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RESEARCH ARTICLE

INCIDENCE OF JAPANESE ENCEPHALITIS VIRUS INFECTION IN WEST BENGAL, INDIA – A TWO YEARS COMPREHENSIVE STUDY.

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Abstract

Japanese Encephalitis Virus (JEV), is a mosquito-borne pathogen, which causes Japanese Encephalitis (JE); a neurotropic disease with high mortality and can be accounted to be the main player in the increasing global trend of viral encephalitis with prime concern on general public health. JE was initially documented in the East Indian state of West Bengal in the year 1973. From that point forward it is being reported every year consistently from various regions of the state, although the vaccination programme has already being procured. In this way, it shows that there may be either an incomplete coverage of the vaccination approach or the gradual rise of transformed new strains of JEV. Considering this reality, to comprehend the frequency of JEV dissemination and its endemicity in the region, we apprehended a pilot scale epidemiological study on an aggregate of 159 positive patient samples gathered from the 533 clinically presumed patients with Acute Encephalitis Syndrome (AES), admitted in the different district health centres and hospitals of West Bengal, India, 2015-2016.

Introduction:-

Japanese Encephalitis (JE) is a well-known mosquito borne viral disease. Currently it is one of the most dreaded and intriguing viral infections concerning the Indian health scenario. It is one of the leading varieties of viral encephalitis globally, mostly prevalent in south-eastern Asia, covering an area with a few billion population (1). Most JE infections are asymptomatic in nature, but if clinical symptom builds up rapidly it naturally effects with significant mortality and morbidity. Though not officially well documented but it can be estimated that, JEV causes approximately 50,000- 55,000 cases and 12,000-15, 000 deaths annually. Japanese Encephalitis is a disease with severe epidemic potential and high fatality rate and hence monitoring its prevalence status is considered to very important especially in countries like India. JEV mainly affects young adults and children areas with high endemicity. Japanese Encephalitis Virus (JEV) is a member of the Flaviviridae family and Flavivirus genus (2). The JEV virion is made up of three structural proteins - nucleocapsid, glycosylated envelope protein and non-glycosylated membrane protein as well as seven non-structural (NS) proteins. JEV exists as a zoonotic pathogen with mosquitoes and pigs as hosts (3). This deadly virus is accounted for severe neurotrophic disease with fever, acute flaccid paralysis, aseptic meningitis and meningo-myleoencephalitis in humans (4, 5).This virus has spread worldwide at an alarming rate and currently it has established itself as a major health concern in India.

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Approximately 30% of the infected patients die and about 50% of the surviving individuals suffer from severe neuropsychiatric sequelae (6, 7). In West Bengal, the first major outbreak of JEV infection took place in 1973 in the districts of Burdwan and Bankura where more than 700 cases and 300 deaths have been reported (8).Since then many outbreaks have been reported; every year sporadic cases are continuously being reported from different districts of West Bengal (9,10).This article presents a comprehensive analytical profile of JE cases from 2015-2016 and intends to specifically establish the current status of JEV endemicity in West Bengal.

Materials and methods:-

Sample collection:-

Different district hospitals, medical colleges and ID & BG Hospital in Kolkata, West Bengal, were selected for this study from 2015–2016. Patients admitted with AES, having high grade fever (≥39 ◦C) for 2–15 days with any two of the following symptoms: headache, vomiting, unconsciousness, convulsions, abnormal movements, stupor, delirium, altered sensorium, neck rigidity, presence of Kernig's sign, aphasia, drowsiness and rigor, were considered for this study. Details of the clinical event, investigations, treatment given and the prognosis of the patients during hospitalization were provided by the concerned clinicians. A short case history along with the results of CSF study of each case was recorded. Most of the patients had moderately high sugar levels (45–65 mg/dL), slightly higher protein levels, varying from 50–70 mg/dL, and a white blood cell (WBC) count of ≥6×106/L. Cerebral malaria and bacteriological etiology were ruled out by the hospitals concerned. Informed consent was obtained before the collection of samples. A total of 533blood samples were collected from the clinically suspected AES cases in sterile gel-line vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA) and also in sterile test tube (Gujarat Borosil Ltd., Bharuch, Gujarat, India) by vein and transported on dry ice to the Indian Council of Medical Research (ICMR) Virus Unit, Kolkata, where the serum was separated and both the serum and CSF were stored at -80°C till tested.

Cell and virus strain culture:-

JEV P20778 strain (GenBank Accession No. **AF080251**) was obtained from the National Institute of Virology (NIV), Pune, and was used as a positive control throughout the study. The virus was reconstituted and inoculated on Aedes albopictus C6/36 mosquito cell lines obtained from National Centre for Cell Science (NCCS), Pune in minimal essential medium (MEM; GIBCO BRL-Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum(FBS; GIBCO BRL-Invitrogen) and penicillin streptomycin antibiotics (PenStrep; GIBCO BRL-Invitrogen) in 75 cm2 tissue culture flask (Nunc, Roskilde, Denmark).The flask was incubated at 28◦C in an incubator under 5% carbon dioxide concentration. It was observed regularly for the appearance of cytopathic effect (CPE) up to 7–8 days. On appearance of the CPE, the tissue culture fluid was centrifuged at 1000 g for 5 minutes and the supernatant was used as positive control for RT-PCR or stored at -80 ◦C.

Isolation of virus:-

Attempts were made to isolate the virus using C6/36cell lines. For this purpose, only IgM negative to JEV samples with a history of ≤3 days fever along with any two of the symptoms stated earlier, were selected. 200 □l of selected serum samples were spread over the monolayer of C6/36 cell line and allowed to adsorb for120 minutes in an incubator at 28◦C under 5% CO2 concentration. After adsorption, the excess sample materials were discarded and 1 ml MEM supplemented with 2% FBS and PenStrep were added in 24 well tissue culture plate (Tarsons Products Pvt. Ltd., Kolkata, West Bengal, India) and were incubated again at 28 ◦C under 5% CO2 concentration. It was observed regularly for the appearance of cytopathic effect (CPE) up to 7–8 days. For those samples which did not produce CPE, the tissue culture fluids were again passaged up to five times to facilitate the isolation of the virus, if any. After the appearance of CPE, the tissue culture fluid was collected by centrifugation at 1000 g for 5 minutes and the supernatants were kept in aliquots at -80 ◦C till isolation of RNA, followed by RT-PCR test.Non-infected C6/36 cell culture was used as a negative control.

Serology:-

For the detection of JE IgM antibody in the collected samples, ELISA tests were performed with the kit, obtained from the NIV following the prescribed protocol.

RNA extraction:-

Those serum samples that produced prominent CPE and the JEV P20778 strain (used as the positive control throughout the study), were subjected for RNA isolation, using 140 µl of tissue culture fluid. The QIAamp® RNA viral kit (Qiagen, GmbH, Hilden**,** Germany) was used, following the manufacturer's protocol.

Reverse transcriptase PCR for envelope gene of Japanese encephalitis virus:-

For the detection of JEV by RT-PCR method, both serum and CSF samples with a history of ≤3 days fever were selected. Qiagen one step RT-PCR kit (Qiagen) was used according to the manufacturer's protocol, using RNA (50 pg to 1 g) and 0.6 M of primer pairs; forward primer: JEnvF (w) 942-ACCATCCTCCTGCTGTTGGTCGCT-965 and reverse primer: JEnvR (w) 2506-CTTGTGATGTCAATGGCACATCCAGTGTCA-2477 which anneal to the conserved region of structural envelop protein (E)specific for Japanese encephalitis virus.

Results:-

Figure 1

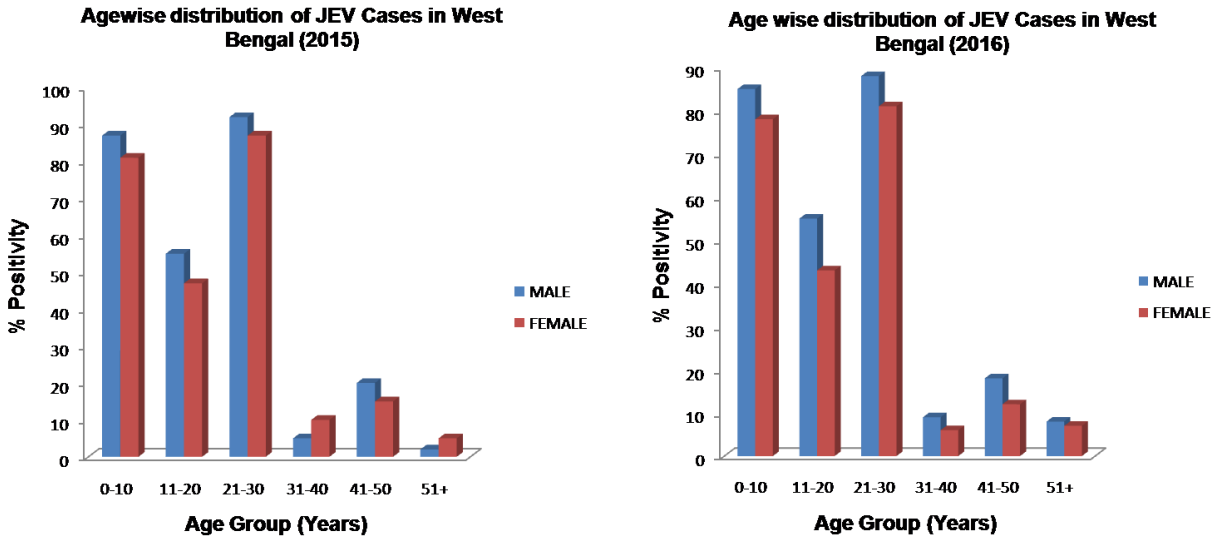


Figure 2

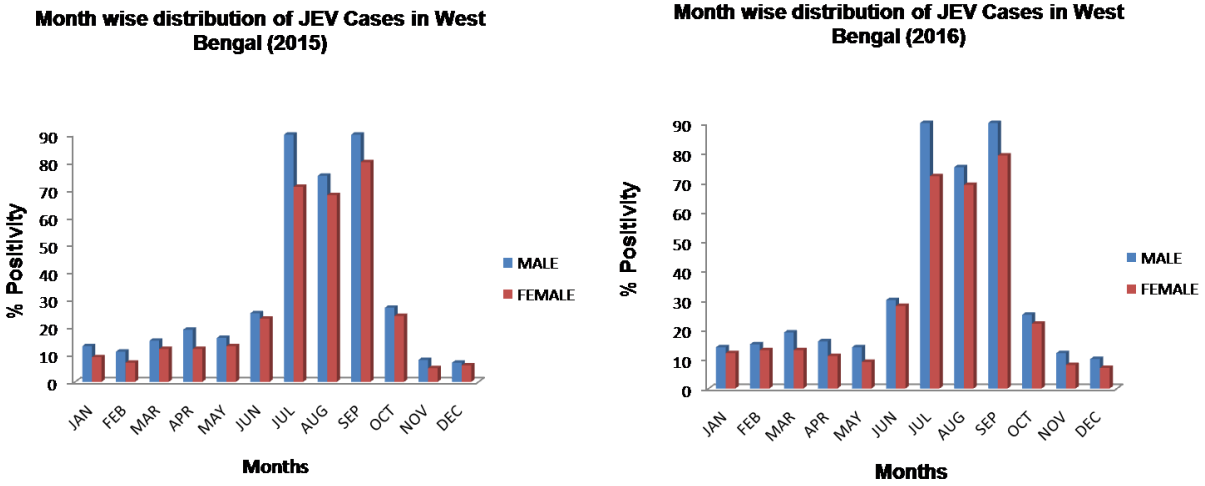
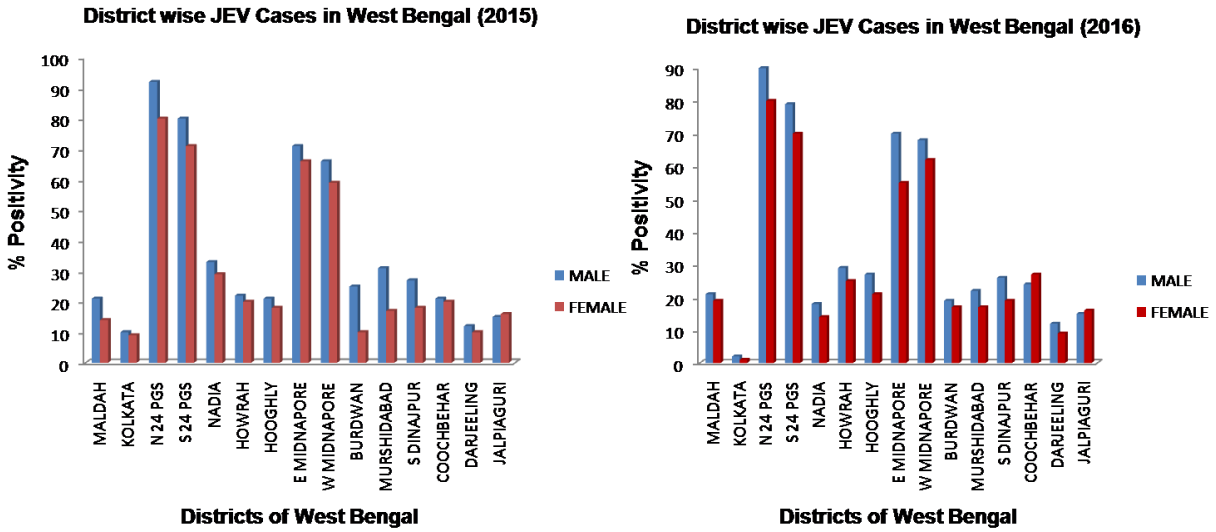


Figure 3



Discussion:-

As most JEV infections are subclinical in nature, a major portion of them remains undiagnosed (11,12). This study was particularly aimed to accurately identify the variable JE cases among the patients with Acute Encephalitis Syndrome (AES) in the East Indian state of West Bengal and to understand the current status of JEV in the state (13,14).JEV infection can be detected either directly or indirectly with the aid of several techniques. The conventional IgM specific capture ELISA is the approach for analysis of many infections including JEV as the primary screening test (15). The ELISA negative samples which had been amassed in the early clinical phase were additionally subjected to the reverse transcriptase (RT)-PCR for the identification of the virus (16). To fulfil this purpose, the collected clinical samples were preserved without disrupting the cold chain. In the present study, serum and CSF were chosen as the clinical specimens for serological and molecular diagnosis of JEV infection in the suspected cases. In India, JEV is a severe public health issue and mainly affects the young and adults up to the age of 30 years. In the present study, out of 533 samples tested in the two years, only 159 (29.8%) were positive to JEV. Percentage positivity of males during this time span was 31.68 % and females were 26.45%. All JEV positive patients had a history of illness for ≥10 days, indicative of active immune response at this stage of illness. This observation is proof of infection in the immediate past. Although, JE cases have been observed from all age groups, the highest numbers of positive cases have been recorded in the age group 21-30, followed by 0-10, in both male and female individuals almost identically (Figure 1) during 2015-2016, which almost corroborates with earlier studies. Only a few cases were detected in the age groups above 21-30 (elderly/higher age groups). The highest number of cases was observed in the 21-30 age groups of both male individuals and female individuals which are most likely because people from this age group actively take part in the cultivation of crop fields in rural areas. The vector usually breeds in the stagnant water in the cultivation fields and hence the majority from this age group are directly exposed to the vector. The next higher numbers were observed for the age group 0-10 mainly due to their underdeveloped immunity. The low number of JE cases in the higher age group is possibly due to the development of effective immunity, either by sub clinical infections or due to earlier vaccination.During both the years the maximum number of cases was reported during the months of July to September which corroborates with the warm, rainy season in West Bengal, an ideal environment for the growth of mosquitoes and disease spread (Figure 2). The major cases were observed from the districts of North 24 parganas, South 24 parganas, East Medinipur and West Medinipur (Figure 3). The reason may be due to the fact that these are sea connected and bordered districts with major chances of immigration carrying infected individuals through ports, as well as huge abundance of migratory birds which carry the virus with them. As males and females have been equally affected in almost all the cases it suggests that JEV does not have any sex preference.

Conflict of interest:-

There exists no conflict of interest among the authors.

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