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A Study of Detection Of Japanese Encephalitis in Pediatric Patients by IgM Elisa in Government General Hospital, Kurnool

KEYWORDS

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ABSTRACT *Japanese Encephalitis Virus (JEV) an arthropod borne pathogen can be found throughout the tropical zones of Asia. Increase in population density, Deforestation and increase in irrigation of agricultural areas contribute to rise in JE incidence. World studies report the mortality due to JE ranging from 23-36% and 18% of cases end up with complications. The major burden of JE is seen in children. Diagnosis depends on high degree of clinical suspicion and confirmation by serology or culture.CSF analysis ,CT and MRI also play an important role. Laboratory confirmation of acute cases of Japanese encephalitis was done by IgM-capture ELISA, which detects anti-JEV immunoglobulin M in serum samples. In the period january2014-December2014, a total of 170 serum samples were submitted to Department of Microbiology, Government General Hospital, Kurnool, and of these, 3 were positive for anti-JEV IgM. Positive cases came from patients aged 6-10 years.*

Introduction

Japanese encephalitis virus (JEV) belongs to the Genus Flavivirus , Family Flaviviridae, and is the causative agent of Japanese encephalitis, a mosquito-borne disease transmitted by Culex spp. (C. tritaeniorhyncus and C. vishnui groups) breeding mostly in flooded rice fields (Kurane, 2002). Pigs are an important vertebrate amplifying host, facilitated by significant viremia following JE infection, large population size, high turnover rate and preferential feeding of the vectors (Burke et al, 1986). Humans are infected through the bite of an infected mosquito. The mosquito vector for JEV is widely distributed across Southeast Asia and the Western Pacific.

The Japanese encephalitis virus affects the membranes of the brain. Pathologic changes, such as perivascular congestion and hemorrhage, may be diffuse or focal, but are seen predominantly in the cortical gray and deep gray matter (WHO, 1988). In an epidemic of Japanese encephalitis, up to 10% of the susceptible human population may be infected. Most JE cases are mild infections accompanied by fever and headache, or without apparent symptoms. However, severe disease exhibits a rapid onset of headache, neck stiffness, disorientation, coma, seizure, and spastic paralysis, and may lead to death (WHO, 2005). One in 300 infected develops clinical encephalitis, and 20-40% of these cases become fatal, while half of the survivors develop neuropsychiatric sequelae (Burke et al, 1985).

Materials and Methods

Collection of Serum samples

One Seventy Samples (170) serum samples extracted from patients showing clinical encephalitis was taken from paediatric department, Government General Hospital, Kurnool.

IgM-capture ELISA (ELISA kit from National Institute of Virology, Pune) for Japanese encephalitis

The IgM- capture ELISA protocol used was modified from the procedure of Bundo and Igarashi (1985). A 96-well flat-bottom microtiter plate was coated with 100 ml of affinity-purified goat anti-human IgM overnight at 4˚C. Each well was blocked with 100 ml of Blockace at room temperature for 60 minutes,Washed 3 times with PBS-0.5% Tween20 for 3 minutes each time. After this, 100 ml of the test and control sera, each diluted to 1:1,000 with PBS-0.5% Tween20, were added to each duplicate well and incubated for 60 minutes. The wells were washed and 100 ml of assay antigen were added and incubated for 60 minutes. After washing, the wells were reacted with 100 ml of horseradish peroxidase (HRPO)-conjugated anti-flavivirus immunoglobulin G, prepared from anti-dengue high-tittered human sera, at 1:4000 dilutions for 1 hour. The plate was washed, and 100 ml of substrate solution containing o-phenylenediamine dihydrochloride (OPD) and 30% hydrogen peroxide were added to each well and kept at room temperature in the dark for 60 minutes. All the preceding steps were done at 37˚C unless otherwise indicated. The reaction was stopped by adding 100 ml of 1N hydrochloric acid, and absorbance at 492 nm was measured by ELISA plate reader. A positive- to-negative ratio (P/N) was obtained by dividing the A492 of the test specimen by the A492 of the negative control. Samples showing a P/N ratio greater than or equal to 2.0 were considered positive.

Results

Clinical specimens, collected from pediatric patients were submitted to Government General Hospital, Department of Microbiology, Kurnool for testing.

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This study involved the development and application of a molecular assay, IgM-capture ELISA, for laboratory confirmation of acute cases of Japanese encephalitis. Anti-JEV immunoglobulin M was detected in serum samples taken from patients exhibiting symptoms of CNS infection.

In the period January 2014-December 2014, a total of 170 serum samples was submitted, and of these, 3 were positive for anti-JEV IgM, whose ages ranged from 6-10years (Table-1).

TABLE-1

Distribution of JE cases by age.

Age	IgM-positive
0-5y	0
6-10y	3
11-14y	0

Discussion

In Kurnool, Japanese encephalitis was found to occur in persons of all ages and quite a number of cases developed in school-age children. Japanese encephalitis has been reported to be endemic in all areas of Kurnool.

The method of IgM-capture ELISA is useful diagnostic test for the early detection and confirmation of acute Japanese encephalitis in patients diagnosed with a viral infection of the central nervous system. This assay is capable of distinguishing Japanese encephalitis cases from other viral encephalitic infections exhibiting similar symptoms. It does not require more than one sampling, unlike the Hemagglutination inhibition test, and is superior to culture in terms of speed and ease in getting a result. The use of this method to support a clinical diagnosis will facilitate treatment of the disease and management of potential outbreak events.

Conclusion

According to the obtained statistics, the incidence of JE in Kurnool District is less, because of good awareness in population. Good Vector control is achieved with extensive usage of insecticides and Vaccination in children.

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