CLINICAL MANIFESTATIONS OF DHF/DSS AND THE CLINICAL RISK FACTORS FOR DSS IN PATIENTS IN RATCHABURI HOSPITAL, THAILAND

VANNYDA NAMVONGSA

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.....Vannyda Namvongsa..........
Ms. Vannyda Namvongsa
Candidate

....
Assoc. Prof. Chukiat Sirivichayakul,
M.D., Dip. Thai Board of Pediatrics,
D.T.M.&H.
Major-Advisor

....
Ms. Raweerat Sitcharungsi,
M.D., Dip. Thai Board of Pediatrics,
D.T.M.&H.
Co-Advisor

....
Assoc. Prof. Pornthep Chanthavanich,
M.D., M.Sc. (M.C.H), D.T.C.H
Co-Advisor

....
Ms. Sirilux Songsithichok,
M.D., Dip. Thai Board of Pediatrics
Co-Advisor

....
Asst. Prof. Watcharee Chokejindachai,
M.D., Dip. Thai Board of Pediatrics,
D.T.M.&H., Ph.D.
Co-Advisor

....
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies
Mahidol University

....
Assoc. Prof. Chukiat Sirivichayakul,
M.D., Dip. Thai Board of Pediatrics,
D.T.M.&H.
Chair
Master of Clinical Tropical Medicine
(Tropical Pediatrics)
Faculty of Tropical Medicine
Mahidol University

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on
March 13, 2009

Vannyda Namvongsa
....
Ms. Vannyda Namvongsa
Candidate

Watcharee Chokejindachai
....
Asst. Prof. Watcharee Chokejindachai,
M.D., Dip. Thai Board of Pediatrics,
D.T.M.&H., Ph.D.
Member

Usa Thisyakorn
....
Prof. Usa Thisyakorn,
M.D., Dip. Thai Board of Pediatrics,
Cert. Pediatric Infectious Diseases
Chair

....
Ms. Raweerat Sitcharungsi,
M.D., Dip. Thai Board of Pediatrics,
D.T.M.&H.
Member

....
Assoc. Prof. Chukiat Sirivichayakul,
M.D., Dip. Thai Board of Pediatrics,
D.T.M.&H.
Member

....
Ms. Sirilux Songsithichok,
M.D., Dip. Thai Board of Pediatrics
Member

....
Assoc. Prof. Pornthep Chanthavanich,
M.D., M.Sc. (M.C.H), D.T.C.H
Member

....
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies
Mahidol University

....
Assoc. Prof. Pratap Singhasivanon,
M.B.B.S., D.T.M.&H. (Bangkok),
Dr.P.H. (Epidemiology)
Dean
Faculty of Tropical Medicine
Mahidol University

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Vannyda Namvongsa

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VANNYDA NAMVONGSA 5138426 TMCP/M

M.C.T.M. (TROPICAL PEDIATRICS)

THEMATIC PAPER ADVISORY COMMITTEE: CHUKIAT SIRIVICHAYAKUL, DIP.THAI BOARD OF PEDIATRICS, PORNTHEP CHANTHAVANICH, M.D., M.Sc.(M.C.H.), WATCHAREE CHOKEJINDACHAI, Ph.D, RAWEERAT SITCHARUNGSI, DIP THAI BOARD OF PEDIATRICS, SIRILUX SONGSITHICHOK, DIP THAI BOARD OF PEDIATRICS.

ABSTRACT

This retrospective study was conducted to assess the clinical manifestations and clinical risk factors for dengue hemorrhagic fever/dengue shock syndrome in both children and adults admitted to Ratchaburi Hospital in Ratchaburi province, Thailand.

A total of 273 cases admitted during 1st January 2007 to 5th May 2008 were studied. The median age of studied subjects was 16 years (the range was 6 months-62 years) and the ratio of adults to children was 1.6:1. Forty-eight percent were 16-30 years old. The common clinical manifestations were nausea/vomiting (74.0%), positive tourniquet test (73.0%), anorexia (67.0%), hemoconcentration (58.0%), headache (54.0%), liver tenderness (43.0%), myalgia (39.0%) and pleural effusion (20.0%). Children had anorexia, epistaxis, positive tourniquet test, liver tenderness, convalescent rash, pleural effusion, ascites and drowsiness more frequently than adults. The case fatality rate was 0.73%.

Among children, the presence of anorexia, hematemesis, melena, confusion, drowsiness, pleural effusion, ascites, liver tenderness, hemoconcentration, platelet $\leq$25,000 cells/mm^3, white blood cell count >3,200 cells/mm^3, albumin $\leq$3 g/dl, globulin $\leq$3 g/dl, potassium >3.7 mEq/L, and elevation of liver enzymes was significantly correlated with dengue shock syndrome.

In multivariate analysis, it was found that in addition to age, drowsiness, anorexia, hematemesis, pleural effusion, albumin $\leq$3 g/dl and potassium >3.7 mEq/L were independent risk factors for DSS.

KEY WORDS: DENGUE HEMORRHAGIC FEVER/DENGUE SHOCK SYNDROME/ CLINICAL MANIFESTATIONS/RISK FACTORS

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LIST OF ABBREVIATIONS

Abbreviation or symbol	**Term**
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
CDC	Center for Disease Control and Prevention
WHO	World Health Organization
NS	Non structural protein
C	Core protein
M	Membrane protein
E	Envelope protein
ADE	Antibody-Dependent Enhancement
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
SGOT	Serum glutamate oxaloacetate transminase
SGPT	Serum glutamate pyruvate transaminase
PT	Prothrombine time
PTT	Partial thromboplastine time
CI	Confidence Interval
OR	Odds ratio
WBC	White blood cell count
SD	Standard deviation
BUN	Blood urea nitrogen
HCO_3	Bicarbonate

LIST OF ABBREVIATIONS (cont.)

Abbreviation or symbol	Term
Na	Sodium
K	Potassium
Hct	Hematocrit
NA	Not applicable
MA	Metabolic acidosis
RF	Renal failure
HF	Hepatic failure
DIC	Disseminated intravascular coagulation
HE	Hepatic encephalopathy
CRF	Case fatality rate
GI	Gastrointestinal
etc	Et cetera
Cr	Creatinin
BMI	Body mass index

CHAPTER I
INTRODUCTION

Dengue hemorrhagic fever (DHF)/Dengue shock syndrome (DSS) is a mosquito-transmitted (usually *Aedes aegypti*) disease caused by any of four closely related virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) of the family Flaviviridae but can not be directly transmitted from person to person. These virus and their mosquito vectors have had a worldwide distribution and affected human for more than 200 years (Pan American Health Organization, 1997). Infection with one of these serotypes provides lifelong immunity to the infecting serotype only. Therefore, person can acquire a second dengue infection by a different serotype, and second infection place them at greater risk for dengue hemorrhagic fever, the more severe form of the disease (CDC, 2008).

Globally, it has been estimated that 50–100 million new dengue-virus infections occur annually. Among these, there are 250,000–500,000 cases of dengue hemorrhagic fever, a severe manifestation of dengue, and 24,000 deaths (Gibbons and Vaughn, 2002). Of these, 90% are children less than 15 years of age with the average mortality of 5% of DHF cases (Ahmed et al, 2001).

DHF/DSS is now a significant public health problem in most of the countries in the tropical areas of Southeast Asia and Western Pacific Regions. The disease is among the ten leading causes of hospitalization and death in children in at least eight tropical Asian countries. During the 1960s and 1970s, DHF/DSS progressively increased as a health problem, spreading from its primary location in major cities to smaller cities and towns in endemic countries. It established seasonal and cyclical epidemic pattern, with large outbreaks occurred at 2-3 years intervals. During this period, 1,070,207 cases and 42,808 deaths were reported, mostly in children. During most of the 1980s, in the endemic countries of China, Indonesia, Malaysia, Myanmar, the Philippines, Thailand and Vietnam, DHF/DSS spread peripherally, affecting even rural villages. Exceptionally large outbreaks occurred in Vietnam (354,517 cases in 1987) and Thailand (174,285 cases in 1987). The total number of people contracting and dying from DHF/DSS reported in all countries of the Western Pacific and Southeast Asia region for the decade of the 1980s was 1,946,965 and 23,793, respectively (WHO, 1997).

Dengue infections have been one of the major diseases affecting children in Thailand for more than 40 years. The last two epidemics occurred in two consecutive years, 1997 and 1998, when 101,689 cases and 127,189 cases, respectively, were reported. Although the case-fatality rate has been reduced from 14% (1985) to 0.34% (1998), the number of deaths was higher, from 300 deaths in 1985 to 464 deaths in 1998. Adults were affected more than expected, and their share of deaths was about 20% in 1998 (Kalayanarooj S et al, 1999).

In Lao PDR since 1983, DF/DHF cases have been occurring in big and crowded cities, mostly in five provinces (Vientiane Municipality, Borikhamxay, Khammouane, Savannakhet and Champasak). In 1987, there were 9,699 cases with 295 deaths, mostly in children under 15 years old (Nambanya, 1997).

DHF/DSS is the third most common causes for hospitalization of children in Thailand and one of the major public health problems causing mortality and morbidity in Thailand and the whole Southeast Asia. The incidence rates of DHF increased in Thailand until 1987 when the largest epidemic, 325/100,000 population, was recorded. Whereas the disease used to be confined to large cities, the rate is now higher in rural (102.2/100,000) than in urban areas (95.4/100,000 in 1997). The age of highest incidence has increased, and the age group most severely affected is now those 5-9 years old (679/100,000 in 1997) (Chareonsook et al, 1999).

Every year, at the beginning of February, there is a gradual starting of increase in the incidence of DHF cases, peaks in July and August, with the monthly number declining thereafter. There are four serotypes of dengue circulating in Thailand though the proportion of each serotype varies from year to year. The trend of the incidence of the diseases had continued to increase in a cyclic pattern. The disease mainly affected the younger age-groups of less than 15 years with the highest proportion of cases occurring in the age group 5-9 years, followed by the age group 10-14 years (Prasittisuk et al, 1998).

From August 2000-2001, a dengue outbreak occurred in Muang district, Ratchaburi Province, Thailand. About 800 cases of dengue infection were reported and among them, 49(5%) were clinical diagnosed as DHF according to the WHO criteria. During the outbreak, the incidence rate of dengue infection in Hin Gong sub district was 2.9 per 1,000 populations (Tuntaprasart et al, 2003)

The report of cases and deaths from DF/DHF in nine countries of the Southeast Asia Region for the period of 1985-2006 revealed that the total cases ranged from 46,458 (1986) to 218,821 (1998) and 188,684 (2006). Since 2003, the trend of reported dengue cases has been rising although the case fatality rate has been maintained below 1%. Thailand reported the highest number of dengue cases in the region till 2003. Since 2004, Indonesia reported the highest number of cases in the region. In 2006, there were 42,456 dengue cases reported in Thailand. This was the second rank when compared to Indonesia. Indonesia was the first rank of both dengue cases and deaths cases reported among Southeast Asian countries. Meanwhile there were 59 dengue deaths reported in Thailand and it was the fourth rank for mortality followed Indonesia, India and Myanmar. In 2006, Thailand reported 23% and Indonesia reported 57 % of the total dengue cases in the Southeast Asia Region of WHO (WHO, 2007).

Many countries reported high numbers of dengue infection during 2007. This trend has continued in 2008, concurrently with a large outbreak being reported in Brazil. As of March 28, 2008, a total of 120,570 cases of dengue infection, including 647 DHF cases and 48 deaths have been reported by Brazilian Health Authorities (CDC, 2008).

The pathogenesis of DSS and leakage is poorly understood. Vascular leakage, hemorrhagic diathesis and complement activation are the hallmark of the disease, ultimately resulting in hypovolemic shock (Avirutnan et al, 1998).

DHF/DSS is a leading course of illness and death especially in children in the tropical areas of the Southeast Asia and Western Pacific Regions and is now a significant public health problem in most of the countries especially in Thailand. Even though the case fatality rate in Thailand is low, dengue cases is still high in 2006 as mentioned earlier. At present there is no drug that can kill dengue virus and dengue vaccine still has not been successful. DSS had an impact physically, mentally and economically on the patients and their household. In patients with DSS, if detection and management of shock is delayed, the morbidity and mortality from prolonged shock or massive bleeding will be high. The severity of the disease can be modified by early diagnosis and adequate replacement of plasma loss. Therefore, to study clinical manifestations of DHF/DSS and the clinical risk factors for DSS is crucial. Data and results gain from this study will be useful to improve medical services, early diagnose and prevent dengue shock syndrome in the future.

CHAPTER II
OBJECTIVES

Primary objective

To describe the clinical manifestations of Dengue hemorrhagic fever/Dengue shock syndrome in patients who were admitted to Ratchaburi Hospital during 2007 and 2008

Secondary objectives

1. To study the clinical risk factors for DSS among children and adults patients.
2. To study the differences of clinical manifestations, severity and outcome of dengue virus infection among children and adults patients.

CHAPTER III
REVIEW OF LITERATURE

Dengue is a mosquito born febrile viral illness. Since its first recognition during the last quarter of eighteenth century, outbreaks have been reported from both developed and developing countries with Asia always remaining the area of highest endemic. In humans, dengue infection causes a spectrum of illness raging from relatively mild, non specific viral syndrome known as dengue fever (DF) to severe hemorrhagic disease and death. The severe hemorrhagic form of disease is called DHF/DSS; a leading cause of hospitalization and death among children in Asia (Shah et al, 2006).

Dengue haemorrhagic fever (DHF) is a presentation of dengue virus infection that is most commonly seen in children less than 15 years of age, but it also occurs in adults. DHF is characterized by the acute onset of fever and associated with non-specific constitutional signs and symptoms. There is a haemorrhagic diathesis and a tendency to develop fatal shock (dengue shock syndrome). Abnormal haemostasis and plasma leakage are the main patho-physiological changes, with thrombocytopenia and haemoconcentration presenting as constant findings. Although DHF occurs most commonly in children who have experienced secondary dengue infection, it has also been documented in primary infections (WHO, 1999).

Etiology

Dengue virus belongs to the Family Flaviviridae. The four serotypes of dengue virus (designated DEN-1 to 4) can be distinguished by serological methods. Dengue viruses share many characteristics with other Flaviviruses. This virus having a single stranded RNA genome surrounded by an icosahedral nucleocapsid and covered by lipid envelope. The genome is composed of three structural protein genes, encoding the nucleocapsid or core protein (C), a membrane-associated protein (M), an envelope protein (E) and seven non structural (NS) protein genes. The domains responsible for neutralization, fusion and interactions with receptors are associated with the envelope protein. Each of the dengue viruses caused closely related clinical illness (WHO, 1997).

Dengue fever/dengue haemorrhagic fever (DF/DHF) is caused by infection with any of the four dengue virus serotypes DEN-1, DEN-2, DEN-3 and DEN-4. This results in extensive cross-reactivity in serological tests, but infection with one serotype does not provide cross-protective immunity against the others; thus, persons living in an endemic area can be infected with any of the four dengue serotypes during their lifetime (Gubler, 1997).

Mode of transmission and vector

The dengue virus is usually transmitted by bites of an infected mosquito vector. The female *Aedes aegypti*, the principal vector, is a small black-and-white, highly domesticated mosquito that prefers to lay its eggs in artificial water-containers commonly found in urban areas of the tropics. The ability of eggs to withstand desiccations is clearly advantageous. These facts also explain the difficulties in control programs to apply insecticidal to breeding sites of the *Aedes* mosquitoes. The adult mosquitoes prefer to rest indoors and to feed on humans during daylight hours in an unobtrusive and often undetected way. Infection with dengue viruses occurs when a person is bitten by an infective mosquito (Gubler, 1997). Consequently, persons who spend more time at home during the daytime, i.e., mothers and children, are more likely to be infected than those who leave the home for work (Ooi et al, 2006). After a period of incubation lasting 3 to 14 days (average 4 to 6 days), the person may experience an acute onset of fever accompanied by a variety of non-specific signs and symptoms. During this acute febrile period, which may be as short as 2 days and as long as 10 days, there is a viremia, which may vary in magnitude and duration. If uninfected *Aedes aegypti* mosquitoes bite the ill person during this febrile viremic stage, those mosquitoes may become infected and subsequently may transmit the virus to other uninfected persons after an extrinsic incubation period of 8 to 12 days (Gubler, 1997).

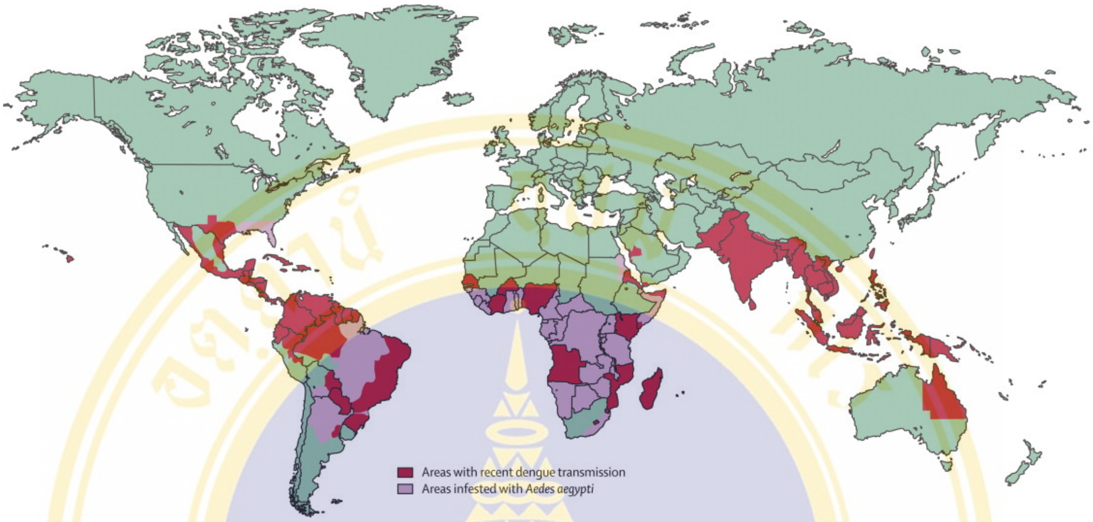

Figure 1: Global distribution of dengue and *Aedes aegypti* in 2005 (Halstead, 2007)

Factors affecting transmission of the virus

In tropical areas, dengue transmission occurs throughout the year. Increased transmission, however, occurs during the rainy season. It is speculated that temperature and humidity favor the survival of adult mosquitoes beyond their extrinsic incubation period thereby increasing the probability that viral transmission occurs. It has also been shown that increased temperature shortens the extrinsic incubation period. Rainfall itself may not be particularly important, as the principal breeding sites for *Aedes aegypti* are present year round. The importance of *Aedes aegypti* breeding sites in the Caribbean and Pacific, where rainfall is less reliable, is highlighted as the most important factor in these locations (McBride and Ohmann, 2000).

Global epidemiology

Epidemic dengue fever is a very old disease, but it was characterized during most of its history by periodic, often infrequent, epidemics. In the past 17 years, however, there has been a dramatic resurgence of epidemic dengue activity in the tropics worldwide. This increased epidemic activity, which has been caused by all four virus serotypes, has been associated with the geographical expansion of both the mosquito vectors and the viruses, the development of hyperendemicity (the co-circulation of multiple virus serotypes in an area), and the emergence of dengue haemorrhagic fever. Hyperendemicity is the most constant factor associated with the evolution of epidemic DHF in a geographical area (Gubler, 1997).

In the past 20 years, dengue fever and a severe form of the disease described for the first time in the mid 1950s, DHF/DSS, have emerged as the most important arthropod-born viral diseases of human. Approximately 250,000 cases of DHF are officially notified, and the true incidence is undoubtedly several-fold higher (Monath, 1994).

Dengue fever and *Aedes aegypti* mosquitoes have a worldwide distribution in the tropical areas of the world, with over 2.5 billion people living in dengue endemic areas (Figure 1). DF/DHF has emerged as the most important arboviral disease of humans, with an estimated 50 to 100 million cases of dengue fever and several hundred thousand cases of DHF occurring each year, depending on epidemic activity. Currently, DHF is a leading cause of hospitalization and death among children in many South-east Asian countries where epidemics first occurred in the 1950s. Epidemic DHF spread to the South Pacific islands in the 1970s, and reached the Caribbean Basin in the 1980s. The pattern of severe haemorrhagic disease has evolved in the American region in 1980s and 1990s in a manner similar to the way it did in South-east Asia in 1960s and 1970s (Gubler, 1997).

The global prevalence of dengue infection has increased dramatically in recent decades, particularly in the Americas, Western Pacific, and South-east Asia (Wichmann et al, 2004). In several Asian countries, about 400,000 cases of DHF are reported annually; this infection is a leading cause of childhood mortality (Hanafusa et al, 2008). Recover from one infection provides lifelong immunity against that serotype but confers only transient and partial protection against heterologous infections and sequential infections may increase the risk of more serious disease (Wichmann et al, 2004).

The epidemiology of DHF in Thailand.

Over the past 40years, the number of dengue infected patients in Thailand has been rising steadily. Case surveillance, mainly clinically based, has been conducted for several years. The epidemic pattern has been changed from one of alternate years to an irregular pattern. The morbidity rate ranged between 209.12/100,000 and 31.8/100,000 during the year 1998 to 1999, respectively (Kantachuvessiri, 2002).

Although many campaigns against it have been conducted, DF and DHF are still the major health problems of Thailand. In 1998, the incidence of DF/DHF was 129,954, of which Sukhothai province alone reported alarming number of 682. It was the second largest epidemic outbreak of dengue after 1987 (Nakhapakorn et al, 2005).

In year 2001, there was a dengue outbreak in an endemic area particularly in Chonburi, Thailand. During this period, 906 patients (582 children and 324 adults) were admitted to Chonburi hospital with suspected DHF (Wichmann et al, 2004).

Pathogenesis of dengue infection

Viral virulence and immune responses have been considered as two major factors responsible for the pathogenesis of DHF. The immunological mechanisms appear to include a complex series of immune responses. A rapid increase in the levels of cytokines and chemical mediators apparently plays a key role in inducing plasma leakage, shock and haemorrhagic manifestations. Sequential infections by two different serotypes of dengue virus may increase the severity of the disease. However, understanding of the DHF pathogenesis is far from complete (Chaturvedi and Shrivastava, 2004).

Antibody-Dependent Enhancement (ADE) of infection has long been thought to play a central role of DHF. The ADE hypothesis was formulated to explain the finding that severe manifestations of DHF/DSS occur in children experiencing a second dengue virus infection by a different serotype from the previous one. Sera obtained before infection from children who later developed DHF/DSS were much more likely to demonstrate ADE in vitro than those who had only DF. Newborn babies less than 1 year old who acquire maternal anti-dengue IgG antibody are also susceptible to developing DHF/DSS post primary infection (Lei et al, 2008).

The risk of DHF/DSS is higher in secondary infections with dengue virus of serotype 2 compared to that with the other serotypes. Furthermore, it was reported that high dengue viremia titer was associated with increased disease severity. Peak viral titers were 10- to 100- fold higher in patients with DSS than those with DF in dengue-infected Thai children. Viral load is a contributing factor in the development of DHF/DSS. However, the virus load has a dynamic change, varying from individual to individual, or even from day to day post infection. It declines quickly at the day fever subsided. Moreover, persons infected with the same dengue virus will have different clinical manifestations, suggesting that host factors must play important roles in the development of the dengue disease (Lei et al, 2008).

Innate immunity in DHF/DSS

The alternate hypothesis on the pathogenesis of DHF/DSS proposed by Noisakran and Perng (2007) divided the pathogenesis into two stages, disease induction or defined stage and protective stage. Innate immune-related parameters, such as natural IgM, platelets, and other factors (cytokines, etc) may directly or indirectly contribute to define the development of DHF/DSS during the course of early dengue virus infection. While in the protective stage, the periods in which fever and viral load are recessed, DHF/DSS may attribute to antigen-induced factors, such as IgG and adaptive immune response (Figure 2).

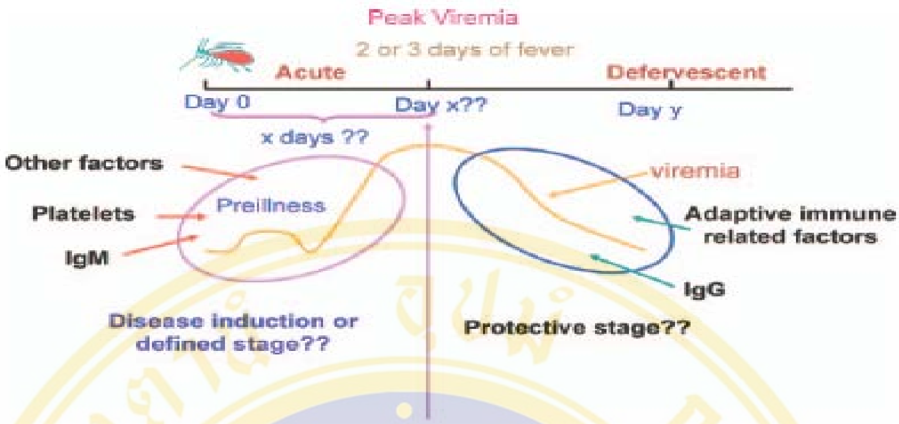

Figure 2: Role of innate immune parameters in DHF/DSS

Clinical manifestation

Dengue virus infection may be asymptomatic or lead to a range of clinical illnesses, even death (Figure 3). The majority of cases are asymptomatic or mild symptomatic.

Clinical profile and outcome of dengue infection cases in hospitalized Hubli children during July 2003 to June 2004 were studied and found that the common clinical features were fever (100%), vomiting (82%), abdominal pain (61%), restlessness (65%), headache (22%) and hepatomegaly (87%). The common bleeding manifestations were gastrointestinal bleeding 22% and petechiae 18% (Ratageri et al, 2005).

A prospective study conducted in Dhaka, Bangladesh during 2000-2001 found that most of the patients were in the age group of 5-15 years and 89% of them presented with DHF/DSS. 85% of cases had secondary dengue infection. Fever, fatigue, headache, retro-orbital pain, myalgia, arthralgia/bone pain were the most common symptoms and occurred in almost equal frequency in DF and DHF/DSS groups. The frequency of constitutional symptoms was much higher in the study as compared to a recent study from Chennai, India. The incidence of bleeding manifestations, tachycardia, hypotention, hepatomegaly, high hematocrit and thrombocytopenia were significantly higher in DHF/DSS group (Shah et al, 2006).

A study of clinical and biochemical profile of DHF in Delhi, India during September 2003 to December 2003 was done in 34 patients. They found that all patients presented with fever and hepatomegaly. Examination also revealed splenomegaly in 11 (32.4%), ascites in 6 (17.6%) and pleural effusion in 3 (8.8%) patients. Common bleeding manifestations were positive tourniquet test in 22 (64.7%) and epistaxis in 8 (23.5%) patients. Most children (56%) had a platelet count between 20,000cells/mm^3 and 50,000cells/mm^3. Bleeding manifestations were not related to platelet count ($p > 0.05$). Serum glutamic pyruvic transaminase (SGPT) >40 IU/L was seen in 22(64.6%) patients, alkaline phosphate (ALP) >400 IU/L in 12 (35.3%) and serum bilirubin >1 mg% in 3 (8.8%) patients. IgM dengue serology was positive in 68.5% cases. There was no statistically significant difference in liver function tests among different age or sex ($p > 0.05$). Clinical features of DHF varied from the previous epidemic. Hepatic dysfunction with increased levels of serum liver enzymes was common in DHF (Faridi et al, 2008).

A relative lymphocytosis with more than 15% atypical lymphocytes is commonly observed towards the end of the febrile phase (critical stage) and at the early stage of shock. Thrombocytopenia and hemoconcentration are constant findings in DHF. Partial thromboplastin time (PTT) and prothrombin time (PT) are prolonged in about one-half and one-third of DHF cases, respectively. Other common findings are hypoproteinemia, hyponatremia and mildly elevated serum aspartate aminotransferase (AST) levels. Metabolic acidosis is frequently found in cases with prolonged shock. Blood urea nitrogen is elevated in the terminal stage of cases with prolonged shock (WHO, 1999).

The study on the effect of DHF on the liver function test in Vietnam in 1995 found that abnormal levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were seen in 97.7% and 37.7% of the patients, respectively. The fact that the level of AST was higher than that of ALT and the elevation of transaminase was mild to moderate in most cases (<5-fold greater than the normal upper limit for AST and ALT) reflected that the involvement of the liver was mild to moderate in most DHF cases (Nguyen et al, 1998).

In a hospital based retrospective study conducted during dengue epidemic in the year 2005-2006, 81 children with DHF were hospitalized in the Ludhiana, India.

They found that hematocrit more than 40% was seen in 27% of cases. All patients had platelet counts less than 100,000/mm^3. Forty-one percent of cases had platelet count more than 50,000/mm^3. Platelet counts was below 20,000/mm^3 in 14.8% of children. Prolongation of the PT and/or PTT was seen in 3 children (3.7%). Hepatic dysfunction with greater than three-fold elevation of hepatic transaminase was seen in 14.8% of children. Hyponatremia (<125 mEq/L) was seen in 5 cases of which 3 had poor outcome (Dhooria et al, 2008).

DSS is a serious complication of DHF which may cause death in more than 50% of cases if not treated properly and promptly. Twenty to thirty percent of DHF patients develop DSS. Early diagnosis of suspicious DSS is essential. With early recognition and appropriate treatment, most patients can recover without sequelaes (Junia et al, 2007).

According to the available data, the risk factors that may be important in developing to DSS are as follows:

1. Age

Age 5-9 years had 1. 6 time higher risk for developing DSS (P=0.036) (Junia et al, 2007).

In Thailand, most of the cases were children under the age of 15 years and the age group at the greatest risk factor for DF/DHF was 5-9 years old (Kongsomboon et al, 2004).

A study conducted in Ho Chi Minh City, Viet Nam during 1991-1996 found that the majority of the DHF cases occurred in children in the age-groups 5-9 years (37.4%) and 10-14 years (28.7%) (Nguyen et al, 1998).

The burden of DHF/DSS was found predominantly in infants 4–9 months of age and children 5–9 years old were a risk factor for severity in children (Hammond et al, 2005).

A comparison of the demographic characters showed that shock was more frequent in younger patients but the difference was not significant (P=0.36) (Narayanan et al, 2003).

2. Sex

The male to female ratio of the total reported cases in Thailand in 1999 appeared to be about 1:1.12 (Kalayanarooj et al, 1999). However, no gender difference was observed in the DF, DHF or DSS patients (Kalayanarooj et al, 2002).

Another study (Junia et al, 2007) found that gender did not show a role in the development of DSS (P=0.32). This is different from the result of Phuong et al in Vietnam who found that 53% of girls required treatment for DSS while only 43% of boys did (Phuong et al, 2004).

The pathogenesis of DHF/DSS is not very well understood, nor is the host conditions that favor the severe disease; however, children and females appeared to be at greater risk (Guzman and Kouri, 2004).

3. Race

The study conducted in Cuba revealed that race was an individual risk factor, since DHF/DSS was more prevalent in white than in black person (Bravo et al, 1987).

Another interesting observation that could explain the severity of the epidemic was the ethnic groups of the people involved. During the 1981 DHF epidemic, white people were considered a risk factor for the severe form of the disease. DHF and DSS, both among children and adults, was significantly higher among the Whites ($p<0.01$). In the second epidemic in 1997, once again, whites were a risk factor for severe form of the disease (Guzman et al, 1999).

4. Nutritional status

Malnourished children are less likely to develop DF or DHF. Conversely, obese children are more prone. However, if malnourished children do develop DHF, they are more likely to experience a severe form, DSS (Kalayanarooj and Nimmanitya, 2005).

Junia et al (2007) also revealed that overweight children had 1.98 times higher risk for DSS (CI=95%=1.29-3.08) compared to well nourished and undernourished children. They also found that malnourished children were more resistant to dengue infection compared to well-nourished ones.

5. Chronic diseases

Bravo et al (1987) in their study in 1986 DHF/DSS epidemic in Cuba stated that chronic diseases such as bronchial asthma, diabetes mellitus and sickle cell anemia were additional risk factors contributing significantly to the development of DSS.

6. Serotype of dengue virus and order of infection

A risk factor for the DHF/DSS was probably the secondary dengue infection with another serotype (Shah et al, 2006).

A study from Thailand suggesting that the risk of developing DSS is greatest following an anamnestic dengue infection, particularly if the most recent infection was with dengue 2 virus. There continues to be debate about the justification for these claims. A prospective study conducted in two townships (suburbs) in Yangon, Myanmar found that the incidence of anamnestic dengue infections in DSS patients was significantly higher than in the community from which they were drawn and there was a significantly higher risk of developing DSS following an anamnestic infection (particularly with dengue 2 virus) than following a primary infection with any serotype (Thein et al, 1997).

Little is known regarding the role of classical HLA-A and -B class I alleles in determining resistance, susceptibility, or the severity of acute viral infections. Both primary and secondary infections with dengue virus (DEN) serotypes 1, 2, 3 or 4, can result in either clinically less severe DF or the more severe DHF. In a case-control study of 263 Thai patients infected with either DEN-1, -2, -3 or -4 to detect the role of HLA class I in secondary dengue infections, it was found that HLA-A*0203 was associated with the less severe DF, regardless of the secondary infecting virus serotype. By contrast, HLA-A*0207 was associated with susceptibility to the more severe DHF in patients with secondary DEN-1 and DEN-2 infections only. Conversely, HLA-B*51 was associated with the development of DHF in patients with secondary infections, and HLA-B*52 was associated with DF in patients with secondary DEN-1 and DEN-2 infections. Moreover, HLA-B44, B62, B76 and B77 also appeared to be protective against developing clinical disease after secondary dengue virus infection. These results confirm that classical HLA class I alleles are associated with the clinical outcome of exposure to dengue virus, in previously exposed and immunologically primed individuals (Stephens et al, 2002).

Serological and virological features of DF and DHF in Thai people were analyzed during 1999-2002. The illness was caused by DEN-1 in 45%, DEN-2 in 32%, DEN-3 in 18% and DEN-4 in 5% of patients. Almost all of the DHF cases caused by DEN-2 and DEN-4 were secondary infection, while approximately 20% of the DHF cases caused by DEN-1 and DEN-3 were primary infection. These results indicate that DEN-1 and DEN-3 may induce DHF in both primary and secondary infections, and suggest that DEN-2 and DEN-4 in Thailand are less likely to cause DHF in primary infections (Anantapreecha et al, 2005).

7. Clinical manifestation

A study was conducted in Chennai, India between October and December, 2001 in children 7months to 12years of age. It was found that the factors related to shock were drowsiness (P=0.0004). Parameter associated with bleeding manifestation was hepatomegaly (P=0.0002). There was no correlation between positive tourniquet test and other bleeding manifestations. Bleeding was related to very low platelet counts (less than 50,000cells/mm^3) but moderately low platelet counts (50,000-100,000cells/mm^3) were not associated with increase in the frequency of bleeding manifestations (Narayanan et al, 2003).

On an analysis of the symptoms, it was found that children with dengue fever who did not have shock were eight times more likely to report body pain (P=0.004). Vomiting was more common in those with shock but the difference was not significant (P=0.29). Children who had complained retro-orbital pain had a significantly higher frequency of bleeding (P=0.045). There were no statistically significant difference in abdominal pain and bleeding between shock and non shock patients. (Narayanan et al, 2003).

Another prospective studies conducted in Dhaka, Bangladesh during 2000-2001 found that the incidence of the bleeding manifestations, tachycardia, hypotension, hepatomegaly, high hematocrit and thrombocytopenia were significantly higher in DHF/DSS group (Shah et al, 2006).

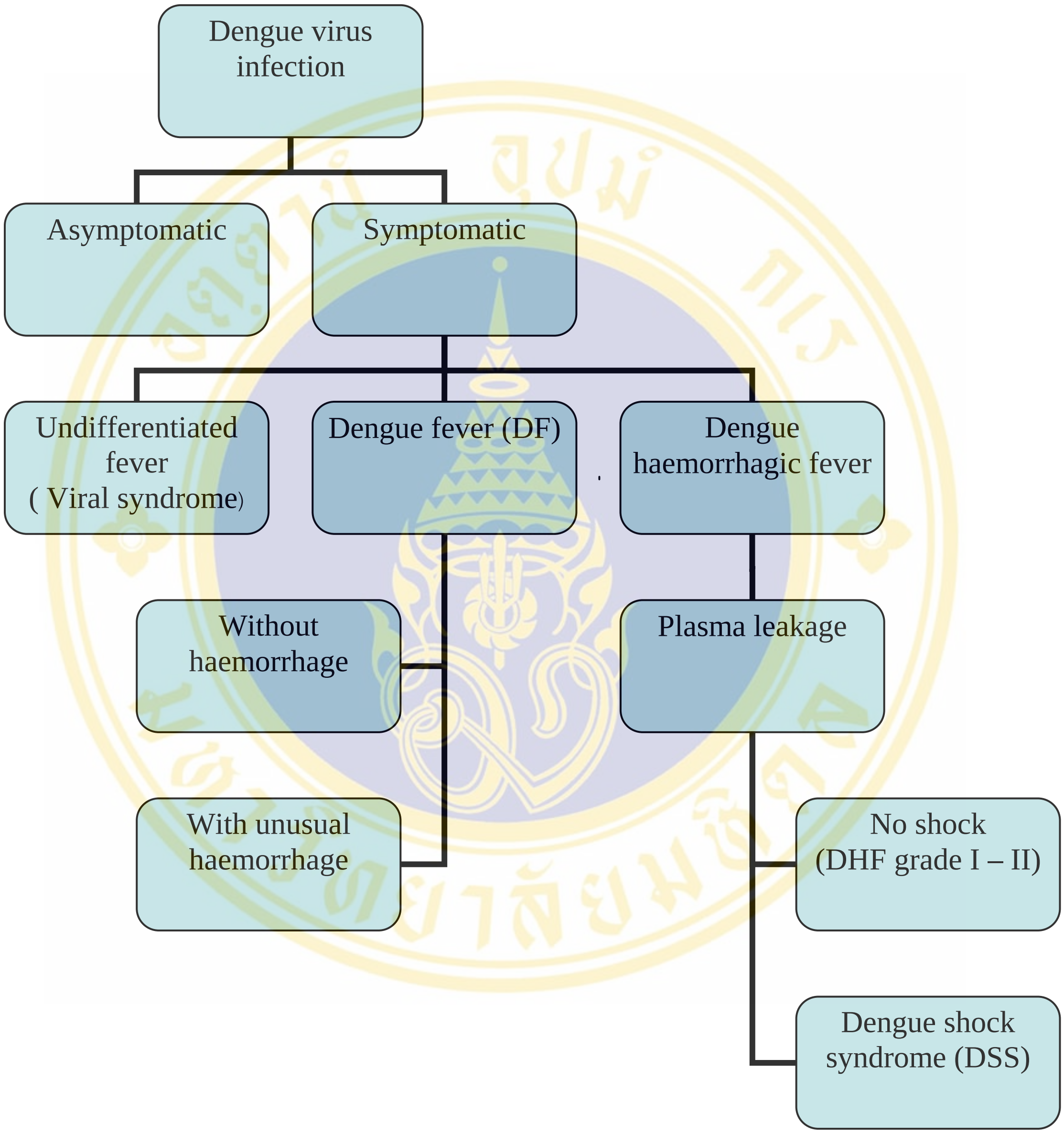

Figure 3: Clinical manifestations of dengue virus infection (WHO, 1997)

In DHF, the severity can be divided into 4 grades. Grade I and II are DHF without shock and grade III and IV are DHF with shock (Table 1).

Table 1: Severity grading of DHF

Grade	Clinical feature	Laboratory finding
I	• Fever • Constitutional symptoms • Positive tourniquet test	• Thrombocytopenia • Hemoconcentration
II	Grade I plus • Spontaneous bleeding of skin, gums and gastrointestinal tract	• Thrombocytopenia • Hemoconcentration
III	Grade II plus • Hypotention • Circulatory failure • Agitation	• Thrombocytopenia • Hemoconcentration
IV	Grade III plus • Profound shock • Blood pressure = 0	• Thrombocytopenia • Hemoconcentration

CHAPTER IV
MATERIALS AND METHODS

Study design

This study was a retrospective descriptive study.

Study site

This study was carried out at Ratchaburi Hospital, Ratchaburi Province, Thailand. Ratchaburi Hospital is a regional hospital, providing tertiary medical care. It has approximately 800 beds including 90 pediatric beds. It is located approximately 100km Southwest to Bangkok, the capital province of Thailand.

Study period

Data collection was started from 6th November, 2008 to 26th December, 2008.

Study population

The medical records of the patients, both adults and children, who were admitted to the Ratchaburi Hospital between 1st of January 2007 and 5th of May 2008 with the clinical diagnosis of DHF/DSS was studied.

The sample size was calculated using the formulation for a descriptive study as followed:

$$n = Z_{\alpha}^{2}\, pq / d^{2}$$

While Z_{α} = 1.96 (95% confident level)

p= proportion of DSS among total DHF= 0.027

q= proportion of DHF= 0.973

d = maximum permissible error = 2%

The calculated sample size was 253. However, there were 273 DHF/DSS cases who were admitted to Ratchaburi Hospital during 1st January 2007 to 5th May 2008 and met the inclusion criteria. All of these subjects were therefore recruited in the study.

Inclusion criteria

All patients who were admitted to Ratchaburi Hospital and were diagnosed as DHF/DSS.

Exclusion criteria

Patients with unfound hospital records were excluded from this study.

Data collection

Data collection was done from all the patients' medical records that meet the inclusion criteria. The following data were collected and recorded into the case record form (appendix A):

Part I: Demographic data consisting of age, sex, race, weight, height, nutritional status.

Part II: Underlying disease of the patient and family members such as: diabetes mellitus, asthma, allergic rhinitis, atopic dermatitis, cardiovascular disease, chronic renal failure.

Part III: Clinical manifestations of DHF/DSS including:

1. Duration of fever before hospitalization, headache, retro-orbital pain, nausea/vomiting, hemorrhagic manifestations (melena, hematemesis, etc.), confusion and drowsiness, etc.
2. Physical examination findings: tourniquet test, liver size, ascites, pleural effusion, liver tenderness, etc.
3. Laboratory findings on the day of admission and during the follow-up period: hematocrit (Hct), white blood count (WBC), AST, ALT, PT, PTT, serology, virology, etc.
4. Complication, outcome and grading of the DHF according to the WHO guideline (WHO, 1997).

All the data were entered and analyzed by using statistical package software SPSS version 11.5.

Data analysis

All the analyses were calculated using a 2-tailed *p*-value < 0.05 as statistically significant cut off point.

1. Descriptive statistics: the clinical data including demographic data, signs and symptoms, and laboratory data were described using descriptive statistics.
2. The clinical manifestations of DHF/DSS in children and adults were compared by using chi-square or Fisher's exact test for the categorical variables and student's t-test for continuous variables that were normally distributed (e.g. highest and lowest hematocrit, sodium, potassium, BUN, albumin, etc.). Some laboratory data that were not normally distributed such as lowest platelet count, lowest WBC, highest atypical lymphocyte, AST, ALT, PT, PTT, HCO3, creatinin, globulin, etc were compared using Mann-Whitney U test.
3. The risk factors for DSS was analyzed by using univariate analysis, Odds ratio (OR) and 95% confidence intervals (CI).
4. Multivariate logistics regression was performed to evaluate the magnitude of risk factors by using enter methods.

CHAPTER V
RESULTS

Among the 273 cases recruited in this study, 105(38.5%) were children and 168(61.5%) were adults. There were 110 subjects in the year 2007 and 163 subjects in the year 2008. Figure 4 shows the proportion of adults and children in each year.

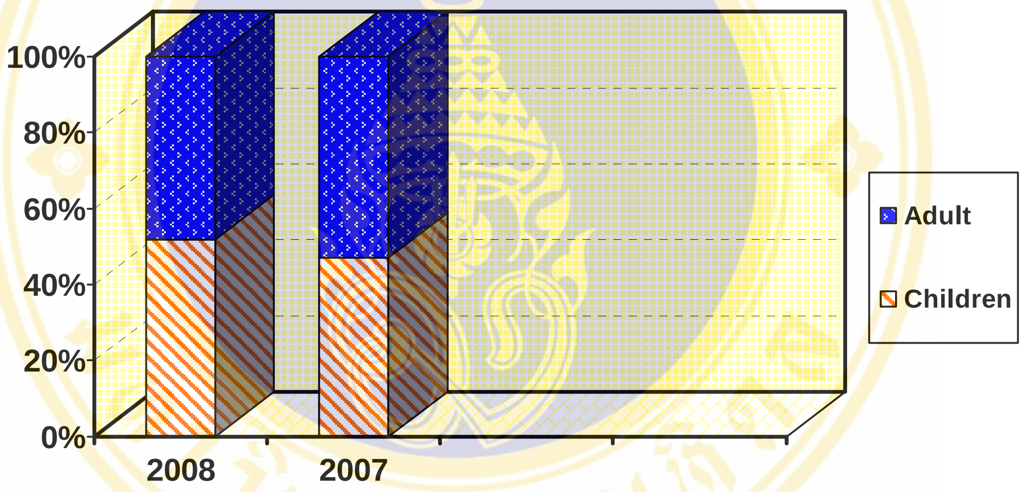

Figure 4: Proportion of adults and children admitted with DHF during the year 2007-2008.

1. Demographic characteristics of the study population

In the children group, 63(60.0%) were male and 42(40.0%) were female. The male to female ratio was 1.5:1. In the adults group, 93(55.4%) were male and 75(44.6%) were female. Males were more than females with a ratio of 1.2:1 (Table 2). The ratio of adults to children was 1.6:1.

The age of the subjects ranged from 6months to 62 years. Most of the subjects (48.0%) were 16-30 years old. The median age of the total study population was 16 years. When we subcategorized the population, then it was 11 years in the children group, and 22 years in the adults group. The age distribution of subjects is shown in (Table 3).

Most of the subjects, 262(96%) were Thai and 11(4%) were not Thai, these included Burmese [7(2.5%)], Korean [2(0.7%)], Japanese [1(0.3%)] and Australian [1(0.9%)] (Table 2).

Table 2: Demographic characteristics of DHF patients.

	Children (%) [n=105(38.5%)]	Adults (%) [n=168(61.5%)]	Total (%)
Sex			
Male	63(60.0)	93(55.4)	156(57.2)
Female	42(40.0)	75(44.6)	117(42.8)
Race			
Thai	101(96.2)	161(95.8)	262(96.0)
Non Thai	4(3.8)	7(4.2)	11(4.0)

Table 3: Age distribution of DHF cases.

	Age group					Total
	0-5y n(%)	6-10y n(%)	11-15y n(%)	16-20 n(%)	≥ 20y n(%)	
DSS	3/13 (23.1)	9/34 (26.5)	17/75 (23.0)	3/62 (5.0)	6/89 (7.0)	38/273 (14.0)
Non DSS	10/13 (77.0)	25/34 (73.5)	58/75 (77.3)	59/62 (95.2)	83/89 (93.3)	235/273 (86.1)

It was found that more males had DSS than females but there was no statistically significant difference between males and females (Table 4).

Table 4: Frequencies of DSS and non DSS in different sex.

Sex	DHF		Total	P-value [a]
	Non DSS (%) n=235	DSS (%) n=38	(%)	
Male	135(57.4)	21(55.2)	156(57.0)	0.8
Female	100(42.6)	17(44.7)	117(43.0)	

[a] Pearson Chi-square test

Underlying diseases

It was found that very few subjects or their family members had underlying diseases. The underlying disease of the subjects and their family are shown in Table 5. It is noted that the presence of asthma, either in the subjects or their family members, may be a risk factor for DSS.

Table 5: Underlying diseases of the subjects and their family members.

Diseases in family members	Non DSS (%) n= 235	DSS (%) n= 38	Total (%)	P-value[a]
Diabetes mellitus				
Yes	5(2.1)	0	5(1.8)	**< 0.001**
No	228(97.0)	38(100.0)	266(97.4)	
ND	2(0.9)	0	2(0.7)	
Asthma				
Yes	2(0.8)	2(5.0)	4(1.4)	**0.007**
No	231(98.3)	36(95.0)	267(98.0)	
ND	2(0.9)	0	2(0.7)	
Allergic rhinitis				
Yes	1(0.4)	2(5)	3(1.1)	0.076
No	232(98.7)	36(95)	268(98.2)	
ND	2(0.9)	0	2(0.7)	
Atopic dermatitis				
Yes	0	0	0	** N/A
No	233(99.0)	38(100.0)	271(99.0)	
ND	2(1.0)	0	2(1.0)	
Diseases of subjects				
Diabetes mellitus				
Yes	2(0.8)	0	2(0.7)	**0.001**
No	231(98.3)	38(100.0)	269(98.5)	
ND	2(0.8)	0	2(0.7)	
Asthma				
Yes	3(1.2)	3(80)	6(2.1)	**0.001**
No	230(98.0)	35(92.0)	265(97.0)	
ND	2(0.8)	0	2(0.7)	
Allergic rhinitis				
Yes	3(1.3)	0	3(1.1)	**< 0.001**
No	230(97.8)	38(100.0)	268(98.2)	
ND	2(0.8)	0	2(0.7)	
Atopic dermatitis				
Yes	0	0	0	** N/A
No	231(98.3)	38(100.0)	269(98.5)	
ND	4(1.7)	0	4(1.5)	
Cardiovascular disease				
Yes	2(0.8)	2(5)	4(1.5)	0.097
No	229(97.4)	36(95)	265(97.0)	
ND	4(1.7)	0	4(1.5)	
Chronic renal failure				
Yes	1(0.4)	0	1(0.4)	1.000
No	230(97.8)	38(100.0)	268(98.1)	
ND	4(1.7)	0	4(1.5)	

[a] Fisher's exact test ** N/A: Not Applicable

Nutritional status of children

The nutritional status (weight for age) of 104 children whose age younger than 15 years was calculated using Epi Info program as Z score. It was found that 82(78.8%), 17(16.3%) and 5(4.8%) had normal nutritional status, over nutritional status and malnutrition, respectively (Table 6).

Table 6: Nutritional status of children with DHF.

Nutritional status	Number (%)
Malnutrition(Z score< -2)	5(4.8)
Normal nutrition(Z score≥ -2 to ≤ + 2)	82(78.8)
Over nutrition(Z score> +2)	17(16.3)

2. Clinical manifestations

Patients with DHF/DSS presented with a wide variety of signs and symptoms as shown in table 7 and 8. Nausea/vomiting were commonly found in both children and adults (74.0%) followed by anorexia (67.0%), headache (54.0%) and myalgia (39.0%).

In this study, children presented more frequently with anorexia [81.0% in children and 58.0% in adults (p-value <0.001)]. Children had epistaxis more often than adults [14.3% in children, 7.1% in adults (p-value= 0.05)]. However, adults more commonly presented with myalgia [48.2% in adults vs. 23.0% in children (p-value< 0.001)] (Table 7).

Liver tenderness was more frequent in children (52.0% in children vs. 36.4% in adults; p-value <0.001). Other clinical findings include drowsiness (11.0% in children vs. 2.4% in adults; p-value= 0.004), convalescent rash (53.4% in children vs. 18.0% in adults; p-value <0.001), pleural effusion (29.0% in children vs. 10.0% in adults; p-value <0.001), ascites (22.0% in children vs. 6.0% in adults; p-value= 0.001) (Table 8).

Table 7: Frequency of clinical manifestations in DHF.

Symptom	Children(%) N=105	Adults(%) N=168	Total (%) N= 273	P-value[a]
Nausea/vomiting	83(79.0)	118(70.2)	201(74.0)	0.108
Anorexia	85(81.0)	97(58.0)	182(67.0)	**< 0.001**
Headache	54(51.4)	93(55.4)	147(54.0)	0.526
Myalgia	24(23.0)	81(48.2)	105(39.0)	**< 0.001**
Abnormal vagina-bleeding	5/42(11.9)	13/75(17.3)	18/117(15.4)	NA
Hematemesis	16(15.2)	15(9.0)	31(11.4)	0.110
Epistaxis	15(14.3)	12(7.1)	27(10.0)	**0.054**
Gum bleeding	9(9.0)	10(6)	19(7.0)	0.408
Melena	8(8.0)	7(4.2)	15(6.0)	0.223
Blood transfusion	4(4.0)	12(7.1)	16(6.0)	0.254
Retro-orbital pain	8(8.0)	7(4.2)	15(6.0)	0.223
Arthralgia	4(4.0)	7(4.2)	11(4.0)	1.000*

*Fisher's exact test

N/A: Not applicable

[a] Pearson Chi-square test

Table 8: Frequencies of clinical manifestations and laboratory findings in DHF.

Findings	Children (%)	Adults (%)	Total (%)	P-value[a]
Liver size				
≤ 2cm	32/41(78.0)	13/13(100.0)	45/54(83.0)	0.094*
> 2cm	9/41(22.0)	0	9/54(17.0)	
Positive tourniquet test	45/57(79.0)	21/34(62.0)	66/91(73.0)	**< 0.001**
Hemoconcentration (Hct increase ≥ 20%)	66/104(63.5)	91/168(54.2)	157/272(58.0)	0.132
Liver tenderness	53/101(52.0)	52/143(36.4)	105/244(43.0)	**< 0.001**
Convalescent rash	31/58(53.4)	11/62(18.0)	42/120(35.0)	**< 0.001**
Day of positive tourniquet test				
≤ 3 days	19/47(40.0)	8/23(35.0)	27/70(39.0)	0.649
≥ 4 days	28/47(60.0)	15/23(65.0)	43/70(61.0)	
Complications	37/105(35.2)	21/168(13.0)	58/273(21.2)	**< 0.001**
Pleural effusion	15/52(29.0)	5/50(10.0)	20/102(20.0)	**< 0.001**
Ascites	11/51(22.0)	3/51(6.0)	14/102(14.0)	**0.001**
Petechia	14/105(13.3)	25/168(15.0)	39/273(14.3)	0.722
Drowsiness	11/105(11.0)	4/168(2.4)	15/273(5.5)	**0.004**
Blood group				
- Group A	4(25.0)	5(29.0)	9(27.3)	
- Group B	5(31.3)	9(53.0)	14(42.4)	0.184
- Group AB	3(18.8)	0	3(9.1)	
- Group O	4(25.0)	3(18.0)	7(21.2)	
Confusion	3/105(3.0)	3/168(2.0)	6/273(2.2)	0.679*
Jaundice	2/105(2.0)	2/168(1.0)	4/273(1.5)	0.640*
Ecchymosis	0	1/168(1.0)	1/273(0.4)	1.000*

* Fisher's exact test

[a] Pearson Chi-square test

Distribution by antibody response

The serological investigations were done in only 8 children and 16 adults. It was found that only 25% of both children and adults had primary dengue infection (Table 9).

Table 9: Frequencies of primary and secondary dengue infection.

Serology	Children N=8(%)	Adults N=16(%)	Total N=24(%)	P-value [a]
Primary dengue infection	2(25.0)	4(25.0)	6(25.0)	
Secondary dengue infection	6(75.0)	12(75.0)	18(75.0)	1.000
Total	8(33.3)	16(66.7)	24(100.0)	

[a] Fisher's exact test

Duration of fever prior to admission and total duration of fever (Table 10)

The median duration of fever prior to admission was 4 days (range 1-11days).

The mean (SD) duration of fever was 6.12 (2.06) days.

The mean (SD) peak of fever during hospitalization was 39.0(2.06) ^{o}C.

There was no difference in the duration of fever prior to admission, total duration of fever, and peak of fever between children and adults.

Table 10: Duration of fever before admission and total duration of fever in children and adults.

	Children N=105(%)	Adults N=168(%)	Total N=273(%)	P-value [a]
Fever prior to admission				
≤ 3 days	48(46.0)	68(40.5)	116(42.5)	0.394
≥ 4 days	57(54.0)	100(59.5)	157(57.5)	
Duration of fever				
≤ 7 days	89(85.0)	141(84.0)	230(84.0)	0.854
≥ 8 days	16(15.0)	27(16.0)	43(16.0)	
Peak of fever				
≤ 38 °C	22(21.0)	51(30.0)	73(27.0)	0.088
> 38 °C	83(79.0)	117(70.0)	200(73.0)	

[a] Pearson Chi-square test

Laboratory findings

Table 11 shows laboratory findings in children and adults. The comparison between children and adults revealed that adults had significantly lower median platelet count 32,000 cell/mm^3 (range 2,900-99,000) than children 46,500 cell/mm^3 (range 6,000-153,000, p-value < 0.001). In contrast, children had lower atypical lymphocyte, lowest hematocrit, sodium, HCO_3, creatinin, albumin and globulin than adults (p-value < 0.001, 0.006, < 0.001, < 0.001, 0.001, < 0.001 and 0.001, respectively). On the other hand, children had higher potassium and AST level (p-value= 0.005 and 0.023, respectively).

Table 11: Laboratory findings in DHF.

Laboratory findings	Children	Adults	P-value[a]
Highest Hct(%)	45.0(5.27)# N= 105	46.2(5.7) # N= 168	0.071
Lowest Hct(%)	36.2(4.22) # N= 104	37.8(5.35) # N= 168	**0.006**
Lowest platelet(cell/mm^3)	46500(6000-153000)* N= 104	32000(2900-99000) * N= 168	**< 0.001[b]**
Lowest WBC(cell/mm^3)	2750(1030-8300)* N= 104	2585(890-8300) * N= 168	0.568[b]
Highest atypical lymphocyte (%)	8.0(0-52) * N= 102	13.0(1-44)* N= 162	**< 0.001[b]**
Sodium(Na)(mEq/L)	132.0(5.66) # N= 68	135.0(3) # N= 116	**< 0.001**
Potassium(K)(mEq/L)	3.7(0.55) # N= 68	3.5(0.58) # N= 116	**0.005**
HCO3(mg/dl)	19.8(3.9-107.7) * N= 68	22.6(9.9-104.5) * N= 116	**< 0.001[b]**
BUN(mg/dl)	12.7(6.31) # N= 29	11.5(4.64) # N= 98	0.341
Creatinin(mg/dl)	0.7(0.2-1.3) # N= 29	0.9(0.1-9.2) * N= 99	**0.001[b]**
Albumin(g/dl)	2.8(0.67) # N= 26	3.4(0.58) # N= 57	**< 0.001**
Globulin(g/dl)	2.7(1.2-4.1) # N= 26	3.2(2.3-4) # N= 57	**0.001[b]**
AST(IU/L)	173.0(37-7480) * N= 27	122.0(15-10841)* N= 61	**0.023[b]**
ALT(IU/L)	87.0(33-2025) * N= 27	77.0(24-3745) * N= 61	0.264[b]
PT(second)	13.0(12-33.7) * N= 10	11.7(9-24.6) * N= 22	**0.002[b]**
PTT(second)	38.4(25.2-73.3) * N= 10	37.5(27.6-56.4) * N= 22	0.597[b]

Mean (standard deviation) #

Median (range) *

[a] Pearson Chi-square test

[b]Mann-Whitney U test

3. Comparison of clinical manifestations between DSS and non DSS.

Children had significantly higher proportion of DSS than in the adults (p-value< 0.001). There was no statistical significant difference between Thai and non Thai (p-value= 0.186) (Table 12).

Table 12: Frequencies of non DSS and DSS in children and adults.

Characteristics	Non DSS N=235(%)	DSS N= 38(%)	Total N= 273(%)	P-value[a]
Children	77(33.0)	28(74.0)	105(38.5)	< 0.001
Adults	158(67.0)	10(26.0)	168(61.5)	
Race				
- Thai	227(96.5)	35(92.0)	262(96.0)	0.186*
- Non Thai	8(3.5)	3(8.0)	11(4.0)	

* Fisher's exact test [a] Pearson Chi-square test

Nausea/vomiting, anorexia, hematemesis, melena and blood transfusion were more commonly presented in DSS group than non DSS subjects (p-value= 0.046, 0.013, < 0.001, 0.042 and < 0.001, respectively) (Table 13).

Table 13: Frequencies of signs and symptoms comparing between non DSS and DSS.

Symptoms	Non DSS (%) N= 235	DSS (%) N= 38	Total (%) N=273	P-value[a]
Headache	130(55.3)	17(45.0)	147(54.0)	0.225
Retro-orbital pain	14(6.0)	1(3.0)	15(5.5)	0.702*
Nausea/vomiting	168(71.5)	33(87.0)	201(74.0)	**0.046**
Anorexia	150(64.0)	32(84.2)	182(67.0)	**0.013**
Myalgia	94(40.0)	11(29.0)	105(38.5)	0.194
Arthralgia	11(5.0)	0	11(4.0)	0.372*
Epistaxis	21(9.0)	6(16.0)	27(10.0)	0.236*
Gum bleeding	15(6.4)	4(10.5)	19(7.0)	0.315*
Hematemesis	16(7.0)	15(39.5)	31(11.4)	**<0.001***
Melena	10(4.3)	5(13.1)	15(5.5)	**0.042***
Blood transfusion	7(3.0)	9(24.0)	16(6.0)	**<0.001**
Abnormal vaginal bleeding	16(7.0)	2(5.3)	18(6.5)	0.931

* Fisher's exact test [a] Pearson Chi-square test

Table 14 shows clinical findings comparing between DSS and non DSS. Confusion was found in 8.0% of DSS but only 1.3% in non DSS group (p-value= 0.037). More DSS subjects had drowsiness, pleural effusion, ascites and liver tenderness (p-value= 0.002, < 0.001, 0.006 and < 0.001, respectively).

Table 14: Frequencies of physical findings comparing between non DSS and DSS.

	Non DSS (%) N=235	DSS (%) N=38	Total (%) N=273	P-value[a]
Jaundice	3(1.3)	1(3.0)	4(1.5)	0.453*
Ecchymosis	1(0.4)	0	1(0.4)	1.000*
Petechia	31(13.2)	8(21.1)	39(14.3)	0.199
Confusion	3(1.3)	3(8.0)	6(2.2)	**0.037***
Drowsiness	8(3.4)	7(18.4)	15(5.5)	**0.002***
Convalescent rash	32(14.0)	10(26.3)	42(15.4)	0.343
Pleural effusion	8(3.4)	12(31.5)	20(7.3)	< **0.001***
Ascites	6(2.5)	8(21.0)	14(5.1)	**0.003***
Positive tourniquet test	57(24.2)	9(24.0)	66(24.2)	0.721*
Liver tenderness	78(33.2)	27(71.0)	105(38.5)	< **0.001***

* Fisher's exact test [a] Pearson Chi-square test

Table 15 reveals laboratory findings in DSS and non DSS subjects. It was found that DSS subjects had higher highest hematocrit and potassium as well as AST, ALT level, comparing to non DSS subjects (p-value= <0.001, 0.039, 0.001, 0.004, respectively). The DSS subjects also had longer prothrombin time and partial thromboplastin time. In contrast, the DSS patients had lower albumin, globulin and platelet value (p-value< 0.001, < 0.001 and 0.034, respectively).

Table 15: Laboratory findings in non DSS and DSS.

Laboratory findings	DSS	Non DSS	P-value[a]
Highest Hct (%)	49.0(6.05)[#] N= 38	45.2(5.4)[#] N=235	**< 0.001**
Lowest Hct (%)	36.3(5.6)[#] N= 37	37.3(4.8)[#] N= 235	0.299
Lowest platelet count(cells/mm^3)	25000(6000-153.000)* N= 37	40.000(2900-118000)* N= 235	**0.034[b]**
Lowest WBC (cells/mm^3)	3600(1100-8300)* N= 37	2550(890-8300)* N= 235	**0.001[b]**
Highest atypical lymphocyte (%)	11.0(1-27)* N= 37	11.0(0- 52)* N= 227	0.725[b]
AST (IU/L)	334.0(37-10841)* N= 26	125.0(15-3707)* N= 62	**0.001[b]**
ALT (IU/L)	148.5(33-3745)* N= 26	72.0(24-470)* N= 62	**0.004[b]**
PT (second)	12.8(11.1-33.7)* N= 12	11.4(9-15)* N= 20	**0.008[b]**
PTT (second)	49.3(27.9-73.30)* N= 12	33.7(25.2-52.30)* N= 20	**0.003[b]**
BUN (mg/dl)	12.7(6.5)[#] N= 26	11.5(4.65)[#] N= 101	0.384
Creatinin (mg/dl)	0.8(0.2-2.2)* N= 26	0.9(0.1-9.2)* N= 102	0.103[b]
Na (mEq/L)	133.6(4.07)[#] N= 12	133.8(4.40)[#] N= 153	0.830
K (mEq/L))	3.9(0.86)[#] N= 31	3.5(0.49)[#] N= 153	**0.039**
HCO3 (mg/dl)	20(9.9-107.70)* N= 31	22(3.9-104.5)* N= 101	**0.032[b]**
Albumin (g/dl)	2.6(0.58)[#] N= 25	3.5(0.52)[#] N= 58	**< 0.001**
Globulin (g/dl)	2.7(1.2-4.1)* N= 25	3.2(2.2-4.8) N= 58	**< 0.001[b]**

[a] Pearson Chi-square test. [b] Mann-Whitney U test. Mean (SD)[#]. Median (range)*

Risk factors for DSS in Univariate Analysis

Table 16 shows that children group had 5.74 times higher risk to develop DSS than adults (95% CI= 2.65-12.43). Patients who had anorexia, hematemesis and melena had 3.02, 8.92 and 3.40 times higher risk to develop DSS (95% CI= 1.21-7.52; 3.91-20.37 and 1.09-10.59), respectively.

Table 16: Univariate analysis on clinical risk factors for DSS.

Clinical data	DSS (%) N=38	Non DSS (%) N=235	OR	95% CI	P-value[a]
Age group					
- Children	28(73.7)	77(32.8)	5.74	2.65-12.43	< **0.001**
- Adults	10(26.3)	158(67.2)			
Race					
- Thai	35(92.1)	227(96.6)	0.41	0.10-1.62	0.186*
- Non Thai	3(7.9)	8(3.4)			
Sex					
- Male	21(55.3)	135(57.4)	0.91	0.45-1.82	0.801
- Female	17(44.7)	100(42.6)			
Headache	17(45.0)	130(55.3)	0.65	0.32-1.30	0.225
Retro-orbital pain	1(3.0)	14(6.0)	0.42	0.05-3.34	0.702*
Nausea/vomiting	33(87.0)	168(71.5)	2.63	0.93-8.04	0.072
Anorexia	32(84.2)	150(64.0)	3.02	1.21-7.52	**0.013**
Myalgia	11(29.0)	94(40.0)	0.61	0.28-1.29	0.194
Arthralgia	0	11(5.0)	-	-	-
Epistemic	6(16.0)	21(9.0)	1.91	0.71-5.09	0.236
Gum bleeding	4(10.5)	15(6.4)	1.72	0.54-5.50	0.315*
Hematemesis	15(39.5)	16(7.0)	8.92	3.91-20.37	**<0.001***
Melena	5(13.1)	10(4.3)	3.40	1.09-10.59	**0.042***

* Fisher's exact test

[a] Pearson Chi-square test

Dengue patients who had confusion, drowsiness, pleural effusion, ascites and liver tenderness had 6.62, 6.40, 9.68, 6.48 and 4.47 times higher risk to develop DSS (95% CI= 1.29-34.15; 2.17-18.90; 3.23-29.00; 1.96-21.45, and 1.94-10.49, respectively) (Table 17).

Regarding the nutritional status, neither malnutrition nor over nutrition was found to be the risk factors for DSS (Table 17).

Table 17: Univariate analysis on clinical risk factors according to physical examination.

Characteristics	DSS N=38 (100%)	Non DSS N=235 (100%)	OR	95% CI	P-value[a]
Jaundice	1(3.0)	3(1.3)	2.09	0.21-20.63	0.453*
Ecchymosis	0	1(0.4)	-	-	-
Petechia	8(21.1)	31(13.2)	1.75	0.74-4.17	0.199
Confusion	3(8.0)	3(1.3)	6.62	1.29-34.15	**0.037***
Drowsiness	7(18.4)	8(3.4)	6.40	2.17-18.90	**0.002***
Convalescent rash	10(26.3)	32(14.0)	1.56	0.62-3.95	0.343
Pleural effusion	12(31.5)	8(3.4)	9.68	3.23-29.00	**< 0.001***
Ascites	8(21.0)	6(2.5)	6.48	1.96-21.45	**0.003***
Positive tourniquet test	9(24.0)	57(24.2)	1.81	0.36-9.06	0.721*
Liver tenderness	27(71.0)	78(33.2)	4.47	1.94-10.49	**< 0.001***
Nutritional status					
- Malnutrition (Z score< -2)	2(7.1)	3(3.9)	1.62	0.18-12.93	0.632*
- Normal nutrition (Z score≥ -2 to ≤ + 2)	23(82.1)	59(77.6)	1.33	0.40-4.67	0.818*
- Over nutrition (Z score> +2)	3(10.7)	14(18.4)	0.53	0.11-2.23	0.550*

* Fisher's exact test

[a] Pearson Chi-square test

Dengue patients with hemoconcentration had 2.55times higher risk to develop DSS compared to those who did not have hemoconcentration (OR= 2.55, 95% CI= 1.16-5.65) (Table 18).

Dengue patients with platelet count less than or equal to 25,000 cells/ mm^3 were more likely to develop DSS compared to those who had platelet count more than 25,000cells/ mm^3 (OR= 2.39, 95% CI= 1.19-4.82) (Table 18).

Patients who had AST and ALT higher than 500 IU/L and 300 IU/L, respectively had 2.34 and 1.91 times higher risk to develop DSS compared to those who had AST and ALT less than 500 IU/L and 300 IU/L, respectively (95% CI= 1.33-4.84 and 1.02-3.61, respectively). In addition, potassium > 3.7 mEq/L had 2.76 times higher risk developing DSS (Table 18).

Patients who had albumin and globulin less than or equal to 3g/dl had 25 and 5.57times higher risk to develop DSS compared to those who had higher albumin and globulin level (95% CI= 7.29-85.69 and 1.93-16.15, respectively) (Table 18).

Table 18: Univariate analysis on laboratory data for the risk factors of DSS.

Laboratory data	DSS		Non DSS		OR	95% CI	P-value[a]
	N	%	N	%			
Blood group	17	100.0	16	100.0			
A	4	24.0	5	31.2			
B	7	41.2	7	44.0			
AB	2	12.0	1	6.2	-	-	0.263*
O	4	24.0	3	18.7			
Hemoconcentration	37	100.0	235	100.0			
Hct increase ≥ 20%	28	75.7	129	54.9	2.55	1.16-5.65	**0.017**
Hct increase < 20%#	9	24.3	106	45.1			
Platelet count	37	100.0	235	100.0			
≤ 25,000cells/mm^3	19	51.4	72	30.6	2.39	1.19-4.82	**0.013**
> 25,000cells/mm^3#	18	48.6	163	69.4			
WBC	37	100.0	235	100.0			
≤ 3,200 cells/mm^3#	15	40.0	165	70.0			
> 3,200 cells/mm^3	22	60.0	70	30.0	3.46	1.694-7.055	**<0.001**
AST	26	100.0	62	100.0			
≤ 500(IU/L) #	16	61.5	57	92.0			
> 500(IU/L)	10	38.5	5	8.0	2.34	1.33-4.84	**0.001**
ALT	26	100.0	62	100.0			
≤ 300(IU/L) #	17	65.4	56	90.0			
> 300(IU/L)	9	34.6	6	10.0	1.91	1.02-3.61	**0.001***
K	31	100.0	153	100.0			
> 3.7(mEq/L)	18	58.0	51	33.0	2.76	1.26-6.09	**0.009***
≤ 3.7(mEq/L)#	13	42.0	102	67.0			
Albumin	25	100.0	58	100.0			
≤ 3(g/dl)	20	80.0	8	13.8	25.00	7.29-85.69	**<0.001**
> 3(g/dl) #	5	20.0	50	86.2			
Globulin	25	100.0	58	100			
≤ 3(g/dl)	19	76.0	21	36.2	5.57	1.93-16.15	**0.001**
> 3(g/dl) #	6	24.0	37	63.8			

Reference group

*Fisher's exact test

[a] Pearson Chi-square test

Table 19 shows the association between bleeding manifestations and low platelet count. Only hematemesis was associated with platelet count lower than 25,000 cells/mm^3 (p-value= 0.014).

Table 19: The association between platelet count and bleeding disorder.

Bleeding manifestation	Platelet count		P-value[a]
	≤ 25,000 cells/mm^3	> 25,000 cells/mm^3	
Positive tourniquet test	16/24(67.0)	50/67(75.0)	0.453
Epistemic	11/91(12.1)	16/181(9.0)	0.398
Gum bleeding	8/91(9.0)	11/181(6.1)	0.407
Hematemesis	16/91(18.0)	14/181(8.0)	**0.014**
Melena	6/91(7.0)	9/181(5.0)	0.581
Petechia	17/91(19.0)	22/181(12.2)	0.147

[a] Pearson Chi-square test

Risk factors of DSS by Mulivariate Analysis

Detection and removal of significant variables from the univariate analysis was performed. In table 20, three factors that demonstrated significant association to DSS, including anorexia, hemoconcentration and potassium higher than 3.7 mEq/L are used for simultaneous analysis. Anorexia and potassium > 3.7 mEq/L were found to be independent risk factors for DSS.

Table 20: Multivariate analysis for risk factors of DSS (1).

Symptoms	β	Standard Error	Exp(β)	95% CI	P-value[a]
Anorexia	1.379	0.641	3.971	1.129-13.958	**0.032**
Hemoconcentration	0.902	0.473	2.465	0.974-6.234	0.057
K > 3.7(mEq/L)	0.985	0.415	2.679	1.188-6.043	**0.018**

In addition, it was found that drowsiness and anorexia were the independent risk factors for DSS when confusion, drowsiness and anorexia are analyzed together (Table 21).

Table 21: Multivariate analysis for risk factors of DSS (2).

Symptoms	β	Standard Error	Exp(β)	95% CI	P-value [a]
Confusion	0.806	1.015	2.238	0.306-16.351	0.427
Drowsiness	1.526	0.650	4.601	1.287-16.445	**0.019**
Anorexia	1.088	0.479	2.967	1.162-7.581	**0.023**

In univariate analysis, it was found that patients who had pleural effusion, ascites, hemoconcentration, liver tenderness, albumin and globulin lower or equal to 3g/dl and potassium higher than 3.7mEq/L were the risk factors for DSS. After entering these factors into the multivariate analysis, it was demonstrated that pleural effusion and albumin lower or equal to 3g/dl were independent risk factors for DSS with p-value< 0.001 and 0.029, respectively (Table 22).

Table 22: Multivariate analysis for risk factors of DSS (3).

Clinical Manifestation	β	SE	Exp(β)	95% CI	P-value [a]
Pleural effusion	17.309	1.859	3.3E+07	860652.368-1257599228	**<0.001**
Ascites	-14.837	0.000	3.6E-07	3.6E-07-3.6E-07	NA
Hemoconcentration	-1.873	2.156	0.154	0.002-10.503	0.385
Liver tenderness	0.123	1.445	1.131	0.067-19.209	0.932
Albumin≤ 3g/dl	3.494	1.603	32.930	1.422-762.551	**0.029**
Globulin≤ 3g/dl	0.471	1.417	1.602	0.100-25.776	0.739
K> 3.7(mEq/L)	2.890	1.938	17.992	0.403-802.491	0.136

NA: Not Applicable

Considering hematemesis and melena together in a multivariate analysis, it was found that only hematemesis was the independent risk factor for DSS with p-value< 0.001 (Table 23).

Table 23: Bleeding disorder in association with DSS.

Bleeding disorder	β	SE	Exp(B)	95% CI	P-value [a]
Hematemesis	1.191	0.441	6.816	2.869-16.192	**< 0.001**
Melena	0.806	0.659	2.239	0.616-8.142	0.221
Platelet≤25,000 cells/mm^3	0.661	0.384	1.936	0.913-4.106	0.085

Complication

Within the study subjects, metabolic acidosis was the most common complication found in this study. Thirty five out of 38 children had metabolic acidosis while 19 out of 21 adults did. Two cases had coagulopathy including one child and one adult. One child had metabolic acidosis and severe hepatitis. An adult had metabolic acidosis, renal failure, hepatic failure and DIC. One child had metabolic acidosis, hepatic encephalopathy, renal failure and DIC (Table 24).

Table 24: Complication of DHF among children and adults.

Complication	Children	Adults	Total
Metabolic acidosis	35	19	54
Coagulopathy	1	1	2
MA and Hepatitis	1	0	1
MA+ RF+ HF+ DIC	0	1	1
MA + HE + RF + DIC	1	0	1

MA: Metabolic Acidosis

RF: Renal Failure

HF: Hepatic Failure

DIC: Disseminated Intravascular Coagulation

HE: Hepatic Encephalopathy

Cases with signs of liver involvement

In this study, we consider increasing of AST for> 5 times of normal level or jaundice as an unusual clinical manifestation of dengue infection.

There were 34cases who had AST> 5times of normal value or more than 200IU/L. Three cases had jaundice. Thirteen cases were children and 21 were adults. The following are three interesting cases (Table 24).

First case: The highest level of AST was found in a boy aged 10years old who was diagnosed as DHF grade 2. At day 7 of fever, it was found that AST= 3997 IU/L, ALT= 506 IU/L, albumin= 2.6g/dl, globulin= 2.2g/dl. His highest hematocrit= 38%, lowest hematocrit= 28.4%, lowest platelet count= 36,000 cells/mm^3, anti HAV, anti HBc and HBsAg were all negative. Dengue IgM was positive, IgG negative. Total duration of fever was 27 days; the largest liver size was 4cm below the right costal margin with liver tenderness, jaundice, drowsiness. Ultrasonography was performed and revealed hepatomegaly, ascites and pleural effusion without biliary tract obstruction. No gastrointestinal bleeding occurred. Within 7days AST and ALT dropped from initially 3997 IU/L and 506 IU/L to 608 IU/L and 195 IU/L, respectively.

Second case: 7 year-old Thai girl presented with signs of shock on admission, she was diagnosed as DHF grade 4 and admitted on day 5 of illness. She presented with hematemesis, melena, liver tenderness, jaundice, drowsiness, pleural effusion and ascites. AST= 502 IU/L, ALT= 156 IU/L, highest hematocrit= 54%, lowest hematocrit= 43%, lowest platelet count= 26,000 cells/mm^3.

Third case: 16year-old Thai boy who was diagnosed as DHF grade 1 and was admitted on day 5 of fever with jaundice. AST= 423 IU/L, ALT= 88 IU/L, albumin= 3.7g/dl, globulin= 2.8g/dl. Ultrasound demonstrated mild hepatosplenomegaly with minimal ascites; suggestive of hepatitis, no biliary tract obstruction. Highest hematocrit= 54%, lowest hematocrit= 43.4%, lowest platelet count= 23,000 cells/mm^3. All cases were survived.

Table 25: Cases with hepatic involvement during dengue infection.

Age, Sex	16Years, Male	7Year, Female	10Year, Male
Grading of DHF	DHF grade I	DHF grade IV	DHF grade II
Drowsiness	No	Yes	Yes
AST(IU/L)	423	502	3997
AST(IU/L)	88	156	506
Total Bilirubin	1.32	-	2.8
Alkaline phosphatase(IU/L)	-	-	464
PT(Second)	-	-	15
PTT Second)	-	-	52.3
Albumin(g/dl)	3.7	3	2.6
Globulin(g/dl)	2.8	2.2	2.2
AST(IU/L) Follow up	-	502	608
ALT(IU/L) Follow up	-	156	195
Hematocrit % (highest-lowest)	54-43.4	54-43	38-28.4
Lowest Platelet count(cells/ mm^3)	23000	26,000	36,000cells/mm^3
Hepatitis serology	-	-	Anti HAV Negative Anti HBC Negative AntiHBS Ag Negative
Dengue serology	No	No	IgM positive, IgG negative
GI bleeding	No	Yes	Yes
Jaundice	Yes	Yes	Yes

Case with signs of encephalopathy

A 14 years old Thai male had fever and watery diarrhea for 3 days. His body weight was 77kg. On day 4 of the illness, he developed shock and was treated at a private clinic with 0.9% normal saline and 500cc of Dextran-40 for two doses. His vital sign was stable. He was well conscious but had melena so he was referred to Ratchaburi Hospital.

During his hospitalization in Ratchaburi Hospital he developed shock again. He was treated again with IV fluid, 2 units of fresh frozen plasma and 2 units of platelet concentration.

His highest and lowest hematocrit was 51% and 36%, respectively. WBC 22,600cells/mm^3, Neutrophil 92%, atypical lymphocyte 2%, platelet count 25,000cells/mm^3, BUN 24mg/dl, Creatinin 1mg/dl, Na 129mEq/L, K 4.3mEq/L, HCO_3 14 mEq/L, calcium 6.3mg/dl, albumin 1.3g/dl, globulin 2g/dl, AST 7480 IU/L, ALT 2025 IU/L, PTT 73.3 second, PT 33.7 second, bleeding time 28.9 second. After fluid replacement, his vital sign was stable but he had confusion, drowsiness and bleeding manifestation at the venesection wound. However, jaundice was not noticed. Superimposed infection was suspected due to marked leucocytosis, therefore, Ceftriaxone was given for 7days.

On day 10^{th} of illness, his laboratory findings was improved: AST 183 IU/L, ALT 326 IU/L, hematocrit 33%, WBC 4540 cells/mm^3, neutrophil 51%, platelet count 108,000 cells/mm^3, albumin 2.4g/dl, globulin 2.5g/dl, BUN 10mg/dl, creatinin 0.6mg/dl, Na 137mEq/L, K 3.3mEq/L, HCO3 22mEq/L. He was survived and diagnosed as DSS with hepatic encephalopathy, renal failure, DIC and metabolic acidosis.

Case fatality rate (CRF)

Two cases in this study died, 1case was a child (1%) and the other was adult (0.6%). The overall case fatality rate was 0.73%.

First case: 8 months old Thai female had fever for 7days prior to admission. She presented with nausea/vomiting, epistaxis, gum bleeding, petechia, hematemesis and drowsiness. On admission, her hematocrit was 35%, platelet count 140,000 cells/mm^3, WBC 3600 cells/mm^3, atypical lymphocyte 5%. She was then referred from the district hospital after endotracheal intubation. Forty-five minutes after admission to Ratchaburi Hospital, the child developed cardiac arrest, blood pressure and pulse could not be detectable. Resuscitation was performed but was not successful. She died one hour after admission because of massive upper gastrointestinal bleeding.

Second case: 15 years old Thai male who had asthma as an underlying disease presented with headache, nausea/vomiting, anorexia, myalgia, epistaxis, petechia, hematemesis, melena, liver tenderness, confusion and drowsiness. The duration of fever prior to admission was 4days. On admission, it was found that his highest hematocrit was 53.5%, lowest hematocrit 42%, lowest platelet count 11000cells/mm^3, lowest WBC 5670 cells/mm^3, highest atypical lymphocyte 11%, AST 10841 IU/L, ALT 3745 IU/L, PT 24.6 second, PTT 53.7 second, Na 136.8 mEq/L, K 7.28 mEq/L, HCO_3 9.9 mEq/L, BUN 21 mg/dl, creatinin 2.2 mg/dl, albumin 2.4g/dl, globulin 3.1g/dl, blood glucose 38mg%, and dengue IgM and IgG were positive. He was given 3 units of fresh frozen plasma and 10 units of packed red cell. He had metabolic acidosis, hepatic failure, renal failure, DIC and massive upper gastrointestinal bleeding. He died 8 hours later after hospital admission.

CHAPTER VI
DISCUSSION

In this study, although dengue infection was serologically confirmed in only 24 cases (9%), the WHO's criteria (WHO, 1997) for clinical diagnosis of DHF/DSS which have high diagnostic accuracy were used in this study.

The clinical features of dengue vary with the age of the patient and, in addition to clinically inapparent infections, can be classified into five presentations: non-specific febrile illness, classical dengue, dengue haemorrhagic fever, dengue haemorrhagic fever with dengue shock syndrome, and other unusual syndromes such as encephalopathy and fulminant liver failure (Innis, 1995; Isturiz et al, 2000). Young children with dengue often have an undifferentiated febrile illness with a maculopapular rash. Most infections in children under 15 years are asymptomatic or minimally symptomatic (Burke et al, 1988). Classical dengue is more commonly seen among older children, adolescents, and adults. They are less likely to be asymptomatic (Sharp et al, 1992).

The DHF in South-east Asia is primarily described as a disease that almost exclusively (more than 95%) affects children under the age of 15 (WHO, 2002). However, in the past two decades a significant shift in the age distribution has been noted. Data from Bangkok have revealed a progressive shift of the median age of children with DHF from 3.8 years in the 1960s to 5.6 years in the 1970s and to 7.4 years in the 1980s (Nimmannitya, 1987a). In Jakarta, Indonesia, the proportion of DHF cases in the age group older than 10 years increased from 11% to 28% between 1975 and 1989 (Hadinegoro and Nathin, 1990). Agarwal et al. (1999) also reported that 53% of the patients with DHF were between 11 and 30 years old. In this study the adults to children ratio among DHF cases was 1.6 to 1. The median age of DHF patients was 16 years. Although age-specific incidence cannot be estimated, this result shows a tendency of increasing DHF incidence in older age group and supports the previous studies.

Many studies found that DSS was highest in the age group of 5-9 years (Hadinegoro et al., 1999; Corwin et al., 2001) but DSS would increase in the age group of 10-14 years old (Prasittisuk et al., 1998; Kalayanarooj et al., 2002; Chairulfatah et al., 2001). The present study found that children younger than 15 years had 5.74 times higher risk in developing DSS.

Many researches found that sex was not a risk for DSS (Kalayanarooj et al., 2002; Nguyen et al., 2005). However, some researches suggested that female had a higher risk to develop DSS (WHO, 1997). In our study, we found that the ratio of male to female in DSS was 1.24 to 1. We did not find a role of gender in the development of DSS. This is similar to previous studies. On the other hand, Nimmannitya (1987a,b) reported that shock and deaths occurred more frequently in females comparing to males. Another study showed that even though the rate of hospitalization in male was higher than in females, there was no significant difference in hospitalization between different genders.

Regarding race, most of the patients in this study were Thai, therefore we cannot clarify the role of ethnicity in the development of DSS.

Overnutrition was found to associate with severe dengue infection. Overweight children had 1.98 times higher risk for DSS compared to well nourished and undernourished children (Junia et al., 2007). This is similar to the results of Kalayanarooj et al. (2005) and Pichainarong et al. (2006). Nevertheless, the studies of Tantracheewathorn et al. (2007) found that the nutritional status was not a significant risk factor. In this study, the nutritional status of patients aged younger than 15 years were analyzed but there was no statistically significant association between nutritional status and DSS. This might be due to too small sample size. Body mass index (BMI) of patients aged 15years or older were not calculated in this study due to the data of weight and height of many patients was not available in medical records.

A previous study by Lee et al. (2002) stated that there was a significant association between DHF/DSS and diabetes mellitus. Another study (Gonzalez et al., 2005) revealed that the most important medical conditions that might associate with DSS were bronchial asthma. Similarly, the study of Bravo et al. (1987) stated that chronic diseases such as bronchial asthma were additional risk factors contributing significantly to the development of DSS. The results of this study reveals that subjects who currently had asthma but not cardiovascular disease and renal failure may have higher risk for DSS. Conversely, no subjects who had diabetes mellitus or allergic rhinitis suffered from DSS. A positive family history for these medical conditions may affect the severity of dengue infection. However, the sample size in this study is very small and therefore we cannot make a conclusion. Further study is warranted to clarify this issue.

All studied patients presented with fever during illness; fever is an essential criterion for the diagnosis of DF or DHF/DSS. However, 7% of patients did not have fever on admission. This may be because some of the patients were referred during the critical phase from other hospitals after being admitted or observed during the febrile phase.

The best known risk factor for development of DHF is pre-existent dengue immunity. A study from (Burke et al., 1988) revealed that secondary infection had 6.5 times higher risk for DSS comparing to primary infection. In a study in Rayong province, Thailand, Sangkawibha et al. (1984) found that DSS was associated with secondary infection. In our study, 4 out of 5 (80%) cases in DSS group whose antibodies were tested showed secondary antibody response. Only one (20%) case had primary antibody response.

In this study, we revealed some differences in clinical manifestations between children and adults. Children tended to have anorexia (81%) and epistaxis (14%), respectively, while adults complained myalgia (48%) more frequently. This result was similar to the previous study by Wichmann et al. (2004) who observed that myalgia were significantly more common in adults (p-value$\leq$ 0.001) and also similar to the study of Hanafusa et al. (2008).

The tourniquet test is a simple clinical procedure reflecting capillary fragility and is recommended as a screening test for dengue infection. The test is incorporated in the WHO case definition for DHF, where it is the only but required bleeding disorder in DHF grade I (WHO, 1999). However, the specificity of the tourniquet test seems to be low (Cao et al., 2002). Moreover, Hanafusa et al. (2008) stated that 85.1% of children and 54% of adults had positive tourniquet test (p-value$<$ 0.001). The sensitivity of the tourniquet test in dengue infection also depends on the day of illness when the test is performed. In our study, positive tourniquet test were found in 79% of children and 62% of adults (p-value< 0.001). Tourniquet test were not repeated in almost all cases, especially when the patients already presented with other evidence of bleeding disorder. Moreover, not all cases were screened by tourniquet test during the hospitalization.

In our study liver tenderness was more common in children. The proportion of children who had this symptom was similar to that revealed in previous studies (Garcia-Rivera and Rigau-Perez, 2003; Wichmann et al., 2004). Convalescent rash with itching was also more common in children than adults.

This study revealed that pleural effusion, ascites and drowsiness (29%, 22% and 11%, respectively) were more common in children than in adults. The presence of pleural effusion and ascites may correlate with plasma leakage and may explain the finding that children had higher risk to develop DSS than adults.

Haemorrhagic manifestations in DHF are usually mild. Massive bleeding that required blood transfusion was not common and usually occurred after the onset of shock (Nimmannitya, 1987b; WHO, 1997). The present study found that bleeding in the early course of the disease before defervescence, including hematemesis was one of the risk factors to develop shock. Bleeding in the early course should alarm the clinicians that the DHF patients are at risk of shock.

Acute abdominal pain was a frequent complaint shortly before the onset of shock (WHO, 1997). Liver tenderness or abdominal pain was found in severe cases and usually significantly associated with DSS. It was an alarming sign for close observation. Dengue patients should be admitted to the hospital if they had this signs. In our study liver tenderness was a risk factor for DSS. DSS occurred 4 times more frequently in patients with liver tenderness comparing to patients without liver tenderness (95% CI= 1.94-10.49, p-value< 0.001). This finding agrees with WHO guideline (1997), which suggested that epigastric discomfort and tenderness at the right costal margin were common in DHF patients. This study was similar to the other studies which found that abdominal pain can be a good predictor for DSS (Suparyatha and Arhana, 2000; Gibbons and Vaughn, 2002). Furthermore, abdominal pain in DHF might be caused by occult GI bleeding or the strain of enlarged liver (Phuong et al., 2004). On the other hand, another literature suggested that in pre-shock or shock condition, the body responded by decreasing blood flow to organs except the vital ones such as the heart and brain, causing tissue hypoxia and subsequent abdominal pain (Ditch, 2005). However, liver tenderness was found to be the risk factor for DSS only in the univariate analysis but when we adjusted the risk by using multivariate analysis there was no statistical significance (p-value= 0.932).

There are many studies comparing the clinical features of DHF and DSS with classical dengue fever. However there are few studies like the one conducted among Vietnamese children (Bethell et al., 2001) comparing the clinical findings in children who developed complication like shock and haemorrhage irrespective of WHO classification. This is important because there are many dengue cases that developed shock and haemorrhage without fulfill WHO criteria for DHF/DSS and these children are at equal risk of death as those who meet all the WHO criteria. In our study we found that patients who had drowsiness had 4.8 times higher risk to develop DSS. This finding is similar to the study of Narayanan et al. (2003) and Bethell et al. (2001) which reported that patients who developed shock had a significantly higher incidence of drowsiness.

In this study, patients who had plasma leakage (pleural effusion) or platelet counts less than 25,000 cells/mm^3, albumin and globulin less than 3g/dl had higher risk to develop DSS (p-value < 0.001, 0.013, < 0.001 and 0.001, respectively). DSS usually had increased vascular permeability leading to plasma leakage including hemoconcentration, hypoalbuminemia, pleural effusion, ascites, and causing threatened shock and profound shock and coagulopathy including thrombocytopenia, DIC and massive hemorrhage (Tangnararatchakit, 2006; Igarashi, 1997). The evidence from many researches found that DHF with plasma leakage might lead to hypovolemic shock in DSS. Because these symptoms (ascites and pleural effusion) were the signs of plasma leakage (WHO, 1997; WHO, 1999), these symptoms are no doubt related to DSS. The present study was similar to the previous study of Srivastava et al. (1990) who found that plasma leakage was associated with shock. Unlike the study of Narayanan et al. (2003) who found that the incidence of fluid leakage into the third space was found to be equal between DSS and non DSS group.

Hemoconcentration (increasing Hct for ≥ 20% from the baseline) and thrombocytopenia are the diagnosis criteria of DHF (WHO, 1997). The present study found that platelet count ≤ 25,000cells/mm^3 and hemoconcentration ≥ 20% were the risk factors for DSS. Thrombocytopenia and hemoconcentration always occurred together. Decreasing of platelet count occurred before hemoconcentration. Both signs occurred before decreasing of fever and shock. This study was similar to the previous study by Narayanan et al. (2003) and Tantracheewathorn (2007). Plasma leakage subsequently causes an elevation of hematocrit and can lead to hypovolemic shock in cases of extensive volume loss (WHO, 1997; Nimmannitya, 1987b). Therefore, frequent hematocrit determinations are essential because they reflect the extent of plasma leakage and the adequacy of volume replacement.

In our study, we found that white blood cell count >3,200 cells/mm^3 was a risk factor for DSS (95% CI= 1.694-7.055). It could be explained that although leukopenia is common finding in viral infection, an increased stress in DSS may induce white blood cells response and therefore DSS patients may have higher white blood cell count. Unlike the previous study, Tantracheewathorn (2007) found that WBC count less than 5,000 cells/mm^3 was associated with DSS by univariate analysis.

ABO blood group frequencies were similar in primary versus secondary dengue virus infections. However, in secondary dengue infection, individuals with blood group AB were likely to have dengue hemorrhagic fever grade 3 rather than grades 1 and 2 combined (p-value, <0.0001) (Kalayanarooj et al., 2007). In our study, blood grouping was performed in only 33 cases. The proportion of the patients who had blood group AB was lower than the other blood group, the sample size is very small and therefore there is no statistical significance. Further study in larger population should be done to clarify this association.

The association of elevated liver enzymes (AST and ALT) and severity of diseases was reported by Pancharoen et al. (2002) and Mohan et al. (2000). In our study, we also found that AST > 500 IU/L and ALT ≥ 300 IU/L were associated with DSS. In addition to elevating of AST/ALT, we found 3 cases out of 273 patients with jaundice. Among these patients, 2 cases had AST level higher than 500IU/L but the other had AST lower than 500 IU/L while one case had ALT ≥ 300 IU/L. The highest levels of AST and ALT found in this study were 10841 IU/L and 3745 IU/L, respectively. There was one patient who had coagulopathy. The present study is similar to the previous study (Dhooria et al., 2008) which reported that the highest value of transaminases found was AST 1500 IU/L and ALT 3000 IU/L, and one child who also had coagulopathy died.

CHAPTER VII
CONCLUSION

This descriptive retrospective study was conducted in 273 patients who were admitted to the Ratchaburi hospital between 1st of January 2007 and 5th of May 2008 with the clinical diagnosis of DHF/DSS. The purpose of this study was to study the clinical manifestations of DHF/DSS and the clinical risk factors for DSS among children and adults patients.

The most common clinical manifestations of DHF were nausea/vomiting, anorexia, headache, myalgia. Children more commonly had anorexia and epistaxis but less myalgia comparing to the adults.

Seventy-three percent of the study subjects had positive tourniquet test. Higher proportion of children had liver tenderness, convalescent rash, drowsiness, pleural effusion as well as ascites comparing to adults.

Seventy-five percent of both children and adults had the evidence of secondary dengue infection

From the univariate analysis we found that DSS had higher occurrence of anorexia, hematemesis, melena, confusion, drowsiness, pleural effusion, ascites, liver tenderness and convalescent rash comparing to non DSS group. However, the multivariate analyses revealed that only hematemesis, anorexia, pleural effusion and drowsiness were independent risk factors for DSS.

Regarding laboratory findings, DSS group had higher highest hematocrit, higher white blood cell count, potassium, AST, ALT, PT, and PTT than non DSS group. The lowest platelet count, HCO_3, albumin and globulin in DSS group were significantly lower than non DSS group.

Platelet count ≤ 25,000 cells/mm^3, white blood count > 3,200 cells/mm^3, AST > 500 IU/L, ALT > 300 IU/L, potassium > 3.7 mEq/L, albumin and globulin < 3g/dl were the risk factors for DSS. However, the multivariate analysis showed that the only risk factor for DSS were albumin < 3g/dl and potassium > 3.7 mEq/L.

Recommendations from the findings of the study

Knowledge about risk factors for DSS is important because it can increase the awareness of clinicians to perform a close monitoring on such patients as mentioned earlier and the necessary intervention can be administered promptly in order to prevent fatal outcome.

Our study has some limitations because of its retrospective nature and hospital-based design. Some data especially the interesting potential risk factors for DSS were missing.

It could be better if serological methods including virus isolation and serotyping of dengue virus were performed in all suspected dengue cases to study the serological and virological risk factors for DSS. In addition, the role of blood group, underlying diseases such as diabetes mellitus and asthma in DSS is another interesting issue for further study.

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APPENDIX

APPENDIX A
CASE RECORD FORM

Clinical manifestations of DHF/DSS and the clinical risk factors for DSS in patients in Ratchaburi Hospital, Thailand

Study Identification number:………………………
Date of hospital visit:………………………………

I. General data.

1. Age:……………year/ ……months (if age < 1 year)
2. Sex:
   Male □
   Female □
3. Race:
   Thai □
   Others: □, define:……………….
4. Weight:……….Kg □ ND
5. Height:…………cm □ ND

II. Underlying disease

- **Family history** (include only parent and siblings)

Diabetes mellitus: Yes□. No □ □ ND

Please specify: ……………….

Asthma : Yes□. No □ □ ND
Please specify: ……………….

Alergic rhinitis: Yes□. No □ □ ND
Please specify: ………………..

Atopic dermatitis: Yes□. No □ □ ND
Please specify: ………………..

Number of sibling:………………………………………….. ND

- **Patient**

Diabetes mellitus: Yes. No ND
Please specify: ……………….

Asthma : Yes. No ND
Please specify: ……………….

Allergic rhinitis: Yes. No ND
Please specify: ………………..

Atopic dermatitis: Yes. No ND
Please specify: ………………..

Cardiovascular disease: Yes. No ND
Please specify: ………………..

Chronic renal failure: Yes. No ND

III. Clinical manifestations of DHF/DSS:

1. Fever duration before admission………..days
2. Peak of fever……………… ̇c

* 3. Headache: Yes No
* 4. Retro-orbital pain: Yes No
* 5. Nausea/vomiting: Yes No
* 6. Anorexia: Yes No
* 7. Myalgia: Yes No
* 8. Arthralgia Yes No
* 9. Epistaxis Yes No
* 10. Ecchymosis Yes No
* 11. Gum bleeding Yes No
* 12. Petechia Yes No
* 13. Hematemesis Yes No
* 14. Melena Yes No
* 15. Jaundice: Yes No
16. Largest liver size:………cm below right costal margin ND
* 17. Confusion: Yes No
* 18. Drowsiness: Yes No
19. Convalescent rash: Yes No   ND
20. Pleural effusion Yes No   ND

21. Ascites Yes □ No □ □ ND
22. Blood transfusion Yes □ No □
23. Total duration of fever:…….. days
24. Tourniquet test: Positive □, day of fever:……
Negative □ □ ND

25. Liver tenderness Yes □ No □ □ ND
26. Abnormal vagina bleeding: Yes □ No □ □ ND

(* If there were no recorded data, it is assumed that the patients had no these symptoms.)

IV. LABORATORY DATA

CBC:

1. Highest Hct……………………………………………%
2. Lowest Hct……………………………………………%
3. Lowest platelet count……………………………../mm^3
4. Lowest WBC………………………………………../mm^3
5. Highest atypical lymphocyte count……………….%

AST………………………………………………………..IU/L†
ALT………………………………………………………..IU/L†
PT…………………...........seconds (control ....seconds) †
PTT…………………... seconds (control ....seconds) †
Na………………………………………………………….mEq/L†
K……………………………………………………………mEq/L†
HCO3……………………………………………………….mEq/L†
BUN………………………………………………………..mg/dL†
Cr…………………………………………………………..mg/dL†
Serum Albumin……………………………………………g/dL†
Serum globulin …………………………………………..g/dL†
Blood group: □ A □ B □ AB □ O □ND
(†If there are >1data, record the worst value.)

V. Result

Complication: Yes □ No □
Define………………………………

□ Outcome Alive □ Die □
Define cause of death………………

VI. Diagnosis

□ DHF gr 1
□ DHF gr 2
□ DHF gr 3
□ DHF gr 4

Laboratory diagnosis

Serological	Yes □	No □			
	If yes, type of infection………□ primary □ secondary				
Virological	Yes □	No □			
	If yes	□ DEN-1	□ DEN-2	□ DEN-3	□ DEN-4
PCR	Yes □	No □			
	If yes	□ DEN-1	□ DEN-2	□ DEN-3	□ DEN-4

APPENDIX B

DEFINITIONS OF SPECIFIC TERM IN THIS STUDY

Age group

Patients aged < 15 years were classified as children.

Patients aged 15 years or older were classified as adults.

Case definition for dengue hemorrhagic fever (WHO, 1997)

*** The following must all be present:**

- Fever, or history of acute fever, lasting 2-7days, occasionally biphasic.
- Hemorrhagic tendencies, evidenced by at least one of the following:
  - A positive tourniquet test.
  - Petechiae, ecchymosis, or purpura.
  - Bleeding from the mucosa, gastrointestinal tract, injection sites or other locations.
  - Haematemesis or melena.
- Thrombocytopenia (100,000 cells/mm^3 or less)
- Evidence of plasma leakage due to increased vascular permeability, manifested by at least one of the following:
  - A rise in hematocrit equal to or greater than 20% above average for age, sex and population.
  - A drop in the hematoicrit following volume-replacement treatment equal to or greater than 20% of baseline.
  - Signs of plasma leakage such as pleural effusion, ascites and hypoproteinemia.

Case definition for dengue shock syndrome (WHO, 1997)

All of the four criteria in appendix B (fever, hemorrhagic tendencies, thrombocytopenia, and plasma leakage) must all be present plus evidence of circulatory failure manifested as:

- Rapid and weak pulse.
- Narrow pulse pressure (< 20mm Hg).
- Hypotension for age (this is defined as systolic pressure < 80 mmHg for those less than five years of age, or < 90 mmHg for those five years of age and older).
- Cold clammy skin and restlessness.

Grading severity of dengue hemorrhagic fever (WHO, 1999)

Grade	Clinical feature	Laboratory finding
I	• Fever • Constitutional symptoms • Positive tourniquet test	Platelet< 100,000cells/mm^3 Hct ≥ 20%
II	Grade I plus • Spontaneous bleeding	Platelet< 100,000cells/mm^3 Hct ≥ 20%
III	Grade II plus • Circulatory failure (rapid, weak pulse and narrowing of the pulse pressure or hypotension) • Restlessness	Platelet< 100,000cells/mm^3 Hct ≥ 20%
IV	Grade III plus • Profound shock with undetectable blood pressure or pulse	Platelet< 100,000cells/mm^3 Hct ≥ 20%

Dengue hemorrhagic fever grade III and IV are also called as dengue shock syndrome (DSS)

BIOGRAPHY

NAME:	Dr. Vannyda Namvongsa
SEX:	Female
DATE OF BIRTH:	04/01/1979
PLACE OF BIRTH:	Vientiane, Lao PDR
MARITAL STATUS:	Married
INSTITUTIONS ATTENDED:	
	Diploma in Tropical Medicne & Hygiene, Mahidol University, 2008-2009
	Master of Clinical Tropical Medicine (Tropical Pediatrics), Mahidol University, 2008-2009
OFFICIAL ADDRESS:	Ministry of Health
	University of Health Sciences
	Department of Medicine
	Tel: 856(21-222883)
HOME ADDRESS	Sihom village, Vientiane, Lao PDR
	Fax: 856(21-215777). PO : 1873
	Tel: 856(205700921) or 856(21314516)
	E-mail: bithmrk@yahoo.com or bithbilly@gmail.com