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Perspectives on Emerging Therapy of Japanese Encephalitis

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ABSTRACT

Japanese encephalitis (JE) is primarily a disease of children and most adults in endemic countries have natural immunity after childhood infection, but all age groups are affected. In most temperate areas of Asia, JEV is transmitted mainly during the warm season, when large epidemics can occur. Only about 1 in 25 to 1 in 1000 humans infected with Japanese encephalitis virus develops clinical features of infection. There is no known specific therapy for Japanese encephalitis. Our treatment was symptomatic and supportive. In this review, we summarize the disease overview along with treatment approach for development of new vaccines and drugs for disease management.

Keywords: - *Flavivirus, Chloramphenicol, Mosquitos, Japanese encephalitis, haemagglutination.*

INTRODUCTION

Japanese encephalitis (JE) is among the most important *viral* encephalitides in Asia, especially in rural and sub-urban areas where rice culture and pig farming coexist. It has also occurred rarely and sporadically in northern Australia and parts of the Western Pacific [1, 2, 3]. It is a vector-borne viral disease. The disease can cause irreversible neurologic damage. The JE virus (JEV) is mainly transmitted by the mosquito *Culex tritaeniorrhynchus*, which prefers to breed in irrigated rice paddies. This mosquito species and members of the *Cx. gelidus* complex are zoophilic. Wading ardeid water birds (e.g., herons and egrets) serve as virus reservoirs, but the virus regularly spills over into pigs, members of the family of equidae (e.g., horses and donkeys), and humans [4, 5]. Humans are generally thought to be dead-end JEV hosts, i.e. they seldom develop enough viremia to infect feeding mosquitoes. Less than 1% of human JEV infections result in JE. Approximately 20–30% of JE cases are fatal and 30–50% of survivors have significant

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neurologic sequelae. JE is primarily a disease of children and most adults in endemic countries have natural immunity after childhood infection, but all age groups are affected. In most temperate areas of Asia, JEV is transmitted mainly during the warm season, when large epidemics can occur. In the tropics and subtropics, transmission can occur year-round but often intensifies during the rainy season [1, 2, 3]. The annual number of human deaths is 10,000–15,000, and the estimated global impact from JE in 2002 was 709,000 disability- adjusted life years (DALYs) [4, 5]. JE is reportable to the World Health Organization (WHO) by its Member States, reporting is highly variable and incomplete. In the late 1980s, Burke and Leake estimated that 50000 new cases of JE occurred annually among the 2.4 billion people living in the 16 Asian countries considered endemic at the time (approximate overall annual incidence: 2 per 100000). In the intervening two decades, despite major population growth, urbanization, changes in agricultural practices and increased use of the JE vaccine in many countries, this figure has been widely quoted, including very recently [6-10]. In 2000, assuming an annual, age-group-specific incidence of 25 cases per 100000, Tsai estimated that in the absence of vaccination 175000 cases of JE would occur annually among Asian children aged 0–14 years living in rural areas [11]. The current study used more recent, published, local or national incidence estimates and current population data to produce an updated estimate of the annual global incidence of JE.

SIGN AND SYMOTOMS:

Most JEV infections are mild (fever and headache) or without apparent symptoms, but approximately 1 in 250 infections results in severe clinical illness. Severe disease is characterized by rapid onset of high fever, headache, neck stiffness, disorientation, coma, seizures, spastic paralysis and ultimately death. The case-fatality rate can be as high as 30% among those with disease symptoms. Of those who survive, 20%–30% suffer permanent intellectual, behavioural or neurological problems such as paralysis, recurrent seizures or the inability to speak [12].

Vomiting from occasional to continuous was noted in 52.2% of the patients, severe diarrhea in 8.2%, and deep ocular pain and photophobia in 31.3%. Diplopia, blurring of vision, and difficulty in focusing were complaints in only 14.2% of the patients [13-17].

EPIDEMIOLOGY:

Neurotropic flavi-viruses: a global Perspective:

Japanese encephalitis virus is transmitted between animals by Culex mosquitoes and occurs across

eastern and southern Asia and the Pacific region. However, related neurotropic flaviviruses are found across the globe. They share many virological, epidemiological, and clinical features [18]. Molecular virological studies suggest that all flaviviruses derived from a common ancestor some 10–20000 years ago, and are rapidly evolving to fill ecological niches [19]. Examples of mosquito borne neurotropic flaviviruses include *Murray Valley encephalitis* virus in Australia, and *StLouis encephalitis* virus in North America. *West Nile* virus, a flavivirus found in Africa, the Middle East and parts of Europe, is traditionally associated with a syndrome of fever arthralgia and rash, and with occasional nervous system disease [20-22].

Enzootic cycle:

Japanese encephalitis virus is transmitted naturally between wild and domestic birds, and pigs by Culex mosquitoes—the most important for human infection being *Culex tritaeniorrhynchus* which breeds in pools of stagnant water (such as rice paddy fields) [23].

Although many animals can be infected with the virus, only those which develop high viraemias are important in the natural cycle. As well as maintaining and amplifying Japanese encephalitis virus in the environment, birds may also be responsible for the spread to new geographical areas.

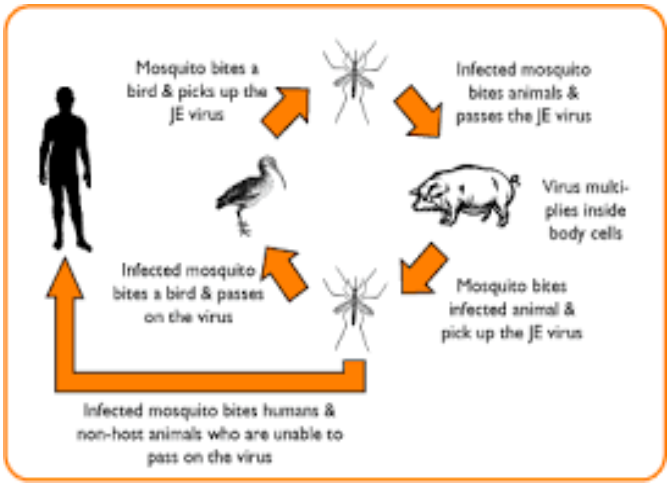

Fig. 1: Enzootic cycle

Pigs are the most important natural host for transmission to humans, because they are often kept close to humans, have prolonged and high viraemias, and produce many offspring—thus providing a continuous supply of previously uninfected new hosts. The virus does not typically cause encephalitis in these natural hosts, athough abortions occur in pregnant sows.

Epidemiology of human disease:

Humans become infected with Japanese encephalitis virus coincidentally when living or travelling in close proximity to the enzootic cycle of the virus. Although most cases occur in rural areas, Japanese encephalitis virus is also found on the edge of cities. Epidemiological studies have shown that after the monsoon rains mosquitoes breed prolifically, and as the numbers grow, so do their carriage of Japanese encephalitis virus and the infection rate of pigs [24, 25]. Human infection soon follows. In sentinel studies,

previously unexposed pigs placed in endemic areas were infected with the virus within weeks. When epidemics first occur in new locations, such as in Sri Lanka, India, and Nepal, adults are also affected [26]. The susceptibility of immunologically naive adults was also demonstrated by the incidence of Japanese encephalitis among American troops during conflicts in Japan, Korea, and Vietnam [27-31].

Broadly speaking two epidemiological patterns of Japanese encephalitis are recognised [32].

In northern areas (northern Vietnam, northern Thailand, Korea, Japan, Taiwan, China, Nepal, and northern India) huge epidemics occur during the summer months, whereas in southern areas (southern Vietnam, southern Thailand, Indonesia, Malaysia, Philippines, Sri Lanka, and southern India) Japanese encephalitis tends to be endemic, and cases occur sporadically throughout the year with a peak after the start of the rainy season [32]. Whereas rainfall patterns are almost identical in northern and southern Vietnam, the temperature is very different, and the number of cases of encephalitis seems to follow temperature closely. In the south, where the temperature remains high through the year, the number of cases each month is constant. In the north a sharp rise in cases of Japanese encephalitis during the summer months corresponds with a rise in temperature above 20ºC. The prolonged mosquito larval development time and longer extrinsic incubation period of Japanese encephalitis virus at cooler temperature, which thus reduces the rate of virus transmission, could be one explanation for these findings.

VIROLOGY:

Japanese encephalitis virus has a small (50 nm) lipoprotein envelope surrounding a nucleocapsid comprising of core protein and 11 kb single stranded RNA (3800 kD). At least five genotypes of Japanese encephalitis virus occur in Asia, which relate roughly to the geographical area of isolation [33-34]. The complete nucleotide sequence has been published, and includes 5' and 3' un-translated regions, and a single open reading frame encoding genes for three structural proteins (capsid protein (C); precursor to the membrane M protein (PrM); and envelope protein (E)) and seven non-structural proteins. The search for genetic determinants of virulence in animal models of flavivirus encephalitis has focused on the E protein [35]. This protein, of about 500 amino acids, is the major component of the surface projections of the virion. As well as eliciting neutralising antibodies and protective immune in common responses in the host [36, 37], it is thought to be the cell receptor binding protein and mediator of membrane fusion and cell entry [38]. A highly sulphated heparin sulphate molecule has recently been identified as the putative receptor of flavivirus cell entry [39].

Various approaches have allowed E gene sequences of flaviviruses to be related to virulence in animal models. These suggest that the E protein has a major role in determination of virulence phenotype, and that single amino acid substitutions are sufficient to cause loss of neuro virulence or neuro invasiveness [40-42]. Whether such differences are important in determining the clinical presentation of Japanese encephalitis virus in humans is unknown.

PATHOPHYSIOLOGY:

Only about 1 in 25 to 1 in 1000 humans infected with Japanese encephalitis virus develops clinical features of infection [32, 43, 44]. These may range from a mild flu-like illness to a fatal meningo encephalomyelitis. The factors determining which of all the humans infected develop disease are unknown, but could include viral factors such as route of entry, titre, and neuro virulence of the inoculum, and host factors such as age, genetic makeup, general health, and pre-existing immunity. After the bite of an infected mosquito, the virus is thought to amplify peripherally, causing a transient viraemia before invading the CNS. Based on data from mice and macaque monkeys, the site of peripheral amplification is thought to be dermal tissue and then lymph nodes. The means by which Japanese encephalitis virus crosses the blood-brain barrier is unknown. In experimental studies with a hamster model of *St Louis encephalitis* virus (a related flavivirus) the olfactory route was shown to be important [45]. Intranasal spraying is also an effective means of experimentally inoculating monkeys [46]. However, immune-histochemical staining of human postmortem material has shown diffuse infection throughout the brain, indicating a haematogenous route of entry [47, 48]. Although experimental evidence suggests that replication within endothelial cells may be an important means of crossing the blood-brain barrier in some flaviviruses, for Japanese encephalitis virus passive transfer across the endothelial cells seems a more likely mechanism [49, 50]. Other factors which compromise the integrity of the blood-brain barrier have also been implicated as risk factors for neuro-invasion. In several studies a disproportionate number of fatal cases had neuro cysticercosis at necropsy [51, 52]. It has also been suggested that head trauma (for example, due to a road traffic accident) during the transient viraemia could facilitate viral entry into the CNS [53]. Electron microscopic studies of the brains of infected mice show that the virus replicates in the rough endoplasmic reticulum and golgi apparatus. There is hypertrophy of the endoplasmic reticulum and degeneration into cyctic structures causing extensive dysfunction.

HISTOPATHOLOGY:

At necropsy, CNS findings in Japanese encephalitis reflect the inflammatory response to widespread neuronal infection with virus [48, 54, 55]. The leptomeninges are normal or hazy. The brain parenchyma is congested with focal petechiae or haemorrhage in the grey matter. When survival is prolonged beyond 7 days blotchy necrolytic zones are seen. The white matter usually appears normal. In some patients, the grey matter of the spinal cord is confluently discoloured, resembling that of poliomyelitis [56]. The thalamus, basal ganglia, and midbrain are heavily affected, providing anatomical correlates for the tremor and dystonias which characterise Japanese encephalitis.

At the histological level, invasion of neurons by Japanese encephalitis virus is followed by perivascular cuffing, infiltration of inflammatory cells (T cells and macrophages) into the parenchyma, and phagocytosis of infected cells [48, 54]. T cells in the brains of fatal cases stained with monoclonal antibodies are CD8+ and CD8- (presumed to be CD4+) and are localised at the perivascular cuff. Both cell types are found in the CSF in acute infection, though the predominant cell type is CD4+ [48].

In patients that die rapidly, there may be no histological signs of inflammation, but immune-histochemical studies disclose viral antigen in morphologically normal neurons [48, 57]. This may explain the normal CSF findings in a proportion of patients with Japanese encephalitis.

TREATMENT APPRROACH:

There is no known specific therapy for Japanese encephalitis. Our treatment was symptomatic and supportive. The headache was managed with aspirin and codeine, while occasionally caffeine administered hypodermically seemed to give some relief. Procaine penicillinin oil, 300,000 units daily, was administered routinely to combat the secondary bronchitis and bronchopneumonia that appeared in the seriously ill patients. Fluid and electrolyte balance was maintained with intravenously administered fluids, whole blood, and vitamin supplements. The basic principles of nursing care were thoroughly exercised.

Fig. 2: Japanese encephalitis medication

Chloramphenicol (chloromycetin®), 2 gm. at the start and 500 mg. every six hours, was given to a

limited number of patients who could take medication orally. No apparent effect was noted. Since the supply of this drug was limited, it had to be discontinued before a thorough evaluation was accomplished. Because of the variable severity of Japanese encephalitis, it would be difficult to say whether any improvement resulted from the use of this antibiotic.

Animal vaccines:-

Live attenuated JE vaccine has been developed in Japan primarily for immunization of pigs to prevent still births. In China, live vaccine prepared in primary baby hamster kidney cell culture has been used for vaccination of horses. In the Republic of Korea live attenuated JE vaccine using baby hamster kidney cells has also been developed and used for the vaccination of pigs. Immunization of pigs may be attempted to control JE epidemics because it has been established that these animals, after vaccination with the live vaccine, showed no viraemia on exposure to infected mosquitos. The immunized pigs must have therefore been unable to "amplify" the virus transmission. However, all investigators engaged in this project pointed out that effective immunization was not easy because of the high turnover in the pig population so that individual pigs might escape being immunized.

Human vaccines:-

(a) Inactivated vaccines. At present two types of inactivated vaccine are in use, one derived from mouse brain and the other from primary baby hamster kidney cells. The mouse brain vaccine is highly purified, well-defined and has been adapted for production in several countries (Table 3). JE virus strains appear to be clustered into two antigenic groups. The inclusion of one strain from each antigenic group (Nakayama and Beijing) in the vaccine has improved its potency. In China, killed monovalent JE vaccine has been produced using baby hamster kidney cell culture; approximately 70 million children are immunized with this vaccine every year. The published data in China on killed vaccine indicates that the vaccine is efficacious and safe when applied to people. In Japan and the Republic of Korea, killed monovalent JE vaccine prepared from infected mouse brain tissue has been produced and used for large-scale vaccination of people. Bivalent killed JE vaccine has also been developed in Japan. In Viet Nam, killed JE vaccine prepared from infected mouse brain tissue has been produced on a small scale. The Government of India decided in 1982 that freeze-dried mouse brain vaccine should be produced with Japanese collaboration during the period 1982-86, so that 2 million doses of vaccine will be available by 1986. Selection of the target human population for this vaccination will be done after completion of morbidity studies. The development of a JE vaccination strategy in India

needs careful consideration before it can be implemented.

(b) Live vaccines. In China, an experimental live attenuated JE vaccine using primary baby hamster kidney cell culture has been developed and has undergone extensive fieldtrials in people. Further studies to characterize candidate strains are needed. Immunization provides a reasonable and practical way to control Japanese encephalitis, but the selection of target populations may present a problem in countries where JE outbreaks cannot be clearly defined in terms of places of infection and affected age groups. However, immunization is recommended for protecting populations where JE virus attacks are highly probable. Monitoring of JE activity will help to identify populations at risk of exposure who can then be given a booster immunization.

It should be mentioned that JE vaccine is not always available because the cost of commercial vaccines is still too high for use in large-scale vaccination. Work on the development of second generation (genetically engineered) vaccines is in progress.

Monitoring and control of vector and amplifier of JE virus:

Monitoring the spread of JE virus in pigs by measuring the antibody titre by haemagglutination inhibition has been conducted in Japan and the Republic of Korea. As already mentioned, in both countries pigs have been immunized with killed vaccine or live attenuated vaccine. In China, Japan, the Republic of Korea, and Viet Nam, monitoring of outbreaks and control of vector mosquitos have been regularly conducted. However, since *Culex tritaeniorhyncus*, an important vector mosquito of JE virus in Asia, emerges mainly from the irrigation water of rice paddy fields covering vast areas, the application of insecticides to these areas for vector control purposes is not practical. One of the main problems is therefore the integration of JE vector control measures at the national level with existing measures to control other infectious diseases, together with the primary health care approach.

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