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JAPANESE ENCEPHALITIS IN GORAKHPUR: A REVIEW

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ABSTRACT
Japanese Encephalitis is mainly a brain fever; hence vernacularly the disease is known by the name "Mastishka Jwar" or "Dimagi Bukhar". Although occurrence of this disease has been reported from several states of India like Bihar, Andhra Pradesh and Orissa, in recent years it has emerged as a serious health problem in rural areas of eastern Uttar Pradesh (UP). The epidemic strikes 135 districts across 17 states every year but more than 70 per cent of these cases are reported from UP. The scourge of the disease is most severe in Gorakhpur Division. In the last 33 years, 50,000 people have died in UP alone, mostly children. Thus compared to adults, children are more susceptible to the disease. Children surviving the disease often develop complex problems relating to the brain. Thus, there is a need to develop a safe, affordable and potent JE vaccine or medicine. This review addresses the current efforts in this direction and the present scenario of JE in Gorakhpur division.

KEYWORDS: Japanese encephalitis; Mastishka Jwar; Dimagi Bukhar; flavivirus; Gorakhpur.

INTRODUCTION
The mosquito-borne Japanese encephalitis virus (JEV) is an enveloped, positive-sense single-stranded RNA virus and member of the genus Flavivirus under the family Flaviviridae.[1] JE is a of major public health concern due to its high epidemic potential, high case fatality and neuropsychiatric sequelae among survivors. The estimated global burden of JE was 709000 disabilities - adjusted life years (DALYs) lost in 2003.[2] Approximately 597,542,000 people in India live in JE-endemic regions and 1,500 to 4,000 cases are reported every year. [3] Gorakhpur is a hyper-endemic region for JE. Medical survey in the state reveals that of the total diseased persons, children represent 80%.[4] Epidemiology of JE is complex due to involvement of several vertebrate and invertebrate hosts. JEV is transmitted naturally between ardeid birds and pigs by mosquitoes species mostly belonging to *Culex* genera. The *Culex vishnui* subgroup mosquitoes comprising *Cx. tritaeniorhynchus*, *Cx. vishnui* and *Cx. pseudovishnui* have been implicated as major vectors of JE in India.[5] Occurrence of JE epidemics is a regular feature in Gorakhpur, Uttar Pradesh (UP), since the first major JE epidemic in 1978.[6]

PREVALENCE OF THE DISEASE
Almost half of the human population now lives in countries where the disease is endemic. The annual incidence of the disease is of 30,000 to 50,000 cases,[1] and the annual number of deaths reported is 10,000 to 15, 000.[7] The disease can cause irreversible neurological damage.[8] A fatality rate of 30% to 50% has been attributed to JE in Southern and Eastern Asia. A large proportion of survivors, 30% to 60% of the cases, suffer from long-term neurological manifestations in the form of convulsions, tremors, paralysis, ataxia, and other such symptoms.

PROBLEM IN INDIA
In India, epidemics of JE are reported from many parts of the country, and it is considered a major paediatric problem. The first recognition of JE based on serological surveys was in 1955, in Tamil Nadu, India. A total of approximately 65 cases were reported between 1955 and 1966 in Southern India.[9] Subsequent surveys carried out by the National Institute of Virology of Pune indicated that approximately half of the population in Southern India has neutralizing antibodies to the virus. Since 1955, many major outbreaks in different parts of the country have been reported. A major outbreak resulting in a 42.6% fatality rate was reported in the Bankura District of West Bengal in 1973. Subsequently, the disease spread to other states and caused a series of outbreaks in different parts of the country. In 1978, cases were reported from 21 states and union territories.[6]

PROBLEM IN GORAKHPUR
In Uttar Pradesh, the first major JE epidemic occurred in Gorakhpur in 1978, with 1,002 cases and 297 deaths reported.[10] Though, many outbreaks were reported in Gorakhpur after the 1978, in intensity and magnitude, the

2005 epidemic surpassed all previous reported epidemic outbreaks in the country.[11] In 2005, Uttar Pradesh faced a devastating epidemic outbreak of JE mostly confined to Gorakhpur affecting 6061 cases with 1500 deaths followed by another outbreak in 2006 with 2320 cases and 528 deaths.[11] Similarly JE cases in Uttar Pradesh were confined predominantly in Gorakhpur during 2007 reporting 3024 cases and 645 deaths.[6][10] As per government records, encephalitis has claimed the lives of 1256 children in 2012 out of which 557 deaths were reported from Gorakhpur division alone. In 2013, death of 350 children has been reported due to encephalitis in Gorakhpur in Eastern Uttar Pradesh.[4] A total of 9693 suspected cases and1490 deaths of JE from India mostly from Gorakhpur were reported in 2014(Source: Website of National Vector Borne Disease Control Programme, New Delhi). These figures are based on total reported cases; it is possible that many cases are unreported and hence the actual magnitude of the threat of JE may be considerably higher, both in the Indian and in the global context. The trend of JE suggests that the problem in Northern India is escalating, and larger epidemics may occur in the future.[11]

Ecological feathers of Gorakhpur favouring JE

Gorakhpur division is located in eastern part of UP. It has a temperate climate with dry winters and wet, hot summers and rainy monsoon. The annual mean temperature is about 30 degree centigrade. The mean summer temperature and mean winter temperature in Gorakhpur division are approximately 33.75 degree and centigrade 24.08 degree centigrade. The annual rainfall is typically between 137.8 to 974.85mm. Gorakhpur division is mainly a paddy growing area, with clay soil and a very high water table. It has seven districts including Gorakhpur district. Gorakhpur district in UP has an area of 3483.8 sq. km with a population of 44, 66,275 (2011 census) and 17, 74,510 bellow 15 years.[4] The village ecosystem of Gorakhpur comprised rivers, lakes, irrigation canals, reservoirs and rice fields during JE transmission season (July-November).

Seasonal occurrence of JE

As Japanese Encephalitis is a mosquito-borne disease with a seasonal distribution, external environmental factors including weather variables may play a significant role in its transmission. Studies in Gorakhpur division and adjoining districts of Bihar and Nepal have suggested that weather variables, such as temperature, rainfall and relative humidity may influence number of cases of Japanese Encephalitis.[11]

Entomological investigations on vectors in Gorakhpur

Entomological investigations were conducted in JE affected villages in the districts namely, Basti, Deoria, Gorakhpur, Kushinagar, Maharajganj, Santkabir Nagar and Sidharth Nagar which are under Gorakhpur Division. A total of 3010 mosquitoes, belonging to 25 species and 5 genera, namely, *Culex tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. quinquefasciatus*, *Cx. gelidus*, *Cx. whitmorei*, *Cx. epidesmus*, *Cx. bitaeniorhynchus*, *Cx. minutissimus*, *Cx. fuscocephala*, *Anopheles subpictus*, *An. annularis*, *An. stephensi*, *An. vagus*, *An. peditaeniatus*, *An. barbirostris*, *Aedes indicus*, *Ae. scatophagoides*, *Ae. lineatopennis*, *Ae. pallidostriatus*, *Ae. vexans vexans*, *Ae. jamesii*, *Mansonia annulifera*, *Ma. uniformis* and *Armigeres subalbatus* were collected. *Cx. tritaeniorhynchus*, the primary vector of JE was the dominant species and comprised 40.8 per cent of the collection.[11]

Cycle of JE virus

JEV is a mosquito-borne, zoonotic flavivirus that infects vertebrate hosts, primarily birds and swine, in an enzootic cycle.[12] Japanese encephalitis virus is maintained in nature in a cycle involving mosquitoes and birds belonging to the family Ardeidae.[13] A fairly high proportion of cattle egrets and pond herons possess neutralizing antibodies to JEV indicating that these birds are the natural reservoir of JEV.[14] Higher seroconversion rates in children were observed in villages with herons when compared with villages without herons.[15] Gorakhpur has several large perennial lakes and swamps which provide a wintering and staging ground for a number of migratory waterfowls and a breeding ground for resident birds. In addition, rice fields also support waterfowl, especially egrets and herons that are the reservoirs of JEV. In India, more than 135 million pig population has been reported, and among the States, 31.7 percent pigs are in (42.84 million) UP.[16] The rural people, who rear pigs, virtually co-exist with these animals. Hence, chances to infect humans increase because pigs are the amplifier vertebrate hosts of JE virus.[17] It appears that environmental factors conducive for the breeding and survival of JE vectors especially *Cx. tritaeniorhynchus*, and the presence of a large number of vertebrate amplifying hosts like pigs may contribute to the persistence JEV in this region.[18]

Mortality and morbidity

JE's mortality rate is approximately 25% to 30%.[19] Although intensive care support can reduce the mortality rate, patients often suffer significant long-term morbidity. Some effects, such as learning difficulties and behavioural problems, can be subtle and may remain undetected for several years.[20] 50% of those who recover suffer from neurological deficit. Over the past 60 years, it has been estimated that JEV has infected more than ten million people, of whom three million died and four million suffered long-term disabilities.[19]

CLINICAL DEPICTION OF JE

Pathogenesis

The incubation period of JEV ranges between six and 16 days. The factors determining who of all the infected develop the disease are unknown, but could include viral factors such as route of entry, titer and neuro-virulence of the inoculum, and host factors such as age, genetic make-up, general health and pre-existing immunity.

After the bite of an infected mosquito, the virus replicates in the skin and is then transported to regional lymph nodes. In most *Flavivirus* infections including dengue virus, and West Nile virus, Langerhans dendritic cells in the skin are reported to support viral replication.[21,22] Next, it amplifies peripherally, causing a transient viremia before invading the central nervous system (CNS).[23] During primary viremia, viral particles are seeded in the extraneural tissues. Major extraneural sites of replication include connective tissue, skeletal muscle, myocardium, smooth muscle, lymphoreticular tissues, and endocrine and exocrine glands. From the blood, the virus penetrates into the CNS. The clinical manifestations of many infections are dependent on whether or not the virus gains access to susceptible cells within the CNS. If the infection is limited to extraneural tissues, the signs may be mild or in-apparent; however, infection of neural tissues by the same agent leads to encephalitis. Therefore, the mechanism by which the virus penetrates the CNS is of prime importance in understanding the pathogenesis of viral diseases.[24,25] How JEV crosses the blood-brain barrier is unknown.[26] However, immunohistochemical staining of human postmortem material has shown diffuse infection throughout the brain, indicating a hematogenous route of entry.[27,28] Although experimental evidence suggests that replication within endothelial cells may be an important means of crossing the blood-brain barrier in some flaviviruses, for JEV, passive transfer across the endothelial cells appears to be a more likely mechanism.[26,27,28] Other factors that compromise the integrity of the blood-brain barrier have also been implicated as risk factors for neuroinvasion. Several studies reported a disproportionate number of fatal cases had neurocysticercosis at necropsy.[29]

Clinical signs and symptoms

Infection due to JEV is most often asymptomatic. On average, only one in 300 cases produces clinical symptoms. The first signs of infection appear after an incubation period between six and 14 days. It usually starts with a fever above 38ºC, chills, muscle pain and meningitis-type headaches accompanied by vomiting. The initial presentation in children usually begins with gastrointestinal symptoms: nausea, vomiting, and abdominal pains similar to those found in an acute abdominal syndrome.[30] These may include confusion, paralysis, Parkinsonian movement disorders, abnormal posturing, seizures, and coma.[31] A proportion of patients with JE have an acute flaccid paralysis that is easily mistaken for poliomyelitis,[32] but the majority present with a reduced level of consciousness, often heralded by generalized convulsions. Fatality is observed in 20 to 30% of the cases, with signs of acute cerebral edema or severe respiratory distress from pulmonary edema. Recovery usually leaves serious behavioural and neurological sequelae, most notably persistently altered sensorium, extrapyramidal syndrome, epileptic seizures, and severe mental retardation in children. The duration of the coma is associated with repetitive seizures, peduncular damage, or intracranial hypertension, which is considered poor prognostic factors, leading to fatality.[33] The course of disease may be divided into four stages. The first is the prodromal stage, which is characterized by an abrupt onset of high fever accompanied by headache, with non-specific symptoms including malaise, anorexia, nausea, and vomiting. The second is acute stage, which includes changes in the level of consciousness ranging from mild clouding to stupors, semi-coma, or coma. Generalized or focal convulsions are common, with neck stiffness and weakness of extremities. In this stage, fatal cases progress rapidly and die. The third is a late stage characterized by defervescence with improved neurologic sequelae in uncomplicated cases. The last stage is the sequelae phase, which includes complete recovery in mild cases, while severe cases also improve, but are left with neurological deficits.

Pathology

Several pathological findings in JE are documented. The main alteration is of the neurological system.[34] In animal models, nonsuppurative encephalitis could be experimentally induced in piglets inoculated with JE.[35] JE predominately affects the thalamus, anterior horn cells of the spinal cord, cerebral cortex, and cerebellum.[36] During the acute stage of illness, congestion, edema, and herniation are found in the brain. Microscopic lesions include meningeal inflammation, perivascular lymphocytic cuffing, neuronal degeneration and neuronophagia, and microglial proliferation forming glial nodules. These changes usually occur in gray matter and predominantly affect diencephalic, mesencephalic, and brainstem structures. Immunohistochemical studies of human fatal cases have shown a different topographic distribution of JEV in the brain.[37] JE virus antigen can be immune-histochemically detected in the cytoplasm of the nerve cells in the cortex of the frontal and temporal lobes, and in the gray matter of the thalamus and midbrain.[35] At necropsy, CNS findings in JE reflect the inflammatory response virus.[28, 35, 37] The brain parenchyma is congested with focal petechiae or hemorrhage in the grey matter. Blotchy necrolytic zones are seen when survival is prolonged beyond seven days. The white matter usually appears normal. In some patients, the grey matter of the spinal cord is confluent discolored, resembling that of poliomyelitis.[38] In humans, a characteristic involvement of bilateral thalami can be seen by diffusion-weighted imaging. Magnetic resonance imaging lesions can also be detected in basal ganglia, midbrain, Pons, cerebellum, cerebral cortex, and subcortical white matter. After recovery from acute encephalitic illness, these cases usually manifest clinically with typical Parkinsonian features.[39] The distribution of cell types does not vary between the first and the last day of hospitalization, is similar in fatal and nonfatal cases and is unaffected by administration of steroids.[40]

Diagnosis

Patients with JE present vivid signs of acute encephalitic syndrome. There are many possible causes of acute encephalitic syndrome; thus, laboratory confirmation is essential for the accurate diagnosis of JE, which is not a simple process due to the very low viremia.[1] Diagnosis of JE can be made by virus isolation in cell/tissue culture, antigen detection and antibody detection.

Culture

Japanese encephalitis virus can be isolated by intracerebral inoculation of clinical specimens in the suckling mouse brain. Various cell cultures that have been used more recently include primary chick, duck embryo cells and lines of Vero, LLCMK2, C6/36, PK and AP61 cells. The virus can be isolated from the blood of patients in the preneuroinvasive and neuroinvasive phases of the illness, usually not later than six or seven days after the onset of symptoms.[41]

Antigen detection

Various studies have proved the efficacy of antigen detection in CSF using reverse passive hemagglutination,[42] immunofluorescence,[43] and staphylococcal coagglutination tests using polyclonal or monoclonal antibodies[44] in rapid diagnosis of JE. Modified techniques, such as M-IGSS, have been successfully used in the detection of antigen in mononuclear cells of peripheral blood and CSF of patients.[45] Immunohistochemistry has been used to identify viral antigens in the CNS. Histopathology examination is also very helpful for clinical correlation and diagnosis of JEV.

Antibody detection

IgM capture enzyme-linked immunosorbent assay (ELISA) has been the most widely used diagnostic methods for JEV antibody detection.[46] At present, much advancement has been achieved with methods for the early detection of JEV, such as the dipstick method[47] and JEVCheX.[48]

PCR diagnosis

Real-time polymerase chain reaction (PCR) assays provide sensitivity and specificity equivalent to that of conventional PCR combined with Southern blot analysis, and since amplification and detection steps are performed in the same closed vessel, the risk of releasing amplified nucleic acids into the environment is negligible. In general, both PCR and amplified product detection are completed within an hour or less, which is considerably faster than conventional PCR detection methods. By reverse transcriptase PCR, the viral genome can be amplified directly from tissue or blood.[49] A novel nested reverse transcription-polymerase chain reaction (RTPCR)-based kit is described for detecting JEV, in which all reagents are lyophilized in reaction tubes and control RNA is included in each reaction to monitor false negative results.[50]

Another study described and evaluated a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for detecting JEV. The sensitivity of the JEV RT-LAMP assay was in concordance with that real-time RT-PCR, and it was more sensitive than that of conventional RT-PCR. The JEV RT-LAMP was highly specific; no cross-reactivity was found with dengue-2 virus, rabies virus, norovirus, astrovirus, and human enterovirus. The JEV RT-LAMP assay was simpler and less time-consuming compared to the conventional RT-PCR and real-time RT-PCR. The results suggest that the RT-LAMP assay can be applied as a practical molecular diagnostic tool for JEV infection and surveillance.[51]

Treatment

There is no cure for JE and treatment is mainly supportive. Patients are not infectious, but should avoid further mosquito bites. A number of antiviral agents have been investigated, including INF alfa-2a[52] and diethyldithiocarbamate (a low molecular weight dithiol).[53] However, none of these have convincingly been shown to improve the outcome of JE. Effective supportive management has been shown to improve the outcome.[36] Mannitol might be used to reduce intracranial pressure. A significant research on minocycline as an anti-JEV drug is an *in vivo* study that showed that minocycline reduces neuronal apoptosis, microglial activation, active caspase activity, proinflammatory mediators, and viral titer markedly on the ninth day after infection.[54] Another compound that has shown inhibition of JEV replication completely *in vitro* is an N-methyl isatin-b thiosemicarbazone derivative.[55] New therapeutics are on the way of development like use of minocycline, short interfering RNA, arctigenin, rosmarinic acid, DNAzymes etc.[56] However, the immune mechanisms that lead to JE are complex and need to be elucidated further for the development of therapeutics as well as safe and efficacious JE vaccines. Supportive nursing care and prevention of infection during hospitalization are important. Close monitoring is necessary for the physiological disturbances during hospitalization and for sequelae after discharge.

PREVENTION AND CONTROL

The prevention of JE is based largely on two interventions; mosquito control, and by an immunization system.

Vector control

Vector control is important in primary prevention. To control the vector population, classical methods such as insecticide and bed nets are widely applied in endemic areas.[36] Thermal fogging with ultra low volume insecticides such as pyrethrum or malathion has been recommended for the prevention of local transmission during epidemics, particularly in peri-urban areas with marshes. However, the vastness of breeding areas makes larvicidal measures currently impracticable. Vector control alone cannot be relied upon to prevent JE since it

is almost impossible to control mosquito density in the rural areas, which are the worst affected due to poor socioeconomic conditions. Thus, JE control through vector control methods is limited by the sustainability and cost effectiveness of the program.

Immunization

To prevent JE, it is necessary to implement a large-scale immunization of the susceptible human population. Vaccination provides active immunity against JEV. There are several groups of vaccines which are currently in use: purified, formalin-inactivated mouse-brain derived, cell-culture derived inactivated and cell-culture derived live attenuated.[4] Formalin-inactivated vaccines have been safe and effective against JEV for at least 30 years.[57] Of these, the most widely produced and internationally distributed is the mouse-brain derived inactivated vaccine. Several vaccines are still in various stages of development. These include: recombinant protein based vaccines, recombinant virus based/chimeric vaccine and DNA vaccines. Second generation recombinant vaccines are in development with the aim of improving immunogenecity and decreasing adverse reactions.[36]

Adverse reactions

There are several side effects of JE vaccination. Local side effects include tenderness, redness and swelling. Sometimes systematic adverse reactions are also noted after vaccination, such as headache, myalgia, abdominal pain, or skin rash.[4] Occasionally local hypersensitivity reactions (erythema or edema at the injection site) can be observed in some children. Other reactions, such as generalized urticaria, facial angioedema and respiratory distress have been reported in a few people from non-endemic zones after vaccination.[4] Some recipients of the vaccine had, very rarely, major neurological side effects (1 to 2.3 per million recipients: encephalitis, seizures, and peripheral neuropathy).[58]

CONCLUSION

Persistence of Japanese Encephalitis in rural areas of eastern UP is a matter of serious concern, it is need of the hour to control and eradicate the disease. The JE has established its dreadful form as endemic in Gorakhpur; cases are reported on the rise every year especially during rainy and post rainy seasons. Environmental and ecological factors are responsible for the spread of JEV. There is no specific treatment for JE; only prevention can control the disease. The disease is usually prevalent in rural areas among the lower socio-economic groups. Patients are treated on the basis of various symptoms. However, diagnosis of the disease in early stage followed by immediate treatment can save the patient's life. As a precautionary measure, nets and repellents should be used regularly to avoid mosquito bite. Since vaccine is available against the disease, 100% vaccination should be ensured to control the disease. Water should not be allowed to stagnate in the surroundings. Temporary ponds and pools created during the rainy season should be destroyed. Permanent ponds should be treated with insecticide from time to time to kill the mosquito larvae. Besides, *Gambusia aphinis*, a mosquito larvae-feeding fish should be allowed to flourish in the wetlands. Similarly, fungi like *Leptolegina caudata* and *Aphanomyces lavis* parasitizing the mosquito larvae should be used as biological control agents to curb the mosquito population. Since pigs are the major reservoirs of the virus, pig farms should shift outside human settlement areas. Unhygienic conditions also favors development of the disease, hence all attempts should be made to maintain neat and clean environment. Thus the disease can be controlled and eradicated by pursuing the policy of 'prevention is better than cure'.

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