ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 03 | March - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SEROPREVALENCE OF JAPANESE ENCEPHALITIS IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Background: Arbovirus are responsible for a significant number of viral encephalitis cases worldwide. Japanese Encephalitis virus is a mosquito-borne flavivirus that causes a major epidemic of acute encephalitis in humans throughout Asia [1]. Scientific literature unequivocally shows the prevalence of Japanese encephalitis in various parts of India [2]. In India, cases have been reported from Tamil Nadu, Andhra Pradesh , Uttar Pradesh , Bihar , Assam , West Bengal , Karnataka , Goa and Maharashtra [3]. **Aims and Objectives:** The present study was performed to estimate the incidence and clinical profile of Japanese Encephalitis among the patients which were clinically diagnosed with Viral Encephalitis at a tertiary care centre. **Material and Methods:** One hundred and sixty five randomly selected cases of clinically diagnosed Encephalitis who were admitted in our hospital, were included in the study. In - house MAC ELISA was used to detect specific IgM antibodies in the Cerebrospinal fluid. **Results:** Anti- JEVirus IgM antibodies were detected in 8 % cases of clinically diagnosed encephalitis who were admitted to our hospital. The mortality rate of the Japanese Encephalitis cases was 12.5 %. No sequalae was recorded in the Japanese Encephalitis cases who survived in our study.

KEYWORDS

Japanese encephalitis virus, Viral encephalitis, IgM antibody capture ELISA.

INTRODUCTION:

A number of neurotropic viruses like Herpes Simplex Virus , St. Louis Encephalitis Virus , Japanese Encephalitis Virus , West Nile Virus and Arbovirus are attributed to be the causative agents of Viral encephalitis worldwide [4]. Japanese Encephalitis which is caused by a mosquito borne neurotropic RNA virus, has emerged as a disease with major epidemics, with a very high mortality and morbidity [5], [6]. All flaviviruses are antigenically related , cross reactions being most evident and hence, the need for tests showing the greatest specificity [7]. Culex tritaeniorhychus and Culex vishnuii species are the main vectors which transmit the disease from the reservoir hosts to man. They generally breed in paddy fields and are invariably found outdoors [10]. The virus is transmitted in the zoonotic cycle among mosquitoes and vertebrate amplifying hosts , chiefly pigs and wading birds [14]. Viral encephalitis is an acute inflammation of brain parenchyma which is characterized by fever, headache, confusion, seizures, altered levels of consciousness and focal neurological disturbances in various combinations. Patients who seek medical attention may have aseptic meningitis or encephalitis, of whom 5 % to 25 % die [2], [15]. The rate of asymptomatic and symptomatic infection varies between 25:1 to 1000:1. The incubation period varies from 1 – 6 days, or as long as 14 days. The onset of illness can be abrupt, acute (< 1 day), subacute (1-3 days) or gradual (> 3 days). Children under 15 years of age were principally affected in endemic areas [19]. Waning immunity or other biological factors associated with aging, have been speculated to be risk factors [12],[13] . This is followed by the encephalitis stage which manifests with altered sensorium, convulsions, neck stiffness, muscular rigidity, mask like facies and abnormal movements (17,18,19) . The principal complications are secondary bacterial infectionsand gastro-intestinal haemorrhage in the acute and subacute phase of the illness [20],[21]. Japanese encephalitis which is acquired during the first two trimesters of pregnancy may lead to foetal infection and miscarriage [15], [22]. The causes of relapse, seizures and sequale were convulsions, frank mental retardation, frank motor deficits, scholastic backwardness, behavioural problems and subtle neurological signs [20], [21].

MATERIALS AND METHODS:

One hundred clinically diagnosed cases of Encephalitis who were admitted in our hospital were included in the retrospective study. The cerebrospinal fluids were randomly selected after excluding bacterial and other viral etiology for meningitis and encephalitis The diagnostic criteria for Japanese encephalitis which was adopted in this study was the demonstration of IgM antibodies in cerebrospinal fluid samples . The Avidin – Biotin system which was used in this test was used to increase the sensitivity and specificity to 100 %. CSF for serology which was received from all cases were collected and stored at – 70 degrees centigrade until they were tested by MAC ELISA. Control group : To check the specificity of the ELISA technique which was employed for the detection of the Japanese Encephalitis Virus infection , Fifteen Cerebrospinal fluid samples which were diagnosed as caused by the Herpes Simplex Virus (5 cases) , Subacute Sclerosing Panencephalitis (5 cases) and Mumps (5 cases) were included in the study . A detailed history and clinical examination was carried out in all cases. Other investigations like Blood count and Haematological and Biochemical Analysis were also compared. IgM Antibody Capture ELISA (MAC ELISA) is the method of choice to demonstrate virus specific antibodies in both in Blood and Cerebrospinal fluid samples [23],[25]. Principle : Solid phase support (microtitre plate wells) were coated with Anti – Human IgM antibodies which were capable of binding all IgM isotype antibodies which were present in the specimen. Specific Antigen was then added, followed by enzyme labeled antigen – specific antibodies. If IgM antibodies which were specific for the antigen in question were present, the sandwich complex would result in an enzymatic colour change which was proportional to the concentration of the IgM specific antibody which was present. This method is highly specific and more sensitive. The assay was considered to be valid only when the controls gave expected results (Quality control) by in house MAC ELISA for the detection of IgM antibodies to the Japanese Encephalitis Virus by an IgM antibody capture ELISA.

RESULTS :

The study was performed over a period of two years among patients with clinically diagnosed Viral Encephalitis at a tertiary care centre in India. IgM Antibody Capture ELISA (MAC ELISA) is the gold standard to demonstrate the presence of virus specific antibodies in cerebrospinal fluid. The number of CSF samples tested = 100 Japanese Encephalitis Positive by MAC ELISA = 8 cases (8 %). Any test sample with more than 100 units is considered to be Positive for IgM antibodies to the Japanese Encephalitis Virus Samples with ELISA units between 30 –99 were considered to be probably positive for the IgM antibodies to the Japanese Encephalitis Virus, but they required further testing with the DengueWest Nile Virus Antigen, as well as with the Normal mouse brain Antigen in order to exclude false positive reactions. A sample with ELISA units less than 30 units was considered to be negative for the IgM antibodies to the Japanese Encephalitis Virus In our study, it was noticed that children younger than 15 years of age were affected with the Japanese encephalitis virus infection, which was also well documented by several earlier reports. The age distribution of the Japanese Encephalitis cases is as mentioned in. Various clinical features which were reported in these cases have been compiled. The mortality rate of the Japanese Encephalitis was 12.5 %. No sequalae were recorded in the Japanese Encephalitis cases who survived in our study.

Table 1: Showing the various clinical manifestations seen among the JE positive AES cases

Clinical feature	Number of JE positive cases showing the below symptoms (n=838)	Percentage
Fever	838	100%
Headache	591	70.5%
Altered sensorium	736	87.8%
Neck rigidity	272	32.4%
Seizure	368	43.9%
Paralysis/Hemeparesis	42	5%
Unconsciousness	297	35.4%

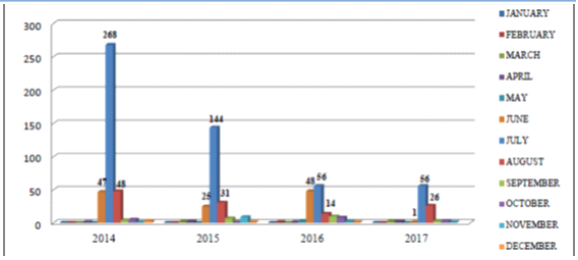

Figure 1: Month wise distribution of JE positives cases from 2015-2018.

DISCUSSION:

Japanese Encephalitis is one of the leading causes of Acute Encephalopathy, affecting children and adolescents in Tropical and Sub – tropical Asia. Japanese encephalitis virus (JEV) is an important cause of encephalitis in most of Asia, with high case fatality rates and often significant neurologic sequelae among survivors. Epidemic outbreaks of Japanese Encephalitis continue to pose a significant public health problem in most parts of India, especially in the Southern states (16). The present study was carried out to diagnose Japanese Encephalitis cases among patients who were clinically diagnosed as Encephalitis. The diagnostic criteria for Japanese Encephalitis which was adopted in this study was the demonstration of the IgM antibodies. From this study, authors have observed that over the period of four years, the JE positivity rate has significantly reduced from 32.9% in 2014 to 13.3% in 2017. Similar findings have also been reported.16,17 Reason for this may be better awareness programs, strengthening of laboratory services, mass vaccination or simply due to herd immunity. According to some authors, there has been a changing epidemiological trend of flavivirus mediated diseases from JE to dengue in recent years possibly due to increased urbanisation of the remote villages.16,18,19 Cross-protection by other flaviviral diseases, namely, dengue, could be a reason for decline of the JE cases to some extent. Although there has been a decline in the number of AES cases due to JE, there are still a large proportion of non-JE AES cases. There has been a shift in understanding of the spectrum of aetiology of AES in India and is now thought to be contributed largely by non- JE aetiology. Hence, it is important that further studies should be done to look for other etiological causes of AES. Although studies have indicated that JE is more common in children as compared to adults, the scenario in Assam is different. Our study shows a higher prevalence of JE in adult. The findings are similar to other studies done from our region.17,20-23 It can be presumably due to the influence of vaccination programme targeting children between 1-15 years and also due to increased exposure of adults to mosquitoes in areas of rice cultivation as explained by some authors.13 No significant difference was seen among the positivity rate of JE among males and females.17,24-26 The present study revealed significant number of JE cases in the monsoon season from June to September which is similar to the findings of higher incidence of JE during similar months.17,20-23,27,29 This can be explained by the fact that the Culex mosquitoes breed abundantly in the paddy fields covered with stagnant water during the rainy season. Fever (100%) was the most predominant symptom followed by altered sensorium (87.8%) and headache (70.5%) similar to the observation of other authors.17,21-23 Our study also indicates that most of the JE cases occurred in the rural districts of Assam, where the main occupation is agriculture. The JE virus is particularly common in rural areas where irrigated rice fields attract the natural avian hosts and provide abundant breeding site for the vector.23,24,27,29.

CONCLUSION:

The disease was of a sporadic nature affecting all age groups, but predominantly, children formed 8 % of the cases which were admitted to our hospital during our study. The mortality rate of Japanese Encephalitis was 12.5 %. No sequalae was recorded in the Japanese Encephalitis cases who survived. No specific antiviral therapy is available for Japanese encephalitis. The specific aetiological diagnosis of Japanese Encephalitis cases helps the patient management protocols and avoids unnecessary use of antiviral therapy. Acyclovir therapy which is of no proven advantage in the cases of Encephalitis which were caused due to the Japanese Encephalitis Virus needs supportive and symptomatic treatment Thus, the management protocol was restricted to temperature control, seizure control, sedation and the control of aggravating intracranial pressure and fluid and electrolyte management.

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