A COMPARATIVE STUDY OF CLINICAL MANIFESTATIONS AND TYPE OF DENGUE VIRUS INFECTION BETWEEN CHILDREN AND ADULT PATIENTS AT PHETCHABUN HOSPITAL

PIYAMARD PITAKARNJANAKUL

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Entitled

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ABSTRACT

Dengue hemorrhagic fever (DHF) is an important public health problem in Thailand, occurring primarily in children less than 15 years of age recently, however, there has been a progressive shift in age towards adults. This descriptive study was conducted in Phetchabun Hospital, Thailand. The objective of the study was to compare clinical manifestations and type of dengue virus infection in children and adults admitted to the hospital between September 2003 and August 2004. Data were collected by interview, medical records, and laboratory reports. Diagnosis of all dengue patients were confirmed by using an immunochromatographic (IC) test. Out of 286 dengue patients, 15 (5.3%) patients had dengue fever (DF) and 271 (94.7%) patients had DHF: of which clinical classifications were DHF I, 40.9%; DHF II, 43%; DHF III or dengue shock syndrome, 10.8%. Among the patients, 231 cases (80.8%) were children and 55 cases (19.2%) were adults. In children the mean age was 8.8 (±3.1) years and male to female ratio was 1.02:1. In adults the mean age was 20.6 (±6.8) years and male to female ratio was 1.4:1. The highest proportion of child cases was DHF I (42.9%) whereas that of adults was DHF II (51%). Some clinical manifestations were more common in adult patients such as petechiae, melena, headache, retro-orbital pain, joint pain, myalgias, and nausea/vomiting. Signs found commonly in children were epistaxis, oliguria, and liver enlargement. These differences in clinical manifestations were of statistical significance (p-value <0.05). Moreover, haemoconcentration and thrombocytopenia, higher levels of alanine aminotransferase, and longer prothrombin time were significantly found to be higher in adults than in children (p-value <0.05). In dengue children, 15.2% were primary infection and 84.8% were secondary infection, while in adults, 7.3% and 92.7% were primary and secondary infections, respectively. Comparing disease severity between child and adult dengue cases, there was no significant difference in primary and secondary infections (p-value = 0.469 and 0.735, respectively). Using fever, positive tourniquet test and leukopenia or thrombocytopenia or haemoconcentration as the screening tests for diagnosis of dengue virus infection provided an accuracy in diagnosis of 95.5% in children and 94.8% in adults.

These findings suggest that clinical manifestations of dengue disease in adults are more severe since nearly all samples had secondary infection. This study could be useful to health care workers for rapid case identification and treatment thus leading to reduction in mortality of DHF.

KEY WORDS: DENGUE HEMORRHAGIC FEVER / CLINICAL MANIFESTATIONS / CHILDREN / ADULTS /
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การศึกษาเปรียบเทียบลักษณะอาการทางคลินิก และชนิดของการติดเชื้อไวรัสเด็งกี่ระหว่าง ผู้ป่วยเด็กและผู้ใหญ่ในโรงพยาบาลเพชรบูรณ์ (A COMPARATIVE STUDY OF CLINICAL MANIFESTATIONS AND TYPE OF DENGUE VIRUS INFECTION BETWEEN CHILDREN AND ADULT PATIENTS AT PHETCHABUN HOSPITAL)

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บทคัดย่อ

โรคไข้เลือดออกเป็นปัญหาสำคัญทางสาธารณสุขในประเทศไทย ส่วนใหญ่เกิดในเด็กที่มีอายุต่ำกว่า 15 ปี แต่ในระยะ 2-3 ปีที่ผ่านมา การเกิดโรคพบในผู้ใหญ่มากขึ้น จึงได้ทำการศึกษาเชิงพรรณนา โดยมีวัตถุประสงค์เพื่อเปรียบเทียบลักษณะอาการทางคลินิกและชนิดของการติดเชื้อไวรัสเด็งกี่ในเด็กและผู้ใหญ่ที่เข้ารับการรักษาในโรงพยาบาลเพชรบูรณ์ ตั้งแต่เดือนกันยายน 2546 ถึงสิงหาคม 2547 เก็บข้อมูลโดยใช้แบบสอบถาม ระเบียนประวัติ ผลการตรวจทางห้องปฏิบัติการ และวินิจฉัยการติดเชื้อไวรัสเด็งกี่ โดยใช้วิธี immunochromatographic (IC) test ผลการศึกษาพบว่า ผู้ป่วยมีการติดเชื้อไวรัสเด็งกี่ทั้งหมด 286 ราย เป็นไข้เด็งกี่ 15 ราย (ร้อยละ 5.3) ไข้เลือดออก 271 ราย (ร้อยละ 94.7) มีความรุนแรงระดับ 1 ร้อยละ 40.9 ระดับ 2 ร้อยละ 43 และระดับ 3 หรือ ไข้เลือดออกช็อค ร้อยละ 10.8 ผู้ป่วยทั้งหมดแยกเป็น เด็ก 231 ราย (ร้อยละ 80.8) และผู้ใหญ่ 55 ราย (ร้อยละ 19.2) อายุเฉลี่ยในเด็ก 8.8 (±3.1) ปี อัตราส่วนเพศชายต่อเพศหญิง 1.02:1 อายุเฉลี่ยในผู้ใหญ่ 20.6 (±6.8) ปี อัตราส่วนเพศชายต่อเพศหญิง 1.4:1 ระดับความรุนแรงของโรคไข้เลือดออกในเด็กสูงที่สุดคือระดับ 1 (ร้อยละ 42.9) ส่วนในผู้ใหญ่พบความรุนแรงระดับ 2 สูงที่สุด (ร้อยละ 51) ลักษณะอาการทางคลินิกที่พบในผู้ใหญ่มากกว่าเด็ก ได้แก่ จุดเลือดออก ถ่ายดำ ปวดศีรษะ ปวดกระบอกตา ปวดข้อ ปวดกล้ามเนื้อ และคลื่นไส้ อาเจียน ส่วนอาการที่พบในเด็กมากกว่าผู้ใหญ่ ได้แก่ เลือดกำเดาไหล ปัสสาวะออกน้อย และตับโต แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (p-value <0.05) ผลการตรวจทางห้องปฏิบัติการ พบว่าผู้ใหญ่มีระดับฮีมาโตคริต และเอนไซม์อะลานินทรานสเฟอเรส ในปริมาณที่สูงกว่า ขณะที่จำนวนเกร็ดเลือดต่ำกว่า และระยะเวลาในการแข็งตัวของเลือดนานกว่าในเด็ก อย่างมีนัยสำคัญทางสถิติ การติดเชื้อไวรัสเด็งกี่ในเด็กเป็นแบบปฐมภูมิ ร้อยละ 15.2 และแบบทุติยภูมิ ร้อยละ 84.8 ในผู้ใหญ่ติดเชื้อเป็นแบบปฐมภูมิ ร้อยละ 7.3 และแบบทุติยภูมิ ร้อยละ 92.7 การเปรียบเทียบระหว่างเด็กและผู้ใหญ่ตามระดับความรุนแรงพบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติในการติดเชื้อแบบปฐมภูมิและทุติยภูมิ (p-value = 0.469 และ 0.735 ตามลำดับ) เมื่อใช้อาการไข้ การทดสอบทูนิเกต์ให้ผลบวก และภาวะเม็ดเลือดขาวต่ำ หรือภาวะเกร็ดเลือดต่ำ หรือภาวะเลือดข้นในการคัดกรองผู้ป่วยสำหรับการวินิจฉัยการติดเชื้อไวรัสเด็งกี่พบว่าให้ผลถูกต้องร้อยละ 95.5 ในเด็ก และร้อยละ 94.8 ในผู้ใหญ่

ผลการศึกษาครั้งนี้ให้ข้อมูลลักษณะอาการแสดงที่สำคัญของผู้ป่วยไข้เลือดออกเด็งกี่ในผู้ใหญ่ซึ่งมีความรุนแรงและแตกต่างจากในเด็ก เกือบทั้งหมดเป็นการติดเชื้อแบบทุติยภูมิ ข้อมูลเหล่านี้มีประโยชน์สำหรับบุคลากรทางการแพทย์ใช้เป็นแนวทางในการวินิจฉัยผู้ป่วยอย่างรวดเร็ว และให้การดูแลรักษาผู้ป่วยได้อย่างเหมาะสม เพื่อลดอัตราป่วยตายจากโรคไข้เลือดออกเด็งกี่ได้

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CHAPTER I
INTRODUCTION

Dengue fever (DF), dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS) are important public health problems in children occurred mainly in South-East Asia, the Western Pacific and the Caribbean (1). The global population at risk is estimated to range form 2.5 to 3 billion individuals living mainly in urban areas in tropical and subtropical regions. There are at least 100 million cases of dengue fever annually and 500,000 cases of DHF require hospitalization. DHF mortality rates average 5%, with approximately 25,000 deaths each year (2, 3). In Thailand, since 1990 the number of the reported cases has been increasing every year but the mortality rate has decreased (4, 5). A large epidemic occurred with 174,285 cases with fatality rate of 0.50 % in the year 1987 (6). DF and DHF continue to occur as an endemic disease with outbreaks every rainy season and large epidemic every two to three years or in consecutive years (7).

DHF is caused by dengue virus which has 4 serotypes (DEN-1, DEN-2, DEN-3, and DEN-4). As the virus shows similarity in some antigens, it can cause the cross-reaction among the 4 types but it is not a permanent action. The primary infection results in the permanent immunity to the infected type and has partial immunity to the rest of other 3 types for 6-12 months (8). Most of the patients normally are secondary dengue infection. *Aedes aegypti* is incriminated as the main vector of DHF (9). It takes blood from a person during the acute febrile (viremia) phase of illness. The infected mosquito bites and injects the salivary fluid into the wound of another person (10). Following an incubation period in humans of 3-14 days, there is often a sudden onset of DHF (3).

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Ninety-five percent of all DHF cases are children under 15 years old (11-13). However, in recent years the annual incidence of dengue in adult has been increased in Thailand. There have been reports of affected adult age over 15 years old of 30% and 35% in 2002 and 2003, respectively. In 2004, the fatality rate of DHF was 0.15% and 38% of this rate was adult age over 15 years old (4). Overt shock in adults was less frequently observed than in children, but was severe when it did occur (3). In adolescents and young adults, where haemorrhage appears to be relatively more severe than in children, histological evidence of intravascular clotting in small vessels has been recognized (14). So far, the data of DHF in adults has been limited since almost DF/DHF patients were children less than 15 years old. Moreover, only retrospective studies of DHF in adults were carried out previously.

Early diagnosis of dengue virus infection is an important control measure for prevention of dengue outbreak. Tourniquet test (TT) positive and leukopenia (white blood cells $\leq$5,000 cells/mm^3) of febrile case have been suggested as the two screening tests for early clinical diagnosis of dengue infection with the sensitivity of 74.21% and specificity of 85.50% (15). The serological methods have been used for the confirmation of dengue virus infection such as haemagglutination-inhibition (HI) (16), IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) (17), indirect IgG ELISA (18), and immunochromatrographic (IC) test (19). Reverse transcriptase-polymerase chain reaction (RT-PCR) is a molecular method to detect dengue virus genome but it is still costly and requires expertise (20).

Phetchabun Province is located in the north of Thailand. It is an endemic area for DHF. High number of cases were reported and the trends of dengue morbidity rate had been increasing from the years 1990 to 1999. Dengue virus infected cases increased from 49 cases (prevalence rate 22.04/100,000 population) in 2000 to 1,113 cases (prevalence rate 492.95/100,000 population) in 2001 and decreased to 496 and 287 cases in 2002 and 2003, respectively. DHF would reemerge and epidemic in this province. Phetchabun Provincial Hospital mainly services in treatment for people in the province and surrounding areas. Since there has been a progressive shift in age-

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distribution towards older children and adults, it is of interest to study the severity of DHF in these groups.

In this research, the prospective study was conducted for comparison of the clinical manifestations and type of dengue virus infection between children and adults admitted to Phetchabun Hospital. All dengue virus infected patients were and confirmed by using IC test.

General Objective

To study the clinical manifestations and type of dengue virus infection in children and adult patients admitted to Phetchabun Hospital, Thailand.

Specific Objectives

1. To compare the clinical manifestations and laboratory findings of children and adult patients infected with dengue viruses.
2. To diagnose dengue virus infection; primary or secondary in children and adult dengue patients by a rapid IC test.
3. To screen dengue virus infection by using the combination of fever, TT positive and leukopenia or thrombocytopenia or haemoconcentration and using the IC test as a confirmation method.

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CHAPTER II
LITERATURE REVIEW

1. History

Dengue fever (DF) had been known in the medical literature for more than 200 years. The first described epidemic attributed to dengue occurred in Cairo, Egypt and Batavia (Jakarta), Indonesia in 1779 and an epidemic in Philadelphia, USA in 1780. Innumerable outbreaks of dengue or probable DF were recorded in the 19th and 20th centuries before the specific diagnostic techniques were available (3, 21-23). Between 1956 and 1989 more than 2.5 million cases of DHF were reported to the World Health Organization with 42,751 deaths (24, 25).

A global pandemic of dengue began in South-East Asia after World War II and has intensified during the last 15 years. Epidemics caused by multiple serotypes (hyperendemicity) are more frequent. The geographic distribution of dengue viruses has expanded and DHF has emerged in Pacific region and the America (8, 21).

The first real outbreak of dengue haemorrhagic fever (DHF) in Asia was recognized in Manila in 1954 and named the disease Philippine haemorrhagic fever (26). In 1958, the first known of Thai haemorrhagic fever occurred in Bangkok-Thonburi and nearby areas (1). Within 20 years it had spread throughout South-East Asia and Western Pacific. Now DF spread in Caribbean, northern South America, Africa, and Eastern Mediterranean (27). Our modern understanding of the transmission of dengue began in 1906 with the suggestion by Bancroft that it was transmitted by *Aedes aegypti* mosquitoes (28). DHF now has become epidemic throughout the tropical and subtropical region (8). Since 1967, the terms DHF and dengue shock syndrome (DSS) has become into general use (29).

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2. Epidemiology

DHF is now widespread in the Western Pacific, South-East Asia and the Caribbean. During the last three decades, more information on epidemiology has been accumulated. In the Western Pacific Region, Viet Nam reported the first outbreak of DHF in 1958 and the number of reported cases has increased every year. The largest number of DHF cases was recorded in 1983, when there was a total number of 149,519 patients (260 per 100,000 population) and a case fatality rate (CFR) of 1.2 percent (30). The highest number of reported cases of DHF in the Philippines was reported in 1966 at /9,384 (28 per 100,000 population), with 250 deaths (CFR, 2.6 percent). The outbreak was mainly confined to Manila and up to 1984 this peak had not been surpassed (31).

In South-East Asia, Thailand ranks first in the number of hospitalized DHF cases. Indonesia ranks second in the region according to the number of cases of DHF. Reported cases have exceeded 10,000 per year since 1983. The highest incidence of DHF was reported in 1987, when case records of 22,765 with 1,039 deaths (CFR, 4.6 percent) were reported. The average number of cases and deaths per year for the whole country of Myanmar is 2,820 and 115, respectively, ranging from 349 and 14 (CFR, 4.3 percent) in 1973 to 7,292 and 222 (CFR, 3.04 percent) in 1975 (32).

In the Americas, the largest number of DHF cases occurred during 1981 in Cuba, when 344,203 cases (220 per 100,000 population) including 159 deaths (CFR, 0.05 percent) were recorded (33). Dengue transmission has spread further in the Americas. A total of 42,424 cases was reported in 1982 and the number of reported dengue patients continued to increase in 1987 for the fifth consecutive year. A total of 128,430 cases with three deaths was reported in 1987 (34).

In Thailand, DHF has been one of the major public health problems. It was first recognized in 1958 (35). After the first outbreak in Bangkok and its suburbs, the disease spread to adjacent provinces in the central regions in 1961 (11). In the next ten years, dengue has spread throughout the country with the total number of 23,769 and 38,768 cases in the year 1972 and 1977, respectively. DHF was originally considered

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primarily an urban disease. The increasing national rate indicates its spread into the provinces including rural areas. The average incidence rate of DHF has increased from 25 per 100,000 population during the period 1968-1977 to 54 per 100,000 during 1978-1987. The large outbreak took place in 1987 with 174,285 cases (325 per 100,000 population) and 1,008 deaths, giving the fatality rate of 0.6% (36). During the period of 1988-1992, total reported cases of DF/DHF declined in 1988 and 1989 and the number of reported cases reached to high peak in 1990 with 113,855 cases (4). Since then, the total reported cases declined, but it increased to 100,095 in 1997. In 1998, total number of cases were 119,743 with 2,169 cases of shock (37). In 1999 and 2000, the number of reported cases declined. In 2001 and 2002, the number of reported cases increased to 139,355 (224 per 100,000 population) and 114,800 (184 per 100,000 population) cases, respectively. Since then the total reported cases declined: in 2003 and 2004 total number of cases were 63,657 (101.36 per 100,000 population) 39,135 (62.04 per 100,000 population), respectively (4).

2.1 Seasonal distribution

After the first severe outbreaks of DHF in Asia between 1954 and 1958, epidemics have occurred every year during the rainy seasons. The highly endemic areas with epidemic seasons occurring in the rainy months almost every year include the Philippines, Thailand, Myanmar, Malaysia, Singapore, Indonesia, and Viet Nam, where the number of patients is correlated with the amount of rainfall. Outbreaks usually start in May and reach their peaks in July and August, before declining in October. However, in an epidemic in Thailand (1985), the disease began in January (38). In Indonesia, unlike other countries, the epidemic begins after September and reaches its peak in December, which correlates with the North-West monsoon season (39). On the contrary, in other countries epidemics are correlated with South-West monsoon seasons.

The outbreak of DHF was studied in City of Palembang, south Sumatra, Indonesia by Corwin *et al.* (40) who found that EL Nino Southern oscillation, rainfall and temperature influenced the outbreak of DHF.

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2.2 Age, sex, ethnic group and occupation

During the early epidemic years in each country the disease mostly affected children, and 95 percent of cases reported occurred in the age group below 15 years old (11-13). However, during subsequent outbreaks in most countries, the number of case reports in older age groups has increased. The high risk groups include children between 5 and 9 years old. In the Philippines and Malaysia during recent years, marked increases in the number of cases in over 15 year olds have been seen, although in Thailand, Myanmar, Indonesia and Viet Nam, the majority of DHF cases are still in the age group of under 14 years (12, 38, 41). More DHF cases have occurred in persons over the age of 15 years in the Americas than in Asia (33).

The study in China showed that most of dengue patients were 21-50 years old with fatality rate of 0.5% and DEN-1 was predominate (42). The average age of DF/DHF patients in Singapore were 14 years old in 1973 and shifted to 27 years old in 1996 (43).

Guzman *et al.* (44) conducted the study in Santiaco de Cuba, in 1997 and found that all 205 DHF/DSS cases were older than age 15 years. The DHF/DSS attack rate for adult of all ages was 420 per 10,000 secondary DEN-2 infection.

The outbreak of DHF in City of Palembang, south Sumatra, Indonesia affected in children age between 10-19 years old (35%), 5-9 years old (25%) and 4 years old (16%), and caused by DEN-3 of 43% (40).

No significant difference has been observed in sex predilection of the disease. In some countries, a slightly higher number of females with DSS has caused a higher case fatality rate in females than in males (11).

A difference in the attack rates among different ethnic groups was noted in Singapore and Malaysia (45). The disease affected Chinese people more than others. This was also observed during the early epidemics in Thailand (11). Black people are less susceptible to shock syndrome than are white and Asia people (46).

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Occupation might also affect the incidence of DHF in some geographical areas (47). This may be due to the fact that people in outdoor professions, such as those of policeman or gardener, are prone to exposure to *Ae. albopictus*, which is highly prevalent in some areas. In areas where *Ae. aegypti* is more prevalent, the disease occurs mostly in children and adult females who stay indoors during the daytime.

Better nutritional status seems to increase the risk of DSS. The disease has rarely occurred in malnourished children (48).

3. Dengue Virus

Dengue virus has four distinct serotypes; DEN-1, DEN-2, DEN-3, and DEN-4 (25). They, previously known as members of the group B arboviruses, are members of the genus *Flavivirus* within the family *Flaviviridae* (49). Dengue viruses have some of the same structures, genomic arrangement and protein types as Japanese encephalitis virus, yellow fever virus, West Nile virus, hepatitis C virus and other flaviviruses (21, 50, 51).

Similar to other flaviviruses, the mature dengue virion consists of a positive single- stranded genomic ribonucleic acid (RNA) surrounded by an approximately icosahedral nucleocapsid about 30 nm in diameter. This nucleocapsid is covered by a lipid envelope 10 nm in thickness. The complete virion has a spherical structure about 40-50 nm in outer diameter with molecular weight of 4×10^6 dalton (52-54).

The dengue virion contains three structural proteins: the capsid or core protein (C); the membrane associated protein (M); and the envelope protein (E) and seven non-structural proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 (52, 54). The envelope consists of a lipid bilayer which is embedded in the E and M parts. The lipid composition of the envelope is dictated by the composition of the host cell membrane from which the virus buds (21, 54).

The complete genome sequences are already known for all four serotypes of dengue viruses. Dengue viral RNA consists of a long open reading frame of

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approximately 10.2 kb to 11.0 kb (50), which codes for gene order 5'-C-prM-M-E-NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5-3' (50, 53).

The major structural envelope protein is involved in the main biologic functions of the virus particle such as cell tropism, acid catalyzed membrane fusion, and the induction of haemagglutination-inhibiting neutralizing, and protective antibodies (54). The first non-structural protein is NS1, a glycoprotein with a function in the virus life cycle that is unknown. NS1 proteins are detected in high titers in patients with secondary dengue infection, but are rarely in primary infections (55). The NS2 region is thought to be implicated in polyprotein processing, whereas NS3 is probably the viral proteinase that functions in the cytosol (56). The NS4 region is involved in the establishment of the membrane bound RNA replication complex. The NS5 region is the most conserved flavivirus protein that believed to be the virus-encoded RNA-dependent RNA polymerase (51).

4. Vector

Dengue viruses are transmitted from person to person by *Aedes (Ae.)* mosquitoes of the subgenus *Stegomyia. Ae. aegypti* is the most important epidemic vector, but other species such as *Ae. albopictus, Ae. polynesiensis*, members of *Ae. scutellaris* complex, have also been incriminated as secondary vectors (57). *Ae. aegypti* can feed any time, although the biting rates are highest during the daytime. After feeding, females typically fly to a resting position in a cool, shaded, interior of a building. Dispersal of fed females is low, usually less than 50 meters daily; in a rural village, females often confine their flight to one or several houses. *Ae. aegypti* usually bites indoors and it is commonly found in crowded and poor areas in the cities. On the contrary, *Ae. albopictus* bites outdoors and it is commonly found in the forest fringe, agricultural areas, and garden or plantation areas around houses. Each of these species has its own particular geographical distribution; however, they are less efficient epidemic vectors than *Ae. aegypti*. While the transovarial transmission of dengue virus has been conducted both in field and the laboratory, the significance of this to maintenance of the virus has not been established. A factor complicating eradication of the vectors is that *Ae. aegypti* eggs can withstand long periods of desiccation,

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sometimes more than years (8, 21). The incidence of DHF cases is related to prevalence and density of the mosquito vectors (58).

The female *Aedes* mosquito usually becomes infected with dengue virus when she takes blood from a person during the acute febrile (viremia) phase of illness. After an extrinsic incubation period of 8-10 days, the salivary glands of the mosquito become infected and virus is transmitted when the infected mosquito bites and injects the salivary fluid into the wound of another person (10). Following an incubation period in humans of 3-14 days (4-6 days average), there is often a sudden onset of the disease with fever, headache, myalgias, loss of appetite, and a variety of nonspecific signs and symptoms including nausea, vomiting, and rash. Viremia is usually present at the time of or just before the onset of symptoms and lasts an average of 5 days after the onset of illness (3).

5. Pathogenesis

5.1 Route of entry and sites of multiplication

Dengue virus infection is transmitted by the bite of the appropriate vector, *Ae. aegypti*, so that the skin represents the route of entry. With the penetration of the skin by the biting parts, the viruses are deposited directly to lymph or blood stream in addition to forming a local part. With early wide dissemination throughout the host, multiplication follows in yet inadequately identified way from target cells and tissues (8).

The dengue virus can gain entry to the host cells via the IgG Fc-receptor on the mononuclear phagocytes (59-61). The site of viral replication during dengue virus infection remains uncertain. Mononuclear phagocytes may be the most likely sites of replication (62). Viral particles or antigens have been detected in monocytes in the kidney, skin tissue, liver, spleen, thymus and lung. Dengue viruses have been isolated from peripheral blood leukocytes as well as from autopsy tissues from liver, spleen, lymph nodes, bone marrow, thymus, heart muscle, kidney, gastro-intestinal tract and lung (8, 25).

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5.2 Hypothesis of pathogenesis

Discussion of pathogenesis and clinical features of dengue virus infection is still complicated because its entity can be recognized as DF, DHF and DSS. There are many theories that explain the causes of severity in dengue virus infection, as followings:

5.2.1 The severity of infection is dependent on the differences in viral virulence between serotypes and/or between strains within serotypes (63, 64). This theory was supported by the study of Vaughn *et al.* (63); they reported that DEN-2 could cause the most severity in dengue virus infection. In addition, difference in the genomic RNA and protein arrangement in the structural and non-structural proteins can cause different severity (65, 66).

5.2.2 The severity of infection is dependent on the number of infected cells. The more infected cells or higher virus titer can cause greater severity of dengue virus infection. Higher peak titers have been associated with increased disease severity (21, 63, 64).

5.2.3 The severity of infection is dependent on the difference in genetic susceptibility or other host factors. A genetic contribution to pathogenesis is suggested by evidence that differences in the frequency of severe outcomes of infection are related to sex and age, while a slight effect is correlated with blood group, chronic diseases and nutritional status. Dengue virus infection can cause more severity in females and children (21, 67).

5.2.4 The severity of infection is dependent on the immunologic enhancement of dengue virus infection. The theory of immune enhancement was developed extensively by Halstead, in 1980 (23). For dengue virus infection, the role of mononuclear phagocytes as target cells and principal sites of replication seem established. The basis for dengue virus infection is an abnormal immunological response involving virus-antibody complex, leukocyte and complement activation. Central to pathogenesis is the concept of IgG antibody-dependent enhancement of

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dengue viral replication in Fc-receptor bearing monocytes. Preformed neutralizing heterotypic IgG antibodies complex with virus promoted both attachment to, and infection of monocytes, leading to progressive amplification of replication in these target cells, resulting in the increased activation of complement and kinins, and the release of mediators of vascular permeability (21, 68). This theory is related to the study of Vaughn *et al.* (63), which reported that secondary infection could cause more severity in dengue virus infection in the patient who has immunological experience as described above.

5.2.5 The severity of infection is dependent on cytokines. The higher levels of the T cell activation markers, soluble (s) CD4, sCD8, and soluble interleukin 2 receptor (sIL2R), were observed in the sera of children with DHF compared with DF, and elevated level of IL-2 and interferon gamma (IFN-γ) in children with dengue infections compared with healthy controls (69). Other investigators found elevated levels of tumor necrosis factor (TNF-α), IL-1, IL-6, and sTNF receptor p75 in patients with severe dengue illness (23, 69, 70).

6. Pathology

In fatal cases, the liver is the tissue most often yielding virus isolation. However, dengue virus has also been isolated from platelets, lung, spleen, heart muscle, bone marrow, lymph nodes, thymus, kidney, gastro-intestinal tract, and mid-brain (8).

At the light microscope level, medium and small blood vessels do not show significant alteration in the vascular wall. Occasionally capillaries and venules in affected organ systems may show perivascular haemorrhage and perivascular infiltration by lymphocytes and mononuclear cells. In the lung, kidney, liver and splenic vasculatures, an increase in the number of young megakaryocytes is observed. In adolescents and young adults, where haemorrhage appears to be relatively more severe than in children, histological evidence of intravascular clotting in small vessels has been recognized (14).

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In most fatal cases, lymphocyte tissue shows an increased activity of the B-lymphocyte system, with active proliferation of plasma cells and lymphoblastoid cells, and active germinal centres. There is evidence indicating that proliferation of large immunoblasts and considerable turnover of the lymphocytes occur. The latter is manifested by a reduction of white splenic pulps, lymphocytolysis, and marked lymphocytic phagocytosis.

In the liver, there is focal necrosis of hepatic cells, swelling, appearance of councilman bodies and hyaline necrosis of kupffer cells. Proliferation of mononuclear leukocytes, and less frequently polymorphonuclear leukocytes, occurs in the sinusoids and occasionally in the portal areas (8, 21).

In the bone marrow, depression of all haematopoietic cells has been observed, which would rapidly improve as fever subsides. Studies in kidneys have shown a mild immune complex type of glomerulonephritis, which would resolve after about 3 weeks with no residual change. Biopsies of skin rashes have revealed perivascular edema of the terminal microvasculature of dermal papillae and infiltration of lymphocytes and monocytes. Antigen-bearing mononuclear phagocytes have been found in the vicinity of this edema. Deposition of serum complement, immunoglobulin and fibrinogen on vessel walls has not yet been described (71, 72).

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7. Pathophysiology

Two main pathophysiologic changes occur:

7.1 Increased vascular permeability resulting in plasma leakage, hypovolaemia and shock. DHF appears unique in that there is selective leakage of plasma into the pleural and peritoneal cavities and the period of leakage is short (24 –48 hours).

7.2 Abnormal haemostasis due to vasculopathy, thrombocytopenia and coagulopathy, leading to various haemorrhagic manifestations (25, 62).

A constant finding in DHF/DSS is an activation of the complement system, with profound depressing of C3 and C5 levels. The mediators that increase vascular

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permeability and the precise mechanism of the bleeding phenomena seen in dengue virus infections have not yet been identified (3).

Platelet defects may be both quantitative and qualitative; some circulating platelets during the acute phase of infection may be exhausted (incapable of normal function). Therefore, a patient with a platelet count lower than 100,000 cells/mm^3 may still have a prolonged bleeding time (21).

8. Immune Response

Infection with dengue virus or any flaviviruses provide long lasting immunity only against the infecting virus. Moreover, because there are numerous flaviviruses infective for man which co-circulate in given geographic local, humans previously sensitized against a heterologus flavivirus may acquire dengue virus infection. Antibody responses to dengue viruses in humans lacking immunological experience with flavivirus antigens are quite distinct from those of humans previously sensitized to a heterologous flavivirus.

Dengue virus infection in the host who has not been infected with any flaviviruses in termed “primary infection”. Anti-dengue virus antibodies’ response in primary infection characteristically evolves slowly, narrowing in specificity over time and usually achieves only relatively low titer. The ratio of anti-dengue IgM to IgG is high for at least three weeks following infection. Dengue virus infection in the host, who is immunologically sensitized to dengue or other flaviviruses is termed “secondary infection”. Operationally, secondary infection is defined by distinctive anamnestic anti-flavivirus antibodies response elicited by epitopes of the infecting viruses that are shared with virus responsible for the past sensitization. Antibody titer rises extremely rapidly, and reacts broadly with many distinct flaviviruses; the ratio of anti-dengue IgM to IgG is low (21).

8.1 Humoral immune response

Persons infected with dengue virus produce IgM and IgG antibodies, both of which appear in 5-7 days after onset of illness in primary infections. The highest titers

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of IgM antibody are produced in primary dengue infections, but it is also produced in secondary and tertiary infections. IgM antibody is transient and disappears in 30-90 days after onset of illness in primary infection and after shorter periods in secondary infections. IgG antibody, in contrast, persists for at least 50 years and probably for the life of the patient. In persons experiencing their first dengue or flavivirus infection, peak IgG titer are reached 14-21 days after onset of illness and seldom exceed 1:1,280. In secondary infection, on the other hand, there is an immediate anamnestic IgG immune response to dengue complex and/or flavivirus-specific antigenic determinants. In these patients both IgM and IgG antibodies neutralize dengue viruses, and infection provides lifelong immunity to that specific dengue virus serotype (73).

8.2 Complement activation

The main action of immune complexes is the activation of complement system. There was marked reduction of serum complement C3 during shock phase of the disease, and the degree of reduction correlated well with the severity of the disease. The complement depression in DHF involved primarily C3, C3 proactivator, C4 and C5 with marked depressions in shock cases. These findings indicated that there was complement activation in both the classical and alternate pathways. The radioisotope studies of complement metabolism support the hypothesis of complement activation as the cause of complement reduction rather than decreased synthesis or extravasation of the complement (74). It was proposed that massive complement activation could produce large amount of anaphylatoxins C3a and C5a which were very potent mediators, causing increased vascular permeability, resulting in decreased plasma volume and hypovolemic shock. Recent measurement of C3a and C5a revealed that C3a and C5a were markedly increased during shock phase and rapidly declined to normal levels in the convalescent stage. The marked complement of consumption was characteristic of DHF/DSS. Complement activation-derived products including fragments of C3, and several pore-forming agents with properties analog to C5b-9 complement complexes induced the synthesis and/or release of interleukin-1 (IL-1), interleukin-6 (IL-6) and possible tumor necrosis factor (TNF) from cultured human monocytes. Attack on platelets by pore-formers because of complement activation

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leaded to acute thrombocytopenia. These reactions are cell reminiscent of the pathological events that characterize DHF/DSS (27).

8.3 Cell-mediated immune response

Cell-mediated immune response (CMIR) is involved in viral clearance. It has been recently reviewed and summarized as follows:

1) circulating killer T cells have been demonstrated in monkeys 5-7 days after dengue virus infection;
2) human peripheral blood mononuclear cells participate in cell-mediated cytotoxicity and antibody dependent cell-mediated cytotoxicity (ADCC) reaction;
3) transformed lymphocytes are found in large number in buffy coat from DHF/DSS patients, and blast transformation in response to dengue antigen occurs in peripheral blood leukocytes from sensitized monkeys;
4) there is pathological evidence for perivascular inflammatory responses in skin and other tissue resembling a delayed-type hypersensitivity (DTH) reaction;
5) the striking lymphocytolysis involving T-cell dependent areas of lymph nodes and spleen in autopsy material may reflect active CMIR and production of cytotoxic factors; and
6) *in vivo* DTH response demonstrated in experimental dengue infection of mice are mediated by T cells requiring H-2 histocompatibility.

Dengue virus-specific $CD4^+$ $CD8^-$ T lymphocytes and $CD4^-$ $CD8^+$ T lymphocytes are present in the peripheral blood mononuclear cell population from donors who are infected with dengue viruses. Most of $CD4^+$ T lymphocytes are dengue serotypes cross-reactive. They lyse dengue virus-infected autologous cells in a human leukocyte antigen (HLA) class II-restricted fashion, and produce IFN-γ. IFN-γ augments anti-dengue virus antibodies by increasing the number of Fc receptors. It increases the number of dengue virus-infected cells in the presence of dengue antibody and activates monocytes to produce various mediators. The mediators released by the monocytes may induce plasma leakage and haemorrhagic manifestations. The

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monocytes are then lysed by dengue virus-specific $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes. Dengue virus specific $CD8^+$ T lymphocytes lyse dengue virus infected autologous cells in an HLA class I-restricted fashion. These $CD8^+$ T lymphocytes are also dengue serotype cross-reactive. The dengue virus-specific $CD8^+$ T lymphocytes and $CD4^+$ T lymphocytes may contribute to recovery by lysing dengue virus-infected monocytes, along with the other immune mechanisms such as natural killer (NK) cells, IFN production and ADCC (81).

9. Laboratory Findings

9.1 White blood cells

The patients with dengue virus infection usually reveal leukopenia. The presence of leukocytosis and neutrophilia may exclude possibility of dengue infection and bacterial infection such as leptospirosis, septicemia etc. should be considered (8, 21).

Toward the end of the febrile phase, atypical lymphocytosis was found in majority of dengue infection both in primary dengue and secondary dengue infections. The percentage of atypical lymphocytes was below 5% at 2 days before onset of shock or subsidence of fever and then rising. There was a significant rise of atypical lymphocytes in both shock and non-shock patients (76).

9.2 Haematocrit and haemoglobin

During the first two to three days of the course of disease, the haemoglobin and haematocrit readings are usually normal or slightly decreased. But later on, the first haematological abnormality noted in DHF is the high haematocrit value, which is usually over 40% and may be as high as 50-60% (77-79). Haemoglobin is also much increased, and reaches more than 14 grams/100 ml. Haemoconcentration is usually observed during the pre-shock and shock phases of the illness and returns to normal during the convalescence stage. The haemoconcentration is due to leakage of plasma into extravascular spaces through damaged capillaries (79).

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9.3 Thrombocytopenia

In dengue infection, thrombocytopenia with platelet count equal or less than 100,000 cells/mm^3 is common. The platelet count may be extremely low in the case of shock (80).

9.4 Haemostasis

Severe liver involvement may complicate the clinical course of dengue infection by causing liver failure and contribute directly to severe bleeding and indirectly to potentiate the severity of disseminated intravascular coagulopathy with markedly prolong prothrombin time (PT) and partial thromboplastin time (PTT) (81, 82).

9.5 Blood chemistry

The number or degree of laboratory abnormalities generally increases in correlation with the severity of illness.

9.5.1 Liver function test

Elevated serum transaminases are observed in dengue infection. Levels of aspartate aminotransferase (AST) are greater than that of alanine aminotransferase (ALT). Increased levels of alkaline phosphatase and serum bilirubin were noted in a smaller proportion of dengue infection cases (83).

9.5.2 Serum protein

There is pronounced hypoalbuminaemia in shock cases of DHF patients with the total serum protein lower than in non-shock cases. The proportion of the various serum proteins, on electrophoresis, is within normal limits (84).

Serum protein of lower than 4.5 gm/dl has been observed in 78.5% and 31% of shock and non-shock cases of DHF, respectively (85). The study of Pongpanich *et al.* (78) showed serum albumin to be 2.55 ± 0.46 gm/dl in severe cases and total protein to be 5.5 ± 0.87 gm/dl.

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9.5.3 Electrolytes, water balance and blood gas

Daily measurements of serum electrolytes in DHF patients show definite decreases in sodium (Na^+) and chloride (Cl^-) levels, especially in shock cases (average 125 mEq/L of Na^+ and 91 mEq/L of Cl^-), which return to normal in the convalescent stage. The level of serum potassium is in the range of high to normal. Water balance studies have indicated that hyponatraemia is the result of salt depletion in excess water available from increased metabolism and, possibly, decreased renal excretion in shock cases (86).

Furthermore, in hyponatraemic patients, hypoosmolarity of urine briefly accompanies plasma hypoosmolarity, raising the question of a transient period of inappropriate anti-diuretic hormone (ADH) excretion. Findings have included low blood carbondioxide (CO_2), somewhat reduced serum base excess and a somewhat elevated serum pH, consistent with alkalosis and mild metabolic acidosis in all cases (86).

9.6 Renal function and urine

Urine is usually normal but occasionally one may encounter a trace to one plus of albumin. Red blood cells are seldom seen unless the urinary tract has been complicated with haemorrhage. In one study, 36% of patients showed transient proteinuria (1+ to 3+) with occasional microscopic haematuria. Mild elevation of serum urea nitrogen and serum creatinine was also observed (87).

10. Laboratory Diagnosis

Dengue virus infection has clinical signs similar to a variety of infectious diseases such as leptospirosis, scrub typhus, melioidosis, etc. So, the diagnosis of dengue virus infection requires laboratory confirmation (8, 21). The two basic methods used for establishing laboratory diagnosis of dengue infection are detection of the virus (antigen, genome) or detection of anti-dengue antibodies by serological methods. Laboratories in many regions of the world are being upgraded to include dengue virus infection in their diagnosis capabilities.

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10.1 Detection of the viruses

There are three methods that are currently used to detect dengue viruses: virus isolation, antigen detection and nucleic acid detection. These methods require the early sample collection due to the amount of dengue viruses being related to the immune response. When the antibodies rise, the virus titer will be decreased, so false negative results can be found (8, 21, 63).

10.1.1 Virus isolation

All dengue virus infected patients can be presumed to experience viremia. Successful isolation requires collection of specimens as early as possible in the course of disease. Mice, mosquitoes and vertebrate cell lines have been used for dengue virus isolation (8, 21, 88).

The first dengue isolation technique was intracerebral inoculation in the brain of suckling mice, where the virus replicates and causes encephalitis. This test can give false positive when specimens are contaminated with other microbial agents. Another partially sensitive assay for virus isolation was mosquito inoculation in mosquitoes in the genus *Aedes*, the vector of dengue viruses and genus *Toxorhynchites* (89). Dengue virus infection of the inoculated mosquito is detected after incubation time by immunofluorescent test of a tissue smear prepared from the crushed heads of the mosquitoes or head squash (90-92) or by complement fixation test (92, 93).

Currently, the most widely used laboratory method for dengue virus infection is inoculation of specimens into the monolayers of the continuous cell lines (88, 93, 94). This is a sensitive method as mosquito inoculation. Animal cell lines currently used are LLC-MK2 from rhesus kidney, VERO cell from gravest monkey kidney and PS cell from pig kidney. Mosquito cell lines currently used are C6/36 from *Ae. albopictus.* AP-61 from *Ae. pseudocutellaris* and TRA-284 from *Toxorhynchites ambionensis.* Infected cells induced cytopathic effect in mosquito cell lines. Culture must be screened for viruses by an immunoassay such as enzyme-linked immunosorbent assay (ELISA) or neutralization test. Animal cell lines can be determined the infected cells by plaque assay (91, 93, 94). However, a study of

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Maneekarn *et al.* (95) reported that virus isolation by inoculation onto C6/36 cell culture had sensitivity of 5-20%.

10.1.2 Detection of dengue virus antigens

The detection of dengue virus antigen in serum showed low sensitivity because dengue immune complexes were present (96). Using sodium thiocyanate to dissociate dengue immune complexes made high sensitivity of dengue antigen detection when identified by dot-blot ELISA (97). Dengue antigens were detected in peripheral blood leukocytes from DF and DHF patients, especially during the febrile phase of illness (98, 99).

10.1.3 Detection of dengue genome

Oligonucleotide fingerprinting analysis have been used to characterize and distinguish dengue virus genotypes (100-102). Genomic RNA from dengue viruses of all four serotypes were subsequently analysed by computer-assisted Rnase Tl-resistant oligonucleotide fingerprinting (103).

Nucleic acid hybridization is a sensitive method which has been applied to both diagnostic and epidemiologic investigation for DHF. The technique could be improved by utilizing a wider selection of dengue virus gene sequences for identification of serotype or genotype-specific probes and by adopting more sensitive nonradioactive probes that have been developed recently (104). Amplification of cDNA and type-specific dot-blot hybridization were applied to identify all four dengue serotypes in serum samples by both virus culture and direct amplification (105, 106).

The reverse transcriptase-polymerase chain reaction (RT-PCR) is a rapid and powerful technique for dengue virus analysis (107-109). The method is extremely sensitive, straightforward and reproducible, making it attractive for the rapid identification of dengue viruses in clinical and field materials. The overall specificity and sensitivity of RT/PCR was 100% correlated with virus isolation (110, 111). The sensitivity of virus detection in serologically confirmed dengue cases was 54% (20). PCR method could identify dengue infection in 3 hours after onset whereas virus

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isolation was positive 3 and 5 days after onset, but negative on day 8, after the appearance of anti-dengue antibodies (112).

10.2 Detection of dengue antibody

Five basic serologic tests are used for the diagnosis of dengue virus infection such as haemagglutination-inhibition (HI), complement fixation (CF), neutralization test (NT), IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA), and indirect IgG ELISA. For HI, CF, and NT, unequivocal serologic confirmation depends upon a significant (4-fold or greater) rise in specific antibodies between acute-phase and convalescent-phase serum samples.

HI test is based on the activity of dengue virus antibodies to inhibit the agglutination of erythrocytes from geese or trypsinized human O red blood cells. This test use antigen prepared by sucrose-acetone extraction of suspensions of the brains of suckling mice infected with prototype dengue viruses. Non-specific inhibitors must be removed from patients' sera by treatment with kaolin or acetone, and then must be absorbed with goose red cells. If the screening test of paired sera is negative, the specimens may be retested against all dengue virus antigens. The HI assay is well standardized worldwide; WHO has issued recommendations for interpretation of the test, including differentiation between primary and secondary infections. While the primary response to dengue virus is characterized by slow evolution of HI antibodies, the secondary antibody response to dengue is characterized by the rapid evolution of broadly flaviviruses reactive antibodies (8, 21, 63).

CF test is not widely used for routine dengue diagnostic serology. The CF test is based on the principle that the complement is consumed during antigen-antibody reactions (113). The test is broadly cross-reactive in secondary infection, and can not be used for serotype identification under these circumstances.

The NT is the most specific and sensitive serologic test for dengue viruses. In secondary infection, it may result in higher neutralization titers to the first of the sequential virus serotypes (80). Although this effect is variable and may not be relied

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upon for serotype identification of sequential dengue infection, the test is also the best measure of true immunity after vaccination (114).

MAC–ELISA is based on detecting the dengue-specific IgM antibodies. The rapid detection of dengue antibodies has been achieved by single serum (115, 116). A study of Kittigul *et al.* (117) revealed that IgM was still detected not only in primary, but also in secondary dengue infection.

IgG-ELISA can also be used to differentiate primary and secondary dengue infection. The IgG-ELISA is very non-specific and exhibits the same broad cross-reactivity among flaviviruses as the HI test; it can not be used to identify the infecting dengue serotype.

Serum IgM and IgG antibodies are usually detected from dengue patients between 3-5 days after the onset of fever and generally persist for 30-90 days, although detectable levels may be present 8 months postinfection. Anti-dengue IgM and IgG antibodies can be detected using commercially available test kits (21, 118-121). The rapid immunochromatographic (IC) test is a new technology used for detection of anti-dengue virus IgM and IgG antibodies. This test is available as a commercial product named “PanBio dengue rapid test”. In this assay, antibody to dengue virus is determined by a rapid colloidal gold-based IC test for the separate determination of IgM and IgG antibodies in a capture assay format. Anti-dengue virus IgM or IgG in the serum combines with anti-human IgM or IgG antibodies, which is immobilized in line across the nitrocellulose strip. Currently, colloidal gold-labeled anti-dengue virus monoclonal antibody (MAb) is reacted with the dengue virus antigens (DEN-1, DEN-2, DEN-3, and DEN-4) which is stabilized on the device. After closing the device, the antigen-MAb-gold complex comes in contact with the captured IgM and/or IgG on the membrane and a color is formed. It demonstrated 100% sensitivity in the diagnosis of dengue virus infection and was able to distinguish between primary and secondary virus infection using single serum (119). This test offers advantages over HI and enzyme immunoassay for the serological diagnosis of

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dengue virus infection, since it provides standardized reagents, which should reduce interlaboratory variation (21, 119, 122-124).

11. Prevention and Control

Prophylaxis consists of avoiding mosquito bites by use of insecticides, repellents protective clothing, screening in house, and destruction of *Ae. aegypti* breeding sites (125). If water storage is mandatory, a tight-fitting lid or a thin layer of oil on all containers may prevent egg-laying or hatching. A larvicide such as Abate available as one percent sand granule formulation and effective at a concentration of one part per million may be added safety to drinking water. Ultra-low-volume spray equipment effectively dispenses malathion from truck or airplane for rapid killing of adult mosquitoes during an epidemic. Only personal antimosquito measures are effective against mosquitoes in the field, forest or jungle.

The use of pesticides introduces further complications concerning public domain and well being of individuals. Biological control agents: larvivorous fish, *Toxorynchites splendens* and bacteria with larvicidal potential e.g. crystal toxins of *Bacillus (Bc.) thuringiensis* and *Bc. sphaericus* have been reported in preference to insecticides in order to preserve environments (126).

Several tetravalent live-attenuated dengue candidate vaccines have been evaluated over the past two decades, most of them were found to have limitations due to under-attenuation (i.e., the vaccines made the volunteer too ill) or over-attenuation (i.e., the vaccines did not induce an immune response) (127). One of the most promising candidate live-attenuated dengue vaccines has been developed by The Mahidol University group from Thailand. After the successful demonstrations of phenotype characteristic changes of viral attenuation by prolonged passages of DEN-4 virus in primary dog kidney cells, Scientists of the Mahidol University group had serially passed DEN-1, DEN-2, and DEN-4 into these cells which produced plaque variants of virus with acceptable immunogenicity and low reactogenicity. However, DEN-3 strain was successfully attenuated through 30 passages in primary African green monkey kidney cells and foetal rhesus lung cells. Each of these 4 passaged

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dengue viruses was tested in Thai male adult volunteers by inoculating the viruses to each individual. The outcomes revealed that nearly 100% had sero-conversion to each of those live-attenuated dengue vaccines (128).

With the lots produced in France, a first clinical trial was performed in the United States using monovalent vaccines and a tetravalent vaccine given in one dose (129). This vaccination induced antibody response predominantly toward DEN-3 virus with low or undetectable titres to other types. A study of Sabchareon *et al.* (127) was able to demonstrate an acceptable safety and immunogenicity with a two-dose vaccination regimen. Vaccination induced in 76% seroconversion against ≥3 serotypes. One major concern in the development of dengue vaccination remains the possibility to induce antibody-dependent enhancements, a phenomenon which is recognized to play a major role in the pathogenesis of dengue (130, 131).

12. Treatment

The treatment of DF is supportive with bed rest, adequate fluid intake, and control of fever and pain with antipyretics and analgesics (e.g. paracetamal). Use of aspirin is contraindicated because of the increased bleeding tendency. Patients can be treated as out-patients if close monitoring is possible (132, 133).

The treatment of DHF during the febrile phase is similar to that of DF. Survival depends on frequent monitoring of patients for a drop in platelet count and a rise in haematocrit and early clinical recognition of plasma leakage. Early volume replacement as the haematocrit rises sharply with plasma leakage can prevent the development of shock. The critical period for the development of shock is the transition from the febrile to the afebrile phase, which occurs from approximately the third day. A reduction in the platelet count to ≤100,000 cells/mm^3 usually precedes a rise in haematocrit. A rise in haematocrit of 20% or more (e.g. increase from 35% to 42%) reflects a significant plasma loss and indicates the need for intravenous fluid therapy. Early volume replacement of lost plasma with Cystalloid solution (e.g. isotonic saline solution) can reduce the severity of the disease and prevent shock. Intravenous fluid therapy before leakage is not recommended. In mild to moderate

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cases of DHF, intravenous fluid therapy may be given for a period of 12-24 hours at out-patient clinic. This is an important life saving measure. Patients should be monitored on an hourly basis by medical personnel. Based on periodic haematocrit/ platelet count determinations and vital signs, the treatment should be reviewed and revised (132, 133).

The treatment of DSS during the afebrile phase of DSS include circulatory failure manifested by rapid and weak pulse, narrowing of the pulse pressure and hypotension, characterised by high diastolic pressure relative to systolic pressure (for example 90/80 mmHg) and the presence of cold clammy skin and restlessness. These complications occur because of thrombocytopenia, abnormal haemostasis and plasma leakage, or also from substantial blood loss. Immediately after hospitalization, the haematocrit, platelet count and vital signs should be examined to assess the patient's condition and intravenous fluid therapy should be started. The patient requires regular and sustained monitoring. If the patient has already received about 1,000 ml of intravenous fluids and the vital signs are still not stable, the haematocrit should be repeated and: (a) if the haematocrit is increasing, intravenous fluid should be changed to colloidal solution preferably Dextran, or (b) if haematocrit is decreasing, fresh whole blood transfusion 10 ml/ kg/ dose should be given.

In case of continued or profound shock when pulse and blood pressure are undetectable, the patient should be given colloidal fluid following the initial fluid bolus. However, in the case of persistent shock when, after initial fluid replacement and resuscitation with plasma or plasma expanders, the haematocrit continues to decline, internal bleeding should be suspected. It may be difficult to recognize and estimate the degree of internal blood loss in the presence of haemoconcentration. It is thus recommended to give fresh whole blood in small volume of 10 ml/ kg bodyweight at one time. Blood grouping and matching should be done for all patients in shock as a routine precaution. Oxygen should be given to all patients in shock. Fluid replacement must be stopped when the haematocrit becomes stable, after the 24-48 hours period of leakage, and vital signs return to normal and a diuresis ensures. Over

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transfusion of fluid at this stage carries the risk of heart failure or pulmonary edema when a return of extravasated plasma takes place (132, 133).

13. Clinical Manifestations

Dengue virus infection may be asymptomatic or may cause undifferentiated febrile illness, dengue fever (DF), dengue haemorrhagic fever (DHF) including dengue shock syndrome (DSS) (Figure 1). Infection with one dengue serotype gives lifelong immunity to that particular serotype, but there is no cross protection for the other serotypes. The clinical presentation depends on age, immune status of the host, and the virus strain.

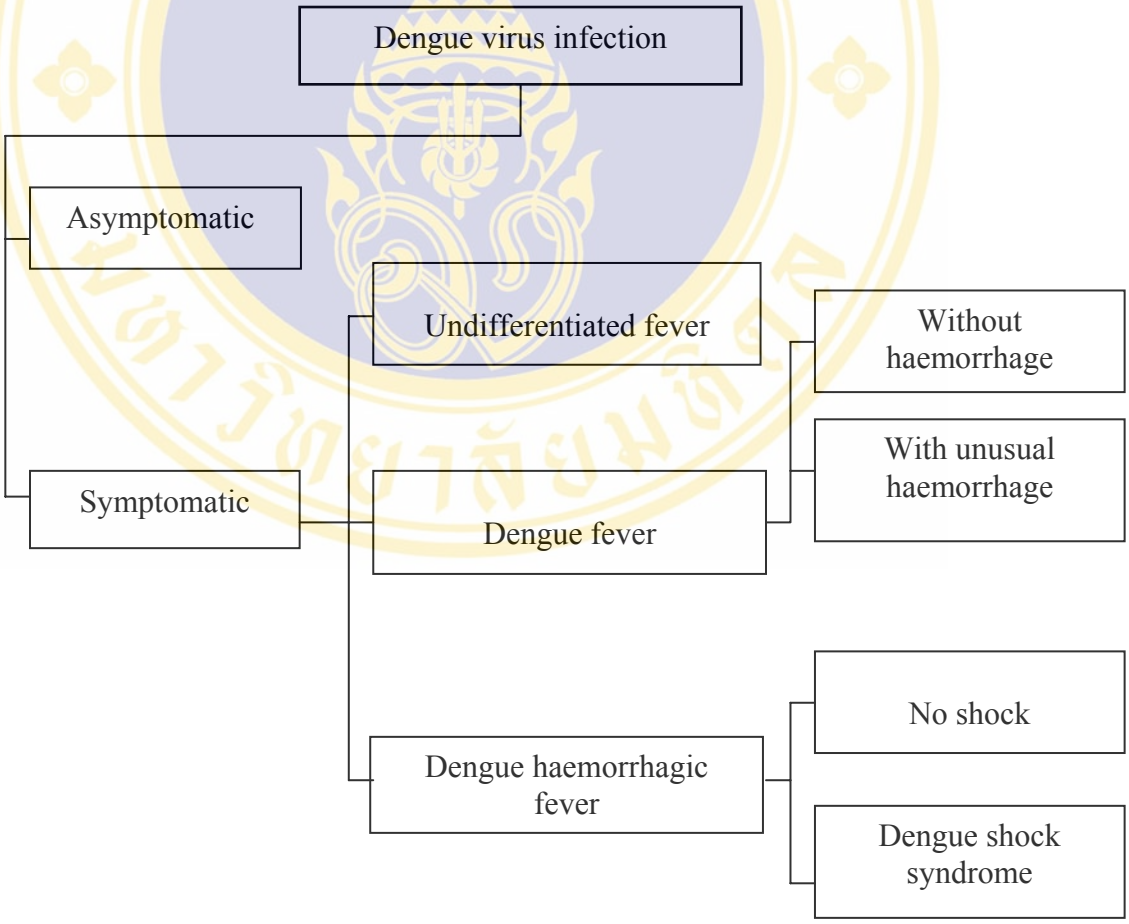

Figure 1 Manifestations of dengue virus infection

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13.1 Undifferentiated fever

Infants and children who are infected with dengue virus at the first time (primary infection) may develop simple fever like viral infection syndrome. Maculopapular rashes may accompany the fever or may appear during defervescence.

13.2 Dengue fever

After an incubation period of 4-6 days (the minimum is 3 days and the maximum is 10 days), various non-specific and undifferentiated symptoms are presented. DF patients may have an undifferentiated febrile disease often with a maculopapular rash, or low to high fever, severe headache, and abdominal or joint pain. Petechiae are not uncommon. Leukopenia is usually seen and thrombocytopenia may be observed and may be associated with prolonged fatigue and depression. DF may be accompanied by bleeding complication such as epistaxis, gastrointestinal bleeding, etc. These symptoms vary in severity and duration depending on the age of patients (8, 21).

13.3 Dengue haemorrhagic fever

Typical cases of DHF are characterized by four major clinical manifestations; high fever, haemorrhagic phenomena, hepatomegaly and often, circulatory failure. Moderate to marked thrombocytopenia with concurrent haemoconcentration is a distinctive clinical laboratory finding of DHF. The major pathophysiological change, that determines the severity of disease in DHF and differentiates it from DF, is the leakage of plasma as manifested by an elevated haematocrit, a serous effusion or hypoproteinaemia.

DHF patients commonly present with a sudden rise in temperature accompanied by facial flush, and other non-specific constitutional symptoms resembling DF patients such as anorexia, vomiting, severe headache, muscle or bone/joint pain and abdominal pain are common. The most common haemorrhagic phenomenon is a positive tourniquet test. Epistaxis and gingival bleeding occur infrequently; mild gastro-intestinal haemorrhage may be observed during the febrile period. The liver is usually palpable to 2-4 cms below the costal margin. Although liver size is not correlated with

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disease severity, an enlarged liver is observed more frequently in shock than in non-shock cases. The critical stage of the disease course is reached at the end of the febrile phase. After 2-7 days of fever, signs of circulatory disturbance of varying severity often accompany a rapid fall in temperature. The patients may sweat, be restless, have cool extremities and show some changes in pulse rate and blood pressure. In less severe cases, these changes are minimal and transient, reflecting a mild degree of plasma leakage. Many patients recover spontaneously, or after a short period of fluid and electrolyte therapy. In more severe cases, when plasma loss is critical, shock ensues and can progress rapidly to profound shock and death if not properly treated (8, 21).

13.4 Dengue shock syndrome

Dengue shock syndrome (DSS) is the condition of dengue virus infected patients who progress to shock suddenly and deteriorate after a fever of 2-7 days' duration. This deterioration occurs at the time of, or shortly after, the fall in temperature. There are the typical signs of circulatory failure: the skin becomes cool, blotchy, and congested: circumoral cyanosis is frequently observed; the pulse becomes rapid. Patients may initially be lethargic, then become restless and rapidly enter a critical stage of shock. Acute abdominal pain is a frequent complaint shortly before the onset of shock.

DSS is usually characterized by a rapid, weak pulse with narrowing of the pulse pressure (≤20 mmHg) or hypotension with cold, clammy skin and restlessness. Patients in shock are in danger of dying if appropriate treatment is not promptly administered. Patients may pass into a stage of profound shock, with the blood pressure or pulse becoming imperceptible. However, most patients remain conscious almost to the terminal stage. The duration of shock is short: typically the patient dies within 12-24 hours, or recovers rapidly following appropriate volume-replacement therapy. Pleural effusion and ascites may be detected by using physical examination or radiography. Uncorrected shock can give rise to a complicated course, with the development of metabolic acidosis, severe bleeding from the gastrointestinal tract and other organs, and a poor prognosis. Patients with intracranial haemorrhages may

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convulse and enter a coma. Encephalopathy, reported occasionally, can occur in association with metabolic and electrolyte disturbances or intracranial bleeding (8, 21).

Criteria for clinical diagnosis of DHF/DSS

- Fever-acute onset, high, continuous, lasting 2 to 7 days.
- Haemorrhagic manifestations, including at least positive tourniquet test and any of the followings: scattered petechiae, confluent petechial rash, epistaxis, gum bleeding, and haematemesis and/or melena.
- Enlargement of the liver.
- Shock- manifested by rapid and weak pulse with narrowing of the pulse pressure (20 mmHg or less) or hypotension, with the presence of cold clammy skin and restlessness.

Laboratory criteria

- Thrombocytopenia (platelet $\leq$100,000 cells/mm^3).
- Haemoconcentration: haematocrit increased by 20 percent or more of base-line value (134).

The first two clinical observations, plus one of the laboratory findings (or at least a rising haematocrit), are sufficient to establish a provisional diagnosis of DHF (8). The physical and laboratory findings associated with the various grades of severity of DHF are shown in Figure 2 (134).

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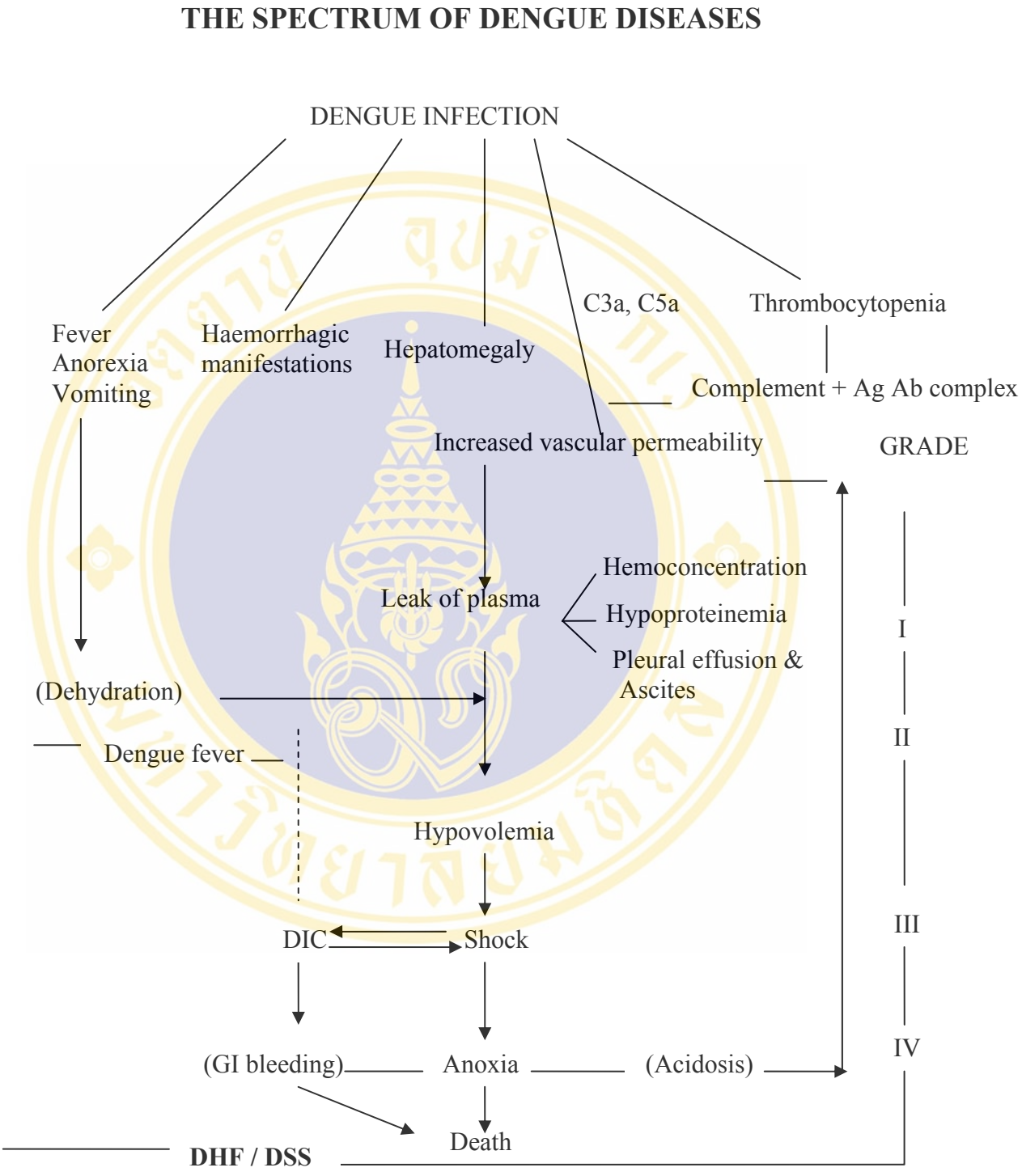

Figure 2 The spectrum of dengue diseases ; DIC = Disseminated Intravascular Coagulation

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Grading severity of dengue haemorrhagic fever

DHF is classified into four grades of severity, where grades III and IV are considered to be DSS. The presence of thrombocytopenia with concurrent haemoconcentration differentiates grades I and II DHF from DF.

Grade I: Fever accompanied by non-specific constitutional symptoms; the only haemorrhagic manifestation is a positive tourniquet test and/or easy bruising.

Grade II: Spontaneous bleeding usually in the form of skin and / or other haemorrhages in addition to the manifestations in Grade I.

Grade III: Circulation failure manifestation by rapid and weak pulse, narrowing of pulse pressure (20 mmHg or less) or hypotension with the presence of cold clammy skin and restlessness.

Grade IV: Profound shock with undetectable blood pressure and pulse (134, 135).

Clinical manifestations of DF/DHF in adults

Cuba's experience in 1981, with 130 adult cases (26 with outcome), showed that the infection was usually manifested by the clinical symptoms of dengue fever (high fever, nausea/vomiting, retro-orbital headache, myalgias and asthenia), regardless of whether the patient had a fatal outcome or not. Less frequently, patients demonstrated thrombocytopenia and haemorrhagic manifestations, the most common of which were skin haemorrhages, menorrhagia, and haematemesis. Overt shock in adults was less frequently observed than in children, but was severe when it did occur. It was found mostly in white adults with a history of bronchial asthma and other chronic diseases. In adolescents and young adults, where haemorrhage appears to be relatively more severe than in children, histological evidence of intravascular clotting in small vessels has been recognized (14). In one series of 1,000 adult cases studied in Cuba, the persons who were severely ill usually showed thrombocytopenia and haemoconcentration (33).

The study in Lucknow, India found that 79% of dengue patients were mainly in the age between 11-30 years old. The onset was abrupt in all the patients, severe frontal headache was observed in 97%, myalgias in 90%, skin rash in 40%, vomiting

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in 29% and arthralgias in knee and hip joints in 9%. Anuria was seen in two patients. Lymphadenopathy was noted in 14%, hepatomegaly in 4%, being associated with mild jaundice in one patient, and splenomegaly in 2% of the patients. Involvement of the heart and lung was seen in one patient each and no case with encephalitis was recorded. Haemorrhages from various sites were observed in 54% of the patients and 17 patients had profound shock (136).

The prospective study was conducted on 110 adult patients in New Delhi, India. The result showed the severity as followings: Grade I; 48 cases (43.6%), Grade II; 40 cases (36.4%), Grade III; 10 cases (9.1%) and Grade IV; 12 cases (10.9%). All of them had fever where as headache, myalgias, and arthralgias were the common symptoms seen in 80.9%, 76.2% and 52.3% of the patients, respectively. Spontaneous bleeding was seen in 62 patients (56.4%) with mucocutaneous bleeding being the most common (46 patients). Gastrointestinal bleeding was seen in 38 (34.5%) patients. In as many as 40 patients, the haemorrhagic manifestations occurred after the fever had come down. Fifty-five patients (50%) required platelet transfusions. Twelve patients died, giving a mortality rate of 0.9% (137).

From the retrospective study, were 347 patients both children and adults with serologically confirmed dengue infection admitted to Chonburi Hospital, Thailand in 2001. The results showed that 128 (37%) patients had DF and 219 (63%) had DHF. Patients with DHF were significantly older than patients with DF (11 years vs 8 years). Clinical bleeding was noted in 124 individuals, both with DF (n = 24) and DHF (n = 100), and significantly more frequently in adults. Twenty-nine (13.2%) of all DHF cases were caused by primary infection. Secondary dengue infection was associated significantly with the development of DHF in children, OR (95% CI) = 3.63 (1.94-6.82), $P < 0.0001$, but not in adults, OR (95% CI) = 0.6 (0.02-6.04), $P = 1$. Unusual clinical manifestations were observed in 23 patients: three presented with encephalopathy and 20 with highly elevated liver-enzymes. In the latter group, four patients were icteric and nine had gastrointestinal bleeding. Dengue should be considered as a differential diagnosis in patients with clinical gastrointestinal bleeding in association with increased liver enzymes (138).

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In 2003, Garcia-Rivera *et al.* (139) studied dengue disease in Puerto Rico, Americas and found that the elderly (≥65 years) appeared to be more likely than youth (2-18 years) and younger adults (19-64 years) to develop severe illness when infected with the dengue virus. The clinical evaluation of elderly patients with dengue must include a careful assessment of increased capillary permeability and occult hemorrhage in order to avoid complications from delayed identification and treatment of severe dengue infection.

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CHAPTER III
MATERIALS AND METHODS

Study Design

The research is a descriptive prospective study of dengue virus infected patients admitted to Phetchabun Hospital, from September, 2003 to August, 2004.

Study Methods

The patients admitted to the Department of Medicine and Department of Pediatrics were enrolled in this study. All patients had three criteria: fever, tourniquet test (TT) positive, and any of the followings: leukopenia, thrombocytopenia, or haemoconcentration. Questionnaire forms including clinical manifestations and laboratory findings were collected by interviewing, observation, medical records everyday, at least 3 days until discharge but not more than 10 days. Blood specimens were collected before patients' discharge and separation of serum samples. Antibodies to dengue viruses were detected by immunochromatographic (IC) test. Data of dengue virus infected patients were analysed.

Ethical Consideration

Research was approved by the Ethical Clearance/ The committee on Human Rights Related to Human Experimentation, Mahidol University. Written consent to participate in the research was obtained from each patient (see appendix B). All patients were allowed to leave the study at anytime, if they want to.

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Study Population

Sample size was calculated by the formula shown in appendix C. Three hundred dengue patients admitted to Phetchabun Hospital including adult, 58 cases and children, 242 cases who participated in this study. All patients had three criteria: fever, tourniquet test (TT) positive, and any of the followings: leukopenia, thrombocytopenia, or haemoconcentration. After laboratory confirmation by IC test, adult 55 cases and children 231 cases (total 286 cases) were analysed for clinical manifestations and type of dengue virus infection.

Collection of Blood Specimens

All patients enrolled in this study were informed of the aims, methods, and anticipated benefits of the study. Blood samples (2-3 ml) were collected by venepuncture before the patients' discharge. The samples were centrifuged at 500xg, room temperature (20-25˚C) for 10 minutes. The sera were separated and stored frozen at –20˚C until use.

Immunochromatographic Test

In the Dengue Rapid Test (PanBio, Brisbane, Australia), antibodies to dengue virus were determined by a rapid colloidal gold-based immunochromatographic (IC) test for the separate determination of immunogloulin (IgM) and immunogloulin G (IgG) antibodies in a capture assay format according to the manufacturer's instruction. Specimens were run blind. Buffer, 3 drops (75 µL) was added to the single glass or plastic tube. Using the loop provided, serum sample was added 1 µL to the tube. The tube was shaken with adequate mixing of the serum in the strip buffer. The test strip was added to the tube containing diluted serum. The red coloured end marked "SAMPLE" was placed into the tube. Whereupon IgM and IgG were captured by lines of either anti-human IgM antibody and anti-human IgG antibody, respectively, striped onto the test strip. At the same time gold-labelled anti-dengue monoclonal antibody (MAb) was rehydrated by the addition of three drops of buffer. After the serum reached the lines of either anti-human IgM antibody and anti-human IgG antibody, gold–labelled anti-dengue MAb to complex with dengue antigens stabilized in the test

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strip. Whereupon it could bind to and reveal captured IgM or IgG antibody that was reactive with dengue virus. After 15 minutes the assay result was visible through the test strip. Captured gold-labelled antigen-antibody complexes appeared as maroon lines (see appendix D).

Data Collection

The data of dengue patients were obtained as follows:

1. Demographic data such as age, sex, occupation, education level were collected from medical records.
2. Clinical manifestations:
   - 2.1 Headache, anorexia, myalgias, joint pain, nausea/ vomiting, retro-orbital pain, abdominal pain, flush face, coryze, and diarrhea were collected from interview and observation.
   - 2.2 Fever, gum bleeding, uterine bleeding, epistaxis, melena, oliguria, haematemesis, and shock were collected from medical records.
3. Physical findings:
   - 3.1 Petechiae, TT positive, and liver enlargement were collected from physical examination.
   - 3.2 Pleural effusion was collected from medical records.
4. Laboratory findings such as haematocrit (Hct), white blood cell count (WBC), platelet count, neutrophils, lymphocytes, atypical lymphocytes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time (PT), partial thromboplastin time (PTT), albumin, creatinine, blood uria nitrogen (BUN), sodium ion (Na^+), potassium ion (K^+) were collected from medical records.
5. Serological test for dengue virus infection by IC test.
6. Outcome of the patients: recovered or dead.

Definition of Terms

Children and adult: Children cases were the patients with age lower than 15 years old. Adult cases were the patients with age equal to or higher than 15 years old.

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Screening method: Fever, tourniquet test (TT) positive and leukopenia or thrombocytopenia or haemoconcentration.

Primary infection: The first dengue virus infection was determined by rapid IC test with a visible IgM line without a visible IgG line.

Secondary infection: The second dengue virus infection was determined by IC test with a visible IgG line with or without a positive IgM line.

Convalescent serum: The serum sample from the patient was collected at convalescent stage that takes 5-7 days after the onset of illness or on the date of patients' discharge.

Clinical manifestations : Signs and symptoms of dengue cases included fever, TT positive, headache, retro-orbital pain, nausea, vomiting, coryze, anorexia, abdominal pain, myalgias, gum bleeding, joint pain, flush face, epistaxis, haematemesis, liver enlargement, melena, petechiae, diarrhea, uterine bleeding, plasma leakage, pleural effusion (observed on a chest x-ray), and shock.

Fever: Patients had temperature ≥38° C (oral).

Liver enlargement: Patients had liver size ≥1 cm below costal margin.

Positive tourniquet test: The number of petechiae count was ≥10 dots/ 2.5 cm^2 (1 $inch^2$) at anticubital fossa after applying a blood pressure of half way between systole and diastole for 5 minutes (3, 15). The tourniquet test was measured when laboratory findings had any of the followings: leukopenia or thrombocytopenia or haemoconcentration,.

Plasma leakage: Patients were considered to have plasma leakage if there was any of the following signs: pleural effusion, haemoconcentration, hypoalbuminemia (serum albumin <3.5 gm/dl), or shock without obvious haemorrhage.

Leukopenia: The number of white blood cells was ≤5,000 cells/mm^3. White blood cell count was performed after the patients admitted to the hospital.

Thrombocytopenia: The number of platelet counts was ≤100,000 cells/mm^3.

Haemoconcentration: Haemoconcentration was considered according to percentage of increased haematocrit in Thai population (see appendix F).

Oliguria: Urine output was less than 0.5 ml/Kg/hr or 30 ml/ 8 hrs.

Diarrhea: Three or more liquid or watery stools in 24 hour period were considered as diarrhea.

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Shock: A patient was considered to have shock if there was any of the following signs:

- Rapid and weak pulse.
- Cold, clammy skin and restlessness.
- Narrow pulse pressure (≤20 mmHg).
- Hypotension for age (for those who were less than 5 years of age: systolic pressure <80 mmHg, and for those who were 5 years of age or more: <90 mmHg).

Data Analysis

1. Descriptive statistics were presented as the percentage distribution of demographic characteristics, clinical signs and symptoms, and dengue virus infected patients.
2. The clinical manifestations and type of dengue virus infection between children and adults were compared by using chi-square test, Fisher' s exact test, t-test, and one way ANOVA. Statistically significant difference was considered with *p*-value less than 0.05.
3. The results of dengue diagnosis by screening tests (fever, TT positive and leukopenia or thrombocytopenia or haemoconcentration) were compared with IC test and presented as percentage.

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CHAPTER IV
RESULTS

Characteristics of Study Population

The total enrollment of patients in Phetchabun Hospital between September, 2003 and August, 2004 were 300 volunteers. All patients had three criteria: fever, tourniquet test (TT) positive, and any of the followings: leukopenia, thrombocytopenia, or haemoconcentration. With clinical and laboratory confirmation, 286 patients were diagnosed as dengue virus infection. Table 1 shows age and sex distributions of the dengue cases. The incidence was highest in the age group of 5-9 years old (37.8%), followed by the 10-14 years old (37.2%) and the 15-19 years old group (11.8%). Only three female cases were aged less than one year, accounting to 1%. Fifty-five cases (19.2%) were more than or equal to 15 years old (adults). The mean age of the total study population was 11 years and ranged from 8 months to 50 years old. There were 149 (52%) male and 137 (48%) female cases with male to female ratio of 1.09:1.

Table 1 Age and sex distributions of the study population

Age (years)	Male (%)	Female (%)	Total (%)
<1	0	3 (1.0)	3 (1.0)
1-4	8 (2.8)	6 (2.4)	14 (4.8)
5-9	56 (19.6)	52 (18.2)	108 (37.8)
10-14	53 (18.5)	53 (18.7)	106 (37.2)
15-19	25 (8.7)	9 (3.1)	34 (11.8)
20-24	3 (1.0)	4 (1.4)	7 (2.5)
25-29	4 (1.4)	5 (1.6)	9 (3.2)
≥30	0	5 (1.6)	5 (1.7)
Range	2-28	0.8-50	0.8-50
Total	149 (52)	137 (48)	286 (100)

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The distribution of dengue patients by month was shown in Figure 3. The majority of cases (89.2%, 255/286 cases) occurred from April to August 2004. The highest peak of dengue cases was found in July within rainy season.

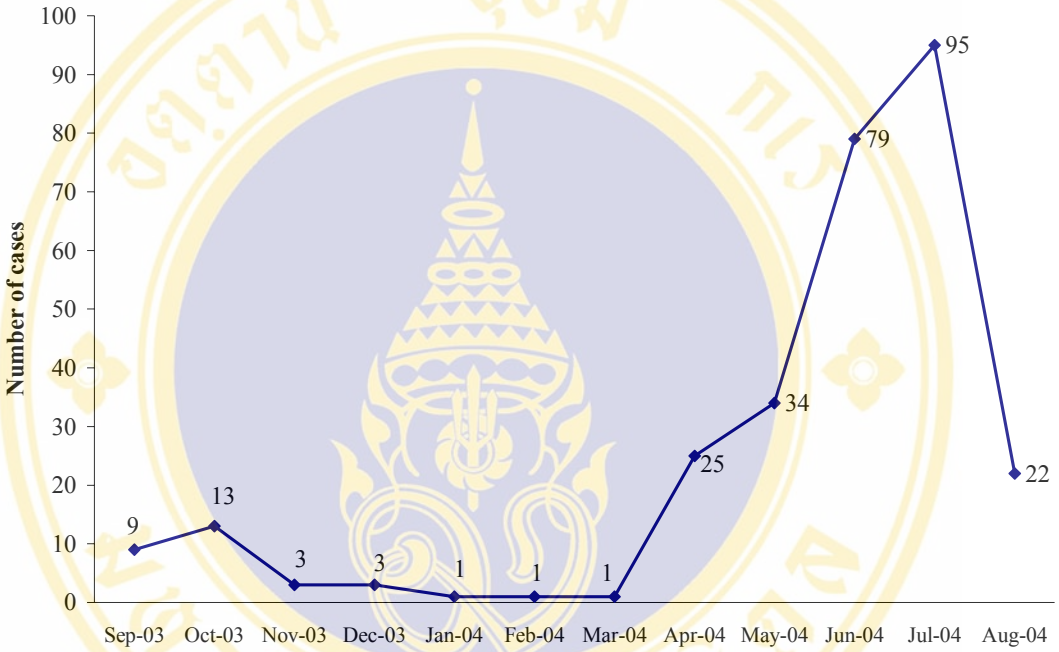

Figure 3 Dengue cases distribution by month between September 2003 and August 2004 in Phetchabun Hospital

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Distribution by Disease Severity

Among 286 dengue patients, there were 15 (5.3%) DF, 117 (40.9%) DHF I, 123 (43.0%) DHF II, and 31 (10.8%) DSS patients.

Duration of fever

Mean duration of fever until defervescence in DF, DHF I, DHF II, and DSS patients were 7.0 ±1.7 (range 4-10) days, 6.9 ± 1.7 (range 3-11) days, 7.6 ± 1.9 (range 3-14) days, and 8.4 ± 3.6 (range 3-19) days, respectively. The more severity of dengue infection, the longer was the duration of fever. The mean duration of fever until defervescence in each group of disease severity was significantly different with *p*-value of 0.001 (Table 2).

Table 2 Duration of fever in dengue patients with disease severity

Duration of fever (days)	DF No. (%) n=15	DHF I No. (%) n=117	DHF II No. (%) n=123	DSS No. (%) n=31	Total No. (%) n=286
3	0	4 (3.4)	1 (0.8)	1 (3.2)	6 (2.1)
4	1 (6.6)	5 (4.3)	4 (3.3)	0	10 (3.5)
5	2 (13.4)	19 (16.2)	10 (8.1)	4 (12.9)	35 (12.2)
6	4 (26.7)	17 (14.5)	19 (15.4)	6 (19.4)	46 (16.1)
7	1 (6.6)	32 (27.4)	29 (23.6)	4 (12.9)	66 (23.1)
8	4 (26.7)	19 (16.2)	26 (21.1)	5 (16.1)	54 (19.0)
9	2 (13.4)	15 (12.8)	16 (13.0)	4 (12.9)	37 (13.1)
≥10	1 (6.6)	6 (5.2)	18 (14.7)	7 (22.6)	32 (10.9)
Mean (±SD)	7.0 (±1.7)	6.9 (±1.7)	7.6 (±1.9)	8.4 (±3.6)	7.4 (±2.2)
Mode	6, 8	7	7	12	7
Range	4-10	3-11	3-14	3-19	3-19

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Duration of hospitalization

The mean duration of hospitalization of all enrolled dengue patients was 4.0 ± 1.0 days (range 3-9). Mean of Hospital days were increased as severity increased i.e. 3.4 ± 0.7 days for DF, 3.8 ± 0.8 days for DHF I, 4.2 ± 0.9 days for DHF II, and 4.7 ± 1.7 days for DSS group. The patients who developed shock syndrome admitted to the hospital significantly longer than the patients with less severe forms of diseas (*p*-value, <0.001), as shown in Table 3.

Table 3 Duration of hospitalization in dengue patients with disease severity

Hospital admission (days)	DF No. (%) n=15	DHF I No. (%) n=117	DHF II No. (%) n=123	DSS No. (%) n=31	Total No. (%) n=286
3	11 (73.4)	50 (42.7)	29 (23.6)	8 (25.8)	98 (34.4)
4	2 (13.3)	46 (39.3)	59 (48.0)	12 (38.7)	119 (41.6)
5	2 (13.3)	16 (13.7)	26 (21.1)	5 (16.1)	49 (17.1)
6	0	5 (4.3)	6 (4.9)	1 (3.2)	12 (4.1)
7	0	0	3 (2.4)	2 (6.5)	5 (1.8)
8	0	0	0	1 (3.2)	1 (0.3)
9	0	0	0	2 (6.5)	2 (0.7)
Mean (±SD)	3.4 (±0.7)	3.8 (±0.8)	4.2 (±0.9)	4.7 (±1.7)	4.0 (±1.0)
Range	3-5	3-6	3-7	3-9	3-9

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Serological diagnosis

All dengue patients were serologically diagnostic confirmed by using immunochromatographic (IC) test. Regarding the antibody response against dengue viruses, the results of IC test showed primary infection of 39 (13.6%) cases, and secondary infection of 247 (86.4%) cases. It was noticed that nearly all DF patients were secondary infection (93.3%). The overall ratios of secondary to primary infection classified according to the disease severity were 14:1 for DF, 6.3:1 for DHF I, 6.7:1 for DHF II, and 4.2:1 for DSS. DSS was found 10.8% of all cases. Comparing the disease severity, there was no significant difference in the distribution of disease severity among the primary and secondary dengue infections observed in this study (*p*-value, 0.677) (Table 4).

Table 4 Types of dengue infection in dengue patients with disease severity

Dengue infection	DF No. (%) n=15	DHF I No. (%) n=117	DHF II No. (%) n=123	DSS No. (%) n=31	Total No. (%) n=286	*p*-value
Primary	1 (6.7)	16 (13.7)	16 (13.0)	6 (19.4)	39 (13.6)	0.677
Secondary	14 (93.3)	101 (86.3)	107 (87.0)	25 (80.6)	247 (86.4)	

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Clinical manifestations

Table 5 shows the clinical manifestations of dengue patients by disease severity. The most severe group (DSS patients) had abdominal pain, hematemesis, melena, headache, anorexia, joints pain, myalgias, oliguria and liver enlargement in higher frequency than other dengue groups. Anorexia was found in every DSS cases (100%) and nearly all of them (96.8%) had liver enlargement. Chance of bleeding tendency was higher in the more severe DHF II and DSS groups.

Table 5 Clinical manifestations in dengue patients with disease severity

Clinical manifestations	DF No. (%) n=15	DHF I No. (%) n=117	DHF II No. (%) n=123	DSS No. (%) n=31
Abdominal pain	5 (33.3)	69 (59.0)	84 (68.3)	25 (80.6)
Gum bleeding	0	0	37 (30.1)	5 (16.1)
Epistaxis	0	0	59 (48.0)	13 (32.4)
Hematemesis	0	0	35 (28.5)	12 (34.0)
Melena	0	0	38 (30.9)	11 (36.6)
Headache	6 (40.0)	57 (48.7)	79 (64.2)	21 (67.7)
Anorexia	11 (73.3)	101 (86.3)	114 (92.7)	31 (100)
Joints pain	0	25 (21.4)	30 (24.4)	12 (38.7)
Myalgias	0	53 (45.3)	59 (48.0)	16 (51.6)
Coryza	3 (20.0)	5 (4.3)	15 (12.2)	8 (25.8)
Oliguria	0	32 (27.4)	42 (34.1)	18 (58.1)
Liver enlargement	9 (60.0)	103 (88.0)	112 (91.1)	30 (96.8)
Shock	0	0	0	30 (96.8)

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Laboratory findings according to disease severity

The percentage of thrombocytopenia (platelet count ≤100,000 cells/mm^3) in DF, DHF I, DHF II, and DSS patients were 20.0% (3/15), 97.4% (114/117), 100% (123/123), and 96.8% (30/31), respectively. There was significantly different in thrombocytopenia and dengue disease severity (*p*-value, <0.001). The mean platelet count of all dengue patients was 67,344 (± 33,094) (range 4,000-175,000 cells/mm^3). The mean (± SD) platelet count in DF, DHF I, DHF II, and DSS patients were 127,000 (± 51,808), 79,095 (± 25,011), 62,507 (± 28,523), and 39,741 (± 25,958) cells/mm^3, respectively. There was significantly different in mean platelet count and dengue disease severity (*p*-value, <0.001).

The percentage of haemoconcentration (haematocrit increase by 20% or more of base-line value) in DHF I, DHF II, and DSS patients were 47.9% (56/117), 43.9% (54/123), and 74.2% (23/31), respectively. There was significantly different in haemocomcentration and dengue disease severity (*p*-value, <0.001). The mean haematocrit (Hct) level of all patients was 42.9% ± 4.9% (normal range 37-54%). The mean (± SD) Hct level in DF, DHF I, DHF II, DSS patients were 39% (± 3.7%), 42.5% (± 4.6%), 42.9% (± 4.7%), and 46.6% (± 5.8%), respectively. There was significantly different in mean Hct and dengue disease severity (*p*-value, <0.001).

The percentage of leukopenia (white blood cells ≤5,000 cells/mm^3) in DF, DHF I, DHF II, and DSS patients were 93.3% (14/15), 72.6% (85/117), 78.0% (96/123), and 58.1% (18/31), respectively. There was no significantly different in leukopenia and dengue disease severity (*p*-value, 0.090). The numbers of white blood cells (WBC) (mean ± SD) in DF, DHF I, DHF II, DSS patients were 3,680 ± 1,475, 4,798 ± 4,290, 5,229 ± 8,344, and 5,029 ± 3,015 cells/mm^3, respectively. There was no significantly different in mean WBC and dengue disease severity (*p*-value, 0.814).

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Comparison between Dengue Virus Infected Children and Adults

Age, sex, and disease severity

Dengue patients admitted to Phetchabun Hospital (286 cases) included 231 (80.8%) children and 55 (19.2%) adults. The child to adult ratio was 4.2:1.The mean age was 8.8 (range 0.8-14) years old in the children group (<15 years old) and 20.6 (range 15-50) years old in the adult group (≥15 years old). In the children group, 117 cases (50.6%) were male and 114 cases (49.4%) were female, the male to female ratio was 1.02:1. In the adult group, 32 cases (58.2%) were male and 23 cases (41.8%) were female, the male to female ratio was 1.4:1. There was no significant difference in sex between children and adult groups (*p*-value, 0.315). Children were found in the highest percentages in DHF I group (99 cases, 42.9%) while adults were most frequently found in DHF II group (28 cases, 51%). Adults had higher percentage of DHF II and DSS than those of children. However, there was no significant difference in disease severity between children and adult groups (*p*-value, 0.449) (Table 6).

Table 6 Dengue severity groups of children and adults

Dengue patients	DF No. (%) n=15	DHF I No. (%) n=117	DHF II No. (%) n=123	DSS No. (%) n=31	Total No. (%) n=286	*p*-value
Children (<15yrs)	13 (5.6)	99 (42.9)	95 (41.1)	24 (10.4)	231 (100)	0.449
Sex ; male	11 (9.4)	51 (43.6)	45 (38.5)	10 (8.5)	117 (100)	
female	2 (1.8)	48 (42.0)	50 (43.9)	14 (12.3)	114 (100)	
Male : female	5.5:1	0.9:1	0.9:1	0.7:1	1.02:1	
Adults (≥15yrs)	2 (3.6)	18 (32.7)	28 (51)	7 (12.7)	55 (100)	
Sex ; male	1 (3.1)	8 (25.0)	19 (59.4)	4 (12.5)	32 (100)	
female	1 (4.3)	10 (43.5)	9 (39.1)	3 (13.0)	23 (100)	
Male : female	1:1	0.8:1	2.1:1	1.3:1	1.4:1	

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The distribution of dengue patients by month was shown in Figure 4. The majority dengue children (87.4%, 202/231 cases) occurred from April to August 2004 with the highest peak in July. The majority of dengue adults (78.2%, 43/55 cases) occurred from May to July 2004 with the highest peak in June. There was no significant difference in distribution by month between children and adult groups (*p*-value, 0.738).

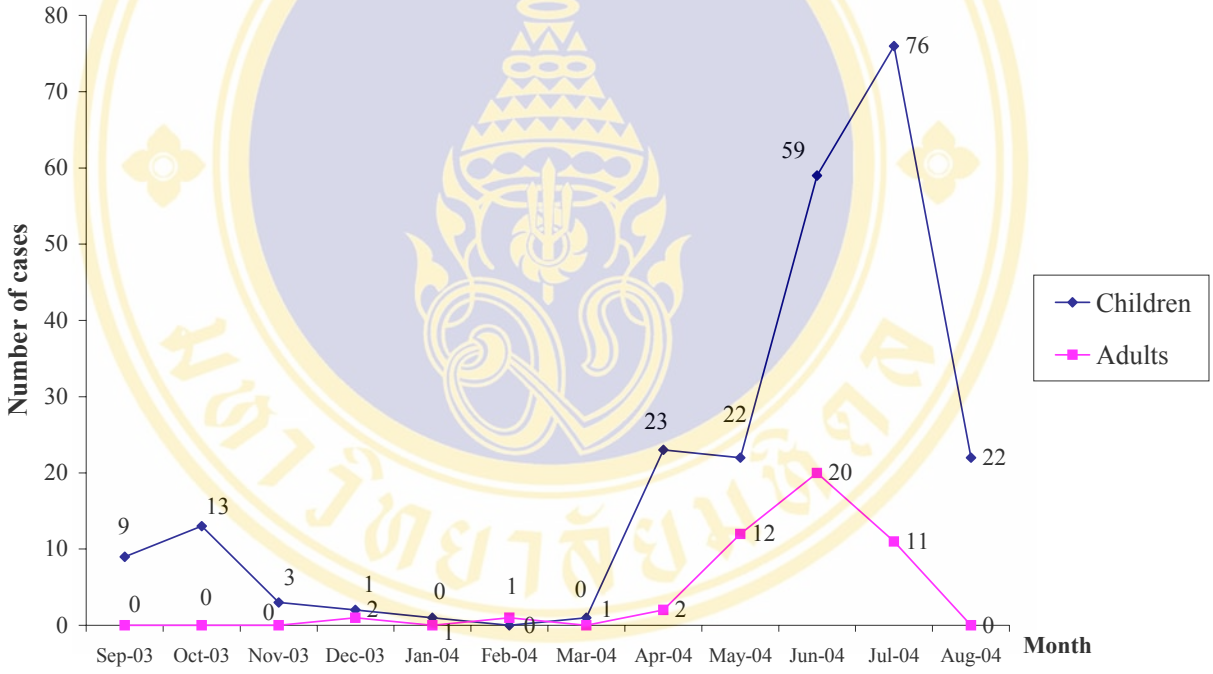

Figure 4 Dengue children and adults distribution by month between September 2003 and August 2004 in Phetchabun Hospital

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Duration of fever

All dengue patients had fever on the hospital admission and the during the hospitalization. The fever ranged from 3-19 days prior to defervescence. It lasted 5-9 days in most dengue patients (83.4%). In adult group, all patients had duration of fever in range of 4-11 days whereas 20 cases (20/231, 8.7%) of children had fever 11 days or more until defervescence. Mean ± SD duration of fever in children (7.5 ± 2.3 days) was not significantly different from that in adults (7.0 ± 1.6 days), *p*-value of 0.186 (Table 7).

Table 7 Duration of fever in children and adults with dengue virus infection

Duration of fever (days)	Children No. (%) n=231	Adults No. (%) n=55	Total No. (%) n=286	*p*-value
3	6 (2.6)	-	6 (2.1)	
4	8 (3.5)	2 (3.6)	10 (3.5)	
5	28 (12.1)	7 (12.7)	35 (12.2)	
6	34 (14.6)	12 (21.8)	46 (16.1)	
7	52 (22.6)	14 (25.5)	66 (23.3)	
8	42 (18.2)	12 (21.8)	54 (18.9)	
9	33 (14.2)	4 (7.3)	37 (12.9)	
10	8 (3.5)	3 (5.5)	11 (3.8)	
11	8 (3.5)	1 (1.8)	9 (3.1)	
12	6 (2.6)	-	6 (2.1)	
13	2 (0.9)	-	2 (0.7)	
14	1 (0.4)	-	1 (0.3)	
15	2 (0.9)	-	2 (0.7)	
19	1 (0.4)	-	1 (0.3)	
Mean (± SD)	7.5 (± 2.3)	7.0 (± 1.5)	7.4 (± 2.2)	0.186
Median	7.0	7.0	7.0	
Range (days)	3-19	4-11	3-19	

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Duration of hospitalization

The mean duration of hospitalization of all enrolled dengue patients was 4.0 ± 1.0 days (range 3-9). Mean ± SD duration of hospitalization in children (4.0 ± 1.0 days) was not significantly different from that in adults (4.1 ± 1.1 days), *p*-value of 0.747 (Table 8).

Table 8 Duration of hospitalization in children and adults with dengue virus infection

Hospital admission (days)	Children No. (%) n=231	Adults No. (%) n=55	Total No. (%) n=286	*p*-value
3	77 (33.3)	21 (38.2)	98 (34.3)	
4	102 (44.2)	17 (30.9)	119 (41.6)	
5	36 (15.6)	13 (23.6)	49 (17.2)	
6	10 (4.3)	2 (3.7)	12 (4.2)	
7	4 (1.7)	1 (1.8)	5 (1.7)	
8	0	1 (1.8)	1 (0.3)	
9	2 (0.9)	0	2 (0.7)	
Mean (±SD)	4.0 (±1.0)	4.1 (±1.1)	4.0 (±1.0)	0.747
Median	4.0	4.0	4.0	
Range	3-9	3-8	3-9	

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Clinical manifestations

Some clinical manifestations were more common in adults than children. The presence of petechiae, melena, headache, retro-orbital pain, nausea/vomiting, joint pain, and myalgias in adults were significantly different from that in children (*p*-value <0.05). In contrast, epistaxis, oliguria, and liver enlargement were more common in children than adults (*p*-value <0.05). Other clinical manifestations were found in both children and adults i.e. gum bleeding, hematemesis, uterine bleeding, diarrhea, anorexia, abdominal pain, coryza, flush face, and shock with no significant difference. Anorexia was predominate symptom in both groups: 88.3% in children and 96.4% in adults with dengue virus infection (Table 9).

Laboratory findings by disease severity on admission

On admission the adult dengue patients (48/55, 87.3%) had thrombocytopenia in higher percentage than children did (153/231, 66.2%) with statistical significance (*p*-value, 0.002). The mean platelet count of children and adult dengue patients were 90,745 (± 45,562) and 57,147 (± 44,183) cells/mm^3, respectively. There was significantly different in mean platelet count in children and adults group (*p*-value, <0.001). There was significantly different in mean platelet count and dengue disease severity in both children and adult group (*p*-value, <0.001 and 0.006, respectively) as shown in Table 10.

Haemoconcentration in adults (36/55, 65.5%) was also significantly different from children (106/231, 45.9%) with *p*-value of 0.009. The mean Hct of children and adult dengue patients were 40.8% (± 4.8) and 45.1% (± 5.7), respectively. There was significantly different in Hct in children and adults group (*p*-value, <0.001). In comparison of mean Hct levels in children and adults with disease severity, there was significantly different in children (*p*-value, <0.001) but not significantly different in adults (*p*-value, 0.534) (Table 10).

Leukopenia in adults (37/55, 67.3%) was not significantly different from children (172/231, 74.5%) with *p*-value of 0.178. The mean WBC of children and adult dengue patients were 6,314 (± 14,651) and 5,378 (± 3,895) cells/mm^3,

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respectively. There was no significant different between WBC in children and adults group (*p*-value, 0.639). There was no significant different between WBC and disease severity in both children and adults group (*p*-value, 0.608 and 0.748, respectively) as shown in Table 10.

The mean neutrophils of children and adult dengue patients were 46% (± 19) and 48% (± 21), respectively. There was no significant different between neutrophils in children and adults group (*p*-value, 0.471). In comparison of mean neutrophils in children and adults with disease severity, there was significantly different in children (*p*-value, 0.004) but not significantly different in adults (*p*-value, 0.711) (Table 10).

The mean lymphocytes of children and adult dengue patients were 46% (± 16) and 36% (± 17), respectively. There was significant different between lymphocytes in children and adults group (*p*-value, 0.027). In comparison of mean lymphocytes in children and adults with disease severity, there was significantly different in children (*p*-value, 0.021) but not significantly different in adults (*p*-value, 0.082) (Table 10).

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Table 9 Comparison of clinical manifestations between children and adult cases

Clinical manifestation	Children (n=231)		Adults (n=55)		*p*-value
	Frequency	Percent	Frequency	Percent	
Petechiae**	90	39.0	30	54.5	0.035*
Melena**	30	13.0	19	34.5	<0.001*
Headache**	117	50.6	46	83.6	<0.001*
Retro-orbital pain**	20	8.7	16	29.1	<0.001*
Nausea/vomiting**	116	50.2	42	76.4	<0.001*
Joint pain**	47	20.3	20	36.4	0.012*
Myalgias**	91	39.4	37	67.3	<0.001*
Epistaxis***	64	27.7	8	14.5	0.043*
Oliguria***	83	35.9	9	16.4	0.005*
Liver enlargement***	212	91.6	42	72.4	0.010*
Gum bleeding	32	13.9	10	18.2	0.415
Hematemesis	39	16.9	8	14.5	0.674
Uterine bleeding†	18	15.8	7	30.4	0.097
Diarrhea	29	12.6	8	14.5	0.692
Anorexia	204	88.3	53	96.4	0.075
Abdominal pain	147	63.6	36	65.5	0.801
Coryza	26	11.3	5	9.1	0.643
Flush face	81	35.1	21	38.2	0.665
Shock	23	10.0	7	12.7	0.547

* Statistical significance, *p*-value <0.05 by chi-square test

** Clinical manifestations were significantly different (*p*-value <0.05) and present in adults with higher frequency than in children

*** Clinical manifestations were significantly different (*p*-value <0.05) and present in children with higher frequency than in adults

† included menstruation (profused)

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Table 10 Haematological profile of dengue patients classified by disease severity and age on the day of admission

Laboratory findings	DF (n=15) Mean (±SD)	DHF I (n=117) Mean (±SD)	DHF II (n=123) Mean (±SD)	DSS (n=31) Mean (±SD)	Total (n=286) Mean (±SD)	*p*-value
Platelet count (cells/mm^3)						
: Children	163,230 (±43,209)	93,949 (±36,088)	87,127 (±41,810)	52,583 (±50,039)	90,745 (±45,562)	<0.001*
: Adults	157,500 (±20,506)	60,722 (±48,350)	47,642 (±33,299)	57,285 (±45,492)	57,145 (±44,184)	0.006*
p-value	0.860	<0.001*	<0.001*	0.825	<0.001*	
HCT (%)						
: Children	37.3 (±4.1)	40 (±4.3)	40.8 (±4.3)	44.4 (±6.8)	40.8 (±4.8)	<0.001*
: Adults	42.5 (±0.7)	45 (±4.8)	44.7 (±6.3)	47.8 (±5.3)	45.1 (±5.7)	0.534
p-value	0.109	0.001*	<0.001*	0.227	<0.001*	
WBC (cells/mm^3)						
: Children	3,938 (±1,548)	5,317 (±5,907)	7,823 (±21,948)	5,745 (±3,271)	6,314 (±14,651)	0.608
: Adults	3,050 (±494)	5,361 (±2,333)	5,753 (±4,938)	4,585 (±2,859)	5,378 (±3,896)	0.748
p-value	0.448	0.975	0.622	0.404	0.639	
Neutrophil (%)						
: Children	32 (±14)	44 (±19)	50 (±17)	45 (±22)	46 (±19)	0.004*
: Adults	52 (±9)	43 (±26)	51 (±20)	47 (±15)	48 (±21)	0.711
p-value	0.088	0.933	0.841	0.829	0.471	
Lymphocytes (%)						
: Children	53 (±9)	44 (±15)	44 (±16)	40 (±17)	46 (±16)	0.021*
: Adults	44 (±6)	37 (±22)	33 (±12)	44 (±21)	36 (±17)	0.082
p-value	0.071	0.110	0.014*	0.724	0.027*	

* Statistical significance, *p*-value <0.05 by ONE WAY ANOVA

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Laboratory findings at day of defervescence

At day of defervescence platelet count was performed in all 286 cases. Two hundred and seventy (94.4%) cases still had thrombocytopenia: 216/231 (93.5%) children and 54/55 (98.2%) adults. The presence of thrombocytopenia was not significantly different between children and adults in thrombocytopenia (*p*-value, 0.317 by Fisher's exact test). Haematocrit was assessed in all dengue cases. One hundred and thirty five (47.2%) cases had haemoconcentration: 102/231 (44.2%) children, and 33/55 (60.0%) adults. Haemoconcentration in adults was significantly different from those of children (*p*-value, 0.034).

Atypical lymphocytes were higher than normal range in adults, 81.8% (45/55) and in children, 71.9% (156/231). There was significant difference between children and adults in atypical lymphocytes (*p*-value, 0.046). There was no significant difference between children and adults in white blood cells count (*p*-value, 0.295). Determination of serum albumin was available in only 25 dengue cases. In adults, 33.3% (6/18) and 57.1% (4/7) in children had serum albumin higher than normal range (3.5-5.5 gm%). There was no significant difference in the level of albumin between children and adults (*p*-value, 0.378). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were done in all 286 cases. It was found that AST was higher than normal range (0-40 U/L) in both children (98.7%) and adults (96.4%). ALT was also higher than normal range (0-40 U/L) in both children (56.7%), and adults (61.8%). There was not significantly different between children and adults in the levels of AST (*p*-value, 0.235) and ALT (*p*-value, 0.491) (Table 11).

Table 12 shows the quantitative blood chemistry values found in children and adults with dengue virus infection. Both children and adults had laboratory values (mean ± SD) in normal range including haematocrit, prothrombin time (PT), lymphocytes, neutrophils, blood uria nitrogen (BUN), sodium ion (Na^+), potassium ion (K^+), and. However, atypical lymphocytes, partial thromboplastin time (PTT), AST, and ALT levels were higher than normal levels in both groups. Platelet count was lower than normal range (72,512 ± 30,932 cells/mm^3) in children and very low level (45,642 ± 33,331 cells/mm^3) was found in adults. Creatinine and albumin were lower

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than normal range in children but normal in adults. There was significantly different between children and adults in the levels of haematocrit, platelet count, PT, and ALT with *p*-value of 0.001, 0.001, 0.047, and 0.002, respectively.

Table 11 Laboratory findings of dengue virus infected children and adults on a day of defervescence

Laboratory findings*	Range of value	Children No. (%)	Adults No. (%)	Total No. (%)	*p*-value
Atypical lymphocytes (%)	0-3	75 (32.5)	10 (18.2)	85 (29.7)	0.046**
	>3	156 (67.5)	45 (81.8)	201 (70.3)	
White blood cells (cells/mm^3)	<5,000	170 (73.6)	35 (63.6)	205 (71.7)	0.295
	5,000-10,000	46 (19.9)	14 (25.5)	60 (21)	
	>10,000	15 (6.5)	6 (10.9)	21 (7.3)	
Serum albumin† (gm/dl)	3.5-5.5	3 (42.9)	12 (66.7)	15 (60)	0.378
	>5.5	4 (57.1)	6 (33.3)	10 (40)	
AST (U/L)	0-40	3 (1.3)	2 (3.6)	5 (1.7)	0.235
	>40	228 (98.7)	53 (96.4)	281 (98.3)	
ALT (U/L)	0-40	100 (43.3)	21 (38.2)	121 (42.3)	0.491
	>40	131 (56.7)	34 (61.8)	165 (57.7)	

* Normal range values:
atypical lymphocytes; 0-3% white blood cell count; 5,000-10,000 cells/mm^3
serum albumin; 3.5-5.5 gm/dl AST and ALT; 0-40 U/L

** Statistical significance, *p*-value <0.05 by chi-square test

† Serum albumin was determined in 25 dengue cases.

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Table 12 Laboratory findings dengue children and adult patients

Laboratory findings	Children		Adults		*p*-value
	n	Mean (± SD)	n	Mean (± SD)	
Haematocrit (%)	231	42.2 (± 4.7)	55	45.8 (± 5.3)	0.001*
Platelet count (cells/mm^3)	231	72,512 (± 30,932)	55	45,642 (± 33,331)	0.001*
PT (sec)	231	14.8 (± 11.2)	55	18.7 (± 17.9)	0.047*
ALT (U/L)	231	90.4 (± 129.5)	55	165.9 (± 241.3)	0.002*
WBC (cells/mm^3)	231	5,770 (± 14,182)	55	4,697 (± 2,911)	0.578
Neutrophils (%)	231	45.9 (± 18.9)	55	48 (± 21.4)	0.471
Lymphocytes (%)	231	44.2 (± 15.9)	55	37.6 (± 17.5)	0.472
Atypical lymphocytes (%)	229	8.8 (± 8.5)	54	13.8 (± 12.7)	0.092
BUN (mg/dl)	5	12.2 (± 3.7)	20	12.5 (± 3.8)	0.897
Creatinine (mg/dl)	6	0.7 (± 0.2)	20	1.5 (± 1.8)	0.312
Albumin (gm/dl)	7	3.2 (± 0.8)	18	3.7 (± 0.5)	0.082
Na^+ (mEq/L)	5	141.4 (± 3.1)	20	141.9 (± 3.2)	0.769
K^+ (mEq/L)	5	3.9 (± 0.5)	20	4.0 (± 0.4)	0.565
AST (U/L)	231	200.4 (± 313.5)	55	299.8 (± 459.7)	0.057
PTT (sec)	231	45.8 (± 31.7)	55	47.1 (± 31.5)	0.782

* Statistical significance, *p*-value <0.05 by t-test

Normal range levels in blood circulation:

haematocrit; 37-54% — platelet count; 100,000-440,000 cells/mm^3

PT; 12-18 sec — WBC; 5,000-10,000 cells/mm^3

neutrophils; 40-75% — lymphocytes; 20-50%

atypical lymphocytes; 0-3% — BUN; 8-23 mg/dl

creatinine; 1-2 mg/dl — albumin; 3.5-5.5 gm/dl

Na^+; 137-150 mEq/L — K^+; 3.5-5.3 mEq/L

AST and ALT; 0-40 U/L — PTT; 25-38 sec

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Diagnosis of Dengue Virus Infection

Serological diagnosis by immunochromatographic test

The total enrollment of patients was 300 volunteers. With laboratory confirmation by immunochromatographic test (IC test), 286 patients were diagnosed as dengue virus infection. Out of 231 children, 35 (15.2%) had primary dengue infection and 196 (84.8%) had secondary dengue infection. The ratio of secondary to primary infection was 5.6:1. In the adult group, out of 55 cases, 4 cases (7.3%) had primary and 51 cases (92.7%) had secondary dengue infection. The ratio of secondary to primary infection was 12.8:1. Comparing between adults and children dengue cases in different severity, there was no statistically significant difference in primary antibody response (*p*-value, 0.469) or secondary antibody response (*p*-value, 0.735) (Table 13).

Table 13 Frequencies of primary and secondary dengue cases in children and adults with disease severity

Dengue infection	DF No. (%) n=15	DHF I No. (%) n=117	DHF II No. (%) n=123	DSS No. (%) n=31	Total No. (%) n=286	*p*-value
Primary						
Children	1 (2.9)	15 (42.9)	13 (37.1)	6 (17.1)	35 (100)	
Adults	0	1 (25.0)	3 (75.0)	0	4 (100)	
Total	1 (2.6)	16 (41.0)	16 (41.0)	6 (15.4)	39 (100)	0.469
Secondary						
Children	12 (6.1)	84 (42.9)	82 (41.8)	18 (9.2)	196 (100)	
Adults	2 (4.0)	17 (33.3)	25 (49.0)	7 (13.7)	51 (100)	
Total	14 (5.7)	101 (40.9)	107 (43.3)	25 (10.1)	247 (100)	0.735

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Screening tests of dengue infection

In children group

Among 300 hospitalized volunteers, 242 cases were children with fever, tourniquet test (TT) positive and leukopenia/or thrombocytopenia/or haemoconcentration, there was 231 cases (95.5%) of dengue infection which confirmed by using IC test.

In adult group

Among 58 cases of adults with fever, TT positive and leukopenia/or thrombocytopenia/or haemoconcentration, there was 55 cases (94.8%) of dengue infection which confirmed by using IC test.

Therefore, using at least 3 indicators as screening tests as employed in this study could identify the dengue infections (positive predictive value) 95.3% (286/300) of cases.

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CHAPTER V
DISCUSSION

Dengue haemorrhagic fever (DHF) is primarily described as a disease that almost exclusively (>95%) affects children under the age of 15 (140). However, in the past two decades a significant shift in the age distribution has been noted. In Thailand data have revealed a progressive shift of the median age of children with DHF from 3.8 years in 1960s to 5.6 years in the 1970s, 7.4 years in the 1980s (11), and 8.5 years in 2000s (138, 141-143). In Jakarta, Indonesia, the proportion of DHF cases in the age-group of patients older than 10 years increased from 11% to 28% between 1975 and 1989 (144).

During September 2002 and August 2003, a total of 300 suspected dengue patients attended in Phetchabun Hospital and 286 patients were confirmed with recent dengue virus infection. The annual reports of dengue patients in Phetchabun Hospital were 496 cases (48.63 per 100,000) in 2002 and 287 cases (37.14 per 100,000) in 2003. In this study, lower number of dengue cases was diagnosed since all cases was laboratory-confirmed by serological method, clinical findings and/or haematological profile, whereas the hospital annual reports were based on only clinical diagnosis. Moreover, some dengue patients may be lost because the early clinical screening (ruled out) of dengue patients was performed by physicians prior to laboratory confirmation.

The demographic distribution of disease showed that DHF affected all age groups and both sexes. The highest attack rate (37.8%) was found in 5 to 9 years age group similar to those reported by Halstead (24) and Nimmannitya (145). However, the attack rate of dengue virus infected cases between 10-14 years (37.2%) was also high indicating a definite shift in the age distribution of DHF from pediatric age to

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teenage groups. This similar evidence was described previously. Through in the early years, mainly children were affected, recently more cases are being seen in 16-20 years age group (146-148).

Over the past ten years a shift in the age distribution of DHF in the South-East Asian countries has been reported. Hadinegoro and Nathin (144) noticed an increase of DHF patients aged more than 10 years from 11% to 28% over a ten years observation in Jakarta, Indonesia. Agarwal *et al.* (136) reported that during the 1996 epidemic in Lucknow, India, 53% of the patients with DHF were aged between 11-30 years. Harris *et al.* (143) reported 25% of DHF cases of the 1998 dengue epidemic in Nicaragua were in patients 15 years or older. In the present study, the attack rate in adult dengue patients with age more than 15 years was 19.2% in Phetchabun Hospital compared to 30% and 35% of the whole country from in 2002 and 2003 (4), respectively. The incidence of dengue cases increased in adults may be due to the virus mutation to new strain, many serotypes co-circulation in endemic area or primary dengue infection occurred in older age. It was found that dengue virus serotype 2 and serotype 3 were predominate in Phetchabun Province in 2003 and 2004, respectively.

Patients with DHF were older than DF cases (11 years vs 9 years) comparable to the study in Chonburi Hospital, Thailand (11 years vs 8 years) (138).

In children with dengue virus infection, male and female were infected equally but in adult, male were infected with dengue viruses in higher numbers than female. This finding was similar to previous reports (35, 42, 138). Moreover, in mild disease like DF, a great numbers of male were infected and higher than female five times whereas the most severity form of dengue (DSS) occurred in female more than male comparable to the study in Vietnamese children (141).

Most dengue children and adults (83.4%) had duration of fever between 5-9 days with higher percentages than that in the previous which reported DHF in children with duration of fever between 5-9 days of 55% (149) and 58% (150).

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All confirmed dengue virus infected cases had fever. Enlargement of the liver is found in most cases. The clinical signs and symptoms in dengue infection are non-specific. The DHF patients had more clinical signs and symptoms than DF cases. Minor haemorrhagic phenomena may be seen during the febrile phase such as petechiae, epistaxis, or liability to bruising. A maculopapular rash or confluent petechiae eruption may be seen after the temperature falls. Abdominal tenderness of right upper quarter and liver enlargement were most frequently found in DSS and correlated with disease severity. Abdominal pain is a well known warning that DSS is impending (151). The present study found abdominal pain was predominate in DSS and significantly different among disease severity groups (DF, DHF I, DHF II, and DSS). Anorexia were found in all DSS cases (100%) and significantly different from other groups. Liver enlargement correlated well with the severity of dengue disease.

Some clinical manifestations including petechiae, headache, melena, retro-orbital pain, nausea/vomiting, joint pain, and myalgias were observed in adult dengue patients more than children. Wali *et al.* (137) reported the common presentation of hospitalized adult dengue patient were headache and myalgia. Possible explanation of more headache and myalgias in adults is that adults can express pain better than children, however other factors can not be excluded. Tantawichien *et al.* (152) carried out the retrospective study of 140 serologically confirmed adult patients with dengue virus infection in a private clinic in Bangkok and found that nausea/vomiting was the most common presentation.

Epistaxis, oliguria, and liver enlargement were significantly more common in children than in adults. This may be due to the difference in anatomy and physiology of organs involved between children and adults. In India, hepatomegaly was presented in 14.9% of adults cases (137) lower than that in the present study (91.6% of children and 72.4% of adults).

In DSS group, the adults (12.7%) showed signs and symptoms of shock in higher frequency than in the children (10.4%) similar to the study in Chonburi

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Province (13% of adults and 11% of children) (153.) Possible cause of increased shock may be due to less intake of fluids and delay diagnosis and treatment in adults.

Thrombocytopenia was more significantly common in adults and children. The high frequencies of dengue patients with thrombocytopenia occur in all adult patients on the day of admission and defevescense (154, 155). Mechanisms of dengue virus induced thrombocytopenia may be due to the bone marrow suppression, destruction by the liver and spleen, immune-mediated (C3) and immune complex injury as demonstration of dengue antibody complexes on the platelet surface, the *in vitro* spontaneous aggregation to vascular endothelial cell pre-infected by dengue virus inducing platelet aggregation, causing lysis and platelet destruction (156).

Out of 286 study subjects, 15 cases had more than 5-fold increased aspartate aminotransferase (AST) and a majority of them presented with hepatomegaly on hospital admission. The impact of dengue viruses on liver function was indicated by abnormal levels of AST, alanine aminotransferase (ALT), bilirubin, alkaline phosphatase. The fact that the level of AST was higher than that of ALT and the elevation of transaminases was mild to moderate in most cases (<5-fold greater than the normal upper limit of AST and ALT) showed that liver involvement was also mild to moderate in most cases of DHF (154).

Liver impairment may be related to the severity of DHF (158). In this study liver abnormality was found in DHF II (55%) and DSS (25%). Pancharoen *et al.* (159) showed a relationship between severity of disease and increased serum transaminase. Although the present study found no significant difference among dengue diseases severity, DHF and DSS adults patients showed gradually increased levels of AST and ALT. AST levels in dengue patients are usually higher than ALT contrast to viral hepatitis (154). Kuo *et al.* (83) mentional that 93% of DF adult patients had abnormal AST, of which 11% >400 U/L, maximum 3,200 U/L different from this study (40.9% of DF adults patients, 48 ± 1.0 U/L).

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Most of dengue patients were secondary infection similar to the previous report (160). However, secondary dengue infection was not only occurred in DHF but also in DF group. In fact, some DF patients were excluded from this study because TT was negative. The previous studies reported that DF patients showed positive TT approximately 40-60% (138, 150). Therefore, the numbers of DF patients in this study might be underestimated. Adult patients showed higher frequency of secondary dengue infection than children (92.7% vs 84.8%). This might cause more severity in the adults. Sequential or secondary dengue virus infection are more likely to produce severe disease explained by the theory of immune enhancement (29, 69). Dengue viruses are flaviviruses which have 4 different serotypes (50). Cross-reactive antibodies from a previous heterologous dengue virus infection bind to the new infecting dengue virus. This immune complex facilitates or enhances virus to enter cells via Fc receptor (59, 62). Antigen presenting cell will activate preexisting cross-reactive dengue virus–specific T lymphocytes from the primary dengue infection (62, 63). This lead to the release of cytokines and chemical mediators which may cause plasma leakage (69, 70).

Among 300 volunteers enrolled in this study under the criteria of fever, TT positive, and any of followings: leukopenia or thrombocytopenia or haemoconcentration, 14 patients were diagnosed negative for dengue virus infection by immunochromatographic (IC) test. Serum samples from these patients were collected after days 5 of illness. These patients might not be recently infected by dengue viruses because the IC test provided 100% sensitivity after days 5 of illness (124). However, they should be confirmed by the more sensitive and specific diagnostic test such as IgM-and IgG-capture ELISA.

In this study, criteria of dengue patients included fever, tourniquet test (TT) positive, and any of the followings: leukopenia, thrombocytopenia, or haemoconcentration. The serum samples from these patients were examined by using IC test for confirmation of dengue virus infection. Based on IC test 74.5% of dengue children and 67.3% of dengue adults had leukopenia similar to the study of Kalayanarooj *et al.* (15). The combination of TT positive and leukopenia was

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recommended for use in initial screening of dengue virus infection (15). Early diagnosis of dengue virus infection is probably helpful because misdiagnosis or delay treatment of DHF patients would cause dead such as a case of a boy aged 13 years recently in Bangkok, Thailand (161).

This prospective study was the first to compare clinical manifestations, laboratory findings, and type of dengue virus infection between children and adults in Phetchabun Hospital. The statistical difference was analysed to achieve the significance of results. The results of this study would help the physicians to treat children and adult dengue patients properly and to speed up the reporting systems so that control actions would start promptly, and be effective for dengue haemorrhagic fever.

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CHAPTER VI
CONCLUSION

1. A descriptive prospective study was conducted to compare clinical manifestations, laboratory findings, and type of dengue virus infection between children and adult groups in Phetchabun Hospital between September 2003 and August 2004. The children group included the patients who were under 15 years old whereas the adult group included the patients who were ≥15 years old. All patients had three criteria: fever, tourniquet test (TT) positive, and any of the followings: leukopenia, thrombocytopenia, or haemoconcentration. Data of clinical manifestations and laboratory findings were collected by interview, observation, and medical records everyday, at least 3 days until discharge but not more than 10 days. During the hospitalization, the blood specimens were collected to confirm dengue virus infection by rapid immunochromatrographic (IC) test. Of 300 patients with clinical diagnosis of dengue virus infection, 286 patients showed dengue virus infection by IC test.

2. For data analysis of comparison, 286 patients were devided into children group (231 patients, 80.8%) and adult group (55 patients, 19.2%). The average age of children was 8.8 (range 0.8-14) years old and that of adults was 20.6 (range 15-50) years old. The ratios between male and female in children and in adults were 1.02:1 and 1.4:1, respectively. There was no significant difference in sex between children and adult groups (*p*-value, 0.315).

3. Regarding clinical manifestations, there were statistically significant difference when the children group was compared with the adult group at *p*-value <0.05. Those signs and symptoms found more in adults than in children were petechiae, melena, headache, nausea/vomiting, retro-orbital pain, myalgias, and joint pain.

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The signs and symptoms found more in children than in adults were epistaxis, oliguria, and liver enlargement.

4. On admission, the higher proportion of adults dengue patients compared with children, had thrombocytopenia (48/55, 87.3% vs 153/231, 66.2%) and hemoconcentration (36/55, 65.5% vs 106/231, 45.9%) with statistical significance, *p*-value of 0.002 and 0.009, respectively. The means platelet counts were 90,745 ± 45,562 in children and 57,147 ± 44,183 in adult dengue group. There was significantly different in means platelet counts in children and adult groups (*p*-value, <0.001). There were significantly different in the mean platelet count and disease severity in both children and adult groups (*p*-value, <0.001 and 0.006, respectively). The means haematocrit were 40.8% ± 4.8 in children and 45.1% ± 5.7 in adults. There was significantly different in Hct in children and adults group (*p*-value, <0.001). The difference in the mean haematocrit and disease severity was also observed in children (*p*-value, <0.001), but not in adults (*p*-value, 0.534).

5. At day of defervescence, the increase numbers of patients with thrombocytopenia were observed in both children and adult groups. There was not significantly different between the children and adult dengue patients (*p*-value, 0.317). High proportion of adult patients (33/55, 60%) had haemoconcentration different from children (102/231, 44.2%), *p*-value of 0.034.

6. In both children and adults, atypical lymphocytes, partial thromboplastin time, aspartate aminotransferase, and alanine aminotransferase levels were above the normal range whereas platelet count was below the normal range. There was significantly different between children and adults in the levels of haematocrit, platelet count, alanine transferase, and prothrombin time with *p*-value of 0.001, 0.001, 0.002 and 0.047, respectively.

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7. By using IC test to confirm dengue virus infection, there were primary infection 15.2% (35/231 cases) and secondary infection 84.8% (196/231 cases) in children. In dengue virus infected adults, there were primary infection 7.3% (4/55 cases) and secondary infection 92.7% (51/55 cases). Comparing between adults and children dengue cases (in disease severity), there was no significant difference in primary antibody response (*p*-value, 0.469) or secondary antibody response (*p*-value, 0.735).

8. Out of 242 children with fever, TT positive and leukopenia or thrombocytopenia or haemoconcentration, 231 cases (95.5%) were positive by using confirmed IC test. Out of 58 adults with fever, TT positive and leukopenia or thrombocytopenia or haemoconcentration, 55 cases (94.8%) were positive by using confirmed IC test.

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APPENDIX

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APPENDIX A

No. 156/2003

Documentary Proof of Ethical Clearance

The Committee on Human Rights Related to

Human Experimentation

Mahidol University, Bangkok

.....

Title of Project: **A Comparative Study of Clinical Manifestations and Type of Dengue Virus Infection between Children and Adult Patients at Phetchabun Hospital**

Principal Investigator: **Miss Piyamard Pitakarnganakul**

Name of Institution: **Faculty of Public Health**

Approved by the Committee on Human Rights Related to Human Experimentation

Signature of Chairman: _____

(Professor Dr. Srisin Khusmith)

Signature of Head of Institute: _____

(Professor Dr. Pornchai Matangkasombut)

Date of Approval: 5 SEP 2003

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APPENDIX B

Consent form

Research title: A comparative study of clinical manifestations and type of dengue virus infection between children and adult patients at Phetchabun Hospital

A Thesis in Master of Science (Public Health) Major in Infectious Diseases and Epidemiology, Mahidol University

Day of consent: _____/_____/_____ (D/M/Y)

Patients H.N …….. Age……years

I understand all statement in the consent form. I also have been given explanation regarding the objectives and methodology of the study and benefit that may occur to myself upon the participation in the study. I understand that the information will be kept confidentially. I can ask for suspicion and problem to the student and requal anytime. My name will not be presented in the study report.

Signature……. Participant
(Mr./Mrs./Ms…….)

Signature…….……..……..Witness
(Mr./Mrs./Ms…….)
Signature…….……..……..Witness

(Mr./Mrs./Ms…….)

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In case of children (< 20 years old)

For Mother/Father or Caretaker

I understand all statement in the consent form. I also have been given explanation regarding the objectives and methodology of the study and benefit that may occur to child upon the participation in the study. I understand that the information will be kept confidentially. I can ask for suspicion and problem to the student and requal anytime. Name of the child will not be presented in the study report.

Signature…… Mother/Father/Caretaker of the patient
(Mr./Mrs./Ms…….)

Signature……..Witness
(Mr./Mrs./Ms…….)
Signature……..Witness
(Mr./Mrs./Ms…….)

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APPENDIX C

Sample Size

The number of patients in this study was calculated by the formula as follows:

Group	Clinical manifestation (coryze)		Sample size
	Yes	No	
Adult	P_1	Q_1	$n_1 = m$
Children	P_2	Q_2	$n_2 = \lambda m$

$$m = \frac{\left[Z\alpha/2 \sqrt{\frac{(\lambda+1)\bar{P}\bar{Q}}{\lambda}} + Z\beta \sqrt{\frac{1}{\lambda}(\lambda P_1 Q_1 + P_2 Q_2)} \right]^2}{[P_2 (RR-1)]^2}$$

$$\bar{P} = \frac{P_2 (RR + \lambda)}{(1+\lambda)}$$

P_1 = 0.37 [Proportion of clinical manifestation (coryze) in adult group = 37% : p = 0.37] (162)

P_2 = 0.56 [Proportion of clinical manifestation (coryze) in children group = 56% : p = 0.56] (163)

Q_1 = $(1- P_1)$ = 0.63

Q_2 = $(1- P_2)$ = 0.44

n_1 = Sample size of DHF in adult group

n_2 = Sample size of DHF in children group

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λ = Anticipated population of DHF in children group at Phetchabun Province = 83/17 = 4.9 fold of anticipated population of DHF in adult group at Phetchabun Province

$$RR = \frac{\text{Incidence rate of clinical manifestation (coryze) in adult group}}{\text{Incidence rate of clinical manifestation (coryze) in children group}} = \frac{36.5}{55.8} = 0.65$$

$1-\alpha$ = Confidence interval 95% ; $Z_{\alpha/2} = 1.96$

$1-\beta$ = Power 80% ; $Z_{\beta} = 0.842$

$$\bar{P} = \frac{0.56\ (0.65+ 4)}{(1 + 4)} = 0.52$$

$$m = \left[1.96\sqrt{\frac{(4 + 1) \times (0.52)\ (0.48)}{4}} + 0.842\sqrt{\frac{(4)(0.37)(0.63) + (0.56)(0.44)}{4}}\ \right]^2 \Big/ \left[\ 0.56\ (0.65-1)\ \right]^2$$

m = 40

Sample size of DHF in adult group = 40

From n_1 = m

n_2 = λm

So Sample size of DHF in adult group (n_1) = 40

Sample size of DHF in children group (n_2) = 4.9 X 40

= 200

Sample size of DHF in children group = 200

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APPENDIX D

Procedure of immunochromatographic test

1. The single glass or plastic tube was required for each sample to be tested.
2. Buffer, 3 drops (75 µL) was added to each tube. The buffer bottle was held vertically when dispensing.
3. Using the loop provided, serum sample was added 1 µL to the tube.
4. The tube was shaken adequate mixing of the serum in the strip buffer.
5. The test strip was removed from the pouch just prior to use.
6. The test strip was added to the tube containing diluted serum. The red coloured end marked “SAMPLE” was placed into the tube.
7. The result was read 15 minutes after adding the test strip. Any trace of a pink/purple line in the area indicates a positive result. If a pink line had not developed after 15 minutes, read the strip again after 30 minutes as some very weak require 30 minutes to develop.
8. The absence of a test band may suggest a negative result. In some strips, a negative result may appear as a white “ghost” line if the background was pale pink.

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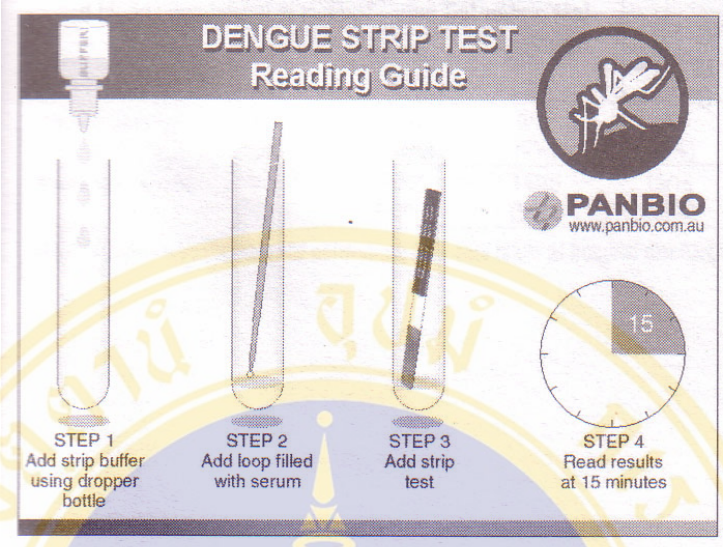

Figure 3 Step of immunochromatographic test

Interpretation of results

- Primary dengue virus infection was defined as a visible IgM line without a visible IgG line.
- Secondary dengue virus infection was defined as a visible IgG line with or without a positive IgM line.
- A negative result was defined as the absence of both IgM and IgG lines: only the control line was visible.

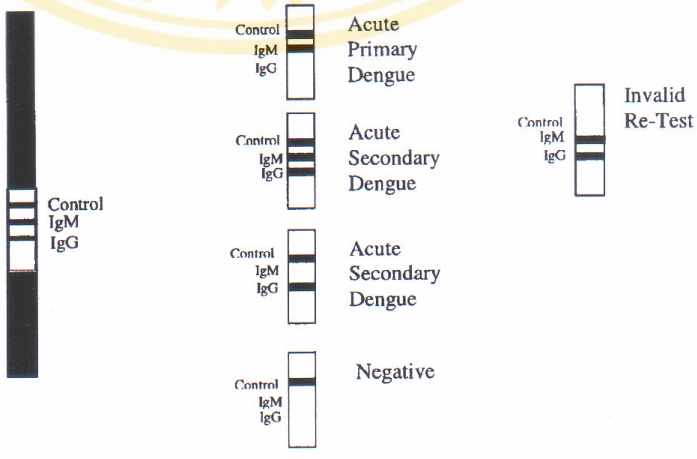

Figure 4 Interpretation of dengue rapid strip test

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APPENDIX E

Code No……

General data

H.N ……..Sex ……. Date of admission……

Ward …….. …… Age…….. ……..…… yrs

Address…….

Date of onset……Fever: duration……..days

Chief complaint ……

Education level

[ ] no education [ ] primary education

[ ] secondary education [ ] college/university

[ ] professional or higher

Occupation

[ ] children < 5 years old [ ] student

[ ] farmer [ ] government official

[ ] private business [ ] government enterprise official

[ ] laborer [ ] housewife

[ ] other (specify)……..

Date of discharge…….....Final Diagnosis: ……

Outcome: [ ] Recovery [ ] Dead

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H.N……..Age…….years

Clinical manifestations obtained from medical records

Clinical manifestations	Date of admission										Remarks
	1	2	3	4	5	6	7	8	9	10	
Max. temperature (° C)											
Pulse											
Min. Blood pressure (mmHg)											
- Systolic											
- Diastolic											
Haemorrhagic manifestations											
- Petechiae/Rash											
- Gum bleeding											
- Epistaxis											
- Haematemesis											
- Melena											
- Uterine bleeding (female)											
Other clinical manifestations											
- Headache											
- Flush face											
- Retro-orbital pain											
- Nausea/Vomiting											
- Anorexia											
- Abdominal pain											
- Myalgias											
- Arthralgias											
- Diarrhea											
- Coryza											

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Code No……

H.N……..Age…….years

Clinical manifestations obtained from medical records

Clinical manifestations	Date of admission										Remarks
	1	2	3	4	5	6	7	8	9	10	
Hypovolemic shock manifestations											
- Oliguria											
- Shock											
- Conscious (good; G, confuse; C, unconcious; U)											
Others											
- Enlarge liver (cm/tenderness)											
- Plural effusion (x-ray)											

LABORATORY FINDINGS

Laboratory finding	Date of admission										Remarks
	1	2	3	4	5	6	7	8	9	10	
Hct (%) - highest											
- lowest											

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Code No……

H.N……..Age…….years

Laboratory findings	**Date**			**Remarks**
CBC				
- Hb (gm%)				
- WBC (cell/mm^3)				
- Platelet (lowest) (/mm^3)				
- Neutrophil (%)				
- Lymphocyte (%)				
- Atypical lymphocyte (%)				
	Date			
BUN (mg/dl)				
Creatinine (mg/dl)				
Serum albumin (gm/dl)				
Serum Na (mEq/L)				
Serum K (mEq/L)				
AST (U/L)				
ALT (U/L)				
PT (sec)				
PTT (sec)				

IC test (Date…….) [ ] Positive [ ] Negative

(……day of onset) [ ] Primary infection

[ ] Secondary infection

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APPENDIX F

Haemoconcentration criteria of Thai population (164-166)

Age	Haemoconcentration, Hct (%)
newborn	53-78
2 wks	50-79
3 -6 mo	37-49
6 mo-3 yrs	40-50
3-6 yrs	42-47
6-10 yrs	43-48
10-15 yrs	44-49
15^{+} yrs (male)	48-64
15^{+} yrs (female)	44-56

From: Hathirat P, *et al*. Haematological finding in normal Thai children. J Med Assoc Thailand 1976; 59: 53-7 (164).

Vichitbandha C, *et al*. Haematological values in health Thai infants and preschool children. J Med Assoc Thailand 1980; 63: 321-9 (165).

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