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Review Article

JAPANESE ENCEPHALITIS: AN OVERVIEW OF THE DISEASE WITH SPECIAL REFERENCE TO TYPICAL THERAPEUTIC MEASURES

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Abstract: Presently, Japanese Encephalitis (JE) is one of the most awful mosquito-borne diseases. It is caused by a virus belonging to the genus *Flavivirus* of the family Flaviviridae and consists of a single stranded positive-sense RNA, wrapped in a nucleocapsid and surrounded by a glycoprotein containing envelope. Amongst the most important vectors of this disease are the *Culex tritaeniorynchus* and *Culex vishnui*. JE infection is a major cause of morbidity and mortality in South East Asia and far east countries. Studies of the viral structure, clinical presentation and classification, basic to advanced diagnostic tools, and primary treatments, are necessary for effective disease prevention and control. This review summarizes current basic knowledge of Japanese Encephalitis virus infection, based on clinical and laboratory studies and embodies the presently available therapeutic measures to counter the disease. This overview should be useful for developing advanced disease control programs and further research.
Key**words:** Japanese Encephalitis, Epidemology, Physiopathology, Treatment and Control

INTRODUCTION

Japanese Encephalitis (JE) is a mosquito-borne disease caused by a group B arbovirus (*Flavivirus*) and transmitted by culex mosquitoes. It is a zoonotic disease infecting mainly animals and incidentally man. The envelope glycoprotein of the JE virus contains specific and cross-reactive, neutralizing epitopes. The major genotypes of this virus have different geographical distribution, but all belong to the same serotype and are similar in terms of virulence and host preference. Following an infectious mosquito bite, the initial viral replication occurs in local and regional lymph nodes. Viral invasion of the central nervous system occurs probably via blood[1]. Japanese encephalitis is the most important cause of viral encephalitis in Eastern and Southeast Asia. Up to 50,000 cases and 15,000 deaths annually are due to JE especially in the rural areas[2,3]. Outbreaks of encephalitis were described in Japan from the 1870s onwards.The virus was first isolated in 1935, and since then it has been recognized all over Asia[4].

The Japanese Encephalitis virus (JEV) virion consists of a single stranded positive-sense RNA, wrapped in a nucleocapsid and surrounded by a glycoprotein containing envelope. The RNA comprises of short and long untranslated region (50 UTR and 30 UTR), between them is a single open reading frame[5] which codes for a single polyprotein which is co- and post-translational cleaved by viral and host proteases into three structural proteins (core—C; pre-membrane—PrM; and envelope—E), and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The C protein being highly basic combines with the RNA to form the nucleocapsid. The PrM which is closely associated with the E protein, forms a heterodimer, and acts as a chaperone to it, impairing its function until after virion release. Prior to virion release, the PrM protein is cleaved by a furin-like protease to form the mature M protein. This allows the formation of E protein homodimers, which are activated[6]. The E protein is the largest structural protein and consists of nearly 500 amino acids with up two potential gylcosylation sites. It is the major target for the humoral immune response, and is important in viral entry into host cells[7].

EPIDEMIOLOGY

Unlike the dengue viruses, Japanese Encephalitis virus infects several extra human hosts, e.g. , animals and birds. The basic cycles of transmission is depicted in **Fig. 1.** The disease is transmitted to man by the bite of infected mosquitoes. Man is an incidental "dead-end" host[1]. Basically three epidemiological patterns are recognized globally:

1. The disease is either endemic with sporadic cases throughout the year or in temperate/northern tropical regions. The endemic region made up of Southern India, Southern Vietnam, Southern Thailand, Philippines, Malaysia and Indonesia. In these regions mosquitoes are more often attracted by birds and pigs and human cases are rare[8].

2. The intermediary subtropical region which includes Northern India, Nepal, North and Central Burma, Northern Thailand, Northern Vietnam, Southern China and Bangladesh where transmission is permanent and of low intensity; however, it increases to higher levels during the rainy season (between April and October). Epidemics in contrast, are severe and concentrated among children[8].

3. The temperate epidemic region, spanning Northern China, Korea, Japan, Taiwan and the Southern extremities of Russia. Transmission is variably coupled with environmental temperature. In winter, mosquitoes are inactive but huge epidemics can be seen in summer and autumn. The geographical area of this disease shows a trend towards expansion. Postulated explanations are migration of birds, certain irrigation projects, animal smuggling and global warming. Development of rice plantations is theoretically foreseeable in other regions (Pakistan, Afghanistan, Nile Valley, Madagascar and Oriental Africa) creating a favourable environment for further vector proliferation. Generally, when the climate favours high mosquito population densities, this coincides with the most significant epidemics[9].

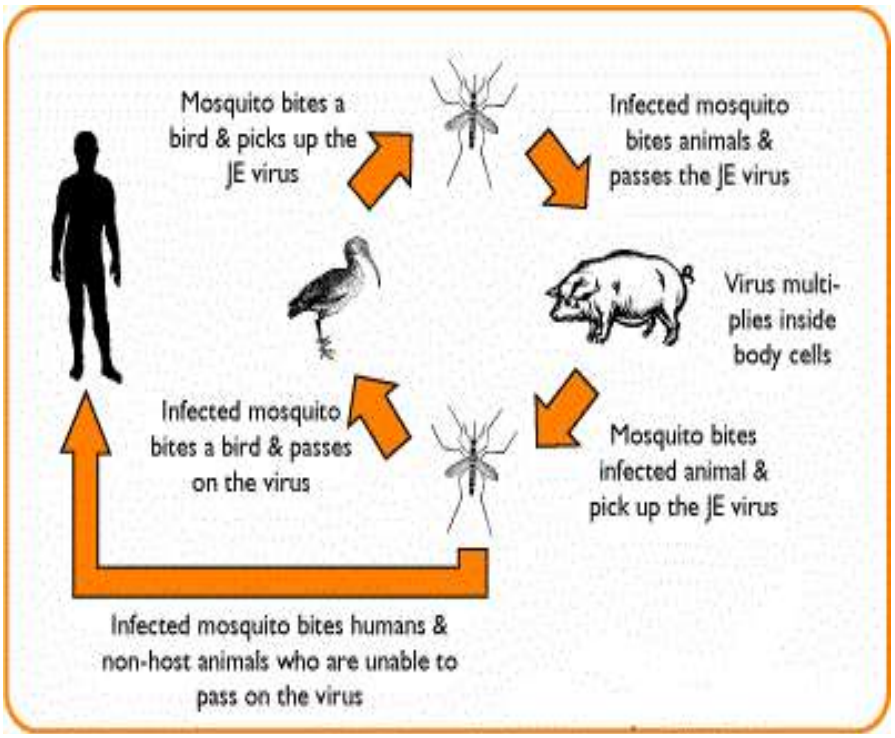

Fig. 1: Transmission cycle of Japanese Encephalitis virus

CLINICAL MANIFESTATIONS AND PHYSIOPATHOLOGY

Encephalitis is frequently preceded by fever, headache, gastrointestinal symptoms, deteriorating state of consciousness, and neck stiffness. Abrupt inception with fever and convulsions may occur in children or infrequently in adults. Patients may reveal cranial nerve palsies, and either flaccid or spastic paralysis. Severe cases may lead to coma with respiratory failure requiring ventilator support. Tremor, cogwheel rigidity, cerebellar ataxia and upper limb weakness are rarely found and about 25% die[9]. Recovery commences after about 1 week, but neurological deficits may take months to cure. Up to 50% of patients show cranial or peripheral nerve palsies, epilepsy, blindness, Parkinsonism and movement disorders. In children residual behavioural, psychiatric disturbances, memory impairment, emotional lability and aggressiveness are commonly seen. JEV infection in pregnancy is rare, but when caused may result in intrauterine foetal infection and death[10]. During the acute stage of encephalitis, seizures have been reported by various investigators and the frequencies range from 6.7 to 67.2%[11,12] .The seizures may be focal or secondary generalized and may be associated with status epilepticus. Seizures are found to be more common in children as compared to that of adults. Patients with JE may manifest with variable focal lower motor neuron signs, which may be as subtle as focal reflex loss or as severe as flaccid quadriplegia. Acute flaccid weakness in JE has been reported in 5–20% of patients[13,14]. JE virus infection is most often asymptomatic[15]. On an average, only 1 in 300 cases produce clinical symptoms[16]. The first signs of infection appear after an incubation period of 6 to 14 days. Initially it starts with fever above 38°C, followed by muscle pain, meningitis-type headaches and vomiting. The initial appearance in children usually begins with gastrointestinal symptoms: nausea, vomiting and abdominal pains. These non-specific signs continue for 2 to 4 days. Nevertheless, the patient's state deteriorates rapidly. Convulsions are experienced by 85% of subjects[17]. The meningeal syndrome predominates with painful neck stiffness and motor paralyses including hemiplegia and tetraplegia. Signs of extra pyramidal symptoms, including tremor, rigidity, and abnormal movements are observed in around 30% of the patients[18]. Decerebration, respiratory distress, hypertonia, opisthotonos related with abnormal oculocephalic reflex indicating infection of the cerebral peduncles appears in comatose forms. Fatality is observed in 20 to 30% of the cases[19] with signs of acute cerebral edema or severe respiratory distress from pulmonary edema. Although recovered still serious behavioral and neurological sequelae are noted alongwith persistently altered sensorium, extrapyramidal syndrome, epileptic seizures and severe mental retardation in children. The duration of coma associated with repetitive seizures, peduncular damage, or

intracranial hypertension are considered poor prognostic factors, leading to fatality[4]. *Culex tritaeniorhynchus* and *vishnuii* are the principal vectors, which breed in rice fields and pools. The disease occurs in rural areas with transmission during April- October in temperate climates or during rainy seasons in tropical/ subtropical areas. However irrigation may allow continuation of transmission into the dry season[20]. Humans are considered dead end hosts to the disease due to insufficient viraemia. Pigs and wading birds the are principal amplifying hosts that are crucial for viral multiplication[21]. When antibody levels in pigs were monitored and the virus in mosquitoes was isolated it showed a rise in mosquito density, alongwith a rise in antibody titres in pigs, followed by infection in humans over a transmission season[22,23,24].

INDIAN SCENARIO

Recognition of Japanese Encephalitis, based on serological surveys, was first made in 1955 in Tamil Nadu[25]. A total of about 65 cases were reported between 1955 and 1966 in South-India[26]. Japanese encephalitis is severe disease and its epidemics are reported from many parts of India[27]. The outbreak of encephalitis have been reported from different parts of India, predominantly in the rural areas of Bihar, Uttar Pradesh, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa and union territories of Goa and Pondicherry[28]. The disease is endemic in 14 states. Assam, Bihar, Haryana, Uttar Pradesh, Karnataka and Tamil Nadu report out-breaks every year and contribute about 80 percent of cases and deaths. The statistical graph depicting affected cases and deaths due to Japanese Encephalitis in India from 2000 to 2011 (as reported by Govt. of India)[29] is shown in **Fig. 2**.

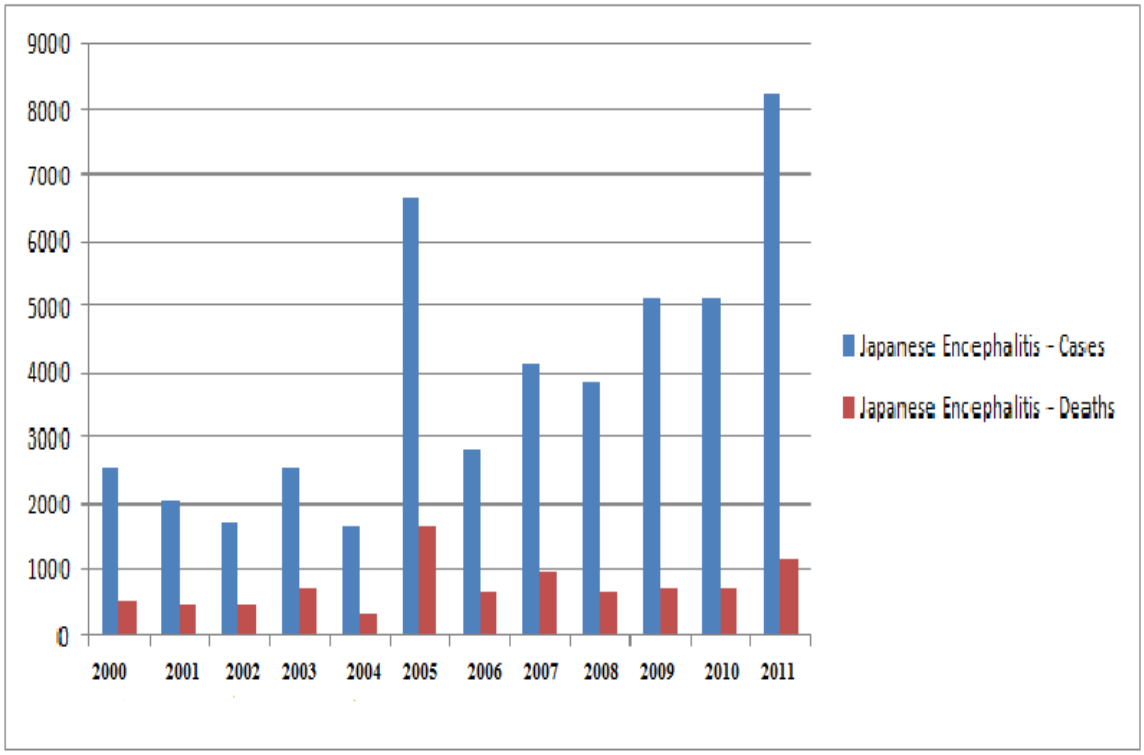

Fig. 2: Statistical report showing cases and deaths due to Japanese Encephalitis from 2000 to 2011 in India

TREATMENT AND CONTROL

Analysis of early Japanese Encephalitis is complicated, as its present symptoms are also shared by other *Flaviviruses*. Viruses responsible for causing cold sores, mumps, measles, and chickenpox may also share encephalitis symptoms. In the absence of specific therapy for JE intensive supportive therapy and symptomatic treatment are indicated. Corticosteroid therapy has been used for a long time without any proven benefit[30]. Fever should be treated with antipyretics based on paracetamol or acetaminophen. Nursing care is must be given in order to prevent decubitus ulcers, infections and phlebitis. Parenteral nutrition, maintenance of proper electrolytic balance, and cautious use of antibiotics are required. Seizures can be restricted with diazepam, clonazepam or phenytoin. Abnormal movements can be treated using haloperidol. Intracranial hypertension, associated with herniation of cerebral peduncles, can be controlled by hyperventilation (pCO2 target between 25–35 mm Hg) and mannitol (0.25 mg per kg for 3 days). RT-PCR and virus isolation from brain tissues have been developed for the detection of Japanese encephalitis, but these have limitations due to specific requirements of laboratory operations, skilled technicians and special equipments and also difficulty in isolating Japanese encephalitis virus from clinical specimens because of low circulating viral numbers and rapid removal of transient viremia by neutralizing antibodies[31]. Recently, antigen capture ELISA method to detect highly conserved E gene in different Japanese encephalitis virus genotypes were developed by Mie et al., 2012, and has been found to be very sensitive diagnostic test against Japanese encephalitis[32].

1. VECTOR CONTROL: Control of both vector and host of Japanese encephalitis is significant in order to

prevent the proliferation of Japanese encephalitis virus. Seasonal epidemics occur during the rainy seasons, when mosquito populations are at maximal[33]. Primarily insecticides and pesticides are sprayed on rice field to eliminate the encephalitis larvae of mosquito but it requires multiple round of spraying of these in one season[34]. Alternating irrigation of rice field also reduce vector breeding. Use of protective mosquito nets and repellents are also important protective tools for the encephalitis. Vector control uses mosquito repellents, mosquito bed nets impregnated with insecticide, clothing that covers the entire body and protects against insect bites. In endemic zones, vector control by spraying of anti larval insecticides around rice paddies is available but it is very expensive[35].

2. VACCINE CONTROL: Vaccination provides active immunity against Japanese Encephalitis virus. The numerous groups of vaccines are :

(a) Purified, formalin-inactivated Mouse Brain-derived JE vaccine (International vaccine): A sequence of protamine sulfate treatment, ammonium sulfate treatment, ultra-filtration and ultra-centrifugation is used to purify this vaccine. It uses either the Nakayama strain or the Beijing-1 strain[36]. This vaccine is effective, safe and is the most frequently used vaccine. Experimental evidences have shown that the Beijing-1 strain produces a higher antigenic yield in cultured brain mouse tissue, therefore, attempts are being made to replace the Nakayama strain by the Beijing-1 strain during the manufacture of this vaccine. The primary vaccination is done between the ages of 1 and 3 at doses of 0.5 ml to 1 ml (0.25 to 0.5 in children under 3) subcutaneously. The dose regimen consists of one injection on day 0, day 7 and day 30 with a booster after 1 year and thereafter every 3 years until 10 years of age. The protective efficacy is above 90%. Local hypersensitivity reactions (erythema or edema at the injection site) can be noted in some children. Other reactions such as headache, myalgia, abdominal pain,skin rash, generalized urticaria, facial angioedema, and respiratory distress have been noticed in some people from non endemic zones after vaccination. In the case of adverse side effects after the first injection the regimen should be stopped. The vaccine is contraindicated in pregnancy or in the persons allergic to mouse or neuronal protein. In case of infants it should be given under 12 months of age. Expatriates spending at least one transmission season in a rural endemic region, travelers staying longer than 2 months during May to October and people working on projects in regions with rice paddies should be vaccinated 2 months before they arrive to endemic regions[37].

(b) Cell culture derived live attenuated vaccine:
It is a live, attenuated vaccine derived from the 17D strain found in the yellow fever vaccine (YF-Vax). This vaccine has proven its efficacy and all phase II clinical studies done in both adults and children (specifically in Australia) have shown high levels of tolerance[38].

i. **Vaccine based on the SA 14-14-2 strain (National vaccine):**

This is an attenuated and genetically stable strain. In large-scale case–control studies in China, it has shown 95% protection after 2 doses with an interval of 1 year. This immunity can persist until the age of 11 years. Recently, in another case–control study in Nepal it has been reported that a single dose of this vaccine induces an efficacy of 98%[39]. Studies on the long-term efficacy of over 5 or 10 years is required to know if the single dose is sufficient or if boosters are necessary for long-term immunization of the targeted population.

ii. **Genetically engineered vaccine**:
A chimeric attenuated Japanese Encephalitis vaccine (ChimeriVax1OraVax) in which the envelope protein genes of attenuated SA14-14-2 JE virus replace the corresponding sequences in a 17D yellow fever (YF) virus cDNA clone[40]. This approach harnesses the replicative apparatus of the attenuated 17D virus to vector the principal structural proteins of the attenuated JE virus. Transcription of Chimeric DNA to RNA and transfection results in a virus that is more neuro attenuated in monkeys than the licensed 17D vaccine. Histopathological scoring system standardized for YF-17D neurovirulence testing, discriminatory and target areas in chimeric virus inoculated animals were scored lower than 17D vaccine inoculated animals. YF/SA14-14-2 chimeric virus-infected animals produced JE neutralizing antibody titers >6400. Immunized monkeys with the chimeric vaccine containing 4.3 or 5.3 dex pfu developed low levels of viremia (<2 dex pfu) for several days and antibody responses peaking to titers of 2560 at 30 days. Intracerebral studies conducted with wild type JE virus were observed 60 days later and were found to produce 1 death in six animals and no detectable viremias and an anamnestic antibody response whereas 6 of 6 unimmunized controls died. The chimeric virus is YF-17D virus-like in the patterns of viremia produced after infection but no neuro virulence is dictated by its JE sequence. Thus a single dose of the chimeric virus is expected to provide life-long immunity to JE, paralleling those attributes of YF-17D vaccine[41].

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In 2013, India will be better armed to combat Japanese encephalitis when a couple of vaccines developed by Indian companies and institutes will become available. Bharat Biotech International, in collaboration with Pune's National Institute of Virology, has developed a vaccine that is effective against the Indian strain causing Japanese encephalitis. The protective vaccine will be launched around March-April, said Krishna Ella, Chairman and Managing Director of the Hyderabad-based vaccine-maker. Another Hyderabad-based company, Biological E, recently launched an 'inactivated' vaccine for the disease, under the brand name JEEV (is a suspension for injection). The vaccine has been priced at Rs 985 a dose. A person has to take two doses. It has minimal side effects and safer than the vaccine India imports from China, according to Mahima Datla, Senior Vice-President, Biological E[34].

3. ANTI-VIRAL TREATMENT: No established antiviral treatment for JEV or any other flavivirus infection have been obtained till now. Range of compounds have shown antiviral activity *in vitro* and or on animal models of infection[42]. Recently, salicylates and nonsteroidal anti-

inflammatory drugs were found to suppress the *in vitro* replication of JEV, and prevent apoptosis of infected cells[43,44] which did not appear to be via suppression of nuclear factor kappa B activation, but may be via mitogen-activated kinase[44]. Interferon α, a glycoprotein cytokine which has been produced naturally in response to viral infections, including JEV[45] is the most promising antiviral candidate. In tissue culture, recombinant interferon is effective against JEV and other arboviruses including West Nile virus[46,47]. In the 1980s, it was given in open trials to a small number of Thai JE patients which gave hopeful results[48]. Recently, in a completed double-blind placebo-controlled trial in Vietnamese children with Japanese Encephalitis showed that although interferon α may have delayed the time to death, it made no force on the overall outcome[49].

Recently, studies were targetted to treat JE with new drugs. Arctigenin, a type of plant lignin was found to reduce viral load and viral replication within the brain. Microglial activation resulted in neuronal death, secondary inflammation and oxidative stress which suggested its potential for treating JE, although unless it is tested in human beings, it cannot be used in treatment of JE[50].Likewise, Interferon α-2a was tested in children with JE, but negative results were obtained Thus there is a need for antivirals that can reduce the toll of death and neurological sequelae resulting from infection with JE virus[9].

4. PLANT EXTRACTS: Recent studies suggest that treatment of Japanese encephalitis virus infections on chick chorio allantoic membrane (CAM), and mice with ultra diluted *Belladonna* preparations showed inhibition of viral growth on CAM. Though no confirmation of the mechanism of these ultra diluted preparations have been obtained[51], the probable action of such preparations may be due to glycosidase inhibitory action of calystegines which compete with the substrate for binding to the active site[52]. Larvicidal activity against *Culex tritaeniorhynchus* with plants like the bark of *Annona squamosa* L. (Annonaceae), leaf of *Chrysanthemum indicum* Linne (Compositae), *Tridax procumbens* Linn (Asteraceae) extracts have showed a result of 100 percent larval mortality[53].

CONCLUSION

Japanese encephalitis. (JE) is evoking and expanding across the globe at an alarming rate. It is virtually evident that all persons residing in the South- East Asia and Far East Regions are at a major risk from Japanese Encephalitis and hence countries should device effective ways to counter the disease and that can be done only through better assessment of the burden of the disease. The review suggests live attenuated JE vaccine as one of the most effective tool to counter the disease. The use of the vaccine in endangered as well as other countries should contribute to decrease in the incidence of this disease. Along with it an extensive research should be carried out in a rational way to develop newer antivirals. Newer effective diagnostic tools should be developed to assess the spread of the disease in succeptible regions. The topological and environmental factors responsible for the spread of JE need further investigations, in order to restrict the spread of this devastating disease.

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