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Prevalence of JE in Meningoencephalitis Patients by IgM Antibody Detection & RT-PCR in a Tertiary Care Hospital

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Abstract
To study the Prevalence of Japanese Encephalitis (JE) by detection of IgM antibody & molecular characterization of JEV by RT-PCR, in association with cytological and chemical changes in CSF of patients admitted with Meningoencephalitis at Thanjavur Medical College Hospital. Approximately 25% of encephalitis patients die while about 50% of the survivors develop permanent neurologic and/or psychiatric sequelae, Most common viral cause for Meningoencephalitis is Japanese Encephalitis. JE is an acute Arboviral infection (Flavivirus group) transmitted by mosquito bite. The most important vector is Culex mosquito. Domestic pigs and wild birds are reservoirs .This is a prospective study extended from June 2015-June 2016. in which 100 clinically diagnosed Meningoencephalitis cases were analyzed for presence of Japanese encephalitis by detection of IgM antibodies in serum & CSF using IgM capture ELISA. CSF was processed for biochemical and cell count analysis & molecular detection of JEV by RT-PCR. Biochemical analysis showed increased protein levels but sugar levels were within normal limits. Cell count analysis shows 2 to 3 lymphocytes in CSF sample. The Prevalence of Japanese encephalitis by IgM ELISA was 1 out of 100 sample (Serum & CSF) which accounts for 1%. CSF sample positive for IgM antibody to JEV by ELISA is further processed by RT- PCR, for genome identification, as well as to assess the reliability of the IgM capture ELISA. IgM positive sample shows positivity in RT- PCR also and the identified gene is E. Real–time PCR is the gold standard method, because it estimates the viral load and genotyping, both plays an important role in treatment strategy. Early diagnosis & appropriate treatment prevents the disease progression and its complications. Moreover knowing about the genotypes in the community helps in the development of future vaccine.
Keywords: JE, IgM antibody, RT-PCR.

Introduction

Acute infection of the nervous system is the most important preventable cause of neurological deficit in the world. Meningoencephalitis is a medical condition that simultaneously resembles both Meningitis (infection or inflammation of the meninges) and Encephalitis (infection or inflammation of the brain). Each one may present with a non specific symptom of fever, head ache, vomiting, altered consciousness, focal neurological deficits and/or seizures.

Acute Encephalitis Syndrome is synonymously used for Meningoencephalitis. It is a group of neurologic presentations caused by various viruses, bacteria, fungi, parasites, chemicals and toxins. Most common viral causes 1.Japanese encephalitis 2.Herpes simplex 3.Enterovirus. Among viral infections Arthropod borne infection by Arbo virus is (Japanese encephalitis) is considered as a main viral etiology of AES.

Japanese encephalitis (JE) is caused by JEV. It has an estimated worldwide[3] annual incidence of 45,000 human cases and 10,000 deaths. Approximately 25% of encephalitis patients die [1,2], while about 50% of the survivors develop permanent neurologic and/or psychiatric sequelae, including memory loss, impaired cognition, behavioral disturbances, convulsions, motor weakness or paralysis, abnormalities of tone and coordination.

Japanese encephalitis is one of the commonest arthropod borne infection in Thanjavur and adjacent Districts. Since the morbidity & mortality of this disease is high, identification of virus in infection prone area by detection of antibodies using IgM capture ELISA in serum &CSF samples for early diagnosis and crucial role in the management and recovery of the patient to prevent complications. In this study IgM capture ELISA is compared with the gold standard molecular test RT-PCR to study about the reliability of the test. Hence the study was undertaken.

Materials and Methods

Study design: Prospective study. Study Period: June 2015 to June 2016.

Inclusion Criteria: Patients clinically diagnosed as acute encephalitis syndrome& suspected as Japanese encephalitis.

Exclusion Criteria: Already diagnosed positive JE cases, clinically diagnosed cause as non-infectious, TB/Fungal/Parasitic meningitis.

Sample: Serum & CSF

1.Serumseparated from blood (3-5ml)collected by venepuncture.Storage-2- 8^0c.If delay in testing at -20^0 c.

2. CSF: Samples collected by lumbar puncture (prior to antimicrobial therapy for highest diagnostic sensitivity) in three sterile calibrated tubes for routine cell count& biochemical analysis (total protein and glucose), serology & for molecular diagnostic testing. The sample should not be refrigerated.

A total of 100 Serum & CSF samples were collected from suspected meningoencephalitis patients.

Serum: was tested for IgM antibody to JEV using IgM capture ELISA

CSF: Processed for Biochemical analysis, cell count & detection of IgM antibody for JE using IgM capture ELISA, at Central Diagnostic Laboratory, Department of Microbiology, Thanjavur medical college, Thanjavur

CSF samples were further processed for molecular characterization of JEV by RT- PCR irrespective of the presence/absence of IgM antibody.

Method

IgM Antibody detection: by ELISA technique. To detect IgM antibody - JE Detect IgM Antibody Capture ELISA (MAC-ELISA)[11] kit with a recombinant antigen called JERA (a marker for JEV infection) is used. The JERA protein consists of a stretch of peptides from different parts of the JEV.

Test was performed using both serum & CSF samples separately. Serum - diluted samples were used. For CSF undiluted samples were used. If there is insufficient sample it can be diluted to 1:2 or higher dilution using the Sample Dilution Buffer provided with the kit. Other steps were similar to serum. Test is a multistep procedure done as per the kit's instruction & readings were taken based on the optical density using a micro plate reader(Absorbance measurement at 450 nanometers).

< 4.0 -- Negative-- No detectable IgM antibody by the ELISA test.

4-6 -- Equivocal-- Need confirmatory test.
>6.0-- Positive--Indicates presence of detectable IgM

Quality Control: positive and negative controls were tested (provided with the kit).

RT-PCR

CSF samples were further processed for molecular characterization of JEV by RT- PCR irrespective of the presence/absence of IgM antibody.

Real-Time PCR Model: AGILENT MX3000P, USA. HELINI JEV Real-time PCR kit is from HELINI Biomolecules, Chennai India. **Viral RNA Purification, PCR Procedure:** Detection Mix.

Components	volume
RT Probe PCR master mix	8µl
RT enzyme mix	2µl
JEV Primer Probe Mix	2.5µl
Internal control Primer Probe Mix[IC PP Mix)	2.5µl
Purified Viral RNA sample	10µl
Total reaction volume	25µl

PCR vials to be centrifuged briefly before placing into thermal cycler.

Negative Control Setup: Included 10µl of nuclease free.

Positive Control Setup: Included 10µl of Positive control.

Amplification Protocol

	Step	Time	Temp
45 cycles	Reverse transcriptase	30sec	42c
	Taq enzyme activation	15sec	95c
	Denaturation	20sec	95c
	Annealing/Datacollection*	20sec	56c
	Extension	20sec	72c

JEV = FAM channel

Internal Control = JOE/HEX/VIC/Cy3 Channel

Results

IgM capture ELISA was done for 100 CSF & serum samples to detect IgM antibody for JEV. Out of 100 samples 1 sample was positive (both CSF&serum) which accounts for 1%.

CSF Gross Appearance[10]: was noted whether it is Clear, turbid, cloudy, purulent or Contains blood/ clots.

CSF: Wet Mount:

After centrifugation of the CSF sample, it was tested for cytological analysis & Gram's Smear Single Neutrophil in the CSF sample is pathogenic. In this study out of 100 samples, cells were present in 6 samples. Among the 6 sample 2 to 3 Neutrophils in 2 samples &4 to 5 lymphocytes in 4samples were observed. Biochemical analysis of CSF samples -Out of 100 samples 7 sample shows abnormal results. Among the 7 samples increased protein levels in 5 samples and increased sugar level 2 samples. CSF samples were further processed for molecular characterization of JEV by RT- PCR irrespective of the presence/absence of IgM antibody, for genome identification & to access the reliability of the IgM antibody by ELISA. IgM positive sample shows positivity in RT- PCR also& the identified gene is E

Table-I JE IgM Positive Cases by Elisa

Result	N=100	Percentage (%)
Positive	1	1%
Negative	99	99%

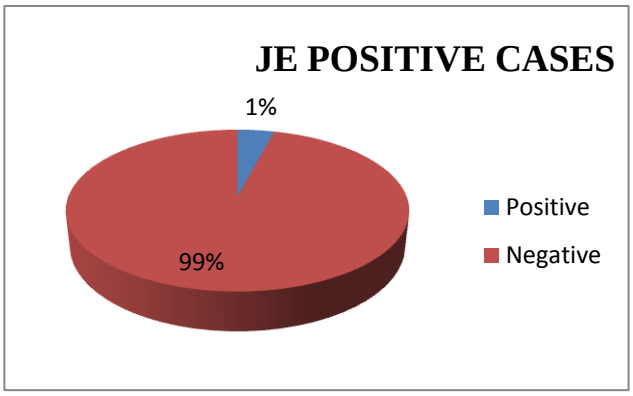
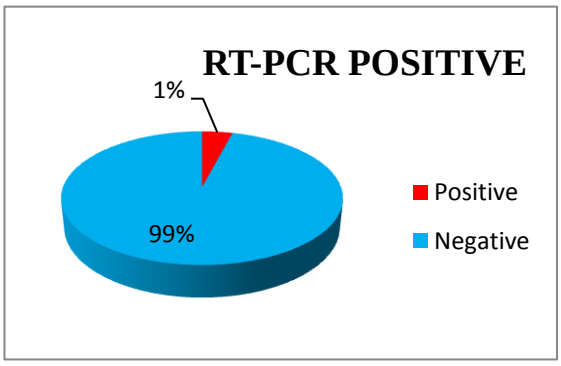
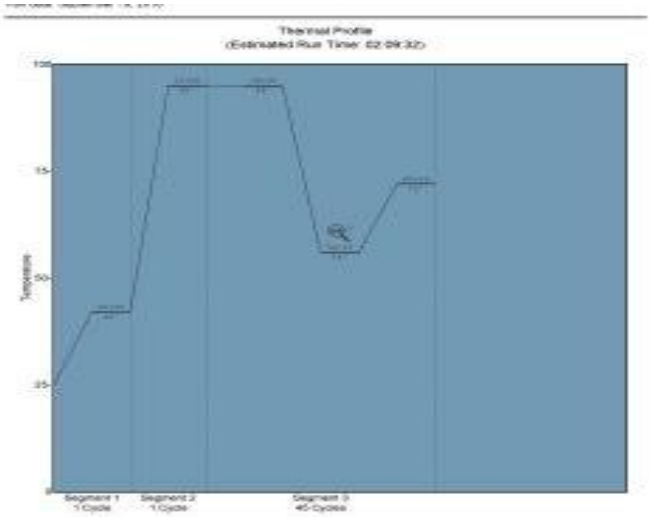


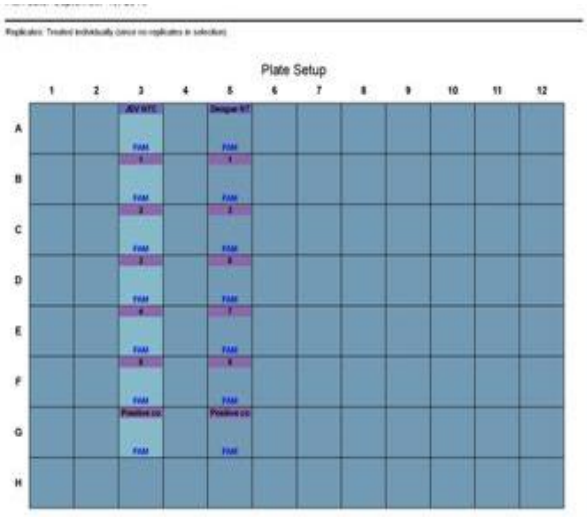
Table- II RT-PCR Positive for JEV

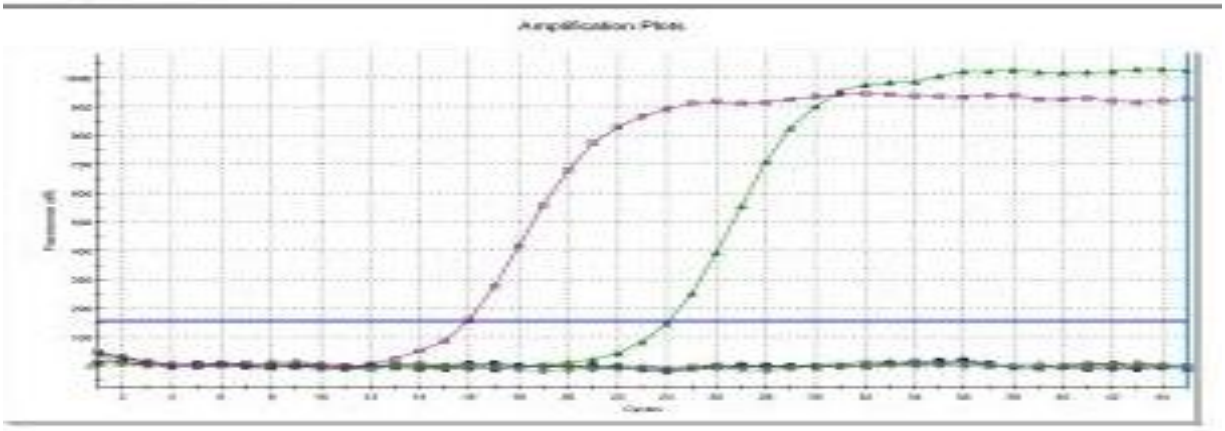
Method	Positive	Negative	Total
Elisa	1	99	100
RT-PCR	1	99	100



Annexure – 1 (RT-PCR)







Thermal Profile Summary

Segment	Cycles	Plateau	Temp. (degrees)	Temp. Inc. (deg/sec)	Duration (min:sec)	Time Inc. (min:sec)	Collect
1	1	Plateau 1	42.0	0.0	30:00	00:00	<none>
2	1	Plateau 1	95.0	0.0	15:00	00:00	<none>
3	45	Plateau 1	95.0	0.0	00:20	00:00	<none>
3	45	Plateau 2	56.0	0.0	00:20	00:00	1 Endpoints
3	45	Plateau 3	72.0	0.0	00:20	00:00	<none>

Replicates: Treated individually (since no replicates in selection)
* Fluorescence form used: dR

Text Report

Well	Well Name	Dye	Well Type	Threshold*	Ct*
A3	JEV NTC	FAM	NTC	157.771	No Ct
B3	1	FAM	Unknown	157.771	No Ct
C3	2	FAM	Unknown	157.771	24.12
D3	3	FAM	Unknown	157.771	No Ct
E3	4	FAM	Unknown	157.771	No Ct
F3	5	FAM	Unknown	157.771	No Ct
G3	Positive control-QS1	FAM	Unknown	157.771	15.99

Discussion

100 CSF & serum samples were collected from suspected meningoencephalitis patients were processed to detect IgM antibody to JEV by IgM capture ELISA and CSF samples were further processed for RT-PCR.

Biochemical analysis of CSFsamples–7 samples show abnormal results which accounts for 7%. Among the 7 samples 5 samples show increased protein levels and other 2samples show increased sugar level. Green et al[1] study explained about the protein elevation in CSF in meningitis & encephalitis correlates with this observation.

Cell count analysis - 6 samples show abnormal results which accounts for 6%. Among the 6 samples 2 to 3 Neutrophils were observed in 2 samples & 4 samples show 4 to 5 lymphocytes. Single Neutrophil in the CSF sample is pathogenic This explains about the infection of the central nervous system strongly associated with the elevated levels of protein and the cell count. This observation is in concordance with Kida S et al[4] study.

IgM capture ELISA was done for 100 CSF samples, out of which one sample exhibits positivity accounting to 1%. Diagana M[11], Preux PM et al, Gourie-Devi M, Ravi V et al[18] also showed seropositivity for IgM by ELISA.

CSF sample positive for IgM antibody to JEV in ELISA alone showed positivity by RT-PCR and the identified gene is E. RT-PCR was done for genome identification as well as to access the reliability of the IgM capture ELISA

Summary

- Prevalence of IgM antibody in JE is 1%.
- Prevalence was high among children.
- CSF sample positive for IgM antibody to JEV

in ELISA alone showed positivity by RT-PCR and the identified gene is E. RT-PCR was done for genome identification as well as to access the reliability of the IgM capture ELISA

Conclusion

This study estimates the prevalence of Japanese encephalitis in a Tertiary Care Hospital. The prevalence of Japanese encephalitis was 1% .The prevalence was high among children when compared to adults for JEV. Early diagnosis prevents the disease progression and further complications.

Real –time PCR was done to estimate the viral load which plays an important role in treatment strategy. Genotyping by RT-PCR identifies the genotypes in the community that helps in the development of vaccine & epidemiological analysis.

Even though RT-PCR is the gold standard method, it is costly compared to IgM by ELISA. Whereas IgM detection by ELISA is as good as RT-PCR in diagnosis. Hence this study suggests IgM detection by ELISA to be an effective & sensitive screening methodology in our Socioeconomic setup. Further RT-PCR may be imparted as an academic & epidemiological tool.

Acknowledgement

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