Japanese encephalitis and vaccines

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Japanese encephalitis (JE) is an inflammatory disease in the central nervous system including cerebrum, cerebellum, and spinal cord caused by viral infection transmitted by Culex mosquitoes. JE is an important disease in East, Southeast, and South Asia, which lead to serious complication and high mortality rate. Complete cure is shown in only one third of the patients. However the ratio of symptomatic infection with neurological manifestation and asymptomatic infection is 1:25 to 1000. There is no any effective treatment however prevention of JE is possible by avoiding visits to epidemic season, avoiding mosquito bites, and receiving vaccine.1

Epidemiology

Though outbreaks of encephalitis attributed to JE virus (JEV) were reported in Japan since 1871, it wasn't until 1924 that JEV was isolated from a clinical case in the first recorded epidemic in Japan. The Nakayama strain of JEV, used in development of mouse brain inactivated virus vaccine was first isolated in 1935. The mode of transmission by mosquito vector was elucidated only 25 years after recognition of JEV. Until 1970, the temperate zone of Asia was the main site of JE transmission. In the last three decades, the focus of viral epidemics has switched over to South and Southeast Asia.

Epidemics and sporadic cases of JE occur in many Asian countries, including Cambodia, China, Indonesia, India, Japan, Malaysia, Myanmar, Nepal, Pakistan, the Philippines, Republic of Korea, Sri Lanka, Thailand, Vietnam, and the south eastern Russian federation.1 Gradual spread to other non-Asian regions including, Torres strait of Australian mainland has been reported.2-4

Patterns of transmission vary countries by countries and from time to time. However there were 2 major epidemiologic pattern of transmission. In tropical zone, occurrence of sporadic JE cases throughout the year is present. In temperate zone and the northern tropical region, JE is outbreak during high temperature season. A probable explanation could be the longer mosquito development time and prolong extrinsic period of JEV at cold temperature, which decrease the viral transmission. In some area, outbreaks were associated with rainfall, floods, and rice fields. The overall incidence of disease ranges from 10-100/ 100 000 population-year.5

JEV is transmitted in a zoonotic cycle among mosquitoes and vertebrate-amplifying hosts, mainly pigs and wild birds. The presence of prolonged and high viremic titers without clinical symptoms, the ability to produce many offspring and rapid amplification of the virus, makes pig to be the most important natural amplifying hosts for human transmission.

Pathogen

JEV belongs to the family Flaviviridae, genus Flavivirus. The genome of JEV is a single positive stranded RNA length about 11 kb. JEV genome encodes an open reading frame (ORF) flanked by about 600- and 100- base-pairs of untranslated regions at the 3’ and 5’ ends, respectively. The uninterrupted ORF codes for 3 structural proteins, the capsid (C), preM which is the precursor to the structural membrane (M) protein, and the envelope (E) protein, and 7 nonstructural proteins including NS1, NS2A-B, NS3, NS4A-B, and NS5. JE virus particle has a round shape with 40-50 nm in diameter. The envelope is a lipid bilayer containing E and M proteins. The nucleocapsid, size about 30 nm in diameter and covered by envelope, is composed of internal genome and C protein. The glycosylated E protein is responsible
for attachment to host cell however there is still-unidentified the specific cellular receptors and fusion process. The E protein contains the major epitopes recognized by neutralizing antibodies. These epitopes include both JEV-specific ones and those cross-reactive for other flaviviruses. The cross-reactivity is high with the JE serocomplex group (neurotropic group) including JEV, West Nile virus, St Louis encephalitis virus, and Murray Valley encephalitis virus. It is generally accepted that neutralizing antibody is a key marker in protective immunity against JEV. Therefore, the E protein should be the most important protein in protective immunity induction. Antibodies against M protein do not possess strong neutralizing activity. The CMI host defense against JEV is not well known, although the E protein includes several epitopes recognized by T lymphocytes. Up to now there are 5 genotypes of JEV, based on nucleotide sequence of the E gene. However, there is only one serotype. Thus, any JE vaccine strain which could induce protective immunity to JEV, it should cover the others.1,6

Vector

The mosquito vector of JE differs in different regions. The major mosquito vector of JE in South East Asia is Culex tritaeniorhynchus. Culex vishnui complex is also incriminated as a vector in India. JEV has been isolated from several species of Culex, Anopheles, Mansonia mosquitoes. Humans are considered as the dead end host, as the brief periods of viremia and low titers of virus which do not facilitate transmission.1, 2

Clinical feature

Children aged less than 15 years, are mainly affected in endemic areas. However adults are also affected. Seroprevalence studies in endemic areas found that most of adult ever exposed to JE. Approximately 10% of the population is infected each year. Infection with JEV is mostly asymptomatic. The ratio of asymptomatic: symptomatic infection varies between 25 and 1000:1. The incubation period in human, after exposure is not exactly documented which could vary from 1–6 days. However, it might be as long as 14 days.1

There are 3 stages of disease including prodromal stage after incubation period. The main symptoms of the first stage are fever which mostly sudden and high grade and headache. Other non specific symptoms such as vomiting, diarrhea, myalgia, etc may be found. Then the second stage (encephalic stage) the clinical is worsen, meningeal irritation and the cerebral symptoms rapidly develop, including alteration of consciousness ranging from mild to coma. Kernig’s sign and babinski’s sign could be found. After several days the illness reaches a peak and the fatality case mostly occurred in this stage. After a couple of weeks (recovery stage), fever gradually subsides and patients become recover from the illness.1

The prognosis of JE is poor. The ratio of fatal cases and neurological sequelae including impaired intelligence and abnormal movement is high. Only one third of the cases were complete recovery. However recently, the trend of fatal cases decreased because of the appropriated treatment, however, that of completely recovered cases is still not increased, only one third. The mortality rate in the elderly patients is high and sometimes over 40%.1, 4

Clinical diagnostic laboratory

Leucocytosis (WBC> 10,000 cell/cumm.) and neutrophilia (PMN> 50%) was found about in most of the cases. The cerebrospinal fluid (CSF) appears clear but rising of pressure, number of cells and amount of proteins should be found. The CSF sugar level is mostly normal. The WBCs in CSF are predominately lymphocytes.5 Cranial MRI appears to sensitive in the detection of brain abnormalities. Typical MRI features consist of either mixed-intensity, predominantly in the thalami, but also in the
basal ganglia, brain stem, cerebellum and cortical areas. Brain autopsy grossly appears edematous with changes mainly involving grey matter including thalamus, substantia nigra, anterior horns of the spinal cord, cerebral cortex, and cerebellum. Microscopy reveals panencephalitis with abundant glial nodules, perivascular cuffing, and necrosis with or without characteristic circumscribed necrotic foci. Neuronal inflammation is typically associated with mononuclear cell infiltration. Diffuse microglial proliferation and formation of glio-mesenchymal nodules in brain parenchyma dominate the histological picture which is compatible to acute encephalitis.7

**Etiologic diagnosis**

**Viral isolation**

Recently JEV isolation was carried out by mosquito inoculation or culture by various cell lines include primary chick, duck embryo cells, and lines of Vero, LLCMK2, C6/36, and AP61 cells. Virus can be isolated from the blood of patients during acute phases, usually not later than first week of symptom onset. However, the yield of viral isolation of virus from clinical specimens is generally poor, probably because of low viral titers, rapid production of neutralizing antibodies, the logistic difficulty of specimen transportation and frequent freezing and thawing of clinical material.

**Genomic detection**

Recently RT-PCR subsequent with nested PCR is the most popular tools for identify and early detection in laboratory and clinical specimen.

**Antibody detection**

IgM/G antibody capture ELISA (Mac-ELISA) is the standard method to demonstrate virus specific antibody in both serum and CSF. Other serological tests such as hemagglutination inhibition, the complement fixation test, and the neutralization test are also available in some laboratories.

However the immunological laboratory interpretation should be caution in the dengue co-endemic area.8

**Treatment**

No specific antiviral therapy is available for JE. Treatment is mainly supportive and symptomatic. Though there is non-available of specific treatment, mortality and morbidity could be decreased by good monitoring and supportive treatment of neurological manifestation such as raised intracranial pressure and convulsions. Therefore the good supportive treatment is the key of treatment for JE.

**Vaccination**

Recently the vaccine is recommended for all children aged greater than 1 year who are living in endemic area. In Thailand this vaccine has been included in EPI program which recommended for

- 3 doses (2 primary and 1 booster) of inactivated mouse brain vaccine/ vero cell derived vaccine or
- 2 doses (1 primary and 1 booster) of live attenuated vaccine/ chimeric Yellow fever-JE vaccine.

These vaccines also should be recommended for the travelers who exposed to high risk area such as journal in rural area, or stay in endemic area for longer than 1 month which estimated risk was equal to the local people.1, 4

There are 4 type of licensed JE vaccines:

1. inactivated vaccine employing mouse brain,
2. inactivated vaccine employing vero cell,
3. live vaccine employing primary hamster kidney cell culture, and
4. chimerivax yellow fever-JE
Inactivated mouse brain vaccine

Among available JE vaccine at present, WHO has recommended a inactivated mouse brain vaccine. Thai government decided to include JE vaccine in EPI program since 1990. Immunization is now recommended for travelers visiting JE-endemic regions longer than 1 month, particularly for those who plan for outdoor activity. The current inactivated mouse brain vaccine of was licensed in Japan since 1965. It had been produced in Japan, Korea, Taiwan, Thailand, India, and Vietnam. In Japan, 2 forms of vaccine, liquid and lyophilized, were produced by many manufacturers. Lyophilized JE vaccine which was higher stability and longer expiring period, was exported for use outside of Japan. Either Nakayama or Beijing-1 strain had been used as a seed virus. The reference vaccine possesses its potency equal to or higher than that which demonstrated over 80% efficacy in the double blind field trial conducted in Taiwan in 1965.

Immunization Schedule

The 3 doses of inactivated mouse brain JE vaccines is recommended for children aged more than 1 year, living in endemic area, at Day 0, 7-28 and 1 year later. In travelers who visit endemic area longer than 1 month, the recommended vaccine schedule is 0, 1 week and then 1 month. At least a two-dose regimen is recommended before visit to endemic area. Booster immunization is made at 1 year after and then every 3 years. High levels of antibody are maintained for at least 3 years after booster vaccination. Hypersensitivity to the previous infection is contraindication for further vaccination. Local pain and tenderness at injection sites are seen in approximately 20% of vaccines. Fever and malaise are reported in 1%. Severe adverse reaction is rare.

Efficacy of Vaccination

Inactivated mouse brain vaccines demonstrated 80-90% efficacy in field trials. Since JEV-specific neutralizing antibody was shown to be the essential immune mechanism to protect JEV infection, seroconversion after vaccination indicates its effectiveness. It was reported that JE vaccine induced no particular adverse reaction in immunocompromized host including HIV-infected children however the lower titer response to JEV. Either Nakayama or Beijing strain induced antibody level high enough to assure the protection against the wild JEV strains circulating. After booster immunization, neutralizing antibody was shown to be above protective level for at least 3 years.

Adverse Reactions of Vaccine

Since JE vaccine used infected mouse brain as a source, concern existed regarding the possible incidence of severe adverse reaction in nervous systems of individuals. Extensive survey of adverse events with a special reference to the incidence of neurological symptoms was conducted in Japan in 1965 and 1966. Some mild symptoms including fever, general malaise, and abdominal symptoms were documented in 1.2%. However there was no evidence demonstrated the etiologic relationship between systemic neurological syndromes and JE vaccination. On the other hand, recently some severe allergic/neurological symptoms have been reported, particularly in European travelers. The systemic reactions, including generalized urticaria, respiratory symptoms, and cardiovascular symptoms have been documented since 1998. Some of them related to serum Ig E against gelatin; however, many remained were unknown in etiology. This claim was reported in Denmark, Australia and Canada, therefore JE recommendation for travelers is on argument in some countries. Severe neurological disorders including acute disseminated
encephalomyelitis (ADEM) were reported in relation to JE vaccination. Patients included children aged 1-7 years and young adults. The ADEM symptoms appeared 1 day to 1 month after vaccination.14-17

Recently, there was an adverse reaction report after vaccination in Japan therefore, BIKEN, the original company decided stopping vaccine production. However some country including Thailand still used this kind of vaccine.

**Vero cell Inactivated JE vaccine**

*Vero cell – derived, inactivated JE vaccines* using Beijing strain and SA14-14-2 had completed phase III study and was submitted for licensure in Japan and China, respectively. The potency of Vero cell - derived vaccine was comparable to the current inactivated mouse brain - derived vaccine.

**Live Attenuated JE Vaccine**

An attenuated strain of JEV was developed in China through several passages of JEV on primary hamster kidney cells. The most common attenuated strain was SA14-14-2 which demonstrated the effective and safety of vaccine since 1989. It has been reported that after more than 100 million children received the SA14-14-2 vaccine, there was no any serious adverse events related to vaccination. Two-dose immunization at 1 year apart is recommended as a routine immunization although a single dose immunization also showed a high efficacy. The SA14-14-2 vaccine is produced mainly in China and used in a limited number of countries. Clinical studies were performed in Nepal and Korea, and many studies showed an excellent efficacy.1, 5, 18-19

**Chimerivax-JE (Imojev)**

Chambers and colleagues developed a chimera virus (*Chimerivax-JE*) by replacing prM and E genes of yellow fever virus, 17D strain, with those of JEV, SA14-14-2 strain. The chimera virus as a candidate vaccine thus constructed induced JEV-specific antibodies in the vaccinees. The chimera vaccine was taken well in yellow fever – immuned individuals and induced antibodies not only to JEV but also to the related flaviviruses. Few vaccinees showed viremia for first week after injection, and some developed fever and malaise as those who had received yellow fever vaccine. The JE chimeric vaccine is expected to be as potent as yellow fever vaccine in term of the duration of specific antibody for years even after a single injection. Development program of this JE chimeric vaccine has finished the phase III study both adult and toddler. Recently it was licensed in Australia and Thailand.21

**Immunization Schedule**

Whether clinical trials revealed high titer and seroconversion rate in toddler vaccinees after a dose of chimerivax-JE, the booster of vaccine is still recommended for children who living in endemic area. The primary vaccine is recommended for children aged more than 1 year, and the booster dose should be prescribed 1-2 years after the first dose. Subcutaneous route was recommended. There was no data about used in pregnancy and lactating woman, therefore it was not recommend for these special circumstances.

**Efficacy of Vaccination**

In a subsequent Phase III study in Thailand and the Philippines involving 1,200 JE vaccine naïve children aged 12–18 months, the seroconversion rate to a single dose of ChimeriVax™-JE was 95% (95% CI 93–96) with a GMT value of 214 (95% CI 168–271). After 2 year follow-up, 80% still had seroprotection however the GMT values
was only 39 (95% CI 34–46). After receiving booster vaccine, the antibody titers increased by 57-fold after 28 days of booster vaccine with a GMT of 2,242 (95% CI 1,913–2,628). One year post-booster, 99% (95% CI 98–100) of children remained seroprotected with a GMT of 596 (95% CI 502–708). In a subgroup who failed to seroconvert after primary vaccination or who were seronegative 2 years post-primary vaccination, the subjects also demonstrated a robust response to a booster vaccine.22-23

**Adverse Reactions of Vaccine**

During clinical trials, the following side effects were reported with the use of Imojev:

**Adults:**

Very Common (>10%): fatigue, malaise, pain on injection site pain, headache, myalgia.

Common (1-10%): chills, dizziness, arthralgia, diarrhea, nausea, abdominal pain, vomiting, sore throat, dyspnea, rhinorrhea, cough, wheezing, nasal congestion, rash and local adverse reaction such as erythema, itching, swelling, bruising.

Uncommon (0.1-1%): Fever

Rare (0.01-0.1%): Viral infections.

**Children:**

Very Common (>10%): fever, malaise, irritability, headache, somnolence, myalgia, vomiting, loss of appetite, abnormal crying and local adverse reaction such as pain/tenderness, erythema

Common (1-10%): swelling on injection site

Uncommon (0.1-1%): local adverse reaction such as induration, itching, bruising, hematoma

Rare (0.01-0.1%): Rash, urticaria, maculopapular rash

Asymptomatic viremia was found in some vaccinees within a week after vaccination, however the level of viremia was very low which not expected to cause adverse environmental impact on transmission in mosquito vectors.22

**References:**