Dengue encephalitis: clinical manifestations and molecular pathogenesis

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Abstract
Background: Dengue encephalitis is an inflammation of the brain parenchyma that occurs due to direct neuronal infiltration by the dengue virus. It is estimated that 50 million dengue infections occur annually and about 2.5 billion people live in dengue endemic countries. When dengue infection is endemic in Brazil, dengue encephalitis is the highest cause of viral encephalitis and also causes meningitis in 10% of cases, in Indonesia is not yet available.
Objective: To know the clinical manifestations and molecular pathogenesis of dengue encephalitis.
Discussion: Neurological manifestations due to the dengue virus are related to differences in viral serotypes that occur in secondary infections with antibodies formed. Dengue virus or DENV will be neutralized by antibodies and immune protection is formed, but when the second infection happens with a different serotype, DENV cannot be neutralized by antibodies which then activate phagocytic cells such as monocytes and carry out viral replication, causing dengue hemorrhagic fever, dengue shock syndrome including neurologic manifestations.
Conclusion: The dengue virus can reach the brain hematogenously. The clinical manifestations of dengue encephalitis are fever, headache, loss of consciousness, meningismus seizures, positive plantar pedis extension pathological reflex, positive signs of frontal lobe symptoms, abnormal posture, cranial nerve paresis, and tetraparesis.

Keywords: dengue encephalitis, clinical manifestation, DENV, dengue virus, encephalitis

Introduction

Dengue encephalitis is an inflammation of the brain parenchyma that occurs due to direct neuronal infiltration by the dengue virus [1]. Dengue virus (DENV) is transmitted through Aedes aegypti and Aedes albopictus mosquitoes. The dengue virus consists of four serotypes, namely DENV 1, DENV 2, DENV 3, and DENV 4 which are members of the flaviviridae family and flavivirus genus [2-5].

There is increasing evidence that dengue virus neurotropism and neurologic manifestations are part of the clinical manifestation of dengue virus infection in 0.5%-7.4% of symptomatic cases [2, 6]. Neurological complications of dengue virus infection are classified into dengue viral encephalopathy, dengue virus encephalitis, immune-mediated syndrome (acute disseminated encephalomyelitis, myelitis, Guillain-Barré syndrome, brachial neuritis, acute cerebellar and others), neuromuscular complications (hypokalemic paralysis, transient benign muscle dysfunction and myositis), dengue-associated stroke and neuro-ophthalmic complications (maculopathy and retinal vasculopathy). Pathogenic mechanisms include systemic complications and metabolic disturbances that result in encephalopathy, direct effects of viruses that trigger encephalitis, and post-infectious immune mechanisms that cause immune-mediated syndromes [7].

Dengue virus is endemic in almost all tropical and subtropical countries. In the last 50 years, the incidence has increased 30-fold with increasing geographical expansion to other countries, development of transportation facilities, and movement of population from urban to rural areas. It is estimated that 50 million dengue infections occur annually and about 2.5 billion people live in dengue endemic countries. The highest incidence is reported in Asia and in Central and South America [8]. Epidemiological studies in India show that 5-21% patients showed manifestation of dengue encephalitis in in endemic area it could affect 4-47% of patients [9].

Data from Vietnam shows 5-6% cases of dengue encephalitis of all cases of encephalitis, which data from India shows 15% of all cases and data from Thailand shows 20% of all cases. When dengue infection is endemic in Brazil, dengue encephalitis is the highest cause of viral encephalitis, namely 47% and also causes meningitis in 10% of cases, data in Indonesia is not yet available [10, 11].

Understanding of clinical symptoms and treatment is needed to establish the diagnosis of dengue encephalitis, so that doctors can provide early and appropriate management, both pharmacologically and non-pharmacologically and prevent further mortality. This literature review aims to review the clinical manifestations and patophysiology of dengue encephalitis.

Generally, the symptoms of encephalitis are fever, headache, focal neurologic deficit, seizures and loss of consciousness beginning with lethargy, confusion, stupor and coma. Encephalitis can also cause behavioral and language disorders, as well as movement disorders, although it is rare [12]. Varatharaj in 2010 reviewed four studies conducted by Kankirawatana *et al* in 2000, Solomon *et al* in 2000, Misra *et al* in 2006 and Kularatne *et al*. in 2008 stated that fever, loss of consciousness, headache, seizures were the most common clinical symptoms in dengue encephalitis. Other clinical symptoms that can appear are meningismus, positive plantar

pedis extension pathological reflex, positive signs of frontal lobe symptoms, abnormal posture, cranial nerve paresis, and tetraparesis [9, 11, 12].

Three of these studies (Kankirawatana *et al* in 2000, Solomon *et al* in 2000 and Kularatne *et al* in 2008) reported that dengue encephalitis can manifest as primary dengue infection and a secondary dengue infection with onset of neurological symptoms within 3-7 days from the onset of fever [9, 11, 12].

The four studies observed clinical outcomes of dengue infection. Research conducted by Kankirawatana *et al*, 2000 stated that all patients (n = 8) had complete recovery with a mean length of stay in hospital for 9 days. Solomon *et al*, 2000 stated that all patients (n = 9) were discharged from the hospital after 1-15 days of treatment with a mean of 6 days with sequelae in 3 patients and complete recovery in 6 patients. Misra *et al*, in 2006 mentioned that from 11 patients with dengue encephalitis, 3 patients died and 3 other patients did not recover completely after 1 month. Kularatne *et al*, 2008 stated that of all dengue encephalitis patients with a total of 6 patients, they regained consciousness within 2 days and had no sequelae [9, 11, 12].

Discussion

Most DENV infections are asymptomatic or mild, but in some cases severe symptoms can occur and involve various organ systems such as gastrointestinal, liver, kidney, cardiovascular, respiratory, musculoskeletal, and neurological. Neurological manifestations of dengue virus include dengue viral encephalopathy, dengue viral encephalitis, post-infectious autoimmune reactions, neuromuscular complications, dengue-associated stroke and neuro-ophthalmic complications [7].

The serotypes DENV-2 and DENV-3 are the main causes associated with cases of encephalitis, meningitis and myelitis, although DENV-1 and DENV-4 have also been identified in cases of encephalitis in some minor cases [10, 22-24]. Neurological manifestations due to dengue virus are related to differences in viral serotypes that occur in secondary infections with antibodies formed. If second infection occur with the same serotype, DENV will be neutralized by antibodies and immune protection is formed, but when the second infection happens with a different serotype, DENV cannot be neutralized by antibodies which then activate phagocytic cells such as monocytes and carry out viral replication, causing clinical manifestations such as dengue haemorrhagic fever, dengue shock syndrome including neurologic manifestations (Figure 1) [10, 11].

The entry of DENV into host cells is mediated mainly by protein E. Virus binds to receptors on the plasma membrane such as the Dendritic Cell-Specific Intercellular adhesion molecule 3-Grabbing Nonintegrin (DC-SIGN) receptor, heparin sulfate receptors, mannose receptors, Cluster of Differentiation receptors 14 (CD14), GRP78, laminin receptor and TAM protein. After binding, the virus becomes internalized via clathrin-mediated endocytosis. Inside the endosome compartment, the acidic pH environment induces a conformational change and rearrangement of the E protein (figure 2) for the fusion of the viral membrane with the endosome membrane releasing positive strand genomic Ribose Nucleic Acid (RNA) into the cytoplasm. RNA genomics are translated into polyproteins. Polyproteins are cleaved into single proteins on the Endoplasmic Reticulum (ER) membrane by viruses and cellular proteases. RNA replication occurs at the ER membrane associated with the viral replication complex consisting of NS1, NS2A, NS2B, NS3, NS4A and NS5. Viral assembly produces immature virus particles. Immature viral particles are transported by secretory pathways. Lower pH in the trans-Golgi network activates host proteases to generate mature DENV particles and release of mature virus into the extracellular space [4].

Several viruses can reach the nerve parenchyma in different ways, namely the entry of infected leukocytes; axonal transport; infection of the olfactory bulb epithelium, entry of other viruses involving damage between endothelial tight junctions and infection of endothelial cells. The latter two ways as well as through infected monocytes are probably the most likely routes used by DENV to reach the nervous system [13]. So far, the most likely mechanism for the spread of the dengue virus to reach the brain is hematogenous. The Blood Brain Barrier plays an important role in supporting viral neuroinvasiveness and neurotropism. The ability of microorganisms to attack the nervous system is known as neuroinvasiveness, while neurotropism is the ability of the dengue virus to infect and replicate in nerve cells [11].

Monocytes are important DENV target cells during secondary DENV infection during the Antibody-Dependent Enhancement (ADE) process, due to the expression of Fc-receptors. The complex formed between the non-neutralizing antibody and the virus can bind to the Fc-receptor and promote infection in surrounding susceptible cells. There is evidence that it is the expression of trypsin-sensitive receptors on monocytes that facilitates DENV infection. DENV can enter monocytes depending on CD14, because lipopolysaccharide (LPS) can inhibit infection. After binding to LPS, Heat Shock Protein (HSP) 70 and HSP90 are clustered around CD14 preventing interaction with DENV. This suggests that HSP70 and HSP90 are part of the receptor complex in monocytes [11].

The primary DENV target cells in the skin are immature Dendritic Cells (DCs) or Langerhans cells. Immature dendritic cells are more sensitive in capturing signals when infection occurs than mature dendritic cells. Dendritic Cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) is the cellular receptor responsible for capturing DENV. DC-SIGN is mainly expressed by immature DCs as a tetramer which is a calcium-dependent C-type lectin family and consists of four domains namely (A) a cytoplasmic domain which is responsible for internalizing signals due to the presence of a dileucine motif; (B) transmembrane domain; (C) seven to eight extracellular repeats involved in DC-SIGN oligomerization; (D) Carbohydrate Recognition Domain (CRD) which can interact with various pathogens. Carbohydrate Recognition Domain (CRD) recognizes high-mannose N-glycans and blood containing fucose antigens. DC-SIGN can bind to various pathogens such as Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Ebola virus and some bacteria, parasites, and yeasts. After antigen capture in the periphery, mature DCs migrate to secondary lymphoid organs. Activated DCs stimulate T cells and produce cytokines and chemokines [5].

In addition to dendritic cells, macrophages also play an important role in the immunopathogenesis of DENV infection as a source of immunomodulatory cytokines. The mannose receptor mediates DENV infection in macrophages by

recognition of glycoproteins on the viral envelope. Mannose receptors are also present on monocyte-derived dendritic cells (MDDC). Anti-MR antibody which can inhibit DENV infection, although to a lesser extent than anti-DC-SIGN antibody. The mannose receptor differs from DC-SIGN in ligand specification and acts as an internalizing receptor for DENV. In addition to type C lectins, CLEC5A (domain of type C lectin family 5, member A) is expressed by the human macrophage channel and thus interacts with DENV and acts as a signal receptor for proinflammatory cytokine release. DC-SIGN and DENV interact in a calcium-dependent manner, CLEC5A binding to the ligand is independent of calcium. Mannose and fucose can inhibit the interaction between CLEC5A and DENV, indicating that the interaction is carbohydrate dependent [5].

Figure 3 describes the pathway of the flavivirus through the Blood Brain Barrier, choroid plexus and glial cells (astrocytes and microglia cells). The journey of the virus through the nerve axons starts from the bite of a flavivirus-infected mosquito on the skin and continues to reach the nervous system. This virus can penetrate the nervous system due to the presence of Sema-7 receptors on nerve axons. The human blood brain barrier (BBB) consists of perivascular astrocytes and microvascular endothelial cells, while the choroid plexus and ependymal cells consist of epithelial cells. The integrity of epithelial barriers such as the Blood Brain Barrier depends on a complex of tight junctions of transmembrane proteins located in the plasma membranes of endothelial cells and adjacent epithelial cells. Tight junctions contain several structural proteins such as claudins (-1, -6, -9) and occludin which are both associated with intermembrane proteins (zone occludens) and junctional adhesion molecules located between epithelial cells and endothelial cells. Flaviviruses can disrupt the BBB indirectly through the effects of systemic inflammatory cytokines or directly by binding to structural proteins such as claudins [14].

Flavivirus can penetrate endothelial cells above through dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin receptors (DC-SIGN) and mannose membrane receptor (MMR) which has a function as an endocytic receptor. MMR is expressed by microglia and astrocytes whereas DC-SIGN is expressed by dendritic cells and central nervous system perivascular cells located in the walls of cerebral blood vessels. DC-SIGN binds to Intracellular Adhesion Molecules (ICAMs) and mannose-associated N-glycans including heparan sulfate [14].

In addition, flaviviruses can also penetrate claudins through the scavenger B1 receptor (SR-B1) which is a type of LowDensity Lipoprotein Receptor (LDLR). Flavivirus also received assistance in the presence of CD81 as a cofactor that causes endocytosis and claudin degradation. This whole process causes the breakdown of tight junctions so that virus-infected lymphocytes and neutrophils can enter the central nervous system, which is known as the Trojan-horse mechanism [14, 15].

Systemic inflammatory cytokines and matrix metalloproteinases (MMP) are a systemic antiviral response process that increases tight junction damage through the ICAM pathway. Damage to the BBB in dengue infection is associated with high levels of metalloproteinase-9 (MMP-9) in plasma. The presence of MMP-9 causes the virus that is free in plasma and in infected cells to enter brain tissue. Flavivirus infection of glial cells (microglia and astrocytes) can be a means for spreading infection to adjacent neurons [11].

Flaviviruses can infect axons at the neuromuscular junction through receptors for Heat Shock Proteins (HSP), Chondritin sulfate (CS) and Glycosaminoglycans (GAG). Sema 7a is a membrane GPI expressed by neurons and axons that can facilitate the entry of viruses into the neuromuscular junction. After the flavivirus is in the axon, axonal transport occurs which ends in the transmission of the virus to neurons through existing synapses, causing nerve cells to undergo a process of apoptosis and neurodegeneration. The dengue virus which occurs in brain would result into dengue encephalitis hematogenously. The clinical manifestations are very important to determine the diagnosis and proper treatment [14].

Conclusion

The dengue virus can reach the brain hematogenously. The clinical manifestations of dengue encephalitis are fever, headache, loss of consciousness, meningismus seizures, positive plantar pedis extension pathological reflex, positive signs of frontal lobe symptoms, abnormal posture, cranial nerve paresis, and tetraparesis. Prognosis in dengue encephalitis depends on the overall condition of the patients during first healthcare encounter, proper diagnosis, and adequate treatment.

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