from 2014 to 2016 (Table 1).

**Table 1. Seasonal variation in AES cases (n=90)**

Monthly distribution of AES	Number	Percentage
OCT – DEC 2014	3	3.33%
JAN – MAR 2015	8	8.89%
APR – JUN 2015	9	10%
JUL – SEPT 2015	10	11.11%
OCT – DEC 2015	18	20%
JAN – MAR 2016	12	13.33%
APR – JUN 2016	13	14.44%
JUL – SEPT 2016	17	18.89%

Fever (100%) was the most common presenting symptom followed by altered sensorium (95.6%), convulsion (30%), headache (6.7%), vomiting (6.7%) and meningeal signs (3.3%) in different combinations (Table 2).

Majority of positive samples (54.5%) presented within 3 days of onset while most others presented within 7 days. Only 1 sample was presented in 9th day of fever (Table 3).

Out of 90 samples tested for both ELISA and PCR, only 1 sample was found to be positive for IgM antibodies

**Table 2. Clinical presentation in AES cases (n=90)**

Clinical Presentation	Number	Percentage
Fever	90	100 %
Change in mental status	86	95.6 %
Convulsion	27	30 %
Headache, vomiting	6	6.7 %
Lethargy, Irritability	5	5.6 %
Change in behavior/ Personality	2	2.2 %
Others (Paresis)	1	1.1 %

**Table 3. Correlation of positivity of ELISA and PCR with date of onset**

Post onset day	No. of samples tested	No. of JEV/HSV positive samples	IgM ELISA Positive	Nested PCR positive
1-3	56	6	4 (67%)	6 (100%)
4-7	29	4	4 100%)	4 100%)
>8	5	1	1 100%)	0 (0%)
Total	90	11	9 (82%)	10 (91%)

**Table 4. Comparison of ELISA with PCR for JEV detection (n=90)**

JEV	PCR Positive	PCR Negative	Total	p-value
ELISA Positive	1	0	1	0.5
ELISA Negative	1	88	89	
Total	2	88	90	

**Table 5. Comparison of ELISA with PCR for HSV 1&2 detection (n=90)**

HSV 1&2	PCR Positive	PCR Negative	Total	p-value
ELISA Positive	7	1	8	0.2397
ELISA Negative	1	81	82	
Total	8	82	90	

against JEV while 2 samples were positive for viral genome in PCR. A comparison is made between the diagnostic tests which showed a p value of 0.5, which is not significant (Table 4).

Out of 9 samples tested positive, 7 samples were posi-

tive in both ELISA assay and nested PCR while 1 each was negative in ELISA and PCR. No viral genome or IgM antibodies were detected in 81 samples. Comparison between the diagnostic tests showed a p value of 0.2397, which is not significant (Table 5).

Out of 90 cases 61 (67.8%) were males and 29 (32.2%) were females. Majority of cases belonged to 1 month - 5years of age in 34 (38%) cases followed by 6-10 years in 23(26%) cases. The season with the highest average AES case occurrence was monsoon and the minimum occurrence was observed in winter. Fever(100%) was the most common presenting symptom followed by altered sensorium (95.6%),convulsion(30%), headache (6.7%), vomiting(6.7%)and meningeal signs (3.3%) in different combinations. A total of 11(12.2%) samples of AES were detected with viral etiology, out of which 9 (10%) were positive for HSV1 & 2 and 2(2.2%) were found positive for JEV. Majority of positive cases belong to <5 years of age group (45.5%) followed by 6-10 years of age group (27.3%). From the 90 samples tested, 1 sample (1.1%) was positive for JEV in ELISA assay & 2 (2.2%) samples were found positive for JEV in PCR assay. A comparison is made between the diagnostic tests which showed a p value of 0.5, which is not significant. HSV specific IgM antibodies were found to be positive in 8 (8.9%) cases and same number of (8) samples (8.9%) were found to be positive for viral genome in PCR assay. Comparison between the diagnostic tests showed a p value of 0.2397, which is not significant. Majority of positive cases (54.5%) presented within 3 days of onset of symptoms while most others presented within 7 days. Only 1 case was presented on the 9<sup>th</sup> day of fever.

**DISCUSSION**

Encephalitis refers to an acute, usually diffuse, inflammatory process affecting the brain. While meningitis is primarily an infection of the meninges, a combined meningoencephalitis may also occur. Encephalitis results in substantial morbidity and mortality worldwide. Specific etiologies are identified in <50% of cases, in part due to lack of consensus on case definition and standardized diagnostic approaches [10]. Of the confirmed viral etiologies, the type of viruses identified in different parts of the world also differs considerably. HSV was shown to be the dominant viral agent for encephalitis in Vietnam (63%, 2012) [12], UK (22.1%, 2003; 19%, 2010) [13], JEV was the predominant cause of AES from Uttar Pradesh (23%, 1990) [11] and northeastern states (Assam, 2004; Arunachal Pradesh, 2011) [12,14] of India, and Cambodia (31%, 2002) [15]. Enteroviruses were reported as the major cause of AES in a prospective study during 2009–2010 in Uttar Pradesh, India [11], and also from China (15.4%, 1996) [16], and USA (25%, 2006) [17]. The present study reports the presence of Herpes simplex virus 1&2, and Japanese encephalitis virus as the etiological agents of viral encephalitis in acute encephalitis syndrome (AES) cases. The case definition

was tested by Solomon et al. who described that this case definition can miss some cases of AES [18] that present only with meningism, paresis, headache or vomiting. By contrast, this case definition can include some of the cases of bacterial or viral meningitis that can present with a short lasting or ill-defined altered sensorium. This could have broadened the denominator, hence reducing the proportion of viral AES cases. The relatively low identification of viral etiologies can be due to possibilities such as: (i) the case definition used was more sensitive but less specific, (ii) delay in investigation because of late admission at referral hospitals in case of sporadic AES cases, and (iii) greater chance of viral identification in an outbreak setting than sporadic AES due to planned and timely investigation along with better case selection due to closeness of case presentations.

This investigation on 90 AES patients enrolled in our study have shown viral etiology in 12.2% of patients, out of which HSV was found in 9 cases (10%) and JEV in 2 (2.2%). This proportion seems to be lower compared to many reports of viral etiology. This can be explained on the basis of case definition and other possible etiologies.

The commonest age group of the patients presented with acute encephalitis syndrome was 1 month to 5 years followed by 6 to 10 years. Majority of cases belonged to < 15 years of age group (80%) which was similar to other studies conducted by Reena et al (2008, 67.5%) and Rathore et al (2014, 81%) [7, 19].

Panagariya et al (2001) found AES was more common in patients of age > 40 years and < 20 years, male: Female ratio was 2:1 which is in accordance to our study where male to female ratio showed 2.1:1 with male preponderance [20]. In our study, commonest age group found to be positive for JEV and HSV was < 5 years which was in accordance with Reena et al while being in contrast to Rathore et al where the commonest age group affected was 11-15 years.

The season with the highest average total AES case occurrence was in monsoon (June, July, August, September, and October) and the minimum occurrence was observed in winter (December, January, February). Herpes simple's virus positivity was seen throughout the year which was in accordance to Whitley et al (1982), which found no characteristic age, sex or seasonal predilection [21]. Only 2 samples were tested positive for Japanese encephalitis during post monsoon period (October- November) which is in accordance to other studies of Reena et al (2008) [7] and Borah et al (2013) [22]. The peak cases of JEV were detected during or just after the rainy season which coincides with heavy rain and flooding which favors the breeding of its vector mosquitoes.

The clinical presentation with fever was seen in all cas-

es (100%) followed by altered sensorium (95%) with or without convulsion (30%), headache (6%) which was similar to other studies of Dwivedi et al [23] and Chaudhuri et al [5], that showed fever (80 and 97%), altered sensorium or convulsion (78 and 98%) and headache (61 and 74%). In the detection of JEV, only 2 (2.2%) samples were tested positive, which is in contrast to other studies like Bandopadhyaya et al in 2011 (22.7%) and 2012 (5%) [24] and Dwivedi et al in 2012 (13.8%) [23].

Only one outbreak of JE was reported from Rourkela city in Odisha, 1989. Our study did not show any endemicity or seasonal variation for JEV infection in our hospital but further studies are needed to confirm the same.

In our study, out of 90 samples, 2 (2.2%) samples were positive in PCR assay for JEV which is in contrast to other studies like Pujhari et al (2010, 30.4%) [25], Reena et al (2008, 69%) [7]. Similarly, only 1 (1.1%) samples were positive in ELISA assay which was in contrast to Reena et al (2008, 53.8%) which could be because of low prevalence of Japanese encephalitis in our centre. HSV is a member of family of Herpes viridae, a DNA virus. HSE is more common in older age group and neonates where it is caused by HSV-1 and HSV-2 respectively. The term HSE is usually applied to focal and severe disease (acute necrotizing encephalitis), which may be insidious to violent in onset. Prodromal phase of 4-10 days with nonspecific symptoms is common in HSE.

In our study, 8 (8.9%) samples were tested positive for Herpes simplex virus 1&2, in PCR assay, which is similar to the studies by Beig et al (2010, 10.5%) [26], while being in contrast to studies conducted by Rathore et al (2014, 16.1%) [19]. Similarly 8 (8.9%) samples were found to be positive in ELISA assay which was in contrast to previous studies by Hanada et al (1988, 22.5%) [27].

Majority of the case which was found to be positive for HSV and JEV presented within 3 days followed by others within 7 days. Only 1 case presented on 9<sup>th</sup> day of fever. In our study, out of 6 samples positive in first 3 days of presentation, 6 (100%) were positive in PCR while 4 (67%) in IgM ELISA followed by 4 samples being positive in 4-7 days from which 4 (100%) were positive in both IgM ELISA and PCR assay. Only one sample presented on the 9<sup>th</sup> day was positive in IgM ELISA assay.

Our study showed less positivity in IgM ELISA assay when tested in the first 3 days of illness which could be due to low level of IgM antibodies in the sample. Viral genome was not detected by PCR in one case which could be due to late sampling. Only 1(1.1%) sample was positive for JEV in ELISA assay while 2 (2.2%) samples were positive for viral genome in PCR. Low detection of the virus could be due to less prevalence of the virus in our centre. A comparison between the two tests

showed a p value of 0.5 which was not significant. More studies are needed to be done to further correlate with the findings and proper monitoring of the disease. Early detection would help the clinician for commencing early treatment for viral JEV. Early detection in an outbreak could also help in initiating preventing measures and starting vaccination of the un-affected children to prevent an epidemic.

Out of 9 samples tested positive, 7 samples were positive in both ELISA assay and nested PCR while 1 each was negative in ELISA and PCR each. No viral genome or IgM antibodies were detected in 81 samples. The comparisons between the two tests showed a p value of 0.239 which was not significant and further studies are needed to be done to confirm the same.

### CONCLUSION

This highlights the efficacy and utility of PCR for the diagnosis of viral encephalitis (HSV&JEV) in early period of the disease. IgM ELISA assay had been shown to be equally efficacious in detection of HSV encephalitis having similar sensitivity to PCR while PCR being more specific owing to detection of viral genome. Finally, viral encephalitis is one of the preventable cause of acute encephalitis syndrome and early detection of the same can be of paramount importance for reduction of morbidity and mortality in AES patients. Further studies needed to be done for proper monitoring and surveillance of Japanese encephalitis and helping the clinician for commencing early treatment for viral encephalitis.

**Conflict of interest:** NIL

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