PEDIATRIC INFECTIOUS DISEASE SOCIETY OF THE PHILIPPINES

PIDSP JOURNAL

Vol.13 No.2
July-Dec 2012

Vol.13 No.2
July-Dec 2012

INVITED REVIEW

Dengue: A Growing Global Health Threat

AUTHORS: Usa Thisyakorn M.D., Chule Thisyakorn, M.D.
Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

KEYWORDS
dengue, dengue fever

CORRESPONDENCE:
Dr. Usa Thisyakorn
Email: usa.thi@mahidol.ac.th

ABSTRACT
Dengue infection, one of the most devastating mosquito-borne viral diseases in humans, is now a significant problem in many 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. Dengue infection with organ impairment mainly involves central nervous system and liver. Consistent hematological Findings include vasculopathy, coagulopathy, and thrombocytopenia. 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. A severity-based revised dengue classification for medical interventions has been developed and validated in many countries. Prevention depends primarily on control of the mosquito vector. The feasibility of a dengue vaccine is high.

Usa Thisyakorn is presently a Professor of Pediatrics at Chulalongkorn University, an advisor of both Faculty of Tropical Medicine, Mahidol University and Department of Health, Bangkok Metropolitan Administration. She is now concentrating on research projects on dengue. In 1989 she received Rockefeller grant for dengue research at the Centers for Diseases Control and Prevention in Atlanta. In 2000, under Professor Thisyakorn's guidance as Chair of the medical committee on the *Save a child's life from AIDS* project, the project was selected as one of the UNAIDS best practices.

She has served as the editorial board of several medical journals and has contributed over 100 indexed publications to date. For her contributions, she has received several awards including 'Woman of the Year' from the Foundation for Thai Society, Scientific Awards from the Elizabeth Glaser Pediatric AIDS Foundation, AmFAR, U.S.A. and the Outstanding Asian Pediatrician Award from Asia Pacific Pediatric Association.

INTRODUCTION

Dengue is the most devastating mosquito-borne viral diseases in humans. The disease, caused by the four dengue virus serotypes, ranges from asymptomatic infection to undifferentiated fever, dengue fever (DF), and 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. Dengue infection with organ impairment mainly involves the central nervous system and liver. The disease is a major public health concern in several countries with the potential spread of the disease to non-endemic areas. It is one of the leading causes of hospitalization in children placing tremendous pressure on strained medical resources with an associated major economic and social impact in countries where dengue disease is prevalent[1-5].

EPIDEMIOLOGY

Dengue is the most common arboviral infection of humans transmitted by Aedes mosquitoes, principally *Aedes aegypti*. These mosquitoes largely breed indoors in clean water, mainly in artificial water containers, and feeds on humans during the daytime. There are four antigenically distinct serotypes of dengue virus (DEN 1, 2, 3 and4) 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) while immunity confers to other dengue serotypes (heterotypic immunity) lasts for a few months, after which patients are susceptible to heterotypic infection.

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 have acquired maternal dengue antibody[6]. Nisalak 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. The authors concluded that the pathogenesis of DHF is complex and a product of host determinants, dengue serotype, and environmental factors[7].

Dengue virus infection in humans causes a spectrum of illnesses ranging from inapparent infection, mild febrile illness to severe and fatal DHF. The clinical spectrum of the infection undermines surveillance activities since the majority of cases are asymptomatic and go undetected. These cases could be an important source of infection for vectors and for risk of developing severe dengue, if a secondary infection occurs.

Exposure to dengue infection generates life-long, serotype specific immunity, but subsequent infection with other serotypes may increase the risk of severe disease. A large proportion of infected individuals have the mild form of the disease which is perceived as not serious enough to warrant health care and may be misdiagnosed and under-reported. Annually, at the global level there are 2.5 billion people, two fifths of the world's population in tropical and subtropical countries at risk. An estimated 50-100 million cases of dengue infections and 500,000 cases are hospitalized with DHF mainly among children with the case fatality rate exceeding 5% in some areas. Dengue is endemic in more than 100 countries with the South-East Asia and Western Pacific regions most seriously affected. The global increase in dengue cases and also the potential spread of the disease to non-endemic areas are due to factors such as atmospheric composition, climate change and human movement. Even with estimates of disease burden increasing,

dengue is widely under-reported due to misdiagnosis and inconsistencies in diagnostic and surveillance systems. Dengue has spread into new geographical areas affecting both children and adults despite being significantly under-reported. Over half of the world's population lives in areas at risk of infection. Complex disease presentation and sudden development of hemorrhagic symptoms in seemingly stable patients can cause fatal outcomes even in well-prepared hospitals[8,9].

INCREASE IN DENGUE INFECTION

The reasons for the apparent upsurge in dengue are probably multifactorial. Vector efficiency of Aedes aegypti increases with increasing temperature for dengue virus . This may explain the increasing dengue patients during the dry hot season. Possibly global warming may contribute to wider spread of dengue infection. The availability of more water and higher humidity, including higher biting rates may augment the epidemic during rainy period. Weather patterns, with average temperatures and increases in rainfall, are classically seen as possible causes. Many factors influence the epidemiologic patterns of dengue. These include the climate, movements of mosquitoes, the type of circulating dengue viruses, environmental factors such as temperature and humidity, and human behavior. Well-targeted operational researches, such as population-based epidemiological studies with clear operational objectives, are urgently needed to make progress in control and prevention. Dengue remains predominantly a pediatric disease but the trend towards higher rates in older children and adults during the last decade is incompletely understood. This may be due to less frequent epidemics in the last few decades so that second exposure to dengue virus is postponed. The trend has important implications for control and prevention[10].

Vertical transmission of dengue virus from mother to child has also been reported[11]. The severity of DF manifestations increases with age. DF causes fever, rash, muscle or joint pain, headache, eye pain but is rarely fatal. DHF is considered a distinct disease characterized by increased vascular permeability leading to leakage of plasma and dengue shock syndrome (DSS)[1-5]. Unusual manifestations of patients with severe organ involvement such as liver, kidneys, brain or heart associated with dengue infection have been increasingly reported in DHF and also in dengue patients who do not have evidence of plasma leakage. These unusual manifestations may be associated with coinfections, comorbidities or complications of prolonged shock. Exhaustive investigations should be done in these cases.

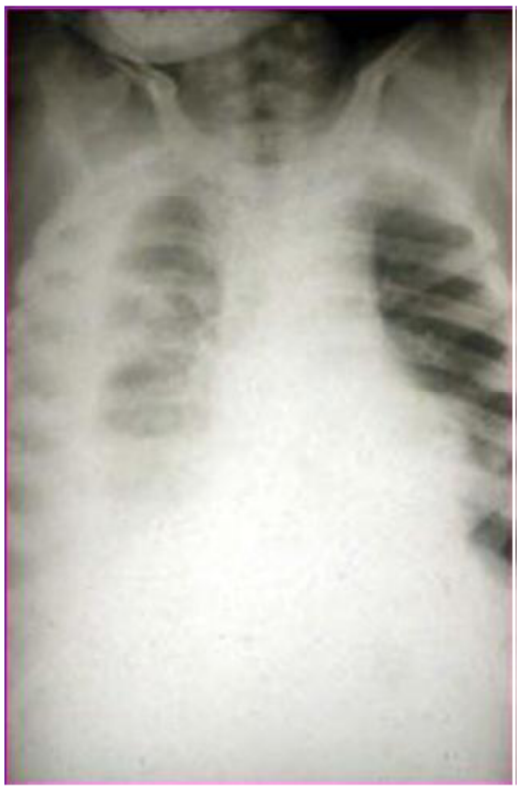
There is a strong association between good nutritional status and an increased risk of developing DSS. DHF and DSS are rarely seen in children with severe malnutrition[12]. Anecdotal records indicate that rare cases of DHF were seen in children with AIDS, while there was no difference in dengue seroprevalence 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[13].

PATHOPHYSIOLOGY OF DHF

The pathophysiology of DHF is an acute increase in vascular permeability resulting in hypovolemic shock. Supporting evidence of plasma leakage includes serous effusions found at autopsy, pleural effusion 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 process 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 which may be due to

gaps in the endothelium. A complex interaction between the dengue virus, immune cells, endothelial cells barrier function in regulating vascular permeability and the activation of the coagulation system occurs in DHF.

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



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.

"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. It was 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 thromboplastin.

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 dengue virus has suggested that genetic variation between strains may be correlated with clinical manifestation and epidemiological characteristics. One study showed that the duration and magnitude of dengue viremia, which did not significantly differ between primary and secondary dengue infection, determine disease severity and the results did not support the immune enhancement hypothesis. This is contrary to another study which found no significant difference in maximal plasma viral RNA levels between children with DHF and those with DF. Another study 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. The 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.

For several decades, 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 outbreak in Cuba showed that almost all cases of DHF/DSS are secondary DEN-2 infection in adults previously infected by DEN-1 during 1997-1979 epidemic. 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. 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 the Southeast Asian strains coincided with occurrence of some severe cases. 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 by an epidemic of DEN-1 five years earlier in the same population. No cases of DHF/DSS were found.

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 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. 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 [1-5, 14,15].

An association between cytokine-related gene expression levels and dengue disease severity has been demonstrated which might serve as a predictor of dengue disease activity, leading to a proper therapeutic plan[16].

DIAGNOSIS

The incubation period of dengue infection is usually 4-7 days but can range from 3 to 14 days. Clinical and laboratory criteria for the diagnosis of DHF/DSS as established by the World Health Organization are shown in List 1.

List 1

Clinical manifestations

- Fever: acute onset, high and continuous, lasting two to seven days in most cases.
- Any of the following hemorrhagic manifestations including a positive tourniquet test, petechiae, purpura, ecchymosis, epistaxis, gum bleeding, and hematemesis and/or melena
- Enlargement of the liver is observed at some stage of the illness in 90%-98% of children. The frequency varies with time and/or the observer.
- Shock, manifested by tachycardia, poor tissue perfusion with weak pulse and narrowed pulse pressure or hypotension with the presence of cold, clammy skin and/or restlessness.

Laboratory findings

- Thrombocytopenia (100,000 cells per mm3 or less).
- Hemoconcentration; hematocrit increase of >20% from the baseline of patient or population of the same age.

The severity of DHF is classified into 4 grades. The presence of thrombocytopenia with concurrent hemoconcentration differentiates grade I and grade II DHF from DF. 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 with narrowing of the pulse pressure, a rapid and weak pulse have DHF grade III. Patients in profound shock without detectable blood pressure and pulse have DHF grade IV. Grade 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[2].

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 leucopenia to mild leukocytosis with an increase in the percentage of lymphocytes and presence of atypical forms[17-19].

Hematological findings include vasculopathy, reduction of several coagulation factors, reduced platelet count, and platelet dysfunction[20]. Thrombocytopenia could be caused by the virus reducing hematopoietic progenitor cell growth and subsequent decrease in thrombopoiesis[21]. Interaction of the virus with the platelets through IgM anti-platelet autoantibody has been demonstrated in dengue patients[22]. 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[9,23]. The tendency toward bleeding should be monitored in any dengue patients since it may cause severe, uncontrollable hemorrhage. The pathogenesis of bleeding in a 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[24]. 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[25].

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[7,26]. Enzyme-linked immunosorbent assay (ELISA) for dengue antibodies is an improvement over the previous hemagglutination inhibition assay for serological confirmation[27]. Commercial kits based on a serological approach to dengue diagnosis are available for routine use. Detection of viral RNA by reverse transcriptase polymerase chain reaction is a highly sensitive technique for the early diagnosis of dengue infection[28]. A pilot evaluation of diagnostic values of ELISA and reverse transcription polymerase chain reaction from oral specimen has yielded promising results. Collection of oral specimens is less invasive and may be more acceptable[29].

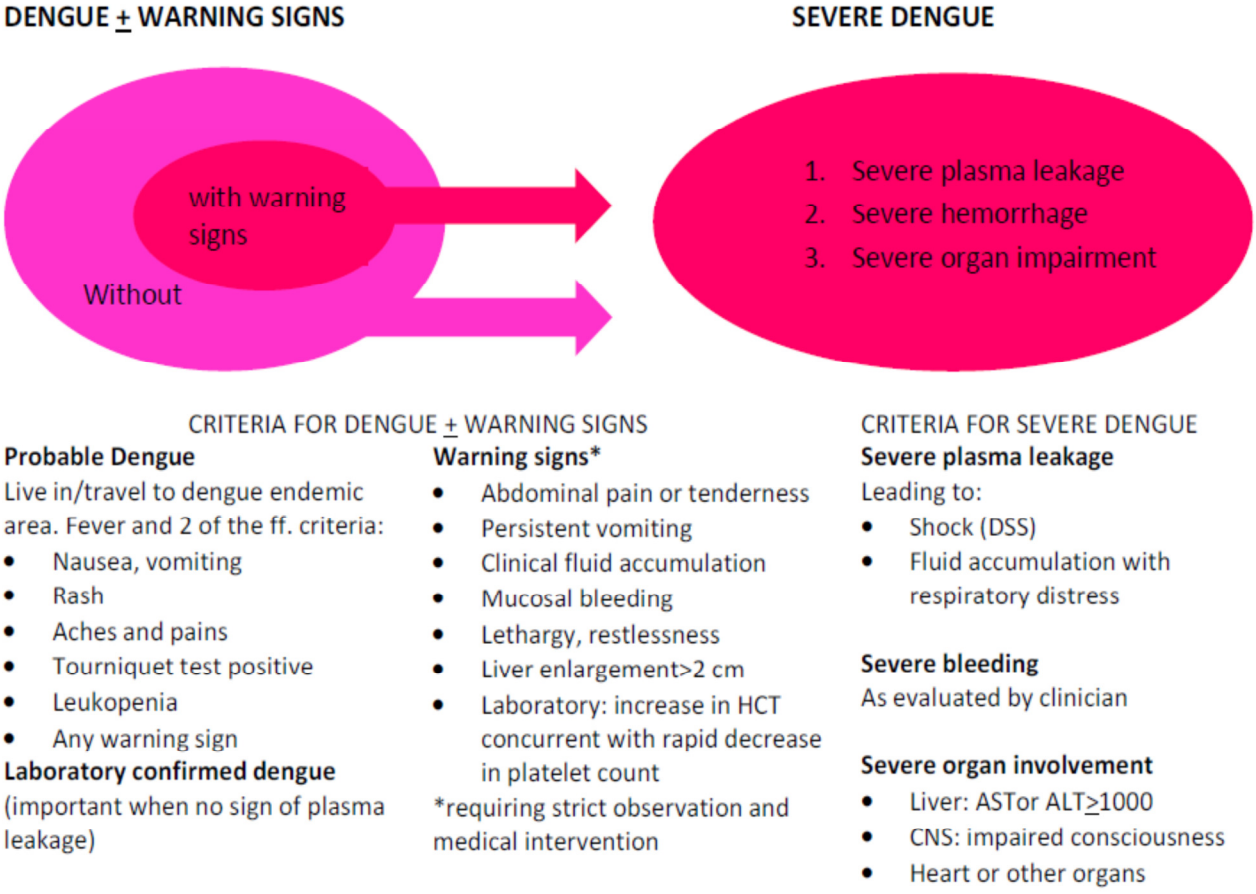
Clinical manifestations of dengue infection vary with age as DSS is more common in children than in adults. Infants with dengue infection present more frequently with convulsions, diarrhea, rash, cyanosis, and splenomegaly [30, 31].

The 1997 WHO dengue case classification according to clinical manifestations and laboratory findings[32] may not clearly correlated with disease severity. It is therefore recommended to establish a validated dengue classification using levels of severity new classification. The new suggested Dengue Case Classification by the World Health Organization is seen in Figure 2. Several studies were done to compare the two classification systems regarding applicability in clinical practice and for surveillance. It was shown that the new classification has a high potential for facilitating dengue case management and surveillance. Further evaluation is necessary[33-35].

EXPANDED DENGUE SYNDROME/ISOLATED ORGANOPATHY (UNUSUAL MANIFESTATION)

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 infection9,[36-38].

Figure 2. Suggested Dengue Case Classification and Levels of Severity



Occasionally, dengue viruses can cross the blood-brain barrier and lead to encephalitis. 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 antigen has been found in the brain in fatal cases, but pathological evidence of encephalitis is rarely seen. Magnetic resonance imaging reveals cerebral edema in most patients evaluated but rarely indicates encephalitis-like alteration. 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[39-42].

Hepatocellular injury manifested by hepatomegaly, elevation in alanine aminotransferase, and mild coagulopathy are common in DHF and even in DF, although hepatomegaly is absent. 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 [43-44].

In all cases of unusual manifestations of dengue infection, a search should be conducted for coinfections, comorbidities particularly in adults with dengue infection or complications of prolonged shock with organopathy[45].

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 favorable outcome. 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 disseminated intravascular coagulation (DIC) causes massive bleeding. Persistent shock despite adequate fluids and a decline in the hematocrit level suggest significant clinical bleeding requiring prompt treatment. DIC occurs in case 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 DIC. Blood grouping and matching should be carried out as a routine precaution for every patient in shock [2, 46].

In moderate DSS, there is no significant difference between crystalloids (Ringer's lactate solution/normal saline solution) and colloids (dextran/hydroxyethyl starch/gelatin) in the initial fluid resuscitation. The decision on choosing the appropriate type of fluid depends on the physician's judgment. Some data suggest colloids as the fluid of choice for the initial resuscitation in severe DSS, but there is no significant evidence to support this data. Moreover, any type of colloid is not significantly different from one another[47].

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 hours after the cessation of shock. Reabsorption of extravasated plasma takes place, manifested by a further drop in the hematocrit level. Excessive fluids during the recovery phase may cause hypervolemia, pulmonary edema, or heart failure. An extremely important point is that a drop in the hematocrit level at this stage not be taken as a sign of internal hemorrhage. A strong pulse and blood pressure, with a wide pulse pressure and diuresis, are good vital signs. They rule out the likelihood of gastrointestinal hemorrhage as is mostly found during the shock stage [2,46].

POST-MORTEM FINDINGS

In DHF, the most frequent gross anatomical post-mortem findings 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[48].

PREVENTION

Prevention of dengue 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. Ultimately, an effective and long lasting vaccine is needed.

Due to the unique challenges of dengue, including the need to provide protection against the four antigenically-distinct serotypes of the viruses, no vaccine has been licensed to protect against this disease, despite more than six decades of research[2,9].

There is currently neither an approved preventive vaccine nor a specific anti-viral treatment against dengue. Main public health preventive interventions consist of mosquito control, which is currently used in endemic countries, and use of vector repellents, both generally with limited results. Development of a dengue vaccine is seen as the best hope to fight this disease[2,9].

Better understanding of new paradigms for a changing dengue epidemiology will not only feed into operational policy for dengue control but also provide fertile terrain for vaccine application strategies in the future. Epidemiological data of this kind will be both valuable for dengue vaccine efficacy trials and for consideration of age group to be vaccinated which will lead to universal dengue vaccine implementation in the future[10,49].

A global strategy aimed at increasing the capacity for surveillance and outbreak response, changing behaviors and reducing the disease burden using integrated vector management in conjunction with early and accurate diagnosis has been advocated. Antiviral drugs and vaccines that are currently under development could also make an important contribution to dengue control in the future[50].

REFERENCES

1. Thisyakorn U, Pengsaa K. Preface Proceedings of the Dengue Symposium at the 9th International Congress of Tropical Pediatrics. Pediatrics and International Child Health 2012; 32: S1
2. Hemungkorn M, Thisyakorn U, Thisyakorn C. Dengue infection: A growing global health threat. BioScience Trends 2007; 1: 90-6.
3. Thisyakorn U, Thisyakorn C. Dengue hemorrhagic fever. In: Textbook of Travel medicine and Health. 2nd ed. Dupont HL, Steffen R, eds. B.C. Decker Inc., Hamilton, 2001; pp. 312-14
4. Thisyakorn U, Thisyakorn C. Studies on flaviviruses in Thailand. In: Progress in Clinical Biochemistry: Proceedings of the 5th Asian-Pacific Congress of Clinical Biochemistry. September 29-October 4, 1991. Kobe, Japan (Miyai K, Kanno T, Ishikawa E, eds.) Excerpta Medica, Amsterdam, 1992; pp. 985-987.
5. Thisyakorn U, Thisyakorn C. Diseases caused by arbovirus-dengue haemorrhagic fever and Japanese B encephalitis. Med J Aust 1994; 160: 22-26.
6. Rigau-Perez JG, Clark GG, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. Lancet 1998; 352: 971-977.
7. Nisalak A, Endy TP, Nimmannitya S, et al. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from1973 to 1999. Am J Trop Med Hyg 2003; 68: 191-202.
8. Guzman MG, Halstead SB, Artrob H, et al. Dengue: a continuing global threat. Nat Rev Microbiol 2010; 8: S7-16.
9. World Health Organization, Regional office for South-East Asia. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded edition 2011. (SEARO Technical Publication Series No. 60).
10. Tanayapong S, Pengsaa K, Thisyakorn U. Changing epidemiology of dengue patients in Ratchaburi, Thailand. (submitted).
11. Thaithumyanon P, Thisyakorn U, Deerojanawong J, Innis BL. Dengue infection during parturition complicated by severe hemorrhage and vertical transmission. Clin Infect Dis 1994; 18: 248-249.
12. Thisyakorn U, Nimmannitya S. Nutritional status of children with dengue hemorrhagic fever. Clin Infect Dis 1993; 16: 295-297.
13. Vithessonthi K, Hemungkorn M, Nisalak A, Stephen T, Thisyakorn U. Seroepidemiology of dengue virus infection in HIV-infected children in comparison to healthy children. (submitted).
14. Martin BEE, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. Clin Microbiol Rev 2009; 22: 564-581.
15. Srikiatkhachorn A. Plasma leakage in dengue haemorrhagic fever. Thromb Haemost 2009; 102: 1042-1049.
16. Waidab W, Suphapeetiporn K, Thisyakorn U. Cytokine-related gene expression in the peripheral blood and dengue infection severity. American Society of Tropical Medicine and Hygiene 57th Annual Meeting. December 7-11, 2008. New Orleans, USA.
17. Wells RA, Scott RM, Pavanand K, Sathitsathein V, Chiamudom U, Macdermott RP. Kinetics of peripheral blood leukocyte alteration in Thai children with dengue hemorrhagic fever. Infect Immun 1980; 28: 428-433.

18. Thisyakorn U, Nimmannitya S, Ningsanond V, Soogarun S. Atypical lymphocyte in dengue Hemorrhagic fever: its value in diagnosis. Southeast Asian J Trop Med Public Health 1984; 15: 32 -36.
19. Wongsawat J, Pancharoen C, Ningsanond V, Thisyakorn U. Kinetics of peripheral blood Leukocytes alterations in Thai children with dengue infection. The1st International Conference On dengue and dengue haemorrhagic fever, Chiangmai, Thailand. November 20-24, 2000. pp.37. (Abstract)
20. Mitrakul C, Thisyakorn U. Hemostatic studies in dengue hemorrhagic fever. In: Proceedings of the 1st International Congress of Tropical Pediatrics; 1989 November 8-12; Bangkok, Thailand (Suvatte V, Tuchinda M, eds.). Ruen Kaew Press, Bangkok, Thailand 1989; pp. 215-217.
21. Murgue B, Cassar O, Guinon M, Chungue E. Dengue virus inhibits human hematopoietic progenitor growth in vitro. J Infect Dis 1997; 175: 1497-1501.
22. Lin CF, Lei HY, Liu CC, et al. Generation of IgM anti-platelet autoantibody in dengue patients. J Med Virol 2001; 63: 143-149.
23. Bunyavejchevin S, Tanawattanacharoen S, Techakraichana N, Thisyakorn U, Tannirandon Y, Limpaphayom K. Dengue hemorrhagic fever during pregnancy: antepartum, intrapartum and postpartum management. J Obstet Gynaedol Res 1997; 23: 445-448.
24. Sosothikul D, Seksarn P, Pongsewalak S, Thisyakorn U, Lusher J. Activation of endothelial cells, Coagulation and fibrinolysis in children with dengue virus infection. Thromb haemost 2007; 97: 627-634.
25. Setrkraising K, Bongsebandhu-phubhakdi C, Voraphani N, Pancharoen C, Thisyakorn U, Thisyakorn C. D-dimer as an indicator of dengue severity. Asian Biomedicine 2007; 1: 53-57.
26. Scott RM, Nisalak A, Chiamudom U, Seridhoranakul S, Nimmannitya S. Isolation of dengue viruses from peripheral blood leukocytes of patients with hemorrhagic fever. J Infect Dis 1980; 141: 1-6.
27. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, et al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am J Trop Med Hyg 1989; 40: 418-427.
28. Deubel V, Laille M, Hugnot JP, et al. Identification of dengue sequences by genomic amplification: rapid diagnosis of dengue virus serotypes in peripheral blood. J Virol Methods 1990; 30: 41-54.
29. Pupaibool J, krajiw S, Arunyingmongkol K, et al. Diagnosis of dengue infection by enzyme-linked immunosorbent assay and severe transcription polymerase chain reaction from oral specimens. Presented at the 16th European congress of Clinical Microbiology and Infectious Diseases. April 1-4, 2006, Nice, France.
30. Hattasingh W, Thisyakorn U, Thisyakorn C. The different manifestations of dengue fever according to age groups. (submitted).
31. Tantawichien T, Thisyakorn U, Pisarnpong A, Israsena S, Suwangool P. Dengue fever and dengue hemorrhagic fever in adults. The first International Conference on dengue and dengue haemorrhagic fever, Chiangmai, Thailand. November 20-24, 2000 pp. 16-17. (Abstract)
32. World health Organization. Dengue haemorrhagic fever: diagnosis, treatment and control. 2nd ed. WHO, Geneva 1997.
33. Barniol J, Gaczkowski R, Barbato EV, et al. Usefulness and applicability of the revised dengue case classification by disease: multi-centre study in 18 countries. BMC Infect Dis 2011; 11:106.
34. Alexander N, Balmaseda A, Coelho IC, Dimaano E, Hien TT, Hung NT, Janisch T, Kroeger A, Lum LCS, Martinez E, Siqueira JB, Thuy TT, Villalobos I, Villegas E, Wills B. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. Trop Med Int Health 2011; 16:936-48.
35. Narvaez F, Gutierrez G, Pérez MA, Elizondo D, Nuñez A, Balmaseda A, Harris E. Evaluation of the traditional and revised WHO classifications of dengue disease severity. PLoS Negl Trop Dis. 2011; 5:e1397.
36. Thisyakorn U, Thisyakorn C. Dengue hemorrhagic fever: unusual manifestation and problems in management. JAMA SEA 1994; 10: 102-103.
37. Thisyakorn U, Thisyakorn C. Dengue infection with unusual manifestations. J Med Assoc Thai 1994; 77: 410-413.
38. Nimmannitya S, Thisyakorn U, Hemsrichart V. Dengue hemorrhagic fever with unusual manifestations. Southeast Asian J Trop Med Public Health 1978; 18: 398-406.
39. Thisyakorn U, Thisyakorn C, Limpitikul W, Nisalak A. Dengue infection with central nervous system manifestations. . Southeast Asian J Trop Med Public Health 1999; 30: 504-506.
40. Pancharoen C, Thisyakorn U. Neurological manifestations in dengue patients. . Southeast Asian J Trop Med Public Health 2001; 32: 341-345.
41. Cam BV, Fonsmark L, Hue NB, Phong NT, Poulsen A, Heegaard ED. Prospective case-control study of encephalopathy in children with dengue hemorrhagic fever. Am J Trop Med Hyg 2001; 65: 848-851.
42. Solomon T, Dung NM, Vaughn DW, et al. Neurological manifestations of dengue infection. Lancet 2000; 355: 1053-1059.
43. Pancharoen C, Rungsarannont A, Thisyakorn U. Hepatic function in dengue patients with various severity. J Med Assoc Thailand 2002; 85: S298-S301.

44. Innis BL, Myint KSA, Nisalak A, et al. Acute liver failure is one important cause of fatal dengue infection. . Southeast Asian J Trop Med Public Health 1990; 21: 695-696.
45. Pancharoen C, Thisyakorn U. Coinfections in dengue patients. Pediatr Infect Dis J 1998; 17: 81-82.
46. Thisyakorn U. Current status of the knowledge on dengue/ dengue hemorrhagic fever/ dengue shock syndrome in Thailand. IV. Clinical aspects. Mosquito Bourne Disease Bulletin 1994; 11: 61-63.
47. Permpalang N, Pitisuttithum P, Torvorapanit P, Kittithamvong P, Thisyakorn U. initial fluid resuscitation for children with dengue shock syndrome; a systemic review. Asian Biomedicine 2009; 3: 579-588.
48. Bhamarapravati N, Tuchinda P, Boonyapaknavik V. Pathology of Thailand hemorrhagic fever; a study of 100 autopsy cases. Ann Trop Med Parasitol 1967; 61: 500-510.
49. Sabcharoen A, Lang J, Chanthavanich P, Yoksan S, et al. Safety and immunogenicity of a three dose regimen of two tetravalent live-attenuated dengue vaccines in five- to twelve-year-old Thai children. Pediatr Infect Dis J 2004; 23: 99-109.
50. Thisyakorn U. ADVASC – New regional initiative supporting transition from dengue vaccine to vaccination in Southeast Asia. Vaccine 2012; 30: 5587-5588.