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JAPANESE ENCEPHALITIS: A CONTINUING GLOBAL THREAT

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Abstract- Japanese encephalitis virus (JEV) is the main mosquito borne pathogen that causes viral encephalitis in South and South-East Asia. The JEV has spread all over the world. Around 30% of the people infected with JEV die every year and a high percentage of survivors are left with irreversible neurological damage. At present, there is no cure for JE and treatment is only for support. Many antiviral agents are being tested but no convincing result has been obtained yet.

Keywords- JE, JEV, Flavivirus, CNS, RT-PCR, vaccines

Introduction

Japanese encephalitis virus (JEV) is a prominent participant of the mosquito transmitted *flaviviridae* family which has small single stranded RNA viruses [1]. This virus mainly affects children below 15 years and also elderly people in whom the immune system is not much developed or weakened [2-4]. This virus causes nervous system diseases with irreversible neurological damage in humans [5]. The diseases caused by this JEV started from Southeast Asia but now it is spreading worldwide [6,7]. The people who are affected by this virus, approx. one-third of them die and half of the survivors are left with permanent neurological damage [8]. JEV genome is a single stranded positive strand RNA of approximately 11 kb in size [9]. The viral genome [Fig-1] acts as a mRNA and encodes 3432 aminoacid polyprotein that is successively cleaved into three structural proteins- nucleocapsid or core protein (C), non-glycosylated membrane protein (prM/M), and glycosylated protein (E) and number of small non-structural proteins (NS1, NS2A, NS2B, NS4A and NS4B), a helicase (NS3) and a RNA directed polymerase (NS5) [10]. JEV NS1 is involved in viral replication and regulation of the innate immune response [11,12]. The function of NS3 and NS4 is eminent, they code for serine protease and RNA dependent RNA polymerase (RdRp) [13]. There is a high rate of mutation in JEV because RdRp is likely to have some error and so that leads to huge differences in genomic sequences of JEV all over the world [14]. Since all flaviviral NS proteins are requisite for viral replication, any of them may be taken as a capable target for selective inhibitors of viral replication for therapeutic intervention [15,16]. Several attempts have been made to target the virus genome to constrain the viral replication using different techniques with varying accomplishment. Recent studies have recognized that JEV replicates solely in the cytoplasm of damaged cells, in a perinuclear location, and matures on intracellular membranes however not on plasma membranes of infected cells. Protein-protein interactions are vital to various cellular functions and hindering such interactions using synthetic composites is a very remarkable idea for formulation of new pharmaceuticals [17].

History

The first epidemic of Japanese encephalitis (JE) was stated in Japan in 1871. Major outbursts have been seen in about every 10 years; in 1924, more than 6,000 cases were documented in a major outbreak in Japan.

Epidemiological Features

JE leads to major outbreaks in tropical regions of Asia with Japan, China, Korea, Philippines, all of South-eastern Asia and India [18,19]. But the virus was able to spread all most all over the globe.

Vector and Transmission

Several species of Culex mosquitoes can spread JE. For Sothern Asia, Eastern Asia and Southeastern Asia, the key vector is *C. tritaeniorhynchus* [20]. For Northern Australia, the key vector is *C. annulirostris.* Still, several other secondary vectors may be significant. From India's perspective there are several secondary vectors such as *Anopheles subpictus, A. peditaeniatus, C. pseudovishnui*, *C. whitemorei*, *C. gelidus, C. epidesmus, Mansonia indiana, and M. uniform* [21]. Pigs and water birds [Fig-2] have been anticipated to be the natural hosts of JEV and Culex mosquitoes spread the disease to humans who are the dead hosts [22,23]. There are two epidemiological forms of transmission: an endemic form in tropical areas with viral circulation almost throughout the year, however there are few months in which the transmission is at very high rate possibly during irrigation periods; and an epidemic pattern in more temperate areas with long summer season [24,25].

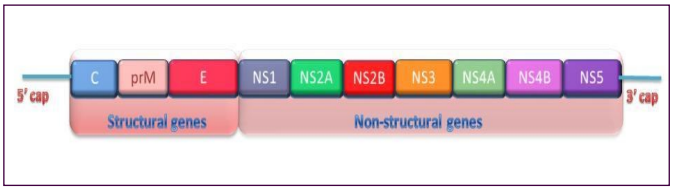

Fig. 1- Structural and non-structural genes in the organization of the Japanese encephalitis virus (JEV) genome.

Mortality and Morbidity

Around 30 million people are at risk of JE infection [26]. JE's mortality rate is nearly 25-30% [27]. Even if intensive care and support can lessen the death rate, patients continue to suffer from this disease for a long period of time. Some effects, such as learning difficulties and behavioral problems can remain hidden for many years. 50% of the patients who recover suffer from neurological defects [28]. From last 60 years, JEV has likely infected at least 10 million people, of whom only 7 million could recover and 4 million people suffered from long term disabilities.

Pathogenesis

The incubation time of JEV varies from 6-16 days. The factors which determines that out of all the people infected who will develop the disease are unidentified, however some viral factors could be taken into consideration such as path of entry, titer and neurovirulence of the inoculum along with the host factors like age, genetic make-up, general health and pre-existing immunity. After the infected mosquito bites the person, the virus replicates in the skin and then it is transported to regional lymph nodes. There it amplifies peripherally, causing a transitory viremia before attacking the central nervous system (CNS). During primary viremia, virus particles are scattered in the extraneural tissues. Main extraneural sites of replication include connective tissue, lymphoreticular tissues, skeletal muscle, smooth muscle, myocardium, endocrine and exocrine glands. The virus infiltrates from the blood into the CNS. The severity of many infections is dependent on whether or not the virus was able to gain access to susceptible cells within the CNS. If the infection is restricted to extraneural tissues, the signs may be minor or unnoticeable; still infection of neural tissues by the same virus causes encephalitis. So the route and mechanism by which the virus enters the CNS is crucial in understanding the pathogenesis of viral diseases. How JEV is able cross to the blood brain barrier is still unknown.

Clinical Signs and Symptoms

The symptoms usually begin with fever, muscle pain, headache along with vomiting. In children JE generally starts with gastrointestinal symptoms like nausea, vomiting and abdominal pains. These may also include confusion, paralysis, seizures and even coma.

Diagnosis

Patients suffering from JE show different symptoms of acute encephalitic syndrome. There are many probable sources of acute encephalitic syndrome, thus laboratory confirmation is needed for the precise diagnosis of JE, which is a tough task as the concentration of JEV is very low in the blood. Diagnosis of JE can be done in many ways such as antigen detection and antibody detection.

Antigen Detection

Several studies has proved the value of antigen detection in Cerebrospinal fluid (CSF) using reverse passive hemagglutination, immunofluorescence and staphylococcal coagglutination tests using polyclonal or monoclonal antibodies in swift diagnosis of JE. Improved methods include Immunogold silver staining (IGSS), have been effectively used in the detection of antigen in mononuclear cells of peripheral blood and CSF of patients. Immunohistochemistry has been used to detect viral antigens in the CNS. Histopathology analysis is also very supportive for clinical correlation and diagnosis of JEV.

Antibody Detection

IgM capture enzyme linked immunosorbent assay (ELISA) has been the most extensively used diagnostic technique for JEV antibody detection [29,30]. Now, much improvement has been done with techniques used for the early detection of JEV including the dipstick method and JEVCheX.

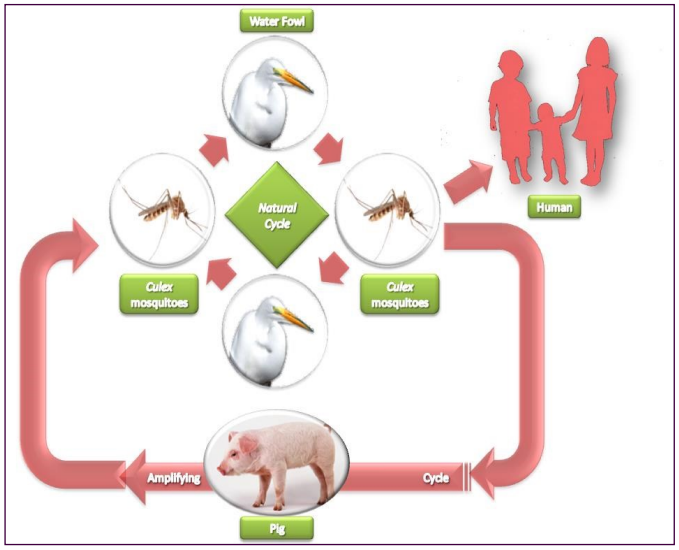

Fig. 2- Transmission cycle of Japanese encephalitis virus. Infected Culex mosquitoes play as vector for spread of JEV. Pigs and birds (egret) act as reservoirs of JEV. Humans are the dead-end host in this transmission cycle.

PCR Diagnosis

Real-time polymerase chain reaction (PCR) assays deliver sensitivity and specificity corresponding to that of conventional PCR combined with Southern blot analysis and as amplification and detection phases are performed in the same closed container, the risk of leaking amplified nucleic acids into the environment is insignificant [31]. Generally, both PCR and amplified product detection are finished within an hour or less, which is significantly faster than conventional PCR detection techniques. With the use of reverse transcriptase (RT-PCR), the viral genome can be amplified directly from tissue or blood [32-34]. An innovative nested RT-PCR based kit is defined for detecting JEV, in which all the reagents are lyophilized in reaction tubes and control RNA is contained within in each reaction to observe false negative results.

One more study suggested a reverse transcription loop mediated isothermal amplification (RT-LAMP) assay for detecting JEV. The sensitivity of JEV RT-LAMP assay was the same as that of real-time PCR and it was more sensitive as compared to conventional PCR. The JEV RT-LAMP assay was highly specific, simpler and fast as compared to conventional RT-PCR and real-time RT-PCR and so it is proposed that the RT-LAMP assay can be used as a diagnostic apparatus for JEV infection [35].

Treatment

There is no therapy for JE and treatment is mostly for care and support [36]. Maintenance of fluid and electrolytes and good nursing care is needed. Patients are not infectious but should prevent further more mosquito bites. Many antiviral agents have been tested such as INF alfa-2a and diethyldithiocarbamate [26]. But still none of them have shown substantial. Mannitol can be used to lessen the

intracranial pressure. An important study on minocycline as an anti-JEV drug is an *in vivo* study that revealed that minocycline decreases neuronal degeneration, microglial activation, active caspase activity, proinflammatory mediators and viral growth mainly on the ninth day after infection. Additional composite that has revealed inhibition of JEV replication wholly in vitro is an N-methyl isatin -b thiosemicarbazone derivative. Maintenance of fluid and electrolytes and support and care are important.

Prevention and Control

The prevention of JE is mostly based on two systems; vector control and by a vaccination system.

Vector Control

Vector control is vital in major prevention. To limit the mosquito population, conventional methods such as insecticides and bed nets are broadly used. During epidemics, particularly in peri-urban regions with marshes, thermal fogging with ultra-low volume insecticides such as pyrethrum or malathion is used for the prevention of local transmission. Still the breeding areas are too vast making it unpractical. In certain countries, to stop the larval development operative actions such as novel water management and irrigation practices like timely dropping the water level, intermittent irrigation and continuous flow systems are carried out. Only vector control cannot stop JE, as it nearly impossible to control mosquito density in rural regions.

Vaccination

To prevent JE, it is essential to apply a large scale vaccination for the human population in JE prone areas [37]. Immunization provides active immunity against JEV. There are many groups of vaccines which are currently in use such as purified, formalin-inactivated mouse-brain derived, cell-culture derived live attenuated [38]. For last 30 years, formalin-inactivated vaccines have been safe and effective against JEV. Mouse-brain derived inactivated vaccine is the most extensively produced and internationally distributed [39]. Many vaccines are still in different stages of development including recombinant virus based/chimeric vaccine and DNA vaccines [40]. Second generation vaccines are in the process of development with the purpose of increasing immunogenicity and deceasing adverse reactions.

Adverse Reactions

There are many side effects of JE vaccination. Side effects which are seen after vaccination are tenderness, redness and swelling. Occasionally systematic adverse reactions are also seen after vaccination like headache, myalgia, abdominal pain and skin rash. Sometimes local hypersensitivity reactions can be seen in some children. Other reactions include generalized urticarial, facial angioedema and respiratory distress has been observed in few people from non-endemic zones after vaccination. Some recipients of the vaccine had very rare major neurological side effects [41].

Conclusions

Japanese encephalitis is a serious health concern, not limited to Asia but has spread all over the world. There is no treatment for JE, so prevention is the only cure. Control over the spread of JE can only be done by developing an active monitoring scheme with effective vaccination program. For reducing the incidences of JE cases, implementation of immunization program for young children, modification in the irrigation practices, pig immunization, strong surveillance, vector control and better living standards can be done.

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Conflicts of Interest: None Declared.

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