Dengue infection: A growing global health threat

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SUMMARY **Dengue infection, one of the most devastating mosquito-borne viral diseases in humans, is now a significant problem in several tropical countries. The disease, caused by the four dengue virus serotypes, ranges from asymptomatic infection to undifferentiated fever, dengue fever (DF), and severe dengue hemorrhagic fever (DHF) with or without shock. DHF is characterized by fever, bleeding diathesis and a tendency to develop a potentially fatal shock syndrome. Consistent hematological findings include vasculopathy, coagulopathy, and thrombocytopenia. There are increasing reports of dengue infection with unusual manifestations that mainly involve cerebral and hepatic symptoms. Laboratory diagnosis includes virus isolation, serology, and detection of dengue ribonucleic acid. Successful treatment, which is mainly supportive, depends on early recognition of the disease and careful monitoring for shock. Prevention depends primarily on control of the mosquito vector. Further study of the pathogenesis of DHF is required for the development of a safe and effective dengue vaccine.**

Key Words: Dengue virus, dengue fever, dengue hemorrhagic fever, dengue shock syndrome, DF, DHF, DSS

Introduction

Dengue hemorrhagic fever (DHF) is an acute febrile illness found mainly in children. It is characterized by fever, bleeding diathesis, and a tendency to develop a potentially fatal shock syndrome. The disease is a major public health concern in South and Southeast Asia, the Western Pacific, and Central and South America and is now being reported in other tropical regions. It is one of the leading causes of hospitalization and death in children in countries where dengue disease is prevalent (*1*).

Epidemiology

Dengue is one of the most infectious human viral diseases transmitted by arthropod vectors. Annually, there are an estimated 50-100 million cases of dengue fever (DF) and 250,000 to 500,000 cases of DHF in the world. Over half of the world's population lives in areas at risk of infection. The resurgence of DF and the emergence of DHF is due to unprecedented population growth, unplanned and uncontrolled urbanization, increased air travel, the lack of effective mosquito control, and the deterioration of public health infrastructure (*2*).

The disease's etiologic agents include all four dengue serotypes, which belong to the genus flavivirus of the family *Flaviviridae*. Primary infection with a particular dengue serotype confers long-lasting immunity for that serotype (homotypic immunity). Immunity to other dengue serotypes (heterotypic immunity) lasts for a few months, after which patients are susceptible to heterotypic infection. The principal vector is the mosquito, *Aedes aegypti*, which largely breeds indoors in clean water, and mainly in artificial water containers, and feeds on humans during the daytime.

Extensive epidemiological studies in Southeast Asia have shown that DHF occurs when two or more dengue serotypes are simultaneously endemic or sequentially epidemic and where ecological conditions favor efficient virus transmission via the mosquito vector. Serological studies demonstrate that there is an association between DHF and a secondary antibody response in most cases. These epidemiological and serological observations clearly link DHF to individuals who have had previous dengue infection or alternatively

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have acquired maternal dengue antibody. Nisalak A *et al*. reviewed dengue virus incidence from 1973 to 1999 in Bangkok and demonstrated that all four dengue serotypes can be found circulating in any one year with one predominant serotype emerging and re-emerging as the cause of the epidemic over time. The authors concluded that the pathogenesis of DHF is complex and a product of host determinants, dengue serotype, and environmental factors (*3*).

Dengue virus infection in humans causes a spectrum of illnesses ranging from inapparent or mild febrile illness to severe and fatal hemorrhagic disease. Vertical transmission of dengue virus from mother to child has also been reported (*4*). The severity of DF manifestations increases with age. Infants and children with DF have symptoms ranging from an undifferentiated fever to mild febrile illness, sometimes associated with a rash. Older children and adults frequently suffer a more severe form with the triad of high fever, pain in various parts of the body, and a maculopapular rash. The infection is rarely fatal.

DHF is considered a distinct disease characterized by increased vascular permeability leading to hemoconcentration and dengue shock syndrome (DSS). DHF mostly affects children under 14 years of age and causes significant mortality (*1,5,6*). There is a strong association between good nutritional status and an increased risk of developing DSS. DHF and DSS are rarely seen in children with severe malnutrition (*7*).

Anecdotal records indicate that rare cases of DHF were seen in children with AIDS, while there was no difference in dengue seroprevalence between HIV-infected and healthy children. Further study and clarification is needed to determine whether the protective effect of immune suppression in HIV-infected persons prevents them from acquiring severe dengue disease (*8*).

Pathophysiology and Pathogenesis

The pathophysiological abnormality of DHF is an acute increase in vascular permeability without an inflammatory response, ultimately resulting in hypovolemic shock. Supporting evidence of plasma leakage includes serous effusions found at autopsy, pleural effusions and ascites on chest and abdominal roentgenograms (Figure 1), hemoconcentration, and hypoproteinemia. Immunological response plays a central role in disease pathogenesis since there is little or no viable virus in the circulation during the occurrence of increased vascular permeability, lending further credibility to the position that these events are mediated by processes not directly related to infection but rather to mediators such as cytokines. Elevated levels of cytokines and other markers of activated T cells support the role of cytokines in increased capillary permeability (*9-11*). Increased capillary permeability may be due to gaps in the endothelium, and a recent study suggests that endothelial cells are a target for dengue virus infection and dengue virus-induced IL-6 and IL-8 production by endothelial cells, which may contribute to the increased capillary permeability (*12,13*). An association between cytokine-related gene expression levels and dengue disease severity has been demonstrated. This might serve as a predictor of dengue disease activity, leading to a proper therapeutic plan (*14*).

Activation of the complement system with profound depression of C3 and C5 levels in serum and the formation of immune complexes are found in all cases. The peak in complement activation and the presence of C3a and C5a anaphylatoxins coincide with the onset of shock and plasma leakage. Levels of C3a correlate closely with disease severity (*15*).

"Immune enhancement" of virus infection has been proposed in the pathogenesis of DHF. Dengue virus shows enhanced replication in human and simian peripheral blood leukocytes (most likely monocytes) in the presence of subneutralizing concentrations of specific antibody (*16,17*). Halstead proposed that an immune elimination response, probably mediated by T-lymphocytes, activates these dengue-infected monocytes to release a variety of factors that cause hemorrhage and shock. These include vascular permeability factor, complement activating factors, and

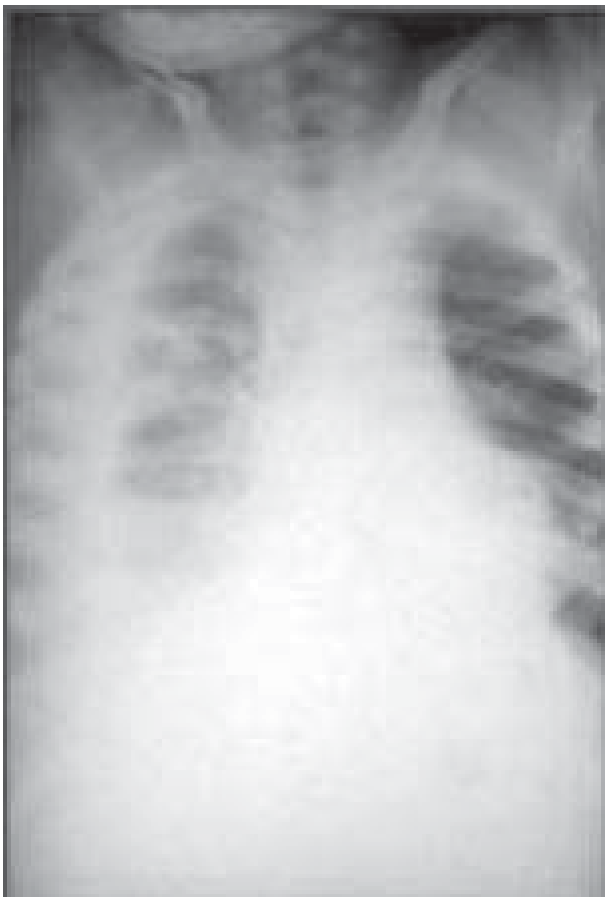

Figure 1. Chest roentgenogram indicating right pleural effusion in a patient with dengue hemorrhagic fever.

thromboplastin (*18-20*).

Other hypotheses suggest that DHF results from infection by a more virulent serotype or strains within serotypes of the virus. DHF has been diagnosed in patients with primary dengue infection that lack pre-existing dengue virus antibodies. Molecular characterization of the dengue virus has suggested that genetic variation between strains may be correlated with clinical manifestation and epidemiological characteristics (*21*). Murgue *et al*. showed that the duration and magnitude of dengue viremia, which did not significantly differ between primary and secondary dengue infection, determine disease severity. Their results did not support the immune enhancement hypothesis (*22*). On the contrary, Sudiro *et al*. did not find a significant difference in maximal plasma viral RNA levels between children with DHF and those with DF (*23*). Vaughn *et al*. determined the duration and magnitude of dengue viremia in serial plasma samples by viral culture and showed that viremia during primary infection was prolonged compared to secondary infection. Their study also showed that the rate of virus clearance was faster in patients experiencing secondary infection than in those with primary infection and was faster in those with DHF than those with DF (*24*).

For the last thirty years, two hypotheses concerning the mechanism of DHF have been debated. Some evidence points to secondary infection or viral virulence. The most plausible explanation is a combination of both hypotheses. Examples of significance of both viral and immunologic factors in dengue pathogenesis come from key studies performed during dengue outbreaks. An investigation of the recent outbreak in Cuba showed that almost all cases of DHF/DSS are secondary DEN-2 infections in adults previously infected by DEN-1 during a 1977-1979 epidemic (*25*). This supports the immune enhancement hypothesis. However, other investigations provide additional interesting information. An outbreak in 1980 in Rayong, Thailand demonstrated that despite the high rate of DEN-1 infection, only DEN-2 virus was recovered from DSS cases, including pre-illness serum specimens from two DEN-1 immune children. A seroprevalence survey prior to the outbreak also revealed that DEN-1 antibodies were the lowest, and yet children with this type were unmistakably prone to developing DSS in comparison to other children (*26*). This suggests that viral factors play a significant role in severe cases. Another good example is the introduction of the Southeast Asian genotype of the virus into some countries in the Americas, *i.e*. Venezuela, Brazil, Columbia, and Mexico. While the native DEN-2 of these countries had not been known to cause DHF/DSS, invasion of Southeast Asian strains coincided with occurrence of some severe cases (*27*). In addition, confirmation of this finding comes from a report of an outbreak in 1995 in Peru due to native strains of DEN-2; this followed an epidemic of DEN-1 five years earlier in the same population. No cases of DHF/DSS were found (*28*).

Certain ethnic groups may be more susceptible or resistant to the dengue virus since DHF is more common in Southeast Asia than in Africa or the Americas. Blacks were found to be relatively resistant to DHF/DSS during the 1981 Cuba outbreak, and there is speculation about a “resistance gene” present in the African population (*29*). Further epidemiological studies are needed to evaluate the effect of immune enhancement with risk factors such as viral virulence, other environmental or infectious agents, genetic susceptibility, or unknown host factors.

Progress in dengue pathogenesis research has been partly hampered by a lack of animal models. The recent development of a mouse model simulating human disease should help accelerate research progress in the field (*30*).

Diagnosis

The incubation period of dengue infection is usually 4-7 days but can range from 3 to14 days (*31*). Clinical and laboratory criteria for the diagnosis of DHF as established by the World Health organization are as follows (*32*).

Clinical criteria

- Fever - acute in onset, high, continuous, lasting for 2-7 days.
- Hemorrhagic manifestations - a positive tourniquet test, petechiae, purpura, ecchymosis, epistaxis, bleeding gums, hematemesis, melena.
- Hepatomegaly - observed in 90-96 and 60 percent of Thai children and adults, respectively.
- Shock - a rapid, weak pulse with a narrow pulse pressure; hypotension with cold, clammy skin and restlessness.

In patients with DHF grade I, a positive tourniquet test is the only hemorrhagic manifestation, whereas spontaneous bleeding occurs in DHF grade II. Patients with circulatory failure (narrowing of the pulse pressure and a rapid and weak pulse) have DHF grade III. Patients in profound shock (no detectable blood pressure and pulse) have DHF grade IV. Grades III and IV DHF are also referred to as DSS. In the initial febrile period, flushing of the skin is common and a centrifugal maculopapular rash is less common. In the convalescent stage, a confluent petechial rash with round pale areas of normal skin is commonly seen.

Clinical manifestations of dengue infection vary with age as DSS is more common in children than in adults. Infants with dengue infection present more frequently with convulsions, diarrhea, rash, cyanosis, and splenomegaly (*33-35*).

Laboratory criteria

- Thrombocytopenia (platelet count < 100,000/mm^3)
- Hemoconcentration (hematocrit increased by > 20%)

These criteria provide conclusive diagnosis in 90 percent of patients. The presence of the first two or three clinical criteria with thrombocytopenia and hemoconcentration is sufficient to establish the diagnosis of DHF. The diagnosis is highly likely to have DHF when shock occurs with high hematocrit levels, except in patients with severe bleeding (*32*).

Other common laboratory findings are hypoproteinemia, hyponatremia, and elevation of hepatic enzymes and blood urea nitrogen levels. Metabolic acidosis may be found in patients with prolonged shock. White blood cell count is variable, ranging from leukopenia to mild leukocytosis with an increase in the percentage of lymphocytes and the presence of atypical forms (*36-38*).

Hematological findings include vasculopathy, reduction of several coagulation factors, reducted platelet count, and platelet dysfunction (*39*). Thrombocytopenia could be caused by the virus reducing hematopoietic progenitor cell growth and a subsequent decrease in thrombopoiesis (*40*). Interaction of the virus with the platelets through IgM anti-platelet autoantibody has been demonstrated in dengue patients (*41*). Disseminated intravascular clotting can occur in all grades of dengue infection. However, only in severe cases and in those with prolonged shock is disseminated intravascular coagulopathy a cause of uncontrolled bleeding and death (*39*). The tendency toward bleeding should be monitored in any dengue patients since it may cause severe, uncontrollable hemorrhaging (*4,42*). The pathogenesis of bleeding in dengue patient is not fully understood. The extent of endothelial cells, coagulation, and fibrinolysis activation in children with dengue infection seems to be correlated with dengue disease severity (*43*). The d-dimer, a specific marker for cross-linked fibrin, is often used as a marker for DIC and is significantly correlated with dengue disease severity (*44*).

The etiological diagnosis of dengue infection can be confirmed by serological tests or by isolation of the virus from blood specimens. Virus isolation is easier during the early febrile phase (*3,45*). Enzyme-linked immunosorbent assay (ELISA) for dengue antibodies is an improvement over the previous hemagglutination inhibition assay for serological confirmation (*46*). Commercial kits based on a serological approach to dengue diagnosis are available and need further testing with regard to sensitivity and specificity before they can be recommended for routine use. Detection of viral RNA by reverse transcription polymerase chain reaction is a highly sensitive technique for the early diagnosis of dengue infection (*47*). A pilot evaluation of diagnostic values of ELISA and reverse transcription polymerase chain reaction from oral specimens has yielded promising results (*48*). Collection of oral specimens is less invasive and may be more acceptable.

Unusual Manifestations

There have been increasing reports of dengue infection with unusual manifestations including encephalopathy, encephalitis, and fulminant hepatitis. Patients with these manifestations tend to be younger and have a significantly higher mortality rate than those with the more common form of the infection (*49-57*). Implications of severe infection such as cerebral edema, acidosis, fulminant hepatic failure, and bleeding may lead to encephalopathy (*51,52*).

Occasionally, dengue viruses can cross the blood-brain barrier and lead to encephalitis (*50,52-55*). Neurological manifestations of dengue include alteration of consciousness, seizures, pyramidal tract signs, meningeal signs, and headaches. Cerebrospinal fluid (CSF) examination shows lymphocytic pleocytosis in 20 percent of patients while the presence of anti-dengue IgM antibodies in CSF is detected in few patients. Dengue antigens have been found in the brain in fatal cases, but pathological evidence of encephalitis is rarely seen (*50,52*). Magnetic resonance imaging reveals cerebral edema in most patients evaluated but rarely indicates encephalitis-like alterations (*54*). In endemic areas, dengue should be considered in patients who present with clinical features of encephalitis, regardless of whether classical manifestations of dengue are present (*55*).

Hepatocellular injury manifested by hepatomegaly, elevation in alanine aminotransferase, and mild coagulopathy are common in DHF and even in DF, although hepatomegaly is absent (*56*). Acute liver failure is a major cause of death. Virus culture, immunocytochemistry, and electron microscopy confirm that dengue virus replicates in the liver. Whether liver injury is a direct effect of virus replication or a consequence of host response to infection is still unclear (*57*).

In all cases of unusual findings or unusual manifestations of dengue infection, a search should be conducted for a coinfection that may modify the clinical presentation. Such a coinfection could result in missed or delayed diagnosis and treatment of dengue infections and possibly be misinterpreted as an unusual manifestation (*58*).

Treatment

Treatment of dengue infection is symptomatic and supportive. In most cases, early and effective replacement of lost plasma with fluid and electrolyte solutions, plasma, and/or plasma expander results

in a favourable outcome. A single high dose of methylprednisolone does not reduce mortality in severe DSS and is not required for conventional critical care (*59*). The outcome depends on early recognition of infection and careful monitoring. Serial determinations of platelet and hematocrit levels are essential for the early recognition and prevention of shock. In rare cases, blood products are required. Blood transfusion is indicated for patients with significant clinical bleeding mainly from the gastrointestinal tract. Fresh frozen plasma and/or platelet concentrate are required when consumptive coagulopathy causes massive bleeding. Persistent shock despite adequate fluids and a decline in the hematocrit level suggest significant clinical bleeding requiring prompt treatment. Disseminated intravascular coagulation occurs in cases with severe shock and may play an important role in the development of massive bleeding and irreversible shock. Coagulation tests should be monitored in all cases of shock to document the onset and severity of disseminated intravascular coagulation. Blood grouping and matching should be carried out as a routine precaution for every patient in shock.

The rate of fluid infusion needs to be carefully tailored according to the patient's vital signs, hematocrit, and urine output. In general, there is no need for fluid therapy beyond 48 h after the cessation of shock. Reabsorption of extravasated plasma takes place, manifested by a further drop in the hematocrit level after intravenous fluids have been halted. This drop may cause hypervolemia, pulmonary edema, or heart failure if more fluids are given. An extremely important point is that a drop in the hematocrit level at this stage not be taken as a sign of internal hemorrhaging. A strong pulse and blood pressure, with a wide pulse pressure and diuresis, are good vital signs. They rule out the likelihood of gastrointestinal hemorrhaging as is mostly found during the shock stage (*60*).

Post-mortem Findings

In DHF, the most frequent gross anatomical findings post-mortem are petechial hemorrhages especially of the mucosa of the gastrointestinal tract, atrophy of the thymus and an increase in extravascular fluid with effusions in serous cavities, increased weight of organs, and edema most commonly in the retroperitoneum. Microscopically, there is no vasculitis. There is widespread evidence of diapedesis of red blood cells around blood vessels and interstitial edema in all tissues of the body. In capillaries and precapillary arterioles, swelling of some endothelial cells suggests that functional alterations are accompanied by structural derangements. Evidence of intravascular thrombosis is seen in some cases. There are degrees of coagulative necrosis of hepatocytes, varying from scattered cells within liver lobules to submassive and massive involvement. Necrotic areas contain cells identical to the Councilman bodies seen in yellow fever that are accompanied by activation of Kupffer cells (*61*).

Prevention

Prevention of DHF depends on the control of the mosquito vector by limiting its breeding places and treatment of stored water with larvicide. These measures against dengue are effective only with a high level of government commitment, education, and community participation (*2*).

An effective, safe, affordable vaccine against the dengue virus is not an immediate prospect since pre-existing heterotypic antibodies within the host increase the risk for DHF and DSS. An effective vaccine will have to offer protection against all 4 serotypes of the virus. Dengue vaccines under development include the first generation live attenuated tetravalent vaccine developed at Mahidol University in Thailand, a second-generation attenuated vaccine created by genetic engineering, and vaccines created using new molecular approaches (*62-65*).

DHF has exhibited geographical expansion in Thailand and other tropical countries, where maintenance of well-documented clinical, epidemiological, and virological descriptions of the syndrome is crucial. Biological and social studies are essential to the development of effective mosquito control, treatments such as medications to reduce capillary leakage, and a safe vaccine.

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