Facing dengue in pediatrics: studies in a highly endemic region

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(met een samenvatting in het Nederlands)

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Contents

Chapter 1

General introduction

Dengue is arguably the most important mosquito-borne viral disease in tropical and subtropical countries. The global burden of dengue has been rising for over 60 years and affects populations in over half of the world.[1] Dengue virus (DENV) consists of 4 dengue serotypes which can be transmitted from human to human by several species of the Aedes genus of mosquitoes. Any of the four virus serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) can induce specific but not cross-protective long-term immunity and all may cause severe dengue.[2]

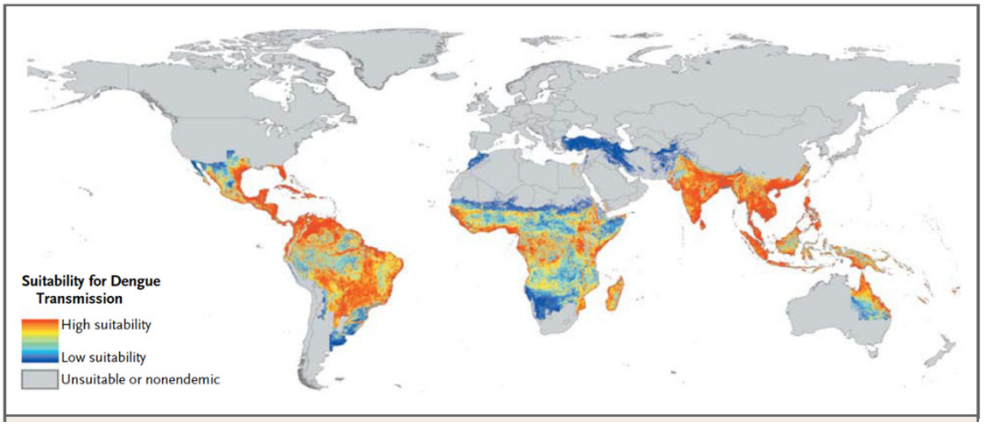

Figure 1. Global dengue risk.[3]

Humans become infected with dengue through the bite of DENV-carrying female *Aedes* mosquitoes, including the species *Aedes aegypti* and *Aedes albopictus*.[4] When a mosquito bites a person who has dengue virus in their blood, the mosquito can become infected with the dengue virus. An infected mosquito can later transmit that virus to other people. Dengue cannot be spread directly from one person to another; the mosquitoes are necessary for transmission of the dengue virus.[5]

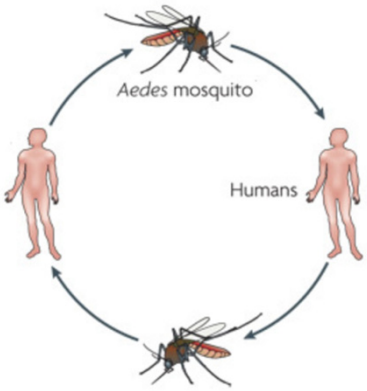

Figure 2. Dengue transmission cycle: dengue virus is spread through a human-to-mosquito-to-human cycle of transmission.[4]

The primary vector of the dengue virus is the species *Aedes aegypti*. They dwell in tropical and subtropical regions all over the world, and are small, dark mosquitoes that can be identified by the white bands on the legs and a silver-white pattern of scales on the body.[5]

Clinical manifestations of dengue infection

Dengue infection may present a wide spectrum of clinical manifestations ranging from asymptomatic, mild simple febrile illness to severe dengue in the form of plasma leakage, bleeding and shock that can eventually result in death.[3] Dengue infection mostly has a self-limiting non-severe clinical course, but a small proportion progresses to severe disease. The course of dengue infection typically consists of three phases: febrile, critical and recovery. The febrile phase may last 2-7 days, the critical phase may occur anywhere from 3-7 days after the first symptoms, and the recovery phase often occurs 2-3 days after the critical phase. In the critical phase, hematology findings will show thrombocytopenia and rising hematocrit levels (hemoconcentration). For severe dengue, early recognition of vascular permeability leakage is crucial, because timely and adequate fluid treatment can be lifesaving. Intravenous rehydration can reduce the case fatality rate to less than 1% of severe cases.[6] Unfortunately, cases that will progress from non-severe to severe disease are difficult to recognize early.

Global changes in the epidemiology of dengue create problems in applying the existing WHO 1997 dengue classification, for example because dengue expansion to additional tropical regions and older age groups becoming affected. Difficulties in performing clinical laboratory tests requiring repetition in limited resource healthcare settings may complicate classifying dengue without bleeding manifestations. In addition, severe dengue cases with organ involvement are not always captured, and there are difficulties of using tourniquet and hematology tests because they are not always available in the field.[7, 8] The WHO 1997 dengue classification divided symptomatic dengue infections into three categories: undifferentiated fever, dengue fever (DF) and dengue hemorrhagic fever (DHF).[9, 10] The category DHF was further classified into four severity grades, with grade III and IV defined as and dengue shock syndrome (DSS).[11] For the diagnosis of DHF, a case must meet all four of the following criteria: fever 2-7 days, hemorrhagic tendency or positive tourniquet test, thrombocytopenia equal or less than 100 000 cells/mm^3 and evidence of plasma leakage (shown by increase hematocrit equal or more than 20% above age, gender and population or signs of plasma leakage such pleural effusion, ascites or hypoproteinemia, This was reported as being difficult to fulfill in the field.[12, 13] The term DHF is considered to emphasize hemorrhagic aspects, but the hallmark of dengue is the plasma leakage leading to shock. Difficulties in applying this dengue classification in clinical settings, together with a clear increase in clinical severe dengue cases which did not fulfill the strict dengue criteria, led to the request for the dengue classification to be re-considered.[13, 14] The WHO in 2009 classified dengue infection into dengue without warning signs, dengue with warning signs and severe dengue. Severe dengue consisted of severe plasma leakage, severe bleeding and severe organ impairment.[15] The WHO 1997[11] and 2009[15] dengue classifications are shown in table 1.

Table 1. Comparison of WHO 1997[11] and 2009[15] dengue classification.

WHO 1997	WHO 2009
Dengue fever	Dengue without warning signs
DHF grade I	Dengue with warning signs
DHF grade II	
DHF grade III	Severe dengue: 1. Severe plasma leakage 2. Severe bleeding 3. Severe organ impairment
DHF grade IV	

Incidence and prevalence of dengue infection

It has been estimated that 50-100 million cases occur annually, with about 500,000 cases developing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which are potentially life-threatening.[16, 17] Dengue occurs in urban and rural parts of the tropical and subtropical countries worldwide. About 2.5% of dengue infections was reported to be fatal and the majority of fatal dengue cases occur in children < 15 years.[2, 16] According to the WHO report 2021, there has been an eight times increase in the number of dengue cases in the last two decades, from 505,430 cases in 2000, to 2.4 million cases in 2010 and 5.2 million cases in 2019. The global burden of dengue was estimated at 3.9 billion dengue virus infections of which 96 million with clinical dengue, spreading to 128 countries. Dengue viral infection has become a leading cause of pediatric morbidity and mortality in some Southeast Asian countries.[18] The WHO reported the highest dengue incidence is Brazil, followed by Indonesia.[16]

Importance and barriers of a timely diagnosis

Early detection of dengue cases is crucial to guide timely and adequate management and prevent a fatal course. In the early years, dengue infection affected mostly children. Dengue infection has now spread globally and often also affects adults. In view of these shifts, more epidemiological data are needed to assess the spread and course of dengue to guide

resources and preventive measures. In addition, it is important to identify mild dengue cases that can be safely sent home in order to prevent unnecessary hospitalizations, and prioritizing the recognition of more severe dengue cases that require intense monitoring in the hospital.[19]

Prevention with vector control worldwide has not reduced dengue incidence globally. A dengue vaccine has been licensed and other candidates dengue vaccines are in phase 3 trials.[20] They may be instrumental in preventing a further increase in the global dengue burden. Although the licensed dengue vaccine has become available in several countries, early diagnosis and prompt treatment, disease surveillance and vector control remain the mainstay of dengue prevention and management.[21]

Are easily applicable diagnostic tools for dengue infection available?

A specific diagnostic tool to confirm dengue infection is a rapid serological diagnostic test called the non-structural (NS)-1 dengue antigen test. It is an important tool when dealing with acute febrile illness in endemic countries, and can be used in field settings, to distinguish dengue from other infectious diseases. Rapid NS1 antigen and NS1 ELISA seem promising to specifically confirm the diagnosis of dengue, especially in the early stages. However, the disadvantages of such tests come to light in cases where the patients present themselves after more than three days of fever, since the accuracy of NS1 antigen test decreases considerably in the subacute phase. The sensitivity and specificity of NS1 ELISA were 66.6% and 89.1%, while sensitivity and specificity of rapid NS1 antigen were 55.5% and 92%, respectively on day 1-3 of fever.[22] Other study which evaluate 5 commercial NS1 antigen showed sensitivity ranging 73% to 80%, and specificity of 100%, during the first 4 days of fever for DENV-3 and in primary infections.[23] Other serological tests to detect dengue infection are aimed at IgM and IgG dengue antibodies, which can be detected in the acute phase on day 5 and later.[24] These tests are available in healthcare facilities for clinical practice in the field.

Gaps need to be addresses

The increased growth of the human population, urbanization and modern transportation, and increases in range and density of the mosquitoes (influenced by global warming) affect dengue incidence worldwide over the years. Epidemiological studies in endemic areas are needed to determine the change of incidence and case fatality rate of dengue, and the age groups who are most at risk. This will benefit dengue diagnosis and management as well as the preventive measures for vector control in endemic countries. Tools to recognize clinical and laboratory profiles of dengue cases in young children at risk of developing severe dengue are especially important, for example for general physicians working in endemic areas. Studies focusing on the high-risk group of infants with dengue are still scarce. Now that the revised WHO 2009 dengue guidelines are implemented world-wide, studies evaluating the warning signs in children are needed but are rare. Longer follow-up evaluating long term effects of childhood dengue are even more scarce, while an increasing body of evidence suggests dengue may influence long-term cardiovascular risks .[25, 26] The general aim of this thesis is to address these gaps in tools and understanding.

Objective and outline of this thesis

In line with the challenges involved in dealing with dengue in children, as made clear by the Case study at the start of this chapter, the objective of this thesis is to contribute to understanding dengue in children. To set the scene of dengue in Indonesia, where the data for the studies originate, a comprehensive overview is given of the epidemiology of dengue in this country. In the heart of the thesis, several clinical profiles are described of dengue in hospitalized pediatric patients. In addition, Warning signs from the WHO 2009 dengue classification were evaluated to detect severe dengue in children of different age groups. Finally, long-term consequences of DHF were studies by assessing the change of carotid intima-media thickness in children with a history of dengue hemorrhagic fever.

Chapter 2 starts the thesis with a description of the changing incidence of dengue hemorrhagic fever in Indonesia over the last 45 years. A registry-based analysis was performed to evaluate changes in incidence, as well as in age groups affected.

Chapter 3 presents the clinical manifestations and hematological findings in children with dengue infection, with the aim to provide guidance on which information is most valuable for clinicians facing children with dengue infection in dengue endemic areas.

Chapter 4 describes a study of the clinical course and disease burden of hospitalized paediatric dengue patients in Jakarta, Indonesia, applying data from a 5-year prospective cohort study. This study describes which signs and symptoms are most useful to prevent hospitalized dengue progressing to dengue shock syndrome (DSS).

Chapter 5 is devoted to infants. Infants with dengue are a high-risk group but the group is small and studies among infants are very rare. Chapter 5 describes the dengue profile and clinical signs in infants based on a 10-year cohort study of infants presented in a tertiary referral hospital in Indonesia. This study presents clinical profiles in infants with dengue.

Chapter 6 presents a study on the value of warning signs from the WHO 2009 dengue classification in detecting severe dengue in children.

Chapter 7 presents a study on changes in carotid intima-media thickness in children with a history of dengue hemorrhagic fever. In DHF, endothelial dysfunction occurs, leading to plasma leakage. It is unknown whether this vascular damage is transient or persistent.

Chapter 8 includes a discussion of the main findings and conclusions of this thesis, with reference to the current literature. In addition, recommendations are provided likely to facilitate a more timely and accurate diagnosis and prognoses in daily practice of the course of dengue infection.

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Chapter 2

The Changing Incidence of Dengue Haemorrhagic Fever in Indonesia: A 45-Year Registry-Based Analysis

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Abstract

Background: Increases in human population size, dengue vector-density and human mobility cause rapid spread of dengue virus in Indonesia. We investigated the changes in dengue haemorrhagic fever (DHF) incidence in Indonesia over a 45-year period and determined age-specific trends in annual DHF incidence.

Methods: Using an on-going nationwide dengue surveillance program starting in 1968, we evaluated all DHF cases and related deaths longitudinally up to 2013. Population demographics were used to calculate annual incidence and case fatality ratios (CFRs). Age-specific data on DHF available from 1993 onwards were used to assess trends in DHF age-distribution. Time-dependency of DHF incidence and CFRs was assessed using the Cochrane-Armitage trend test.

Results: The annual DHF incidence increased from 0.05/100,000 in 1968 to ~ 35-40/100,000 in 2013, with superimposed epidemics demonstrating a similar increasing trend with the highest epidemic occurring in 2010 (85.70/100,000; $p<0.01$). The CFR declined from 41% in 1968 to 0.73% in 2013 ($p<0.01$). Mean age of DHF cases increased during the observation period. Highest incidence of DHF was observed among children aged 5 to 14 years up to 1998, but declined thereafter ($p<0.01$). In those aged 15 years or over, DHF incidence increased ($p<0.01$) and surpassed that of 5- to 14-year olds from 1999 onwards.

Conclusions: Incidence of DHF over the past 45 years in Indonesia increased rapidly with peak incidence shifting from young children to older age groups. The shifting age pattern should have consequences for targeted surveillance and prevention.

Keywords: dengue haemorrhagic fever, epidemiology, incidence, age, Indonesia

BACKGROUND

Dengue infection is the most rapidly spreading mosquito-borne viral disease in the world.[1] The World Health Organization (WHO) reported that the incidence increased dramatically over the last 50 years and that dengue virus infections expanded to new countries, and from urban to rural settings.[1] Approximately 2·5 billion people live in endemic countries of which about 1·8 billion (more than 70%) in Southeast Asia and the Western Pacific Region.[1-4] Annually, about 50 million dengue infections occur,[2,3] and approximately 500,000 patients are hospitalized because of dengue haemorrhagic fever (DHF), of whom a large proportion are children.[2-7]

Demographic and societal changes such as population growth, urbanization, and modern transportation appear to play an important role in the increased incidence and geographical spread of dengue virus.[8] Furthermore, travellers from non-endemic countries to endemic dengue areas are at risk of contracting dengue disease and pose a health threat to non-endemic regions where competent mosquito vectors are currently found.[9-12]

Historically, DHF was predominantly observed in children. Over the past decades however, changes have been observed in the age-distribution of DHF cases in most countries both in Southeast Asia and Latin America.[13-17] Nowadays it is reported that a significant proportion of DHF cases occur among adolescent and adult patients in Southeast Asia,[15,17-19] and also in Latin American countries.[20] However, many studies describing shifting age patterns of DHF fail to report continuous observations over longer periods of time or report on specific locations and outbreaks.[14-17]

Indonesia is one of the largest countries in the dengue endemic region, with a population of 251 million. The first 58 dengue cases in Indonesia were reported from Jakarta (DKI Jakarta) and Surabaya (East Java) in 1968.[21-24] Since then increasing numbers of cases and geographical locations affected by dengue have been reported. [21,22,25-30] Dengue epidemiology in Indonesia has been described mostly in the form of case series, reporting on single outbreaks, or clinical and virological studies on DHF patients in confined geographical locations and selected years.[31] To date, there have been no comprehensive studies describing the incidence of dengue epidemiology over time in Indonesia, and new data from recent years are lacking. Furthermore, data on age-specific dengue incidence in

Indonesia are scarce, even though such information may have important implications for preventive measures. In only one study was the age distribution of DHF reported, showing that between 1975 and 1984 the median age of patients increased by 9 months.[32] The availability of the continuous nationwide Indonesian dengue surveillance registry, enabled us to describe the evolution of DHF incidence and case-fatality rates over a period spanning 45 years and to evaluate age-specific trends over time.

METHODS

Surveillance system and case definition

In 1968, dengue became a notifiable disease in Indonesia,[21,22] and was included in the national disease surveillance system run by the Communicable Disease Center of the Indonesian Ministry of Health. This means that all suspected DHF cases presenting to healthcare facilities or hospitals must be evaluated within 24 hours by healthcare providers and reported to the district health authority while awaiting serological confirmation. This is followed by epidemiological investigation and a vector control program according to National guidelines when indicated based on serologic, virologic or epidemiological confirmation of dengue.[33,34] In addition, the dengue surveillance included state government and WHO training programs for medical officers in diagnosis and case management from 1968 onwards.[32]

Since its inception, the national surveillance-training program applies the same WHO dengue classification system from 1968,[32] which classifies symptomatic dengue into dengue fever (DF) and DHF. Although several changes have been made in the WHO Dengue classification since 1968[32] these have not been adopted in the Indonesian national surveillance system, such that definitions and criteria for reporting have remained stable over the entire observation period, apart from a minor change in dengue serology testing where the haemagglutination inhibition test was replaced by rapid diagnostic tests for serologic IgM and IgG dengue that were available in the field. DHF is defined as having at least the first two of the following four clinical manifestations: 1) sudden onset acute fever of 2 to 7 days duration, 2) spontaneous haemorrhagic manifestations or a positive Tourniquet test, 3) hepatomegaly, and 4) circulatory failure, in combination with haematological criteria of thrombocytopenia (=<100.000 cells/mm3) and an =>20% increased haematocrit. Dengue

shock syndrome is defined as DHF plus a rapid, weak pulse with narrow pulse pressure or hypotension with cold, clammy skin, and restlessness.[29,32,34-36]

Case ascertainment

Every suspected case of DHF based on clinical and haematological criteria requires further investigation to support the diagnosis of dengue. DHF is classified as probable when additional supportive dengue serology from a single blood specimen is available or when there is an epidemiological link to a confirmed dengue case. Supportive dengue serology is defined as positive anti-DENV IgM in acute or convalescent serum sample and/ or a fourfold increase in IgG between the acute and the convalescent samples. DHF cases are classified as confirmed through virus isolation, or detection of viral antigen or RNA in serum. [32-34] This classification has continually been used nationwide by all governmental and private hospitals. Of all initially identified, suspected cases of DHF, only probable and confirmed cases are subsequently reported to the Communicable Disease Center of the Indonesian Ministry of Health by district health authorities and captured in the surveillance database. This database covers all 33 Indonesian provinces. From 1993 onwards data collected in the surveillance database on DHF cases also included the following age categories: less than 1 year, 1-4 years, 5-14 years, and older than 15 years. [23,32,35,37]

Annual geographical mapping of Indonesian provincial incidence rates of DHF was available for the years 2010-2013 and is included.

Population

Population demographic data for 1968 to 2013 were based on civil registration records of village authorities,[38] and obtained from the official national census data, the Central Bureau of Statistics in Indonesia.

Statistical analysis

The DHF incidence by year and age group was calculated by dividing the number of new DHF cases identified from surveillance data by size of the population at risk. Dengue case fatality ratios were determined by the number of deaths from DHF divided by the number of DHF

cases. Trends in annual incidence and case fatality ratios were analysed using Cochrane-Armitage trend tests.[39]

Subgroup analyses were performed to study trends according to age group. In an attempt to rule out that any observed change of incidence is partly due to increasing awareness and reporting in the early stage of the registry, the most recent period (1986-2013) was analyzed separately. The Health Research Ethics Committee Medical Faculty University of Indonesia Cipto Mangunkusumo Hospital approved the study.

RESULTS

Overall annual DHF incidence increased significantly from 0.05/100,000 in 1968 to to ~ 35-40/100,000 in 2013 in 2010 (p-value trend test: < 0.001). Superimposed epidemic peaks occurred with irregular intervals and a progressive increase in intensity. The highest epidemic peak was observed in 2010 with 86 DHF cases per 100,000 person-years. Figure 1 shows the incidence of DHF since 1968. Outbreaks were observed in the years 1973, 1988, 1998, 2007, and 2010. By contrast, the DHF case fatality ratio decreased from 41% in 1968 to 0.73% in 2013 (figure 2, p-value for trend-test < 0.001).

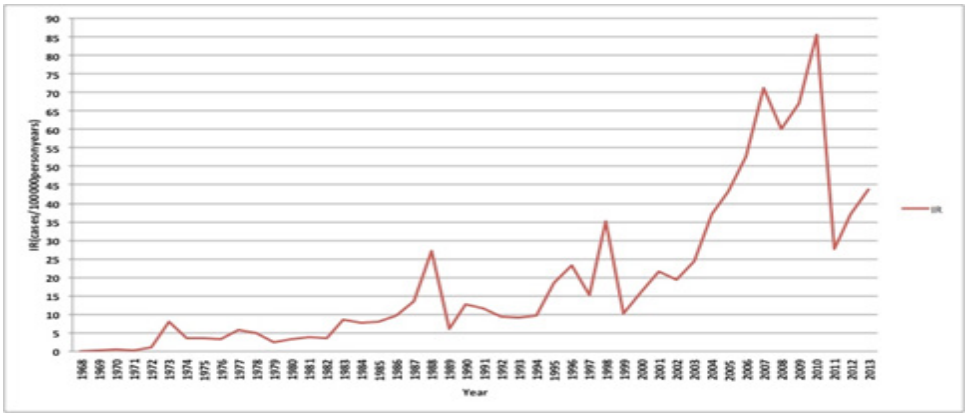

Figure 1. Trends in incidence rate of DHF cases in Indonesia from 1968 to 2013, measured in numbers of cases per 100,000 person years.

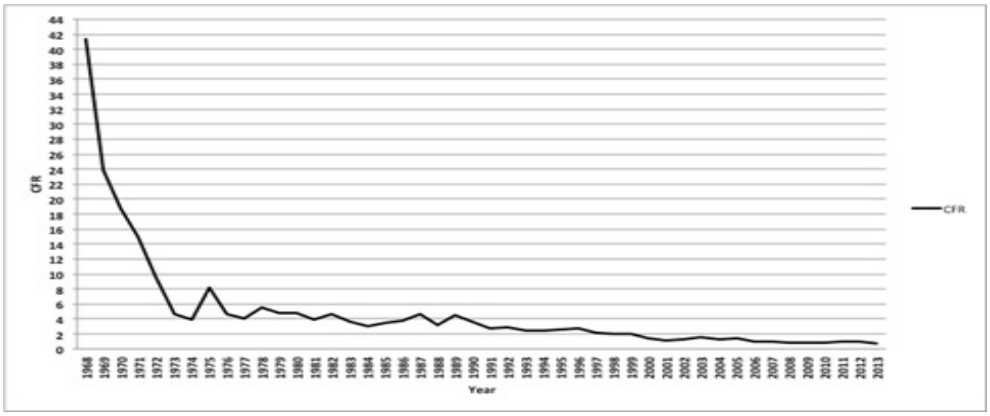

Figure 2. Case fatality ratios of DHF cases in Indonesia from 1968 to 2013.

A separate analysis of the more recent 1986 to 2013 data revealed results compatible with the total period. The annual incidence of DHF increased significantly over time (p-value for trend-test < 0.01), while the case fatality ratio of DHF decreased considerably (p-value for trend-test < 0.01).

From 1993 onwards, when age categories were also being recorded, the highest initial annual DHF incidence was observed in 5 to 14 year olds, but it steadily decreased from then on (p-value for trend-test < 0.01, figure 3). In contrast, while the DHF incidence in 1993 in those aged over 15 years was much lower than in 5 to 14 year olds, a steady increase

(p-value for trend-test < 0.01) in this age category was observed and the incidence surpassed that of young children around the year 1999. Throughout this period, the incidence in children aged less than 5 years was relatively low and remained stable.

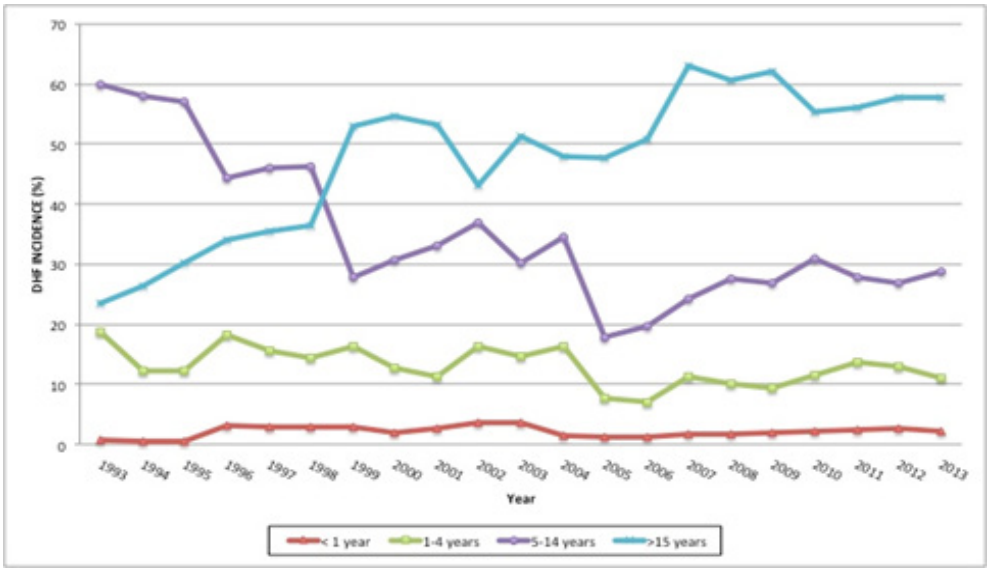

Figure 3. Incidence (%) of DHF in the different age groups from 1993 to 2013.

The geographical mapping of rates of DHF in Indonesian provinces over the years 2010-2013 is shown in figure 4. Bali and DKI Jakarta had the highest incidence of DHF. In 2013, the five highest provincial incidences were observed in Bali (168.5/100,000), DKI Jakarta (104.0/100,000), DI Yogyakarta (96.0/100,000), East Kalimantan (92.7/100,000) and Sulawesi Tenggara (66.8/100,000). The geographical distribution did not change substantially over 2010-2013.

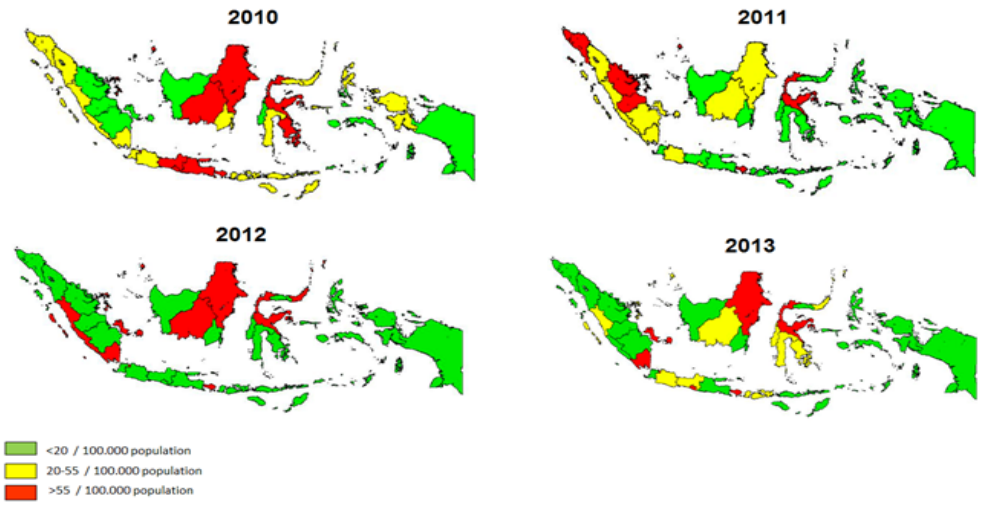

Figure 4. Geographical mapping of Indonesian provincial incidence rates of DHF in 2010-2013.

DISCUSSION

Our results showed that in the last 45 years, the DHF incidence increased rapidly in Indonesia with a pattern of intermittent hyperendemic years. The case fatality ratio, however, decreased over the same period. Based on age-stratified data available from 1993 onwards, there appears to be an important shift in the age of affected individuals; a steady decline in DHF incidence was observed over the years for children aged 5 to 14 years (the age group with highest DHF incidence historically), while the incidence in those aged over 15 years steadily increased and surpassed the decreasing incidence in younger children since 1999.

The strengths of our study are that annual DHF incidence and case fatality ratios have been documented continuously for 45 years, using the same WHO case definition and case classification of dengue, based on both clinical and laboratory diagnostic criteria without any substantial modification throughout the surveillance period. Furthermore, the data offered the opportunity to study trends by age groups.

Some potential limitations should also be discussed. Firstly, specific incidences according to DHF disease severity grade could not be reported since such information was not available from the reports studied. In addition, cases of severe dengue disease

can present with atypical clinical manifestations such as massive hemorrhage and organ failure, neurologic disease, myocardiopathy, hepatic and renal failure, and may therefore not be reported as DHF, a problem also recognized in the WHO 2009 guidelines.[40] Secondly, mild DHF cases not presenting to healthcare facilities will not have been captured by the surveillance. Therefore, our findings likely reflect more severe symptomatic DHF cases requiring medical attention. Thirdly, cases of suspected DHF without serological testing performed do not end up in the surveillance database and this will have resulted in some underreporting.

In the early years of the surveillance program, limited communication and logistic facilities in several parts of Indonesia may have hampered DHF reporting to some extent. From the initial stage of surveillance onwards, the DHF training program will have gradually increased awareness among healthcare providers. We cannot completely rule out that the observed change of incidence is partly due to such increasing awareness and reporting. However, our sub-analysis of the most recent 20 years, clearly confirms our overall findings, thus rendering increased reporting an unlikely alternative explanation.

Increases in DHF incidence may be explained by increased vector density or abundance because of lack of effective mosquito control,[41,42] by increased human mobility,[41,42] and by altered virus-host interaction leading to increased infectivity and therefore more secondary infections.[41] The population in Indonesia is growing fast with an increase of almost 25% (from 206 to 251 million) between 2000 and 2013. Demographic and societal changes such as population density, urbanization and modern transportation probably contributed substantially to the increased incidence and geographical spread of dengue in Indonesia.[31] Over the most recent years of registry, there is a clear annual geographical distribution of DHF incidence with concentrations mainly in high-density populated areas. Possibly for that reason, this distribution does not seem to change much over time.

The DHF incidence in Indonesia has been increasing in over 15 year olds, while in the under 5 year olds it remained relatively low and stable, a pattern that has been observed in other high endemic South East Asian countries.[6-8,13,15,16,43-46] Demographic changes, i.e. changes in birth and death rates, may induce changes in the age distribution of cases and the periodicity of incidence.[15] Lower birth rate decreases the flow of susceptible individuals into

the population and lower infant mortality increases the longevity of immune individuals.[15] Older age groups in endemic areas will be exposed to secondary dengue infection which mostly manifests as DHF, rather than the primary dengue infections that are predominantly found in younger age groups.[4,47] Both births and infant mortality indeed decreased in Indonesia, i.e. from 22 births and 38 deaths per 1000 inhabitants in 2003 to 17 births and 26 deaths per 1000 inhabitants in 2013 and family size decreased to less than 3 children per family in 2010.[48] These demographic changes will most likely have contributed to the upward shifting of age for DHF cases in Indonesia.

Epidemic outbreaks of DHF occurring every 8–10 years have also been reported in other countries, and might be the result of cross-protective immunity.[43] Adams et al, [24] showed that DENV-4 was responsible for this epidemic pattern in Thailand which has an immunological cross-reaction with DENV-1 and, possibly, with other serotypes.[43]

In Southeast Asian countries where dengue is endemic, only Malaysia and Singapore have active surveillance systems. Dengue surveillance programs in other Southeast Asian countries, including Indonesia, remains largely passive.[17,21,32] and it is estimated that incidence rates of dengue cases, based on reports from passive surveillance systems, are underestimated.[42]

Further professional upgrading of dengue surveillance in Indonesia should be considered, including registration of more than only probable and confirmed DHF and including the expanded dengue syndromes with unusual manifestations according to the revised 2011 WHO Southeast Asia Region Office (SEARO) dengue guidelines.[49] Extending the registry to include more detailed demographic data and information on social economic status, and disease severity will likely contribute to our further understanding of dengue epidemiology. Secondly, we believe that awareness about the shifting age-pattern is essential for clinical and public health vigilance and for the efficiency of preventive strategies. Education to create public awareness about the clinical signs of dengue in the adolescent age group and when to seek professional healthcare could improve timely medical interventions. Furthermore, vector-control programs should not only be aimed at houses, but also at schools and working areas.[34]

CONCLUSION

In conclusion, the incidence of DHF has increased substantially over the past 45 years, with superimposed epidemic outbreaks and requires re-enforcement of current surveillance practices. In contrast, the case fatality ratio clearly decreased during the same period. The shifting age pattern towards older age groups (above 15 years of age) should have consequences for targeted prevention strategies.

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A supplementary table of incidence and CFR of DHF

Year	IR (cases	CFR (%)
1968	0.05	41.3
1969	0.14	23.9
1970	0.4	18.8
1971	0.22	14.9
1972	1.14	9.6
1973	8.14	4.6
1974	3.57	3.9
1975	3.47	8.1
1976	3.38	4.7
1977	5.69	4.1
1978	4.96	5.5
1979	2.37	4.8
1980	3.39	4.8
1981	3.96	3.9
1982	3.53	4.7
1983	8.65	3.6
1984	7.86	3
1985	8.14	3.4
1986	9.79	3.7
1987	13.5	4.6
1988	27.09	3.2
1989	6.09	4.5
1990	12.7	3.6
1991	11.56	2.7
1992	9.45	2.9
1993	9.17	2.40
1994	9.72	2.51
1995	18.50	2.52
1996	23.22	2.71
1997	15.28	2.22
1998	35.19	1.96
1999	10.17	2.00
2000	15.99	1.41
2001	21.66	1.08
2002	19.24	1.32
2003	24.30	1.55
2004	37.01	1.20
2005	43.31	1.36
2006	52.48	1.04
2007	71.18	1.00
2008	60.02	0.86
2009	67.00	0.86
2010	85.70	0.87
2011	27.67	0.91
2012	37.11	0.90
2013	43.01	0.73

Chapter 3

Clinical manifestations, hematologic and serologic findings in children with dengue infection

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Abstract

Introduction: Dengue haemorrhagic fever (DHF) is still endemic in Indonesia and remains a public health problem, with highest incidence in children. There have been few reports on the clinical, hematological and serological data in children with dengue.

Objectives: To assess the clinical and laboratory profiles of children with dengue infection in Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Methods: Clinical, hematological and serological information of children diagnosed dengue infection in Cipto Mangunkusumo Hospital were collected in 2007 to 2009.

Results: Of 611 children with dengue were included of whom 143 (23.4%) had dengue fever (DF), 252(41.2 %) had DHF I and II; and 216 (35.4%) had DHF III and IV. Of the 81 cases where dengue serotypes identified, 12.3 % were DEN-1, 35.8% were DENV-2, 48.2% were DENV-3 and 3.7% were DENV-4. Mean age of subjects was 8.9 years (SD 4.4), and 48.4% cases were boys. The mean length of fever before hospital admission was 4.2 days (SD 1.1) and mean length of stay in the hospital was 4 days (SD 2.7). Common symptoms were ptechiae, hepatomegaly and epistaxis. Complications mostly found in those with dengue shock syndrome (DSS) were hematemesis (30 cases, 4.9% of all patients), encephalopathy (19 cases, 3.1%) and melena (17 cases, 2.8%)

Conclusions: Signs and symptoms of fever, bleeding manifestations and thrombocytopenia were present in children with DF and DHF, while signs of increased vascular permeability were found only in those with DHF. Encephalopathy and gastrointestinal bleeding were found mostly in DSS cases. At admission, leukopenia was found in more DF patients than in DHF patients. Absence of leukopenia may be a sign of more severe dengue infection.

Keywords: dengue haemorrhagic fever, children

INTRODUCTION

Dengue infection is considered to be the most important mosquito-borne viral disease in the world. The world Health Organization (WHO) reported that around 2.5 billion people living in hospital and subtropical countries are infected with one or more dengue viruses.[1] About 50-100 million individuals are infected every year, and about 500,000 people are admitted to a hospital annually. Many factors are thought to be responsible for the increasing incidence of both DF and DHF, such as changes of demographics, climate, virulence of the dengue virus, and population growth. [1-4]

Children have a 40 times higher risk of severe dengue than adults, due to vascular permeability during secondary infection.[6] DHF is still a leading cause of death in children. In past years, DHF has occurred primarily in children aged less than 15 years, with its highest attack rate in the 5-9 years. [7-12]

Since clinical and laboratory data in children in Indonesia have been scarce, this study will report the clinical presentations, hematological and serological findings of all children admitted with suspected dengue infection to Cipto Mangunkusumo hospital in Jakarta from 2007 to 2009.

METHODS

A retrospective cohort study was carried out in all children suspected of having dengue and admitted to the Department of Child Health, Cipto Mangunkusumo Hospital, Jakarta between 2007 to 2009.

The diagnosis of DF/DHF/DSS was made according to WHO criteria (WHO 1997).[4] Dengue fever is an acute, febrile illness with the following typical symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations (e.g. epistaxis, petechiae or gingival bleeding) with thrombocytopenia.

Dengue haemorrhagic fever was diagnosed by the presence of fever and haemorrhagic manifestations including at least a positive tourniquet test and minor or major bleeding phenomena, hepatomegaly, with thrombocytopenia (< 100000 platelets/mL^3) and haemoconcentration (increases > 20%) or objective evidence of capillary permeability in the form of pleural effusion.

Dengue shock syndrome was diagnosed by the presence of criteria listed for DHF in combination with hypotension or narrow pulse pressure (<20 mmHg).

Severity of DHF cases were further graded as I to IV, according to WHO guidelines 1997.[4] Those with grades III and IV were diagnosed as DSS.

For all the patients, baseline haemograms, hematocrite by microcentrifuge technique, and absolute platelet counts by cell counters were measured. Other investigations such as blood electrolytes, serum transaminases, prothrombin time and right lateral decubitus chest X-ray, were done when indicated. Serum was collected for virus isolation and Dengue Duo IgM and IgG rapid serological tests to confirm the diagnosis.

Data were collected using the medical records of the Department of Child Health in Cipto Mangunkusumo Hospital. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia.

Statistical Analysis

Descriptive statistics were used to describe the distribution of the patient characteristics, signs and symptoms, and the immunological and serological parameters. Clinical and laboratory findings in DF, DHF and DSS cases were compared by Chi-square tests and ANOVAs. All analyses were performed with SPSS version 17.

RESULTS

A total of 611 children suspected of having dengue were admitted to Cipto Mangunkusumo Hospital from January 2007 to December 2009. After clinical and serological confirmations, 415 (68%) children were diagnosed to have dengue virus infection. The peak of dengue cases was found in the month of April, after the rainy season. The mean age of subjects was 8.9 years (SD 4.4). Dengue fever (DF), , DHF without shock (DHF I and II); and DHF with shock (DHF III and IV, or DSS) was diagnosed in 143 (23.4%), 252 (41.2 %), and 216 (35.4%) children, respectively. The mean duration of fever before hospital admission was 4.2 days (SD 1.1). The mean length of stay in the hospital was 4 days (SD 2.7). Table 1 shows the basic characteristic of the dengue patients classified as dengue fever, DHF without shock and DHF with shock.

Table 1. Basic characterisctics of inpatients with DF, DHF and DSS

Clinical features	Dengue fever	DHF without shock	DSS
Number. of cases, n (%)	143(23.4)	252(41.2)	216(35.4)
Mean age, years (SD)	8.6 (4.2)	10.4(4.3)	7.4(3.9)
Gender (female/male)	74/69	128/124	113/103
Mean duration of fever, days (SD)	4.06(1.2)	4.10(1.2)	4.36(1.0)
Mean duration of hospitalization, days (SD)	3.8(1.7)	3.9(2.7)	4.4(3.2)

Clinical manifestations of the dengue patients are shown in Table 2. Fever, bleeding tendencies and thrombocytopenia were present in children with DF and DHF, whereas pleural effusion, hepatomegaly and gastrointestinal bleeding were only reported in those with DHF. Of children experiencing epistaxis, positive tourniquet test and petechiae, most were in the category of DHF without shock (comprising 56.9%, 48.6% and 44.5%, respectively). However, In children experiencing melena, hematemesis and encephalopathy, most were in the DSS category (comprising 94.1%, 90% and 89.5%, respectively). Of patients with gum bleeding, there were significantly more DSS patients compared to DHF without shock (61.9% vs 19%, P=0.030). Similarly, in patients with haematemesis`(90% vs 10%, P=0.001), melena (94.1% vs 5.9%, P=0.001), hepatomegaly (58% vs 30%, p=0.001), and encephalopathy (89.5% vs 10.5 %, P=0.001), DSS patients were significantly greater.

Table 2. Clinical manifestations in in patients with dengue

Clinical features	Dengue fever	DHF without shock	DHF with shock
Number of cases, n	143	252	216
Petechiae, n(%)	37(25.9)	121(48)	114(52.8)
Epistaxis, n(%)	11(7.7)	29(11.5)	11(5.1)
Gum bleeding, n(%)	4(2.8)	4(1.6)	13(6)
Hematemesis, n(%)	0	3(1.2)	27(12.5)
Melena, n(%)	0	1(0.4)	16(7.4)
Positive tourniquet, n(%)	62(43.4)	138(54.8)	84(38.9)
Hepatomegaly, n(%)	18(12.6)	45(17.9)	87(40.3)
Pleural effusion, n(%)	0	3(1.2)	5(2.3)
Encephalopathy, n(%)	0	2(0.8)	17(7.9)

Figures 1 A-D show the levels of hemoglobin, hematocrite, leukocytes, and platelet in our subjects. At the time of admission (4th day of fever), the mean hematocrite of DSS was 41.4 %, DHF without shock 40.7 % and DF 36.8 %. Thus, at admission (4th day of fever), the mean hematocrite were significantly higher in DSS group compared to other groups (P=0.003). The mean leukocyte counts at admission in DSS, DHF without shock and DF were 5925, 4214, and 4497/mm^3, respectively.

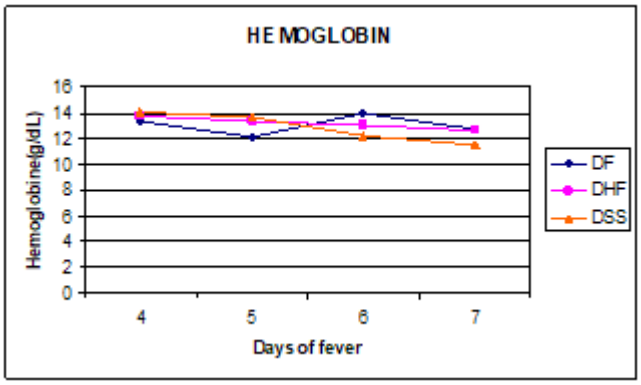

Figure. 1A. Hemoglobin levels

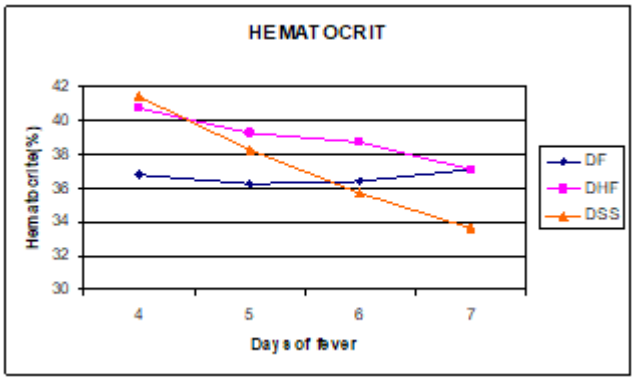

Figure 1B. Hematocrite levels

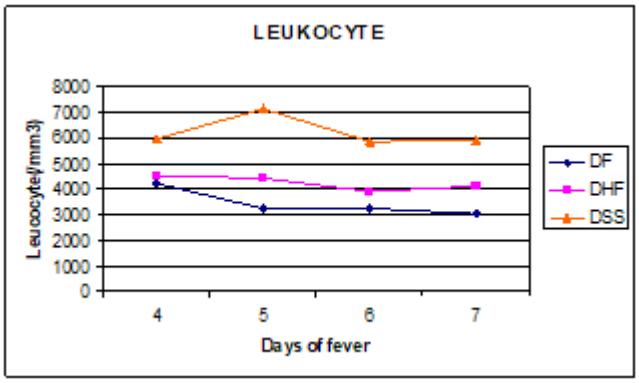

Figure 1C. Leucocyte counts

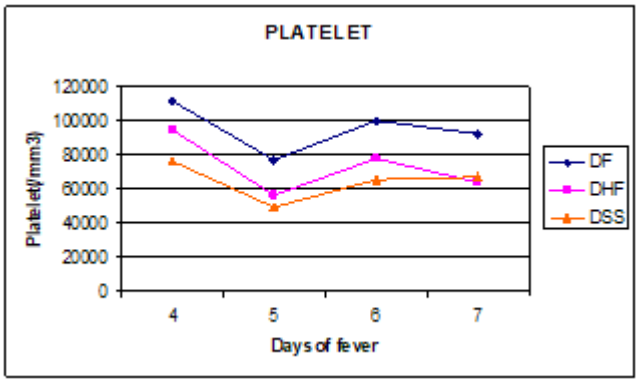

Figure 1D. Platelet counts

Furthermore, at admission, the mean platelet counts in children with DSS, DHF without shock, and DF were 76,216; 94,273 and 111,429./mm^3, respectively.

The majority of children requiring blood or blood products were diagnosed with DSS. Out of 611 children admitted due to suspected dengue,[7] (1.15%) died. Of the deaths, all were admitted to the hospital late in the course of disease, and all suffered from severe gastrointestinal bleeding.

The results of the positive serological tests (n=415) are presented in Table 3. The test showed that most patients with dengue fever mostly had a primary infection, whereas patients with both DHF and DSS more often had a secondary infection.

Table 3. Serological test

Serological test	DF	DHF without shock	DHF with shock
Primary infection	26(38.8%)	21(31.3%)	20(29.9%)
Secondary infection	63(18.1%)	146(41.8%)	139(39.8%)

Table 4 shows the results of positive virus isolation in the 81 cases out of 209 isolates in 2007-2009. Dengue serotype 3 (DENV-3) was found from 39 (48.2%) of the positive isolates, followed by DENV-2 in 29 (35.8%) of the positive isolates, DENV-1 in 10 (12.3%) of the positive isolates, and DENV-4 in 3 (3.7%) of the positive isolates.

Table 4. Virus isolation in 2007-2009

Serotype	Total n=81
DENV-1	10 (12.3%)
DENV-2	29 (35.8%)
DENV-3	39 (48.2%)
DENV-4	3 (3.7%)

DISCUSSION

In the last 3 years, of 611 patients admitted for dengue in Cipto Mangunkusumo Hospital, there were 143 (23.4%) dengue fever (DF) cases, 252 (41.2 %) DHF without shock (DHF I and II) cases and 216 (35.4%) DSS (DHF III and IV) cases. In our pediatric population, the mean age of dengue patients was 8.9 years (SD 4.4). The patients came to the hospital with a mean length of fever of 4.2 days (SD 1.1). Mean length of stay was 4 days (SD 2.7). Similar results were also found in two Thai studies. [5,13] The mean age in our study was similar to that in previous studies in Nicaragua and Thailand. [14,15]

In Taiwan and Saudi Arabia most dengue patients were adults.[16,17] Report of rising age in DHF cases may be explained by demographic transition, decreased birth rates and mortality. [15]

The tourniquet test was positive in 62 (43.4%) DF cases, 138 (54.8%) DHF without shock cases and 84 (38.9%) DSS cases. In DSS group, there were fewer positive tourniquet tests, possibly due to hypotension. However, tourniquet tests may become positive after restoration of depleted intravascular volume.[4] Low sensitivity of the tourniquet test even in DHF was also reported due to difficulties in performing the test on sick and irritable young children. Also, pediatric blood pressure cuffs may not be available. An investigation into the usefulness of slow capillary filling as a surrogate marker for low blood pressure as measured with a sphygmomanometer may be warranted.[4]

Severe gastrointestinal bleeding usually occurred after patients developed shock. This finding contrasts with other studies[10,18,19] that showed upper gastrointestinal bleeding occurred before onset of shock and without hemoconcentration. It has also been suggested that this latter type of hemorrhagic condition probably has a different pathogenesis from classical DHF/DSS.[20]

Some findings in our study were similar to reports from Asia[13] while others were comparable to those of the Americas.[1,14] These differences highlight the need to study region-specific clinical features in dengue infection. We found gum bleeding, hematemesis, melena, hepatomegaly, and encephalopathy were more commonly associated with serious illness. Dengue encephalopathy in DHF/DSS may be due to intracranial hemorrhage, electrolyte imbalance or hypoxic ischemic encephalopathy due to profound circulatory failure. In an endemic area, dengue virus should be considered as a possible etiology agent in children presenting with encephalopathy.[12] However we did not attempt viral isolation or measure IgM antibodies in cerebrospinal fluid.

At the time of admission, the mean hematocrite was higher in the DSS group. The mean platelet count was lower in the DSS group, while the mean leukocyte count was lower in the DF group. In children, the higher the degree of thrombocytopenia or haemoconcentration, the greater the severity of dengue observed.[5] Early severity prediction using clinical features is difficult, but peripheral blood counts can predict severity, which would be more useful in smaller, rural hospitals, where resources are limited. A study in Thailand showed that absence of leucopenia and a low percentage of typical lymphocytes are factors that may predict severe dengue illness. Simple hematologic parameters may be

used to reduce unnecessary admission of patients with suspected dengue infection in the absence of more sophisticated predictors. [21]

In our study, a greater percentage of DF was caused by primary infection, compared to DHF or DSS. In all three groups, secondary infection was more common than primary. Indonesia is endemic for dengue virus and many children have experienced dengue infection earlier in life. This observation is in agreement with other studies that noted increased severity to be correlated with secondary infection[13] Secondary immune status was a risk factor for severe dengue disease.[6,16] There are numerous theories describing potential contributing factors to dengue disease severity, one of which is the antibody-dependent enhancement of infection theory. This theory suggests that secondary infection with a dengue virus of a different serotype increases the risk of developing DHF. In Nicaragua, the great majority of dengue cases were due to secondary infection: 59% of children 1 year old had secondary infection, and by age 3, more than 90% of confirmed DEN-positive cases were secondary infections. [14]

Virus isolation was positive in 81 out of 209 samples, predominantly with dengue serotype 3 (DENV-3). A negative RT-PCR test result does not exclude a dengue infection diagnosis, since blood samples taken after the viremia state (after 5 days of fever) may give a negative result. This finding was similar to a Thai study where the majority of dengue case were DENV-3. [5] In contrast, studies in Nicaragua, Delhi and Taiwan showed that DENV-2 was predominant and associated with secondary infection.[12,14,16]

We have provided information comparing dengue infections in children and highlighted differences in clinical manifestations, hematological and serological findings which can be applied in rural settings, where resources to do further testing may be limited. However, as a retrospective study, some limitations need to be addressed. First, the study was conducted at a single hospital and the patient population may have been biased by referral pattern. Second, laboratory testing and imaging studies may biased by clinician selection based on personal recognition of clinical dengue severity, leading to unavailability of follow-up information.

CONCLUSION

The signs and symptoms of fever, bleeding manifestations and thrombocytopenia were present in children with DF and DHF, while signs of increased vascular permeability (such as haemoconcentration, pleural effusion, hepatomegaly) were only found in DHF. Encephalopathy and gastrointestinal bleeding were mostly found in those with DSS. At admission, signs of leukopenia were more often found in DF compared to DHF patients. Absence of leukopenia may, therefore, be a sign of more severe dengue infection.

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Chapter 4

Clinical course and management of dengue in children admitted to hospital; a 5 years prospective cohort study in Jakarta, Indonesia.

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Abstract

Background: Dengue incidence is rising globally which was estimated 100 million per year, whereas in Indonesia was estimated 7.5 million per year. Dengue clinical course varies from mild dengue fever (DF) to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Patients, clinicians and care facilities would benefit if reliable predictors can determine at admission which cases with clinically suspected dengue will progress to DHF or DSS.

Methods: From 2009 through 2013, a cohort of 494 children admitted with clinically suspected dengue at a tertiary care hospital in Jakarta, Indonesia, was followed until discharge. We evaluated the clinical course and disease outcome of admitted patients, and estimated the burden of dengue cases hospitalized over time.

Results: Of all 494 children, 185 (37%) were classified at admission as DF, 158 (32%) as DHF and 151 (31%) as DSS. Of DF patients, 52 (28%) progressed to DHF or DSS, 10 (5%) had other viral diseases. Of DHF patients, 9(6%) progressed to DSS. Of 33 routinely collected parameters at admission, duration of fever ≤ 4 days was the only significant predictor of disease progression (P= 0.01). Five cases (3%) admitted with DSS died. Between 2009 and 2013, annual dengue admissions declined, while distribution of disease severity remained stable.

Conclusions: Almost a third of children admitted to tertiary care with clinically suspected DF progress to DHF or DSS. Among routinely collected parameters at admission, only fever duration was significantly associated with clinical progression, emphasizing unpredictability of dengue disease course from parameters currently routinely collected.

INTRODUCTION

Dengue continues to increase globally[1] and now reaches an estimated 100 million clinically apparent infections annually.[2] Dengue infection is a major international public health concern[1], with infection transmission occurring in 128 countries and almost 4 billion people at risk, of which at least 70% live in Asia-Pacific region.[2,3] In Indonesia, annual incidence of dengue has increased from 0.05/100,00 in 1968 to 35-40/100,000 in 2013 with a peak at 85/100,000 in 2010.[4]

Dengue virus causes a spectrum of clinical disease ranging from self-limiting, mild symptoms classified as dengue fever (DF) to severe disease including dengue hemorrhagic fever (DHF) or life-threatening dengue shock syndrome (DSS).[5] Children are at the highest risk of developing severe clinical manifestations and represent about 90% of dengue related hospitalizations].[2] In some Southeast Asian countries, including Indonesia, a shift to older, adolescent age-groups has been observed in recent years, which was accompanied by a changing pattern of clinical presentations of dengue.[4] This illustrates that dengue epidemiology is not stable over time and a high level of clinical suspicion is therefore warranted in all pediatric age groups. For this reason, and due to seasonal incidence pattern, dengue poses a substantial burden on pediatric healthcare facilities during peak epidemic months.

A minority of symptomatic dengue cases are reported to progress to severe disease.[6] A detailed description of patients, covering full spectrum of pediatric dengue presenting to clinical care and including their outcomes, can support such awareness and help understand course of the disease. Importantly, it might unravel patient and clinical characteristics that, at early admission, predict dengue course severity, and thus support timely treatment and help reduce dengue care burden by optimizing the triage stage.

Most prognostic studies of dengue have been performed in adults and evaluated predictors for the outcome of severe dengue or mortality.[7-9] Studies on early predictors in pediatric patients admitted to hospital mostly focused on differentiating dengue infection from non-dengue or discriminating between non-severe dengue and severe dengue. Therefore, current prognostic evidence on children hospitalized with dengue is rather undetermined.

This study provides a comprehensive description of a large cohort of pediatric dengue patients admitted to a large tertiary hospital in Jakarta, to provide insights into disease course, treatment and outcome prediction. In addition, to characterize the burden of dengue on hospital facilities during epidemic peaks, we quantified contribution of dengue to all-cause and infectious diseases (IDs) pediatric admissions.

MATERIALS AND METHODS

Study location

This study was conducted in Cipto Mangunkusumo hospital, a public tertiary hospital located centrally in Jakarta Province, that is a national referral center for a range of medical specialty services. It serves a population of 10 million in the urban region of Jakarta and is 1 of 14 national tertiary hospitals in Indonesia. The Child Health Department hosts 157 beds, including a 4-bedded pediatric intensive care unit (PICU) and a 20-bedded ID unit that accommodates children with clinically suspected dengue and other infections. Attending patients are a mix of self-referrals from nearby neighborhoods and referrals from regional primary or secondary healthcare centers. National protocols describe referral indications for dengue cases according to the level of healthcare required. The presence of at least one of the following characteristics is considered an indication for hospitalization at a tertiary care facility: age < 1 year, obesity (Body Mass Index≥ 95th percentile Center Disease Control and Prevention graph), co-morbidities like thalassemia or heart disease, organ involvement or a diagnosis of DSS.[1,5]

Study population

According to local protocol, each patient is initially assessed in a triage unit. There, suspected dengue infection patients can be monitored for 24 hours, followed by either discharge or admission to ID unit depending on expected risk of progression to DHF. Patients with clinical DHF are immediately admitted to ID unit or, in case of DSS, to intensive care unit.

Admitted patients with (suspected) dengue are routinely monitored daily for physical signs and symptoms, as well as hematology (hemoglobin, hematocrit, leucocyte, platelet

count) until normalization and defervescence. In case of encephalopathy or bleeding manifestations, additional diagnostics are performed to rule out other potential causes. For dengue serology the presence of dengue IgM and IgG in acute-phase serum is assessed using a rapid immunochromatographic test (Panbio Dengue Duo Cassette). Dengue serology is routinely performed in blood samples on admission and, if indiscriminate, during convalescence. Patients with negative IgM on admission are retested after 24-48 hours. Disease course is scored daily throughout hospital stay based on 1997 WHO Dengue classification and severity grading (supplementary Table1).

In brief, suspected DF is classified when there is acute febrile illness and two or more of the following: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations or leukopenia.[1] DHF is classified if (1) fever lasting 2-7 days; (2) hemorrhagic manifestations or positive Tourniquet test; (3) thrombocytopenia and 4) evidence of plasma leakage with laboratory findings of hemoconcentration or signs of pleural effusion, ascites or hypoproteinemia.[5] DHF has four severity grades according to presence or absence of spontaneous bleeding and severity of plasma leakage. DSS refers to DHF grades 3 and 4. Although more common in adults,[1] dengue in children may also present as atypical severe disease with organ failure, but without the typical transient increase in vascular permeability seen in DHF and DSS. Presence of severe organ involvement was therefore assessed, irrespective of DHF or DSS classification.[5]

Conclusive dengue infection diagnosis is based on serology testing for combinations of anti-dengue IgM antibody and anti-dengue IgG antibody. Positive IgM and negative IgG indicates primary dengue infection. Positive IgG and positive IgM indicates secondary infection. Positive IgG and negative IgM indicates indeterminate serology (prior infection)[5].

Standard dengue fluid management follows the WHO 1997 guidelines. This includes (1) for suspected DF: oral maintenance fluid or intravenous crystalloids if not able to receive fluids orally; (2) for clinically defined DHF: crystalloids with volume of maintenance plus 5-7% to compensate intravascular fluid deficits, followed by titration to maintenance based on hemodynamic condition; (3) for patients with (imminent) shock or DSS: initial fluid resuscitation (20 ml of crystalloids/kg body weight), and additional fluid loads with colloids or crystalloids if indicated, followed by titration to maintenance within 48-72 hours based

on hemodynamic condition. Blood products, including packed red cells and thrombocyte suspension, (with or without fresh frozen plasma or cryoprecipitate) are indicated for dengue related gastrointestinal bleeding, regardless of the thrombocyte level.

Data collection

For our study, children aged between 1 month to 18 years with clinically suspected dengue infection, admitted from January 2009 to December 2013, were enrolled. History for duration of fever was obtained from parents' answer, but a few older children answered as well. Physical signs and symptoms, hemodynamic parameters, diagnostic testing results, medical interventions and dengue severity grading were recorded daily on a standardized case report form until discharge and entered into a database.

Statistical analysis

We used descriptive statistics for patient demographics and disease grade upon admission. Next, given their disease grade at admission, patients were classified by disease progression status. Progression was defined as moving from DF to DHF/DSS, or as a change from a DHF non-shock (DHF grade 1 and 2) to DHF shock (DHF grade 3 and 4). Subsequently, among patients with DF (n=185), possible prognostic markers (demography, medical history, clinical signs and symptoms at admission) for progression to DHF or DSS, were univariably evaluated using Chi-square, Fisher exact, Student t test or Mann-Whitney U tests, where appropriate.

Treatment over disease course and hospital stay was assessed for each patient and summarized by final disease grade as assigned upon discharge. Serology results were compared between different final disease grades and tested using ANOVA.

Seasonal and time trends between 2009-2013 in dengue admissions in Cipto Mangukusumo hospital were analyzed by calculating number of cases of DF, DHF and DSS by month and year. To assess healthcare resource utilization and impact on facilities, we determined proportion of all-cause pediatric hospitalizations attributable to dengue and total number of bed-days per year, and separately for the peak dengue months in each year. We used dengue infected cases (DF, DHF and DSS) as numerators and number of all-cause pediatric hospitalizations (excluding neonatal admissions < 1 month of age) per year and per

month, as respective denominators. All data were analyzed using SPSS version 22.

Ethical approval for the study was obtained from the ethics committee of the Faculty of Medicine, University of Indonesia. Both parents signed Informed consent for collection of clinical data for study purposes.

RESULTS

Between January 2009 and December 2013, 494 patients met the clinical case definition for dengue. Mean age was 9.0 (4.4) years; most frequently admitted age-class was 10-14 years (35.8%). Median duration of fever was 4 days (IQR 1). Sixty three percent had signs of increased vascular permeability at admission (classified as DHF or DSS), 37% were classified as DF (Table 1).

Table 1. Characteristics of patients meeting the clinical dengue case definition at admission.

	Diagnosis at admission			
	DF	**DHF**	**DSS**	**Total**
	185 (37%)	158 (32%)	151(31%)	494 (100%)
Age-group year(n, %)				
< 1	7 (3.8)	10 (6.3)	4 (2.6)	21 (4.3)
1-4	28 (15.1)	13 (8.2)	29 (19.2)	70(14.2)
5-9	58 (31.4)	47 (29.7)	58 (38.4)	163 (33.0)
10-14	67 (36.2)	59 (37.3)	51 (33.8)	177(35.8)
≥15	25 (13.5)	29 (18.4)	9 (6.0)	63(12.8)
Gender (n, %males)	91(49.2)	85(53.8)	79(52.3)	255(51.6)
Comorbidity present (n,%)*	9 (4.9)	2 (1.3)	3 (2.0)	14 (2.8)

* Comorbidities are thalassemia, anemia, tuberculosis, diabetes mellitus, leukemia, HIV and epilepsy.

Table 2 shows that 52 of 185 patients (28.1%) with initial DF developed DHF or DSS and that nine of 158 patients with initial DHF (5.7%) progressed to DSS. Five cases diagnosed with DSS at admission died from refractory shock (3.3%). The mean age of these children was 5 years (IQR 4).

Table 2. Dengue diagnosis at admission and final dengue diagnosis (n)

Diagnosis at admission / Final outcome	DF	DHF	DSS	Total
DF	123(66.5%)	-	-	123(24.9%)
DHF	44(23.8%)	149(94.3%)	-	193(39.1%)
DSS	8(4.3%)	9(5.7%)	146 (96.7%)	163(33.0%)
Other viral disease	10(5.4%)	0(0.0%)	0(0.0%)	10(2.0%)
Dead (1 other virus and 4 DSS proven dengue serology)	-	-	5 (3.3%)	5 (1.0%)
Total	185(100%)	158(100%)	151(100%)	494(100%)

Table 3. Signs and symptoms at admission of 185 DF cases with or without disease progression.

	DF, no progression N=123	DF to DHF/DSS N=52	P
Age-group (n, %)			0.78
< 1 year	4 (3.3)	1(1.9)	
1-4 years	20 (16.3)	6(11.5)	
5-9 years	39(31.7)	16(30.8)	
10-14 years	43(35.0)	23(44.2)	
>15 years	17(13.8)	6(11.5)	
Male (n, %)	62(50.4)	24(46.2)	0.61
Comorbidities (n)*	5 (4.1)	0 (100)	0.32
Days with fever prior to admission (n, %)			0.01
≤4	75 (61.0)	42 (80.8)	
>4	48 (39.0)	10 (19.2)	
Symptoms on admission**			
Anorexia	49(45.4)	16(37.2)	0.36
Nausea	64(59.3)	25(58.1)	0.90
Vomiting	52(48.1)	21(48.8)	0.94
Abdominal pain	46(42.6)	22(51.2)	0.34
Diarrhea	18(16.7)	2(4.7)	0.05
Headache	54(50.0)	18(41.9)	0.37
Myalgia	28(25.9)	16(37.2)	0.17
Arthralgia	16(14.8)	5(11.6)	0.61
Retro-orbital pain	11(10.2)	3(7.0)	0.76
Shiver	10(9.3)	0(0.0)	0.06
Malaise	27(25.0)	9(20.9)	0.60
Sore throat	5(4.6)	6(14.0)	0.08
Cough	29(26.9)	9(20.9)	0.45
Signs on admission(n, %)**			
Positive tourniquet test	48(39.0)	25(48.1)	0.34
Distended abdomen	0(0.0)	1(2.3)	0.29
Hepatomegaly	15(12.3)	10(19.6)	0.21
Petechial	48(39.0)	17(32.7)	0.43
Epitaxis	21(17.1)	6(11.5)	0.35
Gum bleeding	1(0.8)	0(0.0)	1.00
Purpura	16(14.8)	2(4.7)	0.08
Hematemesis	0(0.0)	2(3.8)	0.09
Melena	0(0.0)	1(1.9)	0.30
Encephalopathy	0(0.0)	1(2.1)	0.08
Hematology findings			
Mean hematocrit day 3 (cut-off>40 %)	6(4.9)	1(1.9)	0.81
Mean leucocyte day 3 (cut-off < 5000/µL)	23(18.7)	12(23.1)	0.22
Mean thrombocyte day 3 (cut-off < 50,000 /µL)	4(3.3)	3(5.8)	0.62

Final diagnosis as other viruses were not included in the analysis

* Comorbidities are thalassemia, anemia, tuberculosis, diabetes mellitus, leukemia, HIV and epilepsy.

**Percentages are calculated based on available data.

Table 3 shows signs and symptoms at admission by in-hospital progression status among 185 patients with DF at admission. Only duration of fever of <4 days was statistically significantly associated with progression from DF to DHF or DSS (P=0.01). This was the critical phase when plasma leakage occurred which may lead to shock hypovolemic, after the febrile phase (3 days after the onset of fever). Hematemesis (n = 2), melena (n = 1) and encephalopathy (n = 1) at admission were only present in DF patients that subsequently progressed to DHF or DSS.

Table 4 describes treatment and hospitalization course by final disease grade, excluding the children who had another viral disease (n = 10). Of all admitted patients, 123 (25.4%) had a final diagnosis of clinical DF, 193 (39.9%) as DHF and 163 (33.7%) as DSS and 5 (1.0%) deaths (included 4 DSS proven dengue serology and 1 other virus). Overall median length of hospital stay was 4 days (IQR 2) and differed significantly by disease severity (p<0.001). There was no severe organ involvement in children without DHF or DSS. Hematemesis, melena and encephalopathy were more diagnosed in children with DSS compared to children diagnosed with DF or DHF.

Table 4. Disease course and treatment by final diagnosis.

	Final diagnosis				P
	DF (n=123)	DHF (n=193)	DSS (n=163)	Death cases (n=5)	
Complications (n, %)					
Hematemesis	0 (0.0)	3(1.6)	18 (11.1)	0 (0.0)	<0.001
Melena	0 (0.0)	3(1.6)	15 (9.3)	1 (20.0)	<0.001
Encephalopathy	0(0.0)	7 (3.6)	14 (8.6)	3 (60.0)	0.007
Length of stay (median days, range)	4 (1)	4 (2)	4 (2)	3(1.5)	<0.001
Therapy					
Supportive Fluid treatment (n, %)					<0.001
Oral only	6 (5.6)	3(1.7)	2(1.3)	1(25.0)	
IV Maintenance only	87 (80.6)	75(43.4)	57(38.0)	0 (0.0)	
IV Maintenance + deficit 5-7%	13 (12.0)	95(54.9)	88(58.7)	3 (75.0)	
Others	2 (1.9)	0(0.0)	3(2.0)	0 (0.0)	
Fluid resuscitation					
Crystalloid (yes) (n, %)	0(0.0)	1(0.5)	134(96.4)	4(100.0)	<0.001
Colloid (yes) (n, %)	0(0.0)	1(0.5)	59 (40.1)	2(50.0)	<0.001
Blood products(%)					
Any PRC (n,%)	0(0.0)	1(0.5)	7(4.3)	1(20.0)	
Any TC (n,%)	0(0.0)	1(0.5)	2(1.2)	0(0.0)	
Any FFP (n,%)	0(0.0)	0(0.0)	10(8.1)	0(0.0)	
Any Cryo (n,%)	0(0.0)	0(0.0)	3(1.8)	0(0.0)	

note: prc=pack red cells, ffp=fresh frozen plasma, tc=thrombocyte components, cryo= cryoprecipitat

Four hundred eighty (97.2%) patients received intravenous fluid solution, whereas only 14 (2.8%) received oral fluid. There were some deviations from standard fluid management protocol: of DF patients, 13 (12%) received additional 5-7% fluid replacement because of mild to moderate dehydration on admission, while 75 (43.4%) of DHF patients with grade 1-2 were only treated with maintenance without replacement. Among DSS patients, 138 (96.5%) received crystalloid for initial fluid resuscitation. Additional colloids were necessary in 61(40.4%) DSS patients because of insufficient hemodynamic response to crystalloid fluid resuscitation. Blood components were given to 13 children with final diagnosis in DSS for 12 times and in DHF 1 time, and additionally to one DSS case who died. The 4 clinical DSS cases who died were resuscitated with crystalloid and 3 cases were

followed by colloid, however 1 DSS case who died received maintenance fluid solution who was resuscitated before referred to our hospital. Only 1 DSS case who died with secondary dengue infection had recurrent shock and received PRC on day 1.

Table 5 shows serology results by final disease grade. Confirmation of primary dengue infection was obtained in 94 patients (19.8%), secondary infection was found in 224 patients (47.2%) and serology was indiscriminate in 121 patients (25.5%). In 36 patients (7.6%), dengue serology was negative for both IgG and IgM. Serology results differed by disease grade (P<0.03). Negative serology was seen more frequently among DF patients (14% versus 5% and 6% among DHF and DSS, respectively), whereas secondary infection was associated with more severe dengue (36% among DF cases, 48% among DHF and 54% among DSS). Dengue serology of 5 dead cases confirmed 1 primary infection, 2 secondary infections and 1 indeterminate, and 1 negative.

Table 5. Serology results by final dengue disease stage

	Final diagnosis				P
	DF (n=118)	DHF (n=190)	DSS (n=162)	Death cases (n=5)	
Serology results (n, %)*					<0.03
IgM+/IgG-	26(22.0)	37(19.5)	30(18.5)	1(20.0)	
IgM+/IgG+	43(36.4)	92(48.4)	87(53.7)	2(40.0)	
IgM-/IgG+	32(27.1)	52(27.4)	36(22.2)	1(20.0)	
IgM-/IgG-	17(14.4)	9(4.7)	9(5.6)	1(20.0)	

* percentages are calculated based on available data.

Table 6. Proportion of dengue cases during peak months per year.

Year	Dengue peak months	Dengue* admissions/ all-cause pediatric hospital admissions (%)		Dengue* admissions/all-cause admissions infectious diseases ward (%)	
2009	June	40/648	4.3	40/70	36.4
2010	March	20/570	3.7	20/48	29.4
2011	January	13/512	1.4	13/70	15.7
2012	April	8/462	1.5	8/68	10.5
2013	July	11/373	2.0	11/63	14.9

Between 2009 and 2011, number of dengue patients declined and remained more or less stable thereafter. Distribution of disease severity did not change over time (Figure 1). Proportion of all-cause hospital admissions (excluding neonatal) attributable to dengue declined from 4.3% in 2009 to 1.5% in 2012 and then increased in 2013 (2.0%). At ID unit, proportion of dengue admission declined from 36.4% in 2009 to 10.5% in 2012 (Table 6). Dengue admissions showed a clear seasonal pattern during the high epidemic year of 2009, while no clear pattern was observed in more recent years and the proportion of admissions for dengue at ID ward in any month varied between 0 and 36.4%, with lower percentages in last three years of the period considered. (Figure 1 and Table 6).

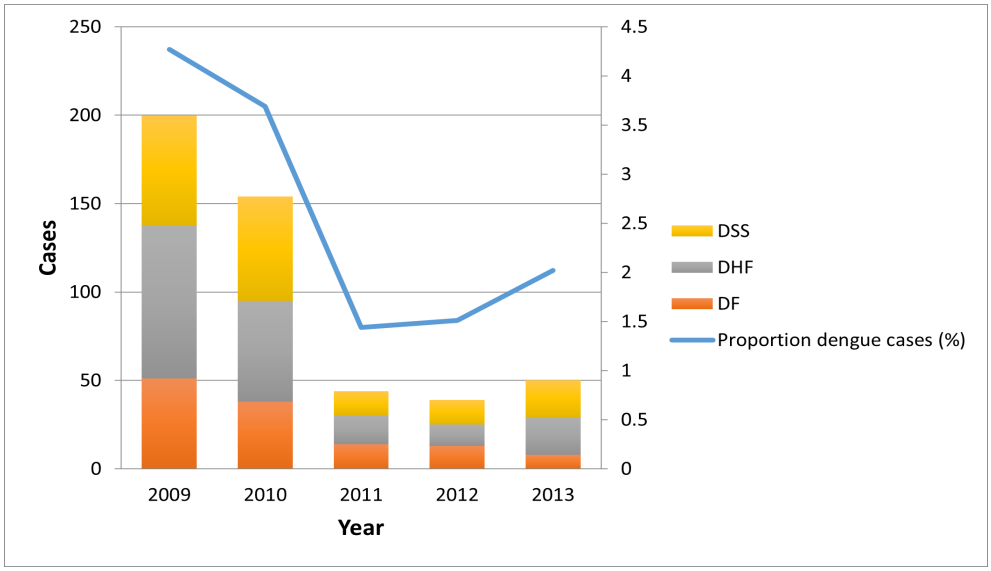

Figure 1. Number of dengue admission by disease grade and proportion of all-cause hospitalizations attributable to dengue (excluding neonatal admissions)

DISCUSSION

This study shows among 494 children with clinical dengue, 52 out of 185 (28%) of patients admitted with DF progressed to DHF or DSS. Except for prior duration of fever, there were no other predictors at admission of disease progression among DF patients, although some rare disease manifestations (hematemesis, melena and encephalopathy) were only observed

in patients that progressed. In addition, 9 out of 158 (5%) of patients admitted with DHF progressed to DSS. This study has emphasized the unpredictability of dengue disease course from parameters currently routinely collected at admission.

Before further discussion of our findings, some limitations need to be addressed. In our study, the WHO 1997 clinical case definitions were used to classify dengue disease.[5] A newer classification was proposed by WHO in 2009, but this was not implemented in routine care for children in Indonesia during the course of this study. Additional training of health workers will be required before nation-wide implementation of the WHO 2009 dengue guidelines in Indonesia can be recommended. The main difference between the 1997 and 2009 WHO dengue guidelines is the classification of dengue severity. The WHO 1997 guideline classifies dengue infection into DF and 4 DHF categories (1-4), based on severity of plasma leakage and presence/absence of shock. The 2009 classification has 3 categories (1) DF without warning signs, (2) DF with warning signs and (3) severe dengue. The latter is defined as dengue with severe plasma leakage, severe bleeding, or severe organ manifestation. Our study assessed bleeding and severe organ involvement separately, in addition to the 1997 disease grade. Importantly, none of our patients had bleeding or severe organ involvement without plasma leakage indicating that the 1997 classification captured all severe cases in our setting. Second, primary or secondary care health centers in Indonesia, usually have limited resources and laboratory facilities. They are managed by primary care physicians, and only few general pediatricians are locally available. The PICU facilities for DSS care are not available in all secondary healthcare centers. Our tertiary care hospital does have PICU facilities and the pediatric dengue population attending our hospital is a mix of referrals from primary and secondary care, and of self-referrals. Consequently, after triage the admitted dengue cases in our hospital are a reflection of that patient mix and generalizability of our findings to primary of secondary care setting may thus be limited. A strong feature of our study is that our cohort is relatively large and patient mix spans all severity levels of dengue, from mild to severe. Inclusion criteria and study monitoring were rigorously applied, and all patients were systematically and thoroughly assessed.

Given common peaks in dengue incidence and the strains on care facilities, it is important for physicians to have evidence-based tools that can discriminate between those

at risk of progression to severe disease, and those that can be safely discharged early. However, from our study it is clear that at time of admission there currently are very few, if any, predictors of in-hospital outcome available. Duration of fever was the only statistically significant predictor, where progression was more likely to occur in those children with ≤ 4 days of fever prior to admission. However, positive predictive value of < 4 days of prior fever was still too low for accurate and safe distinction in practice. Hematemesis, melena and encephalopathy, each very rare, were present only in children with DF that progressed to DHF or DSS, and obviously these are ominous signs in their own right. Therefore, we infer that at admission, using characteristics that currently are clinically available at that moment will not provide doctors in this setting with tools for accurate prediction of disease progression among children with DF.

Although there are comparable studies in adult patients, 7-9 there is limited insight for pediatric patients with dengue available from other studies, and they show substantial heterogeneity in settings (patient mix, referral and care systems, care practice (triage), dengue characteristics), and differ in research objectives. A study from Vietnam included 2301 hospitalized children with laboratory confirmed dengue.[10] Those with a history of vomiting, higher body temperature, a palpable liver, and a lower platelet count had a higher risk of progressing to DSS after admission. It was concluded that a prediction model from such signs was of little clinical use because of very high false positive rates. Another study in 145 Brazilian children hospitalized with confirmed DF or DHF grade I or II, 23 patients progressed to use of advanced life support therapy or death.[11] At admission, lethargy, abdominal distension, pleural effusion and presence of hypoalbuminemia were found to predict such serious outcomes. A more recent Brazilian case-control study of hospitalized children with laboratory confirmed dengue, compared 69 patients with severe dengue (shock, severe bleeding, organ impairment and death) and 164 that did not develop severe dengue.[12] By design this study could not estimate absolute risks but it did show that lethargy, dyspnea and abdominal pain at admission were independent predictors of progression during hospital stay to severe dengue. Furthermore, several studies in Indonesia assessed patient and clinical characteristics of pediatric dengue cases by disease grade, but none assessed how patient and clinical characteristics upon admission correlated with disease

progression thereafter.[13-15]

In summary, to our knowledge only 3 prior studies[10-12] evaluated characteristics at admission in predicting progression to more severe dengue during follow-up, the largest of which showed that the clinical downside of their prediction model was a high false positive rate. Based on the combined results from ours and other studies, we infer that for real gain in triage, treatment and hospital discharge efficiency, further detailed prognostic study of dynamic clinical predictors during hospitalization, rather than at admission, will be necessary.

Another important finding in our study is that all 61 dengue cases who progressed to higher severity during hospital-stay recovered with adequate fluid therapy, using mainly standard first-line treatment regimens with crystalloids. Fluid management for DHF and DSS cases who were referred to our hospital was adjusted according to clinical condition at admission. Additional colloids were required in less than half of DSS cases and blood products were administered only occasionally.

Because of the seasonality of dengue, infection rates typically show peaks that may well burden tertiary care treatment capacity. Over our study period, dengue admissions among all-cause pediatric hospital admissions varied between 1.5% and 4.3%, but severity of hospital admitted dengue was relatively stable. Of note, in some years, there appeared to be a complete absence of a seasonal pattern, with year-round low-grade transmission. In addition, we observed overall a decline over time in the number of admissions for dengue, which is in line with observations from regional [16] and national dengue surveillance.[4] What causes this change in seasonality is unknown, but it could be the result of a climatic trend towards shorter and less intense rainy seasons[17] as well as of improved vector control programs during rainy seasons, resulting in reduced vector populations[18-20] or acquired immunity by the population to the circulating strains in those years.

CONCLUSION

Summarizing, almost a third of children admitted to tertiary care with clinically suspected DF progress to DHF. It proved not possible to accurately and usefully predict progression from DF to more severe dengue, based on routinely collected parameters at admission.

Dynamic prognostic studies using time dependent clinical measurements are needed to better differentiate between dengue patients who can be sent home or referred to lower care, and dengue patients who are likely to progress to severe stages and need close in-hospital monitoring.

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Supplementary table 1. WHO Dengue classification 1997.

Dengue Fever (DF)
Acute febrile illness with two or more of the following manifestations:

- Headache
- Retro-orbital pain
- Myalgia
- Arthralgia
- Rash
- Hemorrhagicmanifestations
- Leukopenia

AND

- Supportive serology of a positive IgM antibody test on acute or convalescent phase serum specimen, OR
- Occurrence at the same location and time as other confirmed cases of dengue fever

Dengue Hemorrhagic Fever (DHF)
All of the following must be present:

- Fever or history of acute fever, lasting 2-7 days, occasionally biphasic.
- Hemorrhagic manifestations; Positive tourniquet test, Petechiae, ecchymosis, purpura, bleeding from mucosa, gastrointestinal tract (Hematemesis / melena), injection sites or other locations
- Thrombocytopenia (≤100,000 cells/mm3).
- Evidence of plasma leakage due to increased vascular permeability, manifested by at least one of the following:
    - a rise in hematocrit ≥ 20% above average for age, sex, and population
    - a drop in hematocrit following volume replacement therapy of ≥ 20% of baseline
    - signs of plasma leakage such as pleural effusion, ascites and hypoproteinemia

Dengue Shock Syndrome (DSS)
The DHF criteria plus evidence of circulatory failure manifested by:

- Rapid and weak pulse, and narrow pulse pressure (<20 mmHg),

or:

- Hypotension for age, and cold, clammy skin and restlessness

Grading the severity of dengue illness:

I. Fever accompanied by nonspecific constitutional symptoms with a positive tourniquet test as the only hemorrhagic manifestation
II. Same as grade I, except with spontaneous hemorrhagic manifestations
III. Circulatory failure manifested by rapid, weak pulse with narrowing of the pulse pressure (<20mmHg) or hypotension
IV. Profound shock with undetectable blood pressure and pulse
V. The term DHF non-shock refers to DHF grades I and II, whereas dengue shock syndrome (DSS) refers to DHF grades III and IV

Severe Organ Involvement

- Liver AST or ALT ≥ 1000
- CNS: impaired consciousness
- Heart and other organs

Chapter 5

Dengue profile and clinical signs in infants: A 10-year cohort study in a tertiary referral hospital in Indonesia

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Abstract

Background: Clinical manifestations of dengue range from mild dengue fever to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). In infants, dengue infection is particularly challenging as the signs and symptoms may be more difficult to distinguish from other febrile illness, but studies in such very young children are scarce.

Objective: To describe the clinical and laboratory profiles of infants with dengue infection.

Methods: This was a retrospective study of all 46 infants aged less than 12 months presented to Cipto Mangunkusumo Hospital with suspected clinical dengue infection between 2009 and 2018. Clinical characteristics of the subjects, as well as information related to their illness, were extracted from their medical records.

Results: In total 42 infant cases of dengue were included, of which 24 (57.1%), were primary dengue cases. The median age of the subjects was 8 months (interquartile range (IQR) 3.27). Eleven cases (26.2%) had DSS (DHF grade III or IV); the 31 non-DSS cases consisted of 11 cases of DF (26.2%) and 20 cases (47.6%) of DHF grade I or II. Atypical signs such as diarrhea (13 cases; 31%), and cough (11 cases; 26%), were common. Based on univariate analysis, liver enlargement (odds ratio (OR) 6.0, p=0.01) and impaired consciousness (OR 7.5, p=0.02) were strongly associated with development of dengue shock syndrome. Low hemoglobin levels (median 9.8 g/dL) were observed at discharge.

Conclusion: The majority of dengue cases in infants were primary infections but some infants manifest as severe DHF/ DSS. Diarrhea and cough as atypical signs occurred in about one third of dengue infants. Liver enlargement and fluid accumulation were two strong indicators of dengue shock syndrome in infants. The majority of dengue infants were anemic at discharge suggesting that hematocrit is not a reliable indicator of hemoconcentration, but rather the result of pre-existing anemia. We therefore suggest radiological examination as alternative to detect plasma leakage in infants with suspected dengue.

Keywords: dengue; dengue fever; dengue hemorrhagic fever, infants

INTRODUCTION

Dengue infection remains a public health burden in many tropical and sub-tropical countries. Infection with any one of the four dengue serotypes may lead to manifestations ranging from mild non-specific febrile illness, classical dengue fever (DF), dengue hemorrhagic fever (DHF), to dengue shock syndrome (DSS). Severe dengue is less common in infancy than in older children and adults, but mortality risk is high. For that reason, infants with dengue infection should be monitored in the hospital.[1] In dengue-endemic countries, children under the age of one year comprise one to five percent of dengue cases admitted to hospitals each year.[2] Severe dengue (DHF/DSS) is associated with secondary heterotypic dengue virus infections but can also occur in primary dengue infection of infants born to dengue-immune mothers. Pre-existing dengue IgG antibodies are thought to play a role in both serotype-specific immunity to infection as well as in the pathogenesis of dengue through the mechanism of antibody-dependent enhancement of infection.[3] Severe dengue occurs predominantly in infants when they are 4-9 months old, when maternal antibodies decrease to a level at which the dengue virus is not neutralized.[3] These non-neutralizing antibodies bind to dengue virus and enhance virus uptake by monocytes/macrophages (antibody dependent enhancement, ADE), thus increasing viral burden and resulting in more severe dengue.[4]

A limited number of studies have documented clinical and laboratory findings in infants with dengue, reporting considerable variation in signs and symptoms,[5,6] while studies with serial laboratory measurements and radiological examinations to detect pleural effusion and monitoring the clinical evolution over time are even more scarce. Infants with dengue have been reported to have unique manifestations and characteristics, such as a higher incidence of shock, platelet counts lower than 50,000/uL, more plasma leakage, and fewer hemorrhagic manifestations compared to dengue in older children and adolescents. Dominicus et al, in Surabaya, Indonesia, found that encephalopathy, liver enlargement and melena were related to DHF in infants.[7] A study by Mariko and Hadinegoro, in Padang, Indonesia reported serial cases of dengue in infants with a median follow-up of 5 months. Signs and symptoms most often reported include vomiting, followed by petechiae, cough and shock.[8] As dengue in infants may be challenging to distinguish from other febrile illness,

a laboratory diagnostic dengue test[3] and radiological examination to find signs of plasma leakage[9,10] are helpful to support the diagnosis.

This study aimed to contribute to the limited body of evidence by describing the unique clinical and laboratory profiles of infants hospitalized with dengue infection and of infants developing severe dengue fever.

METHODS

We included infants, defined here as children aged less than 12 months, admitted with suspected dengue infection to the tertiary national referral center, Department of Child Health, Cipto Mangunkusumo Hospital, Jakarta, from January 2009 to December 2018. Only confirmed dengue cases were included in the analysis. Subjects' data were obtained retrospectively. The standard procedure for admitted clinical dengue patients was followed. This includes daily physical examination and complete peripheral blood analysis until discharge. Clinical and laboratory data were recorded on daily monitoring forms completed during the infant's hospital stay. For the purpose of this study, we scrutinized the medical file collecting patient vomiting, diarrhea, cough, anorexia, convulsion, malaise, nausea, lethargy, abdominal pain, impaired consciousness, liver enlargement and pleural effusion, and on hematology findings including hemoglobin, hematocrit, white blood count and platelet count. In addition, demographical/ epidemiological characteristics collected in the database included age, gender, and dengue serology of mothers who ever had DHF.

The 1997 WHO definitions and criteria were used to classify dengue disease.[11] Final diagnosis refer to case definition DHF consisted of fever, hemorrhagic tendencies, thrombocytopenia and evidence of plasma leakage must all be met. Hemorrhagic tendencies and plasma leakage is evidenced by one of a limited range of options.[11]

Children with DHF were classified into four grades of severity: Grade I as fever accompanied by non-specific constitutional symptoms, with a positive tourniquet test being the only hemorrhagic manifestation; Grade II as spontaneous bleeding in addition to the manifestations of grade I; Grade III as circulatory failure manifested by a rapid, weak pulse and narrowing of pulse pressure or hypotension, with the presence of cold, clammy skin and restlessness; Grade IV as profound shock with undetectable blood pressure or pulse.

Grades III and IV were considered dengue shock syndrome (DSS), whereas grades I and II were considered DHF without shock. Non-DSS in our study refers to cases presenting as DF or DHF grade I or II.

Dengue confirmation was based on either dengue serology antibody IgG/IgM positivity, or non-structural 1 (NS-1) dengue antigen testing. Dengue infection diagnosis was based on antibody dengue serology tests performed on blood samples after five days of fever. Positive dengue serology was defined as positive dengue IgM and/or IgG antibodies. Infection was defined as primary in case of a positive dengue IgM result with negative IgG, and secondary in case both IgG and IgM were positive. Indeterminate (prior dengue infection) was defined when IgG was positive and IgM negative. Diagnostic NS-1 antigen testing was available after 2013 and was performed only in suspected clinically dengue infants who came in the early febrile phase, to help differentiate between dengue and other acute febrile illness in infants. A positive result confirmed dengue infection but did not differentiate between primary or secondary dengue infection. Data of dengue serology IgG and IgM of mothers with previous DHF who delivered neonates included in this study were collected from medical records. Pleural effusion was obtained from chest x-ray in a right lateral decubitus position. Ultrasound examination was not performed on the infants in this study.

Data analysis was done using SPSS version 22.0 (IBM, Chicago). Univariate analysis was used to find signs and symptoms associated with dengue shock syndrome (DSS) versus non-DSS (DHF grade I or II and DF); for dichotomous variables Pearson chi-square tests or Fisher's exact test were applied accordingly. A non-parametric test was used to analyze whether the hematology findings differed between the DSS and non-DSS group of infants. A p-value less than 0.05 was considered statistically significant.

Ethical clearance was obtained from the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No:KET-1345/UN2.F1/ETIK/PPM.00.02/2019).

RESULTS

In total, 699 (suspected) pediatric clinical dengue cases aged 0-18 years were admitted to the department of Child Health, Cipto Mangukusumo hospital in the period 2009-2018. Of these, 46 were infants less than 12 months of age on admission (6.5%). Four of these infants were excluded from further analysis because the medical records were no longer available (two infants) or because dengue was not confirmed serologically (two infants). Clinical and hematological characteristics of the 42 remaining infants included in this study are presented in Table 1. Eleven infants (26.2%) had DF, 20 infants (47.6%) had DHF grade I or II, and 11 infants (26.2%) had DSS.

Among the infants included in the study, the three most common signs and symptoms at admission besides fever were liver enlargement in 21 subjects (50%), followed by pleural effusion in 17 (40.5%) and vomiting in 15 subjects (35.7%). Univariate analysis showed that liver enlargement (OR 6.03, p=0.01) and impaired consciousness (OR 7.53, p=0.02) were associated with DSS. Other signs and symptoms including vomiting, diarrhea, cough, anorexia, convulsion, malaise, nausea, lethargy, abdominal pain were not associated with DSS.

The most frequent bleeding manifestation was petechiae in 24 subjects (57.1%). Hematologic values at the time of admission and discharge are listed in Table 1. At admission, platelets and leukocytes count in the infants with DHF grades III and IV (DSS group) were lower than in the non-DSS group. At discharge, the median hemoglobin and median hematocrit were 9.8 (IQR 2) and 29.5 (IQR 6), respectively, which indicates that at baseline many infants may have been anemic.

Table 1. Clinical and hematology profile of infants with confirmed dengue (n=42).

Parameters	Overall n(%) (n=42)	Dengue classification Non-DSS (DHF 1&2 and DF) n(%) (n=31)	DSS (DHF grade III-IV) n(%) (n=11)	p-value
Median age in months (IQR)	8(3)	8(3)	8(4)	
Male gender(male/female)	19 (%)	10 (%)	9 (%)	
Median length of stay (days) (IQR)	4(2)	4(1)	5(4)	
Median days of fever prior to admission (IQR)	4(2)	5(2)	4(1)	
Clinical signs and symptoms at admission				
Fever	42(100)	31(100)	11(100)	
Vomiting	15(36)	10(32.3)	5(45.5)	0.43*
Diarrhea	13(31)	8(25.8)	5(45.5)	0.23*
Cough	11(26)	7(22.6)	4(36.4)	0.44*
Anorexia	9(21)	6(19.4)	3(27.3)	0.68*
Convulsion	7(17)	4(12.9)	3(27.3)	0.35*
Malaise	6(14)	4(12.9)	2!18.2)	0.65*
Nausea	5(12)	5(16.1)	0(0)	0.30*
Lethargy	4(10)	2(6.5)	2(18.2)	0.28*
Abdominal pain	4(10)	3(9.7)	1(9.1)	1.00*
Impaired consciousness	8(19)	3(9.7)	5(45.5)	0.02*
Liver enlargement	16(38)	8(25.8)	8(72.7)	0.01*
Pleural effusion	17(41)	11(35.5)	6(54.5)	0.50*
Bleeding manifestations				
Petechiae	24(57)	17(55)	7(64)	0.73*
Purpura	15(36)	13(42)	2(18)	0.27*
Epistaxis	2(5)	2(6.5)	0(0)	1.00*
Rumpel leed test	14(33)	9(29)	5(45.5)	0.26*
Hematemesis	2(5)	0(0)	2(18.2)	0.06*
Melena	2(5)	0(0)	2(18.2)	0.06*
Hematology findings				
At admission	Median (IQR)	Median (IQR)	Median (IQR)	
Hemoglobin (g/dL)*	13.1(2)	12.3(3)	13.5(3)	0.05**
Hematocrits (vol%)	37.3(7)	37.0(9)	38.7(6)	0.09**
White blood count (/μL)	6095(4415)	6,280(5100)	5,680(2900)	0.38**
Platelet count (/μL)	44600(45750)	47000(42000)	30000(34500)	0.25**
At discharge				
Hemoglobin (g/dL)	9.8(2)	9.9(2)	9.7(3)	0.26**
Hematocrits (vol%)	29.5(6)	28.9(6)	30.3(9)	0.47**
White blood count (/μL)	8075(6482)	9080(5900)	6790(6730)	0.16**
Platelet count(/μL)	70050(55700)	58900(42000)	94000(117900)	0.37**

Note: *Univariate analysis with DSS versus non-DSS (DHF 1&2 and DF). ** Nonparametric test (Mann-Whitney)

Univariable analysis showed that the major determinants associated with the development of DSS were impaired consciousness (OR 6.03) and liver enlargement (OR 7.52).

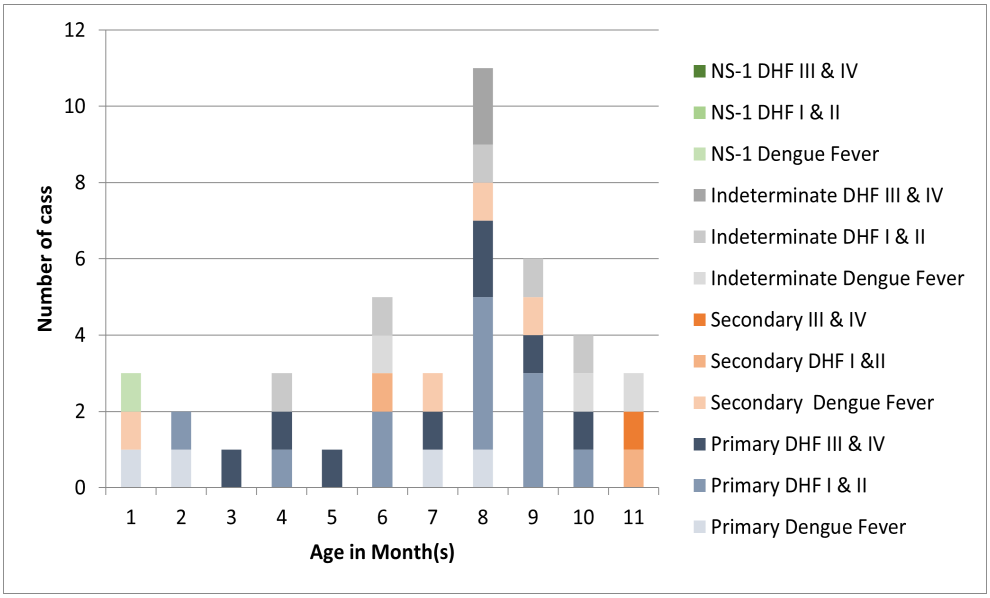

Figure 1. Dengue in infants (n=42) according to age in months based on dengue classification and dengue serology antibody or NS1 dengue antigen testing

Based on serology testing, 24 (57.1%) infants were classified as primary dengue cases, seven (16.7%) as secondary dengue cases, and 10 (23.8%) as indeterminate cases. Six infants were tested with dengue NS1 antigen with all results positive: one infant (2.4%) had a positive NS1 dengue antigen result only, without subsequent dengue antibody serology, the other five infants were tested serologically, with four infants having primary dengue and one infant having secondary dengue infection. Figure 1 shows the age distribution of subjects together with their dengue classification and laboratory dengue serology antibody and/or NS1 dengue antigen test results. Of the 11 DSS cases, eight were classified as primary dengue infection, one as secondary dengue infection and two as indeterminate. The youngest DSS case was 3 months of age and had a primary dengue infection.

Four dengue serological tests of mothers with previous DHF whose infants were included in this study were performed: in one mother with primary infection, one with secondary infection and two mothers with negative IgG and negative IgM.

DISCUSSION

This 10-year retrospective study performed in a tertiary referral hospital describes 42 confirmed cases of dengue in infants, with almost a quarter of the infants having DSS. Dengue-infected infants predominantly had primary dengue infection manifesting as DHF/ DSS. Diarrhea and cough as atypical signs occurred in about one third of dengue infants. Liver enlargement and fluid accumulation were two strong indicators of dengue shock syndrome in infants. The majority of dengue infants were anemic at discharge. In this study, infants comprised 6% (42/699) of all children admitted with dengue to Cipto Mangunkusumo hospital. This compares well with observations in other countries, where an average of 5% was reported from several studies on hospitalized children with DHF.[2, 6, 12]

Diagnosing dengue in infants can be challenging as the infection in this pediatric group is relatively rare and may be clinically similar to other acute febrile illness. Besides fever and vomiting, liver enlargement and fluid accumulation were the most common clinical manifestations with the latter two being strong indicators of dengue shock syndrome. Signs of cough and diarrhea were common, but these atypical symptoms in dengue are not included the WHO dengue guidelines. These symptoms were also reported in other studies in infants.[6,13] The most common bleeding manifestation in dengue-infected infants was petechiae with the laboratory analysis showing decreased platelet counts.

The median hemoglobin and median hematocrit levels were low at discharge in the majority of infants in our study, possibly indicating prior anemia in these children. Radiological examination was important to detect pleural effusion as a sign of plasma leakage in anemic cases.[14] Anemia was not detected at admission, when values were in the normal range due to hemoconcentration, but the overall prevalence of anemia is estimated at 60% in Indonesian children younger than 1 year.[15] Our findings suggest that clinicians should not rely on hematocrit on admission to determine the level of hemoconcentration resulting from plasma leakage in infants, as this can be unreliable in a population with high levels of pre-existing anemia.

Detection of plasma leakage by physical examination in the critical phase of dengue infection, or even at the time of shock, may be difficult. A chest X-ray with right lateral decubitus position can be helpful to detect minimal pleural effusion which can be a sign

of plasma leakage.[16] A study in India showed that plasma leakage in children with DHF can be detected with radiography (pleural effusion) and with ultrasound examination (pleural effusion and/or ascites) in 66% and 91% of the cases, respectively.[9, 17] In our study, chest X-rays were routinely performed in order to detect plasma leakage. Seventeen subjects (42 %) had pleural effusion based on a chest X-ray with right lateral decubitus position. Chest x-rays are practical and helpful in detecting plasma leakage for physicians as the results can be obtained quickly and can be interpreted by any medical doctor in limited resources settings.[10] Ultrasound is more sensitive to detect minimal plasma leakage in dengue.[18-20] However, it is operator dependent and often not available in limited resource health-care settings.

In this study, most of the infants were classified as having primary dengue infection, based on serology, with ages between 2 to 10 months and with median peak age and highest severity manifested as DHF/DSS at 8 months. Infants have been reported to be at high risk for DHF/DSS. In Southeast Asia, the age-specific incidence of infant DHF is 5 per 10,000 infants aged three to eight months.[6] Severe dengue in infants mostly occurred at the age of four to nine months old. In another study, conducted in Thailand, Vietnam, Myanmar, and Indonesia, the distribution of DHF/DSS in infants in all four countries presents a pattern similar to our findings, with DHF/DSS occurring predominantly in infants six to eight months of age.[2]

Our finding of frequent primary infections in infants with dengue agrees with other studies[2, 5, 13] in which infants born to dengue-immune mothers may develop clinically apparent DHF on primary infection with the dengue virus. Such a phenomenon is most prevalent after seven months of age.[6] At birth, infants are protected from dengue virus infection by maternal antibodies. With increasing age, maternal IgG antibodies wane, thus increasing the vulnerability to DHF/DSS in infants. It has been suggested that DHF/DSS predominantly develops when neutralizing antibody maternal IgG has declined to plaque-reduction neutralization test (PRNT) 50 titer of 1:10, which tends to occur between the age of 6 to 10 months. Another study showed that 65% of infants experience DHF when maternal neutralizing antibody titers declined below 1:20.[4] Infants acquire DHF/DSS during the short time period when maternal neutralizing antibodies have degraded to low titers

becoming non-neutralizing antibodies, allowing antibody dependent enhancement (ADE) to occur.[2, 21] The increased viral loads from ADE will promote inflammatory mediators and vasodilatation leading to vascular permeability of DHF in infants.[2, 4]

Our study has several limitations. Dengue serology tests of dengue mother's and dengue serotype in infants were not performed routinely. In mothers who had secondary dengue infection, IgG antibody can pass the placenta and may persist in their infants for several months and protect their infants. Furthermore, this study was performed in a tertiary referral hospital, and results may not be representative for primary or secondary healthcare settings. Finally, although our study included all infants with dengue during a 10-year period, the number of patients was relatively small. A strength of this study is that it included all admitted dengue infants in an endemic region over a 10-year period during which clinical and laboratory data were recorded using a standardized method for all patients.

CONCLUSION

The majority of infants with dengue presented with primary dengue infection which can manifest as DHF/ DSS. Atypical signs of diarrhea and cough occurred in about one third in dengue infants. Liver enlargement and fluid accumulation were two strong indicators of dengue shock syndrome in infants. The majority of the infants had anemia and hematocrit may not be a reliable measure to detect plasma leakage. It is therefore advisable to use radiological examination to detect plasma leakage in infants.

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Chapter 6

The value of warning signs from the WHO 2009 dengue classification in detecting severe dengue in children

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Submitted

Abstract

Background: The World Health Organization (WHO) proposed seven warning signs (WSs) to identify risk of severe dengue in 2009. Predicting or excluding the occurrence of severe dengue infection among children with dengue is challenging, especially when working in healthcare in limited resource settings. This study aimed to evaluate the value of these warning signs in detecting severe dengue among confirmed pediatric dengue cases in Indonesia.

Methods: A cross-sectional study was conducted utilizing data of children with clinical dengue infection obtained from medical records between January 2009 and December 2018 in Jakarta. Children with confirmed dengue were analysed and stratified into three age groups: infants less than 1 year old, children aged 1-14 years and adolescents aged 15-18 years. Positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity of each warning sign present or absent on admission in detecting severe dengue were computed.

Results: Six hundred ninety-nine children with clinical dengue infection were enrolled in the study, among whom 614 (87.8%) had confirmed dengue infection, either by antigen or antibody serological tests. Severe dengue occurred in 211/614 (34.4%) cases. In infants, important warning signs on admission to detect or exclude severe dengue were liver enlargement (NPV 80.8%), and clinical fluid accumulation (NPV 75%). In children and adolescents, the warning sign with the highest NPV (in children 76.6% and in adolescents 91.9%) was increase in haematocrit concurrent with rapid decrease in platelet count. Other warning signs with high NPV values in children were abdominal pain (72%), vomiting (70%), clinical fluid accumulation (69.3%), and in adolescents' abdominal pain (80.7%), vomiting (75.7%), clinical fluid accumulation (82.7%). NPVs increase with more than 1 WS in all age group.

Conclusion: In infants, liver enlargement or clinical fluid accumulation are important warning signs for severe dengue, i.e. when both of these are absent, severe dengue is unlikely. In older children and adolescents, an increase in haematocrit with concurrent rapid decrease in platelet count is most discriminative; in case of absence of this symptom severe dengue is very unlikely. In children and adolescents, absence of abdominal pain, vomiting, or fluid accumulation are unlikely severe dengue.

INTRODUCTION

Dengue virus infection has a wide spectrum with manifestations from very mild to severe, sometimes lethal forms. Severe dengue can lead to intravascular leakage and, without adequate supportive therapy, multiple organ failure and even death.[1] The World Health Organization (WHO) reported that about 3.9 billion people living in 128 countries are at risk of contracting dengue. Annually an estimated, 50-100 million cases of dengue infection occur worldwide and number of deaths increase from 16,957 in 1990 to 40,467 in 2017.[2,3] This makes dengue an important public health problem globally.[4] According to the 1997 WHO Dengue Classification (WHO-1997), symptomatic dengue virus infections are grouped into three categories: Undifferentiated fever, dengue fever and dengue haemorrhagic fever (DHF). The DHF cases are further classified into four severity grades, with grade III and IV being defined as dengue shock syndrome (DSS).[5] Changes in the epidemiology of dengue led to criticism on the usefulness and applicability of this classification in clinical care and in 2009 the World Health Organization issued a new guideline that classifies clinical dengue as 1) dengue without warning signs, 2) dengue with warning signs and 3) severe dengue (WHO-2009).[6]

The new guideline includes warning signs (WSs) that help early identification of (imminent) dengue shock syndrome, severe bleeding manifestations, or severe organ impairment.[7] Delay in diagnosis, referral to healthcare and fluid management during the critical phase in dengue disease progression can lead to higher mortality in severe dengue cases and therefore early detection of disease progression is crucial. The WSs included in the guideline were selected to support healthcare professionals in resource limited healthcare settings in the clinical assessment of dengue infected patients[8]. The selection was based on usability tests conducted in clinical settings across 18 countries[9] and resulted in a more practical and acceptable guideline for clinicians, although not all dengue endemic countries have applied the new guidelines to date.[9-11]

Timely recognition or ruling out of severe dengue among children with dengue is notoriously difficult. Studies on the performance of WSs to predict severe dengue in pediatric care are rare, while WSs might also differ between infants and older children. The diagnostic value of WSs in different age groups thus needs to be explored in larger groups

dengue infected cases, of whom some develop severe dengue. Evidence on the value of WS should be given attention to prevent complications and mortality.[a]

In Indonesia, children with severe dengue need referral to a secondary or tertiary care hospital centre and hence adequate discrimination of children with severe versus non-severe dengue in primary healthcare facilities is essential. The aim of this study was to evaluate the diagnostic value of WHO-2009 WSs on admission to hospital in detecting severe paediatric dengue, with patients stratified by age group into infants (less than 1 year), children (1-14 years), and adolescents (more or equal to 15 years).

METHODS

Study design and population

This study was conducted in a tertiary care hospital, Cipto Mangunkusumo, Jakarta, Indonesia, by collecting data from medical records in a 10-year period from January 2009 until December 2018. The hospital uses standardized daily assessment sheets for all patients with a clinical diagnosis of dengue infection, including results of physical examination, vital signs, laboratory parameters and radiological examinations. Clinical data and laboratory findings were obtained daily until discharge. All children up to 18 years old diagnosed with clinical dengue infection based on the WHO-1997 dengue criteria were assessed in detail.

Case definition for dengue fever include an acute febrile illness with two or more of the following manifestations of headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, leukopenia and supportive serology positive IgM antibody test. Dengue haemorrhagic fever (DHF) is defined as with following symptoms as acute fever 2-7 days, biphasic, haemorrhagic tendencies, thrombocytopenia < 100 000 cells per mm and evidence of plasma leakage due increase vascular permeability, manifested as a rise of at least one of the following as rise of haematocrit equal or greater than 20% above average for age, sex and population, drop in haematocrit following volume-replacement treatment equal to greater than 20% of baseline, signs of pleural effusion, ascites and

[a] The International code of disease (ICD)10, uses WHO-1997, but WHO revised to ICD 11 using the dengue diagnosis based on WHO-2009 which is planned to be applied in 1st January 2022 (https://www.who.int/news-room/detail/18-06-2018-who-releases-new-international-classifcation-of-diseases-(icd-11)).

hypoproteinaemia. Syndrome shock dengue (SSD) is defined as all above four criteria DHF with evidence of circulatory failure. From these, we selected children with laboratory confirmed dengue infection for inclusion in our study. Patients with non-dengue or proven hematologic disorders or malignancy or incomplete medical record data were excluded.

Laboratory confirmed dengue infection

Serological confirmation tests of non-structural 1 (NS1) dengue antigen or IgM, IgG dengue antibody was performed from acute blood samples. Dengue viral infection was confirmed by positive NS1 detection using Dengue NS1 Ag Strip (Panbio®). Primary dengue infection was defined when only IgM was positive, secondary dengue infection was defined when IgM and IgG were both positive, while indeterminate (prior dengue infection) was defined when IgG only was positive but IgM negative. Non-dengue was defined when both IgG and IgM were negative. For dengue serology, the presence of dengue IgM and IgG in acute-phase serum was assessed using a rapid immunochromatographic test (Panbio® Dengue Duo Cassette).

Outcome definition

Patients were classified as non-severe or severe dengue according to WHO-2009 case definitions. Severe dengue includes; a) Severe plasma leakage with shock (dengue shock syndrome) or presence of hypotension, tachycardia and signs of poor capillary perfusion with or without narrow pulse pressure; b) Severe bleeding including bleeding from the gastrointestinal tract with or without the need for transfusions of blood products or 3) Severe organ impairment defined as elevated levels of aspartate transaminase (AST) or alanine transaminase (ALT) of 1000 IU/L or higher, central nervous system impaired consciousness or heart and other organ involvement.[7] An independent trained physician classified the clinical dengue cases to classify patient outcome as non-severe dengue (NSD) or severe dengue (SD).

Definition of WSs on admission

The WSs considered included: abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, liver enlargement, and laboratory results showing

an increase in haematocrit concurrent with rapid decrease in platelet count. Abdominal pain was defined as abdominal tenderness and continuous (i.e., not intermittent) pain. Persistent vomiting was defined as more than three episodes of vomiting within 12 hours. Fluid accumulation was defined as pleural effusion visible on a chest X-ray or ultrasound, and ascites detected by abdominal ultrasound. Mucosal bleeding was defined as bleeding gums or conjunctiva, epistaxis, vaginal bleeding, haemoptysis, or haematuria. Lethargy was defined as an alteration of consciousness with a Glasgow score of less than 15. Hepatomegaly was defined based on the liver edge palpation of more than 2 cm below the right costal margin. Increased haematocrit concurrent with rapid decrease in platelet count (high HCT/ low platelets) was defined as any increase in haematocrit from baseline at admission in the febrile phase, with a concurrent rapid decrease in platelet count of at least 10,000 cells/ mm^3 in 24 hours or with a drop of platelet count below 100,000 cells/mm.[3 12] Presence or absence of each warning sign on admission was enumerated per patient.

Statistical analysis

All laboratory confirmed cases of dengue infection were included in the analysis. Patients were stratified into three age groups, namely infants (less than 1 year old), children (1-14 years old) and adolescents (15-18 years old). Clinical WSs were compared between patients with and without severe dengue using chi-square tests or Fisher's exact tests where appropriate. We assessed the value of each warning sign to discriminate between severe and non-severe dengue using the following diagnostic test criteria: positive predictive value (PPV), negative predictive value (NPV), sensitivity (Sn) and specificity (Sp), stratified by age group. Next, we computed the diagnostic test criteria for combinations of WSs. Data was analysed by using SPSS version 22 (IBM, Chicago) andMedCalc's diagnostic test evaluation calculator https://www.medcalc.org/calc/diagnostic_test.php).

Ethical review

Ethical approval was obtained from the Medical Research Ethics Committee of Faculty of Medicine, Universitas Indonesia (No.1151/UN2.F1/ETIK/PPM.00.02/2019)

All data analysed were anonymized.

RESULTS

A total of 699 medical records were reviewed of children with clinical dengue admitted to Cipto Mangunkusumo hospital during the study period. Of these, 614 (87.8%) cases with confirmed dengue infection were included in the study (Figure 1). The 85 cases excluded consisted of 75 non-dengue patients and 10 patients with incomplete medical records. Of the confirmed dengue cases, 139/614 (22.6%) were classified as primary dengue infection, 304/614 (49.5%) as secondary dengue infection, 159/614 (25.9%) as indeterminate (prior dengue infection) and 12 (2%) as positive NS1 dengue. Based on the WHO-2009 classification, 403 (65.6%) out of the 614 confirmed dengue cases were classified as non-severe dengue and 211 (34.4%) as severe dengue. The prevalence of severe dengue stratified by age group was 16/42 (38.1%) in infants, 181/506 (35.1%) in children and 14/63 (22.2%) in adolescents. The median age for children with non-severe and severe dengue was 10 (IQR=7) and 7 (IQR=7) years, respectively. Baseline characteristics of confirmed dengue cases are shown in Table 1.

Figure 1. Flow diagram to illustrate the flow of participants through the study.

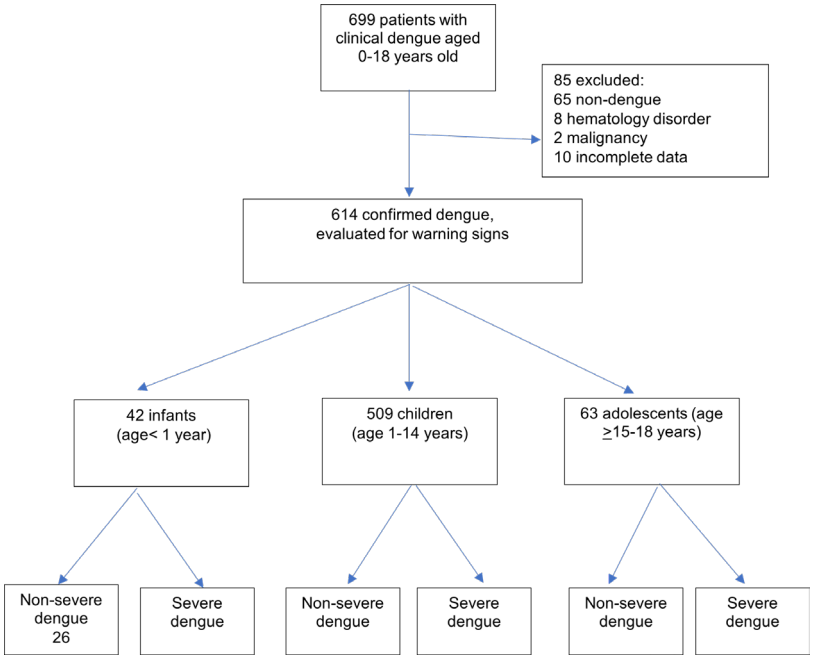

Table 1. Characteristics of children with confirmed dengue infection admitted between January 2009 and December 2018 (n=614)

Characteristics	non-severe dengue (%) N=403 (65.6%)	severe dengue (%) N=211 (34.4%)
Age group (years)		
< 1	26 (6,4)	16 (7,5)
1 – 14	328 (81,3)	181 (85,7)
15 – 18	49 (12,1)	14 (6,6)
Gender		
Male	219 (54,3)	107 (50,7)
Female	184 (45,6)	104 (49,2)
Duration of fever (days) *	4 (1)	4 (1)
Length of stay (days)*	4 (2)	4 (2)
Vital signs at admission*		
Systolic blood pressure	100 (20)	94 (10)
Diastolic blood pressure	60 (10)	60 (10)
Pulse rate	100 (20)	120 (30)
Respiration rate	24 (5)	24 (=8)
Temperature on admission*	37,3 (1,6)	36,8 (1,3)
Haematological findings at admission**		
Haemoglobin (g/dL)	13.2 + 2.0	14.1 + 2.6
Haematocrit (%)	39.3 + 5.8	41.5 + 7.5
Leucocyte (/µL)	4378.8 + 3315.3	5957.5 + 3834.6
Platelet (/µL)	88494.7 + 46364.1	65477.3 + 45971.3
Outcome		
Alive	403 (100)	205 (97,1)
Died	0 (0)	6 (2,8)

IQR= interquartile range

*= median (IQR), **= mean + standard deviation

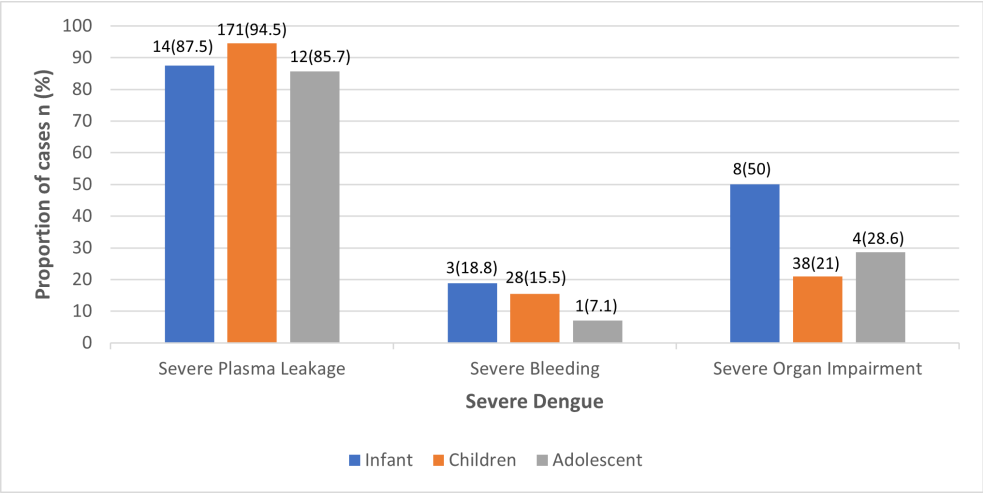

Figure 2. Prevalence of different manifestations of severe dengue by age group (n=614).

The most common manifestation of severe dengue was severe plasma leakage in all age groups (Figure 2). Severe bleeding and severe organ involvement occurred more frequently in infants compared to other age groups. Some patients had more than one manifestation of severe dengue; this is depicted in Figure 3. Severe organ impairment in infants manifested as impaired consciousness, in eight out of 16 infant cases with severe dengue, whereas in children aged 1-14 years it was mostly increased transaminase levels (> 1000 IU/L, seven cases), and cardiac involvement (2 cases). In adolescents, severe organ impairment manifested as increased transaminase levels in 2 cases, and as impaired consciousness in 2 cases. All six deaths occurred in age group 1-14 years: two cases were aged 3 years, two were 5 years of age, one case each were 7 and 11 years.

WSs present at admission and stratified by age group are described in Table 2. Among the infants with severe dengue, the most common WSs were liver enlargement 11/16 (68.6%) and clinical fluid accumulation 10/16 (62.5%). In children 1-14 years with severe dengue, the three most common WSs were increase in haematocrit with rapid decrease of platelet count 122/181 (61.9%), abdominal pain 108/181 (59.7%) and persistent vomiting 95/181 (30.4%). In adolescents with severe dengue, the most common warning sign was increase in haematocrit with rapid decrease of platelet count 11/14 (78.6%).

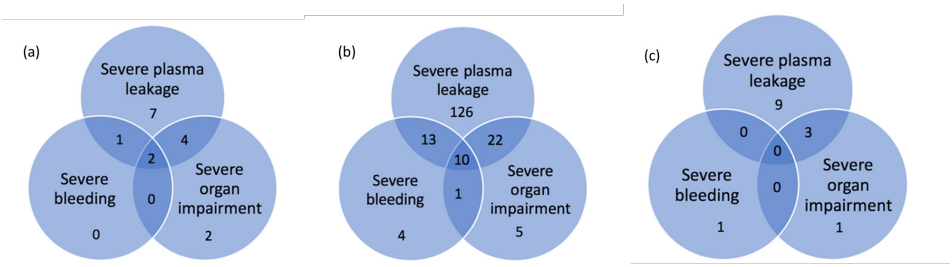

Figure 3: (a) Infants with severe dengue (n=16) (b) Children with severe dengue (n=181) (c) Adolescents with severe dengue (n=14).

Table 2. Warning signs on admission among 614 confirmed dengue patients stratified by age and by severe or non-severe dengue.

Warning Signs	Total dengue cases n (%)	non-severe n (%)	severe n (%)	p value	PPV(%)	NPV(%)	Sn (%)	Sp(%)
Age < 1-year patients (infants)	**42(100)**	**26(61.9)**	**16(38.1)**					
Individual WS								
Abdominal pain or tenderness	4(9.5)	2(7.7)	2(12.5)	0.628	50	63.1	12.5	92.3
Persistent vomiting	15(35.7)	10 (38.5)	5(31.3)	0.636	33.3	59.2	31.3	61.5
Clinical fluid accumulation	18(42.9)	8(30.8)	10(62.5)	0.044	55.6	75	62.5	69.2
Mucosal bleeding	2(4.8)	2(7,7)	0(0)	0.517	50	63.2	12.5	92.3
Lethargy, restlessness	4(9.5)	2(7.7)	2(12.5)	0.628	50	63.2	12.5	92.3
Liver enlargement > 2 cm	16(38.1)	5(19,2)	11(68.8)	0.001	68.8	80.8	68.8	80.8
IHigh HCT/low platelets	13(31)	8(30.8)	5(31.3)	0.974	38.5	62.1	31.3	69.2
Age 1-14 years (children)	**509(100)**	**328(64.4)**	**181(35.6)**					
Individual WS								
Abdominal pain or tenderness	248(48.7)	140(42.7)	108(59.7)	0.003	43.6	72.0	59.7	57.3
Persistent vomiting	222(43.6)	127(38.7)	95(52.5)	0.003	42.8	70.0	52.5	61.3
Clinical fluid accumulation	98(19.3)	43(13.1)	55(30.4)	0.000	56.1	69.3	30.4	86.9
Mucosal bleeding	80(15.7)	54(16.5)	26(14.4)	0.533	49.1	68.0	28.8	83.5
Lethargy, restlessness	16(3.1)	11(3.4)	5(3.1)	0.714	31.3	64.3	2.8	96.7
Liver enlargement > 2 cm	120(23.6)	61(18.6)	59(32.6)	0.000	49.2	68.6	32.6	81.4
High HCT/low platelets	214(42)	102(31.1)	122(61.9)	0.000	52.3	76.6	61.9	68.9
Age 15-18 years (adolescents)	**63 (100)**	**49(77.8)**	**14(22.2)**					
Individual WS								
Abdominal pain or tenderness	32(50.8)	24(49)	8(35.7)	0.590	25	80.7	57.1	51.0
Persistent vomiting	26(41.3)	21(42.9)	5(35.7)	0.632	19.2	75.7	35.7	57.1
Clinical fluid accumulation	11(17.5)	6(12.2)	5(35.7)	0.041	45.4	82.7	35.7	87.8
Mucosal bleeding	5(7.9)	4(8.2)	1(7.1)	1.000	20	77.6	7.1	91.8
Lethargy, restlessness	2(3.2)	1(2)	1(7.1)	0.398	50	78.7	7.1	98
Liver enlargement > 2 cm	10(15.9)	9(18.4)	1(7.1)	0.434	10	75.5	7.1	81.6
High HCT/low platelets	26(41.3)	15(30.6)	11(78.6)	0.001	42.3	91.9	78.6	69.4

Legend: PPV: positive predictive value, NPV: negative predictive value, Sp: specificity, Sn: Sensitivity,

In infants, liver enlargement was most discriminative with NPV of 80.8 %, followed by clinical fluid accumulation (NPV 75%). In older children, all WSs, except for mucosal bleeding and lethargy. restlessness, were significantly more common among patients with severe dengue. NPV was highest for an increase in haematocrit concurrent with rapid decrease in platelet count (76.1 %). Other WSs with good NPVs in children 1-14 years were abdominal pain, persistent vomiting and clinical fluid accumulation. In adolescents, results on discriminative value of individual WS was largely in line with results for 1-14-year-olds.

In infants, the combination of the two most discriminative WSs (liver enlargement and clinical fluid accumulation), had a NPV of 88%. In adolescents, several combinations of WS each had comparable discriminative power (data not shown), including abdominal pain and increase in haematocrit concurrent with rapid decrease in platelet count (NPV: 100%), increase in haematocrit concurrent with rapid decrease in platelet count and clinical fluid accumulation (NPV: 100%). When the number of WSs increases, the discriminatory ability, notably NPV and specificity, improves, especially in infants (data not shown).

DISCUSSION

This study showed that in infants, the most discriminative warning sign for severe dengue is liver enlargement with PPV of 68.8% and NPV of 80.8 %, followed by clinical fluid accumulation with NPV 75%. In older age-groups, an increase in haematocrit concurrent with rapid decrease in platelet count was most discriminative WS with NPV (76.1 % and 91.9% in children 1-14 years and adolescents, respectively). Other WSs with high NPVs in children 1-14 years and adolescents were abdominal pain, persistent vomiting and clinical fluid accumulation. In the absence one of these three WSs in older children, lead to unlikely to severe dengue.

Prevalence of severe dengue in our pediatric cohort was about 34.4%, which was higher compared to another study from Medan, Indonesia, where the severe dengue prevalence was 25% among children aged less than 18 years,[13] but lower than the estimate from another study among pediatric patients, by Pothapregada et al. in India, where severe dengue occurred in 40.6%.[14] The high prevalence obtained in the present study is probably due to the fact that the subjects came from a national tertiary referral hospital,

predominantly attracting severe pediatric dengue cases. Our result was also much higher than studies that included all ages.[15 16 17]

Clinical awareness and assessment of the WSs as proposed in the WHO-2009 guidelines[7] is crucial to monitor the critical phase in dengue infected patients. When specific warning signs with a high NPV are absent, this can be used to guide clinical decision making on watchful waiting versus referral, as the post-test probability of severe dengue is substantially reduced in these subjects. While the specificity of the WS can also be a helpful measure, it's impact on post-test probability may be low if the particular WS is uncommon in both diseased and undiseased (i.e. absence of the WS does not rule out disease). The NPV is therefore the most useful test characteristic to use in this context of clinical decision making.

In infants WSs based on clinical assessment such mucosal bleeding and lethargy each have NPV of 63.2%, whereas when combine 2 WSs of lethargy and liver enlargement occurred, the NPV increase to 76.5%. WSs based on laboratory with increase haematocrit and decrease platelets count; and based on radiology examination to detect clinical fluid accumulation both have high NPVs, but these WSs can only be detected in healthcare facilities equipped with laboratory or radiology facilities. While new diagnostic tools such NS1 serotype-specific IgG measured by ELISA or PCR-based techniques with rapid results to detect dengue virus are being developed to assist the assessment of disease severity, medical management still heavily relies on clinical judgement as the time from onset of WSs to severe illness in most dengue cases is typically less than one day and patients may progress towards hypovolemic shock and even death if adequate fluid therapy is not administered immediately.[18] In addition, access to more advanced diagnostic testing and imaging may be limited in many primary healthcare settings, further stressing the importance of the clinical WSs in early patient assessment to reduce morbidity and mortality in children.[19]

The fact that the dengue cases in this study were from a tertiary hospital is a potential limitation as circumstances and prevalence in primary or secondary healthcare settings may differ, with consequences for the applicability of our conclusions. The strength of this study was that the patients were obtained from a 10-year period with complete datasets of clinical manifestations, laboratory and radiology findings monitored daily until discharge and focus on paediatrics with age stratification.

CONCLUSION

In infants, important WSs to detect severe dengue are liver enlargement and clinical fluid accumulation; when both these WSs are absent in infants, severe dengue is unlikely. In older children, presence of any of the WSs increase in haematocrit concurrent with rapid decrease in platelet count, abdominal pain, vomiting, or fluid accumulation occur, is likely severe dengue.

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Chapter 7

Increased Carotid Intima-Media Thickness in Children with a History of Dengue Hemorrhagic Fever

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ABSTRACT

We assessed carotid intima-media thickness (cIMT) and arterial stiffness in 28 children and adolescents with previous dengue hemorrhagic fever (DHF) (mean interval between DHF and cardiovascular assessment, 8.4 years), and 34 controls in a low-resource setting. Participants with previous DHF had an adjusted increased cIMT of 42.6 μm (95% confidence interval [CI]:10.0 – 75.3, P = 0.01), and of 61.7 μm (95% CI: 21.5 – 102.0, P < 0.01) in a sub-group analysis on dengue shock syndrome. There were no differences in arterial stiffness. In this first exploratory study, children and adolescents with a history of DHF had an increased cIMT, which may be modulated by dengue severity.

INTRODUCTION

Dengue is the most common arthropod-borne viral disease. Fifty to one hundred million cases occur annually and the incidence is increasing.[1] In urban Indonesia, dengue burden is particularly high; 80% of children aged 10 years or older are seropositive.[2] Dengue is usually self-limiting but a minority develop significant plasma leakage and/or hemorrhage, leading to dengue hemorrhagic fever (DHF).[3] There are an estimated 500,000 DHF cases annually, of which 22,000 are fatal.[1]

Considerable evidence supports an association between pediatric infectious diseases and the development of cardiovascular disease in adulthood.[4] Adverse cardiovascular effects of infectious disease are well-described in both chronic infections such as HIV, where adverse vascular changes are evident in childhood,[5] and in severe acute infections. For example, a dose-response association has been reported between the number of acute childhood infection-related hospitalizations and the risk of cardiovascular disease events in adulthood.[6] The mechanism that connects an infectious disease with the development of atherosclerosis has not yet been completely elucidated, although recent evidence suggests that trained immunity, the development of macrophages with a persistent proinflammatory phenotype in response to stimulation by microorganisms, might play a significant role.[7]

The effects of infection-induced inflammation on the cardiovascular system may be most pertinent if the primary infection affects the vasculature. In DHF, endothelial dysfunction occurs leading to plasma leakage, with increased biomarkers indicative of vascular damage.[8] However it is unknown whether this vascular damage is transient or persistent. In this study, we investigated whether previous DHF in childhood was associated with vascular parameters indicative of preclinical atherosclerosis several years later.

METHODS

We performed a cross-sectional study including 29 children previously admitted with DHF between 2009-2015 at a tertiary reference hospital in Jakarta, at a mean follow-up of 8.4 years post-DHF. One participant was excluded because of extensive comorbidities. In addition, 34 healthy controls were recruited by inviting cases to bring acquaintances or siblings without a history of DHF hospitalization. Exclusion criteria were on-going chronic

infectious or inflammatory disease. Because of the low-resource setting, blinding of observers for case and control status was not possible as recruitment and vascular assessment were performed by the same researchers.

Before data collection, a sample size calculation was performed using carotid intima-media thickness (cIMT) as the primary outcome parameter. Based on a study that assessed the effects of pediatric HIV on cIMT, a mean difference in cIMT of 36μm was assumed. An assumed cIMT standard deviation of 34.1μm was derived from a study on 204 healthy children.[5,9] Using a two-sided α of 0.05 and a β of 0.20, the required sample size was 15 participants per study arm.

The study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia. Informed consent was obtained from participants' caregivers, or if they had reached the legal age of majority, from the participants.

Data on the initial DHF hospitalizations were extracted from prospectively collected patient-data in 2009-2015 that included the following: 1) dengue severity by both the adjusted 1997 WHO classification (DHF grade I-IV; grade III and IV indicate dengue shock syndrome [DSS]) commonly used in Indonesia and the 2009 WHO classification; 2) date and duration of hospitalization; and 3) clinical parameters including significant plasma leakage and gastrointestinal bleeding.

Three sets of ultrasonographic measurements of the right carotid artery were performed with the Esaote MyLabOne system based on the wall-track system. The cIMT of the far wall was measured 1.5 cm caudal from the carotid bifurcation. Blood pressure was measured two to three times using an OMRON HBP-1300 automated sphygmomanometer. Values are used to estimate mean carotid pressure, assuming a constant pressure in the whole arterial tree. Measurements of average right cIMT, carotid diameter, arterial distension and blood pressure were used to calculate the elastic properties of the right carotid artery as a hollow structure (arterial distensibility) and of the wall (elastic modulus) as previously described.[10]

All measurements were performed by a single trained researcher (T.V.). Double measurements were performed in five cases and five controls. Using a two-way mixed model, intra-class coefficients for cIMT and arterial distension were 0.94 and 0.91, respectively,

indicating excellent intra-rater reliability.[11]

Data on possible confounders, including participant and parental smoking status, socioeconomic status and other hospitalizations were obtained by questionnaires administered at the cardiovascular assessment to caregivers or older participants.

Data are presented as proportions or means (SD), or in the case of a skewed distribution, as medians. Differences in characteristics between cases and controls were tested using independent samples t-tests, Chi-square tests or Mann-Whitney U-tests, as appropriate.

To assess associations between a previous DHF episode and vascular parameters, we performed univariable and multivariable linear regression with cIMT, distensibility and elastic modulus as dependent variables, respectively and history of DHF as the exposure of interest. Gender and parental education were included as confounders based on observed differences and BMI z-score, as obesity has been previously associated with DHF risk[12]. Participant and parental smoking status were not included due to no observed group differences. Systolic blood pressure was a priori identified as a possible intermediate in the relationship between DHF and cIMT and so was added in an additional explanatory model.

A sub-group analysis using the same design and analysis was performed on children hospitalized with DSS and all controls. All analyses were performed using SPSS version [21].

RESULTS

Participant characteristics are shown in Table 1. Groups were similar in age and no participants were HIV-positive. Participants with a history of DHF were more likely to be female, had a higher systolic blood pressure, had lower self-reported overall health and their caregivers remained in education longer.

Table 1. Characteristics of participants by history of DHF

Characteristics	Cases, n = 28	Controls, n = 34	*P*-value
Age (years, range)	15.1 (5.0 – 24.2)	15.0 (5.1 – 24.9)	0.91
Male gender (*n*, %)	9 (32.1%)	16 (47.1%)	0.23
Weight (kg)	51.7 (4.3)	45.7 (2.3)	0.20
Height (cm)	154.9 (2.4)	151.0 (2.1)	0.23
BMI, crude	21.0 (1.3)	19.7 (0.6)	0.35
BMI, *Z*-score	-0.1 (0.3)	-0.2 (0.2)	0.85
BMI, percentile (%)	48.7 (6.8)	48.4 (5.6)	0.97
Chest circumference (cm)	79.4 (2.7)	77.2 (1.7)	0.48
Abdominal circumference (cm)	72.2 (2.8)	70.1 (1.6)	0.50
Hip circumference (cm)	83.0 (3.0)	79.0 (1.7)	0.24
Systolic blood pressure (mmHg)	116.7 (3.2)	109.5 (2.2)	0.06
Diastolic blood pressure (mmHg)	67.1 (2.0)	64.6 (1.3)	0.29
Mean arterial pressure (mmHg)	83.7 (2.3)	79.2 (1.6)	0.10
Pulse frequency (bpm)	81.3 (2.6)	81.1 (1.9)	0.95
Right carotid arterial distension (µm)	593.5 (120.0)	592.3 (117.7)	0.97
Right carotid diameter (mm)	6.44 (0.11)	6.46 (0.06)	0.88
History of chronic infection/inflammation (*n*, %)	-	-	0.27
HIV/AIDS	0 (0.0)	0 (0.0)	-
Chronic infectious disease	1 (3.6)[b]	0 (0.0)	-
Inflammatory disease	0 (0.0)	0 (0.0)	-
None of the above	27 (96.4)	34 (100.0)	-
History of hospitalization for other causes (*n*, %)[c]	-	-	0.71
None	23 (82.1)	27 (79.4)	-
Once due to infectious disease	2 (7.1)	3 (8.8)	-
Multiple times due to infectious disease	0 (0.0)	0 (0.0)	-
Once due to other causes	2 (7.1)	3 (8.8)	-
Multiple times due to other causes	0 (0.0)	1 (2.9)	-
Multiple times due to infectious disease and other causes	1 (3.6)	0 (0.0)	-
Participant smoking status (packyears, median)	0.0	0.0	0.12[a]
Parental smoking status (packyears, median)[d]	3.1	3.0	0.76[a]
Parental early-onset CVD (*n*, %)	-	-	0.96
None	24 (85.7)	29 (85.3)	-
Father	2 (7.1)	3 (8.8)	-
Mother	2 (7.1)	2 (5.9)	-
Total years of education			
Participant (years)	8.3 (0.8)	7.6 (0.6)	0.53
Father (years, median)	12.0	12.0	0.21[a]
Mother (years, median)	12.0	12.0	0.01[a]
Father and mother combined (years)	24.4 (0.9)	21.2 (1.0)	0.03

DHF = dengue hemorrhagic fever; BMI = body mass index. All values are mean standard deviation, unless otherwise indicated. Independent t-test and Chi-square were used as appropriate. * Recurrent tonsillitis and asthma, 1 case. Last episode was 6 months before the current measurements.

† Excluding the hospitalization for DHF in cases.

‡ Mann–Whitney U-test was used because of non-normal distribution.

§Combined amount of packyears of the parents of the participant

Characteristics of the DHF hospitalizations are shown in table 2. The majority (n = 14; 56%) met the adjusted WHO 1997 DSS definition. Most children (n = 22; 88%) suffered from plasma leakage, whereas severe bleeding (n = 3) or other complications (n = 4) were uncommon. The mean age at DHF hospitalization was 6.9 years (range 2.8-8.8 years), and the mean interval between hospitalization and cardiovascular assessment was 8.4 years (range 0.8-16.5 years).

Carotid IMT of children with a history of DHF was significantly increased compared to controls (Table 3). This effect was more pronounced when corrected for gender, parental education, and BMI z-score (mean difference of 42.6µm, P = 0.01). Systolic blood pressure was considered a possible intermediary, and additional adjustment for systolic blood pressure attenuated the findings slightly (mean difference of 33.2µm, P = 0.05).

Sub-group analysis of children with DSS (n = 14) and controls showed a more pronounced difference in cIMT (adjusted mean difference: 61.7µm, P < 0.01). An additional sub-group analysis was performed (Supplemental Table 1) to assess whether the length of the time interval between DHF and cardiovascular assessment affected cIMT. There was an association between a longer interval (upper meridian) and cIMT. There were no group differences in arterial stiffness parameters.

Table 2. Characteristics of DHF hospitalization

Characteristics of DHF hospitalization (n = 25)*	
Dengue grade (adjusted WHO 1997; n, %)	
Dengue fever	0 (0)
Dengue hemorrhagic fever grade I	6 (24)
Dengue hemorrhagic fever grade II	5 (20)
Dengue hemorrhagic fever grade III (DSS)	7 (28)
Dengue hemorrhagic fever grade IV (DSS)	7 (28)
Dengue grade (WHO 2009; n,%)	
Dengue fever without warning signs	1 (4)
Dengue fever with warning signs	10 (40)
Severe dengue	14 (56)
Age at hospitalization (years)	6.9 (2.8-8.8)
Time between hospitalization and current measurements (years)	8.4 (0.8-16.5)
Duration of hospitalization (days)	4.4 (3–8)
Presence of plasma leakage (n, %)	22 (88)
Presence of mucosal bleeding manifestations (n, %)	4 (16)
Presence of gastrointestinal bleeding manifestations (n, %)	3 (1
Presence of dermal bleeding manifestations (n, %)	12 (48)
Presence of pleural effusion (n, %)	4 (16)
Presence of encephalopathy (n, %)	1 (4)
Presence of other severe complications (n, %)†	3 (12)
Presence of dengue IgM antibodies (%)	56
Presence of dengue IgG antibodies (%)	80
Mean lowest recorded hematocrit (%)‡	32.6 (16.6–45.3)
Mean highest recorded hematocrit (%)‡	43.6 (30.0–56.0)
Mean Δ-hematocrit (%)‡,§	39.7 (4.9–123.8)
Mean lowest recorded thrombocyte count (cells/µL)‡	42,929 (6,000–89,000)
Mean highest recorded thrombocyte count (cells/µL)‡	98,088 (44,100–206,000)
Mean Δ-thrombocyte count (%)‡,§	225.8 (14.6–1,250.0)
Mean highest recorded leukocyte count (cells/mm3)	13,468 (3,300–127,000)
Mean lowest systolic blood pressure (mmHg)	97.9 (70–120)
Mean lowest diastolic blood pressure (mmHg)	58.0 (32–80)
Mean lowest mean arterial pressure (mmHg)	71.3 (50–93)
Crystalloid fluid management (%)	
Maintenance (Ringer's lactate)	6 (24)
Maintenance (dextrose + NaCl)	2 (8)
10% rehydration deficit (Ringer's lactate)	17 (68)
Presence of colloid transfusion (%)	5 (20)

DHF = dengue hemorrhagic fever; DSS = dengue shock syndrome. All values are mean (range), unless otherwise indicated.

* Clinical data on the hospitalization period were not available for three participants and these are, therefore, not included in this table.

† Three of 25 participants had renal impairment as a complication of DHF.

‡ One participant had only one reported value of hematocrit and thrombocyte count and is not included in the mean data of those parameters, but is included in the other parameters.

§ Expressed as "(highest value–lowest value)/lowest value * 100%."

Table 3. Association between previous hospitalization because of DHF and cardiovascular parameters, full and subgroup analysis.

		Mean difference (µm, (95% confidence interval))					
		Crude	*p*-value	Adjusted #1†	*p*-value	Adjusted #2‡	*p*-value
Full analysis							
cIMT (µm, (SD))							
DHF+	429.9 (65.0)	38.9 (7.4 – 70.4)	0.02*	42.6 (10.0 – 75.3)	0.01*	33.2 (0.5 – 65.9)	0.05*
Controls	390.9 (58.8)						
Carotid distensibility (MPa^{-1}, (SD))							
DHF+	46.2 (13.3)	-3.5 (-10.1 – 3.2)	0.30	-3.0 (-10.0 – 4.0)	0.39	-	-
Controls	49.7 (12.8)						
Carotid elastic modulus (kPa, (SD))							
DHF+	373.5 (192.2)	9.4 (-70.8 – 89.5)	0.82	2.7 (-79.6 – 85.0)	0.95	-	-
Controls	364.2 (120.9)						
Sub-group analysis on DSS (n_{cases} = 14)§							
cIMT (µm, (SD))							
DSS+	447.9 (69.1)	57.0 (17.4 – 96.5)	0.01*	61.7 (21.5 – 102.0)	<0.01**	53.5 (15.5 – 91.6)	<0.01**
Controls	390.9 (58.8)						
Carotid distensibility (MPa^{-1}, (SD))							
DSS+	48.2 (12.5)	-1.5 (-9.6 – 6.6)	0.72	-1.5 (-9.9 – 6.9)	0.73	-	-
Controls	49.7 (12.8)						
Carotid elastic modulus (kPa, (SD))							
DSS+	315.8 (89.6)	-48.4 (-120.6 – 23.8)	0.18	-54.4 (-128.0 – 19.1)	0.14	-	-
Controls	364.2 (120.9)						

cIMT = carotid intima-media thickness; DHF = dengue hemorrhagic fever; DSS = dengue shock syndrome; SD = standard deviation.

* P value of less than 0.05 but more than 0.01 (denotes significance).

** P value of less than 0.01.

†Adjusted model corrected for gender, BMI *z*-score and combined educational years of parents as a proxy for socio-economic status.

‡Adjusted model, similar to model 1, additional correction for systolic blood pressure. This model was not used for carotid distensability and elastic modulus, as systolic blood pressure is a part of the composition of these parameters.

§Including only cases that have been hospitalized for DSS (DHF grade III or IV, n = 14) according to the adjusted WHO 1997 definition, and all healthy participants.

DISCUSSION

This study suggests that a childhood history of DHF is associated with an increased cIMT later in life. The current measurements were performed on average 8.4 years after the episode of DHF, indicating a persistent effect. Sub-group analysis of DSS cases showed stronger evidence of an association, possibly compatible with a relationship between dengue severity and cIMT. In addition, we found an association between a longer time interval between DHF and cardiovascular measurements and cIMT, suggesting a cohort effect. However, this finding should be interpreted cautiously because of its low power.

We observed no group difference in arterial stiffness. Interestingly, we observed some evidence of an increased systolic blood pressure in DHF cases. We hypothesized this increase reflected a possible intermediary effect, but adjusting for systolic blood pressure in an additional model still showed a significant increase in cIMT.

An increased cIMT in childhood is considered a possible early biomarker for preclinical atherosclerosis,[13,14] although the long-term clinical implications remain unclear. Increased cIMT is increasingly recognized in children with severe infection, although these reports mainly focus on chronic infectious diseases, such as in HIV.[5] Our findings indicate that lasting vascular structural changes may also occur after a single brief intense inflammatory stimulus, such as DHF.

Certain polymorphisms of genes encoding for pro-inflammatory cytokines are more prevalent in DHF patients.[15] It is possible that by studying DHF cases, we have selected a population more susceptible to an exaggerated inflammatory response more generally, which could contribute to increased cIMT. Alternatively, DHF may have a pathogen-specific effect on vasculature as the magnitude of the cIMT increase was unexpected: a mean adjusted difference in cIMT of 70.4μm has been reported in ART-naïve HIV pediatric patients,[5] comparable with our mean adjusted difference of 61.7μm in DSS.

This preliminary study has several limitations. Although the small sample size of our study allowed us to observe differences in cIMT, it may have had insufficient power to show differences in parameters of arterial stiffness. Also, the lack of blinding might have introduced bias, although the automatic wall-track system minimizes this possibility.

Our findings highlight a possible effect of DHF in childhood on cardiovascular disease.

Dengue hemorrhagic fever seems to be one of several infectious diseases that are linked with vascular structural changes and is, to our knowledge, one of the first non-chronic infectious diseases described in this context. Our study is one of the few long-term follow-up studies performed on patients with DHF. There are reports of dengue-related symptoms months after the episode,[16] and calls for more research on possible long-term effects of dengue have already been made.[17]

CONCLUSION

Our findings suggest that DHF in childhood may be associated with increased cIMT, and the effect may be modulated by DHF severity.

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Chapter 8

General discussion

This thesis focuses on the difficulties in diagnosing dengue in children and on barriers to reach a timely diagnosis. According to the report of the Indonesian Ministry of Health in 2021 dengue incidence rate is 27/100,000 and continues to rise especially after the rainy seasons.[1] With a view to recent scientific evidence and the findings of the studies presented in this thesis, several relevant challenges to further improve dengue management will be discussed in this chapter. These challenges include education to increase awareness in the community and primary care about early recognition of dengue signs and symptoms. Especially community awareness could be instrumental to bring any suspected dengue case to seek help as early as possible at the nearest health care facility for adequate diagnostic tests and, when applicable, prompt treatment, thereby preventing progression to severe dengue.

In this chapter, I first summarize the main findings of the thesis. I then discuss these findings in the context of the challenges in Indonesian clinical settings, including primary care, partly in relation to the motivating dengue case that I described in Chapter 1.

Main findings of the studies included in the thesis

In Chapter 2, an overview of several aspects of dengue epidemiology in Indonesia over a period of 45 years is provided. It was shown that incidence increased and that this increase came with a shift in age of patients from young children to older older children. This increase and shift have consequences for targeted surveillance and prevention. The older age group is at risk of exposure to the day biting Aedes aegypty mosquitoes in school or the work environment. Vector control programs should focus on cleaning the breeding sites of the mosquitoes in the environment of the older age group. In Chapter 3, the clinical manifestations and hematology findings in children with dengue infection are studied. This was a retrospective cohort study carried out in 611 suspected dengue cases admitted to Cipto Mangunkusumo hospital from 2007-2009. The clinical manifestations and laboratory findings with respect to dengue were described and dengue serotypes from 81 dengue cases were identified. Confirmed dengue infection was found in 415 (68%) cases, with dengue fever (DF) occurring in 23.4% of the cases, dengue hemorrhagic fever (DHF) without shock in 41.2% of the cases and dengue shock syndrome (DSS) in 35.4% of the

cases. Of 81 cases, 12.3% were DENV-1, 35.8% were DENV-2, 48.2 were DENV-3, and 3.7% were DENV-4. The manifestations were mostly fever, petechiae, epistaxis, hepatomegaly, and thrombocytopenia. Encephalopathy and gastrointestinal bleeding were found only in DSS. Leucopenia was more prominent in DF cases compared to DHF cases, whereas the absence of leucopenia may be the sign of severe dengue. In a five-year study on the clinical course progression and management of dengue in hospitalized children (Chapter 4), comprehensive information for clinicians facing dengue infection in dengue endemic countries is provided. This was a five-year cohort study evaluating the clinical course and disease outcome of 494 clinically suspected dengue children and estimated the burden of dengue cases hospitalized over time at the tertiary care hospital from 2009 through 2013. As reported earlier, encephalopathy and gastrointestinal bleeding were found only in DSS. This study demonstrated that in our tertiary care setting, 52 (28%) of hospitalized children with dengue fever progressed to DSS, and of DHF cases, 9 (6%) progressed to DSS. Among all parameters collected, only fever duration was significantly associated with clinical progression, emphasizing that the disease course of dengue in children is predictable and stressing the importance of close clinical and laboratory monitoring of these patients as almost one third of dengue cases may progress to DSS. Fever, bleeding manifestations and thrombocytopenia were presented in children with DF and DHF, while increased vascular permeability, which is considered the hallmark of dengue, was found only in DHF. Interestingly, annual dengue admissions declined between 2009-2013, while distribution of disease severity remained stable in our hospital.

Infants in particular are considered a high-risk group for severe dengue in the WHO 2011 dengue guidelines,[2] because infants are more prone to progress to DSS as the capillary fragility in infants is greater than in older children.[3] Shock, plasma leakage and marked thrombocytopenia were more prevalent in infants compared to the older age group.[3] Therefore, we studied the profile and clinical signs in 42 infants with confirmed dengue using patient data of a tertiary referral hospital from a 10-year period (Chapter 5). This study was performed to increase awareness and provide clinicians taking care of infants with dengue further insights in clinical presentations, disease course and management. Although the majority of infant dengue patients had primary infections, some infants developed

severe dengue, which at older age is more typical in secondary dengue infections. Diarrhea and cough as atypical clinical presentations were found in one third of dengue infants. Two strong indicators of dengue shock syndrome in infants were liver enlargement and fluid accumulation. Most of the infants with dengue were anemic at discharge suggesting that hematocrit was not a reliable indicator of hemoconcentration to determine plasma leakage, but rather the result of pre-existing anemia. Radiological examination is then helpful as an alternative to detect plasma leakage in infants with suspected dengue.

Changes in dengue epidemiology led to difficulties in applying the existing WHO 1997 dengue classification worldwide in dengue endemic regions. Difficulties reported were that the DHF case definition was too rigid and too difficult to apply in primary care or resource-limited settings. For example, repeated hematology tests every 4-6 hours, using the tourniquet test to measure capillary fragility and thrombocytopenia, is not practical to be performed in the field. The case definition fails to capture severe dengue cases with dengue encephalopathy, hepatic failure and severe bleeding manifestations. Symptomatic dengue infection in WHO 1997 was classified as undifferentiated fever, DF and DHF. The severity of DHF was classified into four grades, as DHF grade I and II (non-shock) and DHF grade III and IV (shock/ DSS).[4] To overcome these difficulties, an updated WHO dengue guideline WHO was proposed in 2009 by expert consensus groups which divided cases into non-severe dengue and severe dengue.[5] For practical reasons, non-severe dengue was stratified to 'dengue without warning signs' and 'dengue with warning signs'. The warning signs (WSs) consisted of abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, liver enlargement, and laboratory results showing an increase in hematocrit concurrent with rapid decrease in platelet count. The WSs in dengue are useful for early identification of dengue shock syndrome. Delays in diagnosis, referral to healthcare and fluid management during the critical phase in dengue disease progression can lead to higher mortality in severe dengue cases.[5]

The change in guidelines makes it important to explore the diagnostic value of warning signs in different age groups, especially in pediatric dengue infected cases. I addressed this issue in Chapter 6. In infants, liver enlargement (negative predictive value (NPV) 80.8%) or clinical fluid accumulation (NPV 75%) are shown to be important warning signs to

exclude severe dengue and when both of these are absent, severe dengue is unlikely. In older children and adolescents, an increase in hematocrit with concurrent rapid decrease in platelet count was most discriminative (NPV in children 76.6% and in adolescents 91.9%); in case of absence of this symptom severe dengue is very unlikely. In children and adolescents, absence of abdominal pain, vomiting, or fluid accumulation renders severe dengue unlikely.

In DHF, endothelial dysfunction occurs, leading to plasma leakage with increased biomarkers indicative of vascular damage. However, it is unknown if this vascular damage is transient or persistent. The study presented in Chapter 7 evaluated whether previous dengue infection in childhood was associated with vascular parameters indicative of preclinical atherosclerosis several years later. Carotid intima-media thickness (cIMT) and arterial stiffness were assessed in 28 children and adolescents with previous DHF (mean interval between DHF and cardiovascular assessment 8.4 years) and 34 controls, in a low-resource setting. Participants with previous DHF had an adjusted increased cIMT of 42.6µm (95% CI 10.0 – 75.3, $p = 0.01$), and of 61.7µm (21.5 – 102.0, $p < 0.01$) in a sub-group analysis on dengue shock syndrome. There were no differences in arterial stiffness. Studies of long-term follow-up of pediatric patients who experienced dengue infection are scarce. In this first exploratory study, children and adolescents with a history of DHF had an increased cIMT, which may be modulated by dengue severity.

Dengue Challenges in Indonesia

Challenge 1: Reducing delay to diagnosis and preventing progression to severe dengue

Case revisited

A four-months old male infant with confirmed dengue with warning signs of persistent vomiting, diarrhea, petechiae and hepatomegaly was admitted to the hospital. The chest X-ray (shown in Figure 8.1) in right lateral decubitus position revealed the existence of pleural effusion, which is indicative of plasma leakage. On the second day of hospitalization (fourth day of fever), there was a decrease in consciousness accompanied by signs of hypovolemic shock, with blood pressure 90/47 mmHg (50-90th percentile), pulse rate 148 times per minute, weak and regular and temperature of 36,5-degree Celsius, respiratory rate 32 times per minute, cold extremities, capillary refill time more than two seconds, and saturation 98%. The patient was diagnosed with severe dengue and was given oxygen at two liters per minute with a nasal cannula. Initial fluid resuscitation of crystalloid 20 ml/kgBB bolus was administered within 30 minutes, but the vital signs still showed a weak pulse, tachycardia, accompanied by a decrease in blood pressure. The general practitioner in the emergency unit consulted the pediatrician for the next steps, as he was in doubt of the next decision. What fluid solution should be given if the hypovolemic shock persisted? What laboratory examinations should be performed? I will address below in Challenge 3 how the diagnosis and the patient progressed and indicate where the insights from this thesis could assist in future similar cases.

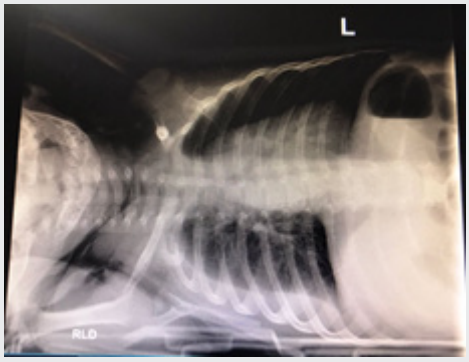

Figure 8.1. Chest X-ray in right lateral decubitus position

Diagnosing dengue in infants can be challenging for general practitioners in endemic countries, as the signs and symptoms are very similar to other acute febrile illnesses. The four-months old infant presented to the clinic three days after onset of fever, with petechiae, diarrhea, persistent vomiting, and hepatomegaly as accompanying signs and symptoms. General practitioners working in endemic dengue countries should be aware of dengue infection especially after the rainy season and infants with suspected dengue infection are considered a high-risk group who should be hospitalized to prevent life-threatening complications. The diagnostic test for non-structural (NS)1 dengue antigen was positive and serologic IgM dengue antibody showed a positive result with IgG dengue antibody being negative. These results confirm the dengue primary infection in this infant. In Chapter 5, an important finding was that infants hospitalized with dengue mostly had a primary infection, but that nevertheless one third progressed to DSS. Therefore, although this infant had primary dengue infection, close monitoring of the clinical status and laboratory values during hospitalization is crucial.

After three days of fever, this infant came in the critical phase of dengue infection when plasma leakage occurred. Our study in Chapter 4 showed that a duration of fever less or equal to four days was the only significant predictor of disease progression. The dengue-in-infants-study in Chapter 5 suggested that hepatomegaly and pleural effusion (sign of clinical fluid accumulation) were two strong indicators of developing DSS. Important warning signs in the infant age group who progress to severe dengue in Chapter 6 were liver enlargement and fluid accumulation, which were also present in this four-month infant case. These findings should increase awareness for clinicians that this infant patient is prone to develop DSS. Furthermore, this patient also had diarrhea, an atypical sign observed in one third of infants with dengue in the study reported in Chapter 5.

After recovering from the dengue shock syndrome, the infant was found to be anemic. In Chapter 5, we described that the majority of infants with dengue were anemic and therefore the hematocrit as indicator of plasma leakage was not reliable. For this reason, a chest X-ray is important as an alternative diagnostic tool to identify fluid accumulation as a sign of plasma leakage. These insights, when applied in clinical practice, can support a timely diagnosis of dengue with plasma leakage and prompt initiation of fluid therapy. In

particular, general practitioners in endemic countries should be aware that these predictors of life-threatening dengue are important, because most children in endemic countries are initially assessed by primary care doctors. Moreover, the general public should be educated to recognize dengue symptoms and seek medical help when appropriate.

Challenge 2: Educating the people in the community about dengue

Awareness of the people in the community about dengue is important, to implement preventive measures and to improve seeking medical care when symptoms of dengue occur. When a febrile illness occurs in dengue endemic countries, one should watch for warning signs, in particular when the temperature declines, typically after three days of fever, and immediately return to healthcare facilities in case any warning sign occurs such as persistent vomiting, abdominal pain, bleeding manifestations, being drowsy or irritable, pale, cold or clammy skin or breathing difficulties.[5] Our studies have identified that the important warning signs in dengue infants were liver enlargement and fluid accumulation, whereas in older children these were an increase in hematocrit with concurrent rapid decrease in platelet count, followed by abdominal pain, vomiting, or fluid accumulation. This important information needs to be provided to the community.

A study in Jakarta6 was conducted about parents' knowledge, attitude, behavior, and related sociodemographic factors regarding symptoms and treatment of suspected dengue in children. This study showed that about half of the respondents in the community (52.1%) had very good to good knowledge on dengue infection symptoms. Indonesia has been endemic with dengue since 1968 and there have already been numerous community campaign programs about dengue. However, there were still a number of typical symptoms of dengue infection that were only recognized by few respondents in this study, such as pain behind the eyes (24.7%), and abdominal pain (28.3%). Another study, in Depok, West Java[7], also evaluated the knowledge of community about dengue signs and symptoms. Warning signs in dengue were answered correctly by 98 respondents, for vomiting 77.6%, abdominal pain 49% and gum bleeding 56.1%. The study in Jakarta only included abdominal pain as one of the warning signs and the study in Depok included vomiting, abdominal pain and

gum bleeding (as mucosal bleeding) as warning signs in dengue. Vomiting and abdominal pain were identified in our studies as important WSs in children who may progress to severe dengue. Low recognition might be because the signs and symptoms asked in the questionnaire were not commonly addressed in the community campaigns about dengue or that younger children may have difficulty to communicate warning sign symptoms, such as abdominal pain, to their parents. A lack of personal experience with dengue itself could also be a factor explaining why the respondents do not recognize these symptoms. Also, when these questionnaire studies about dengue symptoms were performed, the WHO 2009 dengue with warning signs guidelines were not implemented yet in Indonesia.

The study among parents in Jakarta[6] also addressed knowledge on appropriate supportive care for children with dengue. Most of the supportive care interventions for fever were already common knowledge among parents such as providing sufficient water to drink (93.6%), making sure the child urinates a lot (65.5%), giving paracetamol (93.3%), making sure the child gets plenty of rest (87.6%), and taking the child to a healthcare facility (99.6%). On the other hand, only 41.6% of the subjects knew that it is advised to make sure the child does not do any physical activities and 55.1% properly responded that one should sponge the child using warm water, and the rest still applied cold water.6 Studies by Prasetyo[8] also showed that the majority of parents in Jakarta still apply cold water sponging instead of warm water sponging in treating children with fever. Almost half of the respondents (49.8%) still agreed to give ibuprofen to children with suspected dengue infection, which is contraindicated in patients with dengue.[8]

Community campaigns about dengue prevention programs to clean mosquito breeding sites with integrated vector control strategies have been implemented in several endemic countries. However, the above shows that essential knowledge on important aspects of health education about dengue infection is lacking at the community level. Importantly, knowledge about the need to early visit a healthcare facility in case of symptoms and signs suggestive of dengue should be improved. Elements from this thesis about monitoring warning signs that occur on the third day or after onset of fever could and should be emphasized also to the community outside the healthcare setting. Moreover, it should be made clear that infants suspected of dengue should be hospitalized as they are

prone to developed hypovolemic shock or severe dengue. Any warning signs in children, such as vomiting, abdominal pain, lethargy and any bleeding manifestations which occur on the third day of fever should alert the parents of caregivers to seek help at the nearest health care facility. These messages, including important warning signs and initial home care treatments should be emphasized in the upcoming public awareness community campaign programs. Especially after the rainy season early in the year, the community should be aware that dengue infection rates will rise as breeding sites of the Aedes aegypty mosquitoes in containers filled with rainwater increase in the environment. Therefore, vector control with community engagement needs to be strengthened. Education for the community can be delivered through social media, campaign and disseminated by the primary care workers in health care facilities.

Challenge 3: Educating (future) general practitioners about early recognition of disease progression in children with dengue

Universities and other medical schools training future general practitioners as health care professionals are key settings to emphasize the importance of warning signs in dengue. Our study in infants identified specific aspects of dengue infection in this age-group that need to be emphasized in education for GPs and medical students. First, our study showed that hematocrit is an unreliable marker of plasma leakage in infants due to pre-existing anemia. Therefore, assessment of plasma leakage in infants requires alternative diagnostic approaches such as a right lateral decubitus chest X-ray. Second, in infants DHF or DSS occur more frequently among primary infections, while in older age group, this is typically seen in secondary infections. Third, many infants present with atypical dengue symptoms such a cough and diarrhea. These symptoms are not recognized as dengue warning signs, but in infants, should be recognized as a sign of potential dengue infection.[5]

General practitioners working in triage at the primary and secondary care levels (where patients are first seen and evaluated) are critical in determining the clinical outcome of dengue. A well-managed front-line response not only reduces the number of unnecessary hospital admissions but also saves the lives of dengue patients. Furthermore,

early notification of surges in dengue cases seen in primary and secondary care is crucial for identifying outbreaks and initiating a control response.[4, 5] Various forms of severe manifestations may unfold only as the disease progresses through the critical phase; the warning signs in pediatrics identified in our studies were age-specific, which are good indicators of a higher risk of developing severe dengue.

In attempting to fill some of the knowledge gaps in dengue in children, I was able to confirm or identify some important clinical features of dengue in children such as fever, bleeding manifestations and thrombocytopenia (Chapter 3). Encephalopathy and gastrointestinal bleeding only occurred in DSS. In Chapter 4, one third of dengue fever pediatric cases progressed to DHF or DSS (severe dengue) during hospitalization, emphasizing the importance of increasing awareness to closely monitor dengue cases for general practitioners and other health care professionals working in endemic areas.

Continuation of the case

The patient had decreased consciousness; diuresis was 0,9ml/kgBW/ hour. Repeated hematology findings revealed hemoglobin 10.1 g/dL, hematocrit 28.7%, platelets 13,000/ µL, leukocytes 6.720/µL, basophils 0.4%, eosinophils 0.7%, neutrophils 16.3%, lymphocytes 74%, monocytes 9.7%, sodium 128 mEq/L, potassium 3.7 mEq/l, chloride 102.7 mEq/L, blood gas analysis pH 7.382, pCO2 31.8 mmHg, pO2 27.2 mmHg, HCO3 19.1 mmol /L, base excess -4.6 mmol/L, oxygen saturation 50.4%, lactate 4.6 mmol/L, complete stool showed positive occult blood, urinalysis within normal limits. The patient was diagnosed with severe dengue with manifestations of severe plasma leakage accompanied by dengue encephalopathy. The patient was given another crystalloid solution with loading Ringer's Lactate (RL) 10 ml/kgBW in 30 minutes up to two times, but did not respond adequately, with blood pressure 80/40 mmHg (50-90th percentile), weak pulse 152x/minute, capillary refill time more than two seconds, breath 30 breaths per minute, 99% oxygen saturation with oxygen at 2 lpm via nasal cannula. No signs of fluid overload were found. Dengue shock syndrome had not been resolved, therefore a colloid fluid, gelofusine, was given 10 ml/kg body weight in 30 minutes twice. After administration of gelofusine, vital signs showed blood pressure 92/42 mmHg (50-90th percentile), pulse rate 110 beats per minute, good volume, regular, warm extremities, capillary refill time less than two seconds, respiratory rate 28 times per minute. The fluid solution given to the patient was gradually reduced according to the improvement of the patient's clinical and laboratory results. Intravenous fluid therapy was discontinued 24 hours after the shock resolved. Clinical improvement occurred marked by improved appetite, no fever, and at discharge hematology results revealed hemoglobin 8.2 g/dL, hematocrit 23.9%, leukocytes 6,100/µL and platelets 102,000/µL. The patient was discharged after six days of hospitalization.

The infant case was presented with vomiting, diarrhea, petechiae, hepatomegaly and pleural effusion, and was diagnosed with primary dengue infection, that later progressed to DSS. The 'dengue in infants' study in Chapter 5 enriched our knowledge that although infants have primary infections, some of them may manifest as severe dengue as DHF/ DSS. Diarrhea and cough as atypical signs occurred in about one third of dengue infants. Other

studies in India also showed that dengue in infants may be accompanied by gastrointestinal symptoms (vomiting, diarrhea in 28% of patients) and upper respiratory symptoms (cough, coryza in 22% of patients).[9] Liver enlargement and fluid accumulation were two strong indicators of dengue shock syndrome in infants in our studies and these two warning signs were also shown to predict progression to severe dengue in infants. The majority of dengue infants were anemic at discharge suggesting that hematocrit is not a reliable indicator of hemoconcentration, but rather the result of pre-existing anemia. As the prevalence of anemia in Indonesian infants is high chest X-ray to detect pleural effusion was performed in the infant case suspected of dengue infection, as pleural effusion is a sign of plasma leakage in infants with dengue.

As the WHO 2009 dengue classification is now adopted world-wide, knowledge about the value of warning signs stratified by different pediatric age groups is instrumental in achieving a timely diagnosis, and to exclude severe dengue, especially in primary care. Warning signs such as liver enlargement and fluid accumulation in infants, which can be detected after examined by the general practitioner or other health care workers, are important to alert the physician when the infant is at risk to develop severe dengue. In older children and adolescents other warning signs indicating development of severe dengue are important, such as increased hematocrit with a strongly decreased platelet count, vomiting, abdominal pain and clinical fluid accumulation. Health care professionals facing dengue cases in endemic areas should be aware of these age-related warning signs in children.

The study on the possible longer-term (8.5 years) effect on common intima media thickness (cIMT), a measure of atherosclerosis, of experiencing dengue (Chapter 7) revealed that those children indeed had an increased cIMT (adjusted increased cIMT of 42.6 µm (95% confidence interval [CI]: 10.0–75.3, P = 0.01)). The increased to an adjusted cIMT of 61.7 µm (95% CI: 21.5–102.0, P < 0.01) in a subgroup analysis on children having experienced dengue shock syndrome, indicating that the effect on atherosclerosis may be modulated by dengue severity. Until recently, knowledge on long-term effects after a history of dengue in pediatric patients was scarce; most longer-term studies after dengue infection included short follow-up periods upto 6 months and were restricted to adults. [10,11] This research suggested that children and adolescents with a history of DHF have an increased cIMT, which may be caused

by dengue and is influenced by disease severity. It is unknown whether the vascular leakage that occurs in DHF may cause transient or persistent vascular damage. Future research with follow-up periods even longer that 8,5 years are warranted to explore the post-dengue development of cardiovascular diseases into adulthood. An increased cIMT in childhood is considered a possible early biomarker for preclinical atherosclerosis, but the long-term effects need further study to understand the full impact of dengue infection, and this may further fuel dengue prevention policies in children, such as recommendations to implement dengue vaccination in the future.

Concluding recommendations

- Community awareness about, age-specific, warning signs of dengue in children, such as vomiting, abdominal pain, lethargy and bleeding manifestations needs to be embedded in community campaigns, to prevent delays in seeking help at healthcare centers.
- The incidence of dengue in infants is rare, but infants should be considered as a high-risk group, because they are more prone to develop dengue shock syndrome. Infants often present with diarrhea and cough which are atypical signs found in about one third of dengue infants.
- Infants with the warning signs of liver enlargement and clinical fluid accumulation are more likely to progress to severe dengue, while in younger children and adolescents, the warning signs are firstly an increase in hematocrit with concurrent rapid decrease in platelet count, followed by abdominal pain, vomiting, or fluid accumulation. The importance of these age-dependent warning signs should be both communicated to the community and discussed in detail in medical training.
- Follow-up of previous severe dengue cases several years later to evaluate vascular parameters to detect preclinical atherosclerosis is warranted.

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APPENDICES

SUMMARY

Dengue infection remains an important public health problem globally, especially in subtropical and tropical countries. Dengue is a mosquito-borne viral infection caused by any one of four single-stranded, positive-sense RNA viruses (DENV-1 to DENV-4). Dengue infection has a wide spectrum of clinical manifestations from asymptomatic, mild to severe dengue disease. The course of dengue infection typically consists of the febrile phase lasting 2-3 days, the critical phase, which occurs from 3-6 days after the first symptoms, and the recovery phase, which often occurs 2-3 days after the critical phase. In the critical phase, plasma leakage may occur for 24 - 48 hours with rising hematocrit levels (hemoconcentration) and thrombocytopenia. Early recognition of plasma leakage is crucial, because timely and adequate fluid treatment in this critical phase may prevent progression to severe dengue.

This thesis focuses on the difficulties in diagnosing dengue in pediatric patients and on barriers to reach a timely diagnosis. With the recent scientific evidence and the findings of the studies presented in this thesis, several relevant challenges will be discussed to further improve dengue management, which includes education to increase awareness in the community and of primary care physicians about early recognition of dengue signs and symptoms in children.

Chapter 1 contains an introduction to the main topics of the thesis. I then discuss these topics in the context of the challenges in Indonesian clinical settings. In Chapter 2, an overview of dengue epidemiology in Indonesia over a period of 45 years is presented. It was shown that dengue incidence increased from 0.05/100,000 in 1968 to ~35-40/100,000 in 2013 and that this increase came with a shift in the age of patients from young children to older age groups, which has consequences for targeted surveillance and prevention. The older age groups are at risk of exposure to Aedes aegypti mosquitos, that bite during the day, at school or in the work environment. Vector control should be targeted at cleaning the breeding grounds of the mosquitos at the venues frequented by the children in these older age groups. In Chapter 3, the clinical manifestations and hematology findings in children with dengue infection are studied. This was a retrospective cohort study carried out in 611 suspected dengue cases admitted to Cipto Mangunkusumo hospital in the period 2007-2009. The clinical manifestations and laboratory findings with respect to dengue were described and dengue serotypes from 81 dengue cases were identified. Confirmed dengue infection

was found in 415 (68%) cases, with dengue fever (DF) occurring in 23.4% of the cases, dengue hemorrhagic fever (DHF) without shock in 41.2% of the cases and dengue shock syndrome (DSS) in 35.4% of the cases. Of 81 cases, 12.3% were DENV-1, 35.8% were DENV-2, 48.2 were DENV-3, and 3.7% were DENV-4. The manifestations were mostly fever, petechiae, epistaxis, hepatomegaly, and thrombocytopenia. Encephalopathy and gastrointestinal bleeding were found only in DSS. Leucopenia was more prominent in DF cases compared to DHF cases, whereas the absence of leucopenia may be a sign of severe dengue.

Chapter 4 describes a five-year cohort study on the clinical course progression and management of dengue in hospitalized children, with comprehensive information for clinicians facing dengue infection in dengue endemic countries. This study evaluates the clinical course and disease outcome of 494 children with suspected clinical dengue, and estimated the burden of dengue cases hospitalized over time at our tertiary care hospital from 2009 through 2013. This study demonstrated that in our tertiary care setting, 52 (28%) of hospitalized children with dengue fever progressed to DSS, and of DHF cases, 9 (6%) progressed to DSS. Among all variables that were measured, only fever duration was significantly associated with clinical progression, emphasizing that the disease course of dengue in children is difficult to predict and stressing the importance of close clinical monitoring of these patients. Clinicians taking care of hospitalized dengue should monitor closely the clinical and laboratory manifestations as almost one third of hospitalized dengue cases may progress to DSS. Fever, bleeding manifestations and thrombocytopenia were presented in children with DF and DHF, while signs of increased vascular permeability, which is considered the hallmark of dengue, was found only in DHF. Annual dengue admissions declined between 2009-2013, while distribution of disease severity remained stable in our hospital.

The WHO 2011 dengue guidelines include infants. They are considered a high-risk group for severe dengue, because infants are more prone to progress to DSS as the capillary fragility in infants is greater than in older children. Infants were more prone to develop shock and marked thrombocytopenia compared to the older age group. In Chapter 5, we contributed to the knowledge about infants, by performing a study of the profile and clinical signs in 42 infants with confirmed dengue using patient data of a tertiary referral hospital from a 10-year period. This study was performed to increase awareness and to provide clinicians taking care of infants with further insights in to dengue clinical presentations,

disease course and management. Although most infant dengue patients had primary infections, some infants developed severe dengue, which at an older age is more typical in secondary dengue infections. Diarrhea and cough as atypical clinical presentations were found in one third of dengue infants. Two strong indicators of dengue shock syndrome in infants were liver enlargement and fluid accumulation. Most of the infants with dengue were anemic at discharge suggesting that hematocrit was not a reliable indicator of hemoconcentration to determine plasma leakage, but rather the result of pre-existing anemia. Radiological examination is then helpful as an alternative to detect plasma leakage in infants with suspected dengue.

Some of the difficulties in applying the existing WHO 1997 dengue classification worldwide in all dengue endemic regions were related to the DHF case definition being too rigid and too difficult to apply in primary care or resource-limited settings. Symptomatic dengue infection in WHO 1997 was classified as undifferentiated fever, DF and DHF. The severity of DHF was classified into four grades: DHF grade I and II (non-shock) and DHF grade II and IV (shock/ DSS). An updated WHO dengue guideline WHO was proposed in 2009 by expert consensus groups which divided cases into non-severe dengue and severe dengue to overcome some of the difficulties. For practical reasons, non-severe dengue was stratified to 'dengue without warning signs' and 'dengue with warning signs'. The warning signs consisted of abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, liver enlargement, and laboratory results showing an increase in hematocrit concurrent with a rapid decrease in platelet count. The warning signs in dengue are useful for early identification of dengue shock syndrome. Delay in diagnose, delayed referral to health care facilities, and delayed rehydration in the critical phase of the disease may lead to increased mortality in severe dengue cases.

The alteration in guidelines makes it important to explore the diagnostic value of warning signs in different age groups, especially in pediatric dengue infected cases. I addressed this issue in Chapter 6. In infants, liver enlargement (NPV (negative predictive value) 80.8%) or clinical fluid accumulation (NPV 75%) are shown to be important signs to exclude severe dengue, i.e., when both of these are absent, severe dengue is unlikely. In older children and adolescents, an increase in hematocrit with concurrent rapid decrease in platelet count was most discriminative (NPV in children 76.6% and in adolescents 91.9%); in case of absence of this symptom severe dengue is very unlikely. In children and adolescents,

absence of abdominal pain, vomiting, or fluid accumulation are unlikely in severe dengue.

In DHF, endothelial dysfunction occurs, leading to plasma leakage, with increased biomarkers indicative of vascular damage. However, it is unknown whether this vascular damage is transient or persistent. The study presented in Chapter 7 evaluated whether previous dengue infection in childhood was associated with vascular parameters indicative of preclinical atherosclerosis several years later. Carotid intima-media thickness (cIMT) and arterial stiffness were assessed in 28 children and adolescents with previous DHF (mean interval between DHF and cardiovascular assessment 8.4 years), and 34 controls, in a low-resource setting. Participants with previous DHF had an adjusted increased cIMT of 42.6µm (95% CI 10.0 – 75.3, $p = 0.01$), and of 61.7µm (21.5 – 102.0, $p < 0.01$) in a sub-group analysis in dengue shock syndrome. There were no differences in arterial stiffness. Studies of long-term follow-up of pediatric patients who experienced dengue infection are still scarce.

In Chapter 8, the main findings and conclusions of this thesis were discussed and recommendations aimed at reducing delay in diagnosis of dengue infection pediatric cases are provided. Several challenges were discussed in reducing delay to diagnosis and to prevent progression to severe dengue; reinforcing education on warning signs of dengue to inform the community and general practitioners will increase awareness and early recognition of dengue disease progression.

Community awareness about warning signs needs to be increased in community campaigns, besides vector control programs. Implementing education about the important warning signs of dengue for general practitioners in the triage/ healthcare facilities in endemic countries is necessary, thus helping to prevent dengue cases progressing to severe dengue. In infants, cases with an absence of warning signs of liver enlargement and clinical fluid accumulation were less likely to progress to severe dengue. Whereas, in children and adolescents, cases with an absence of warning signs of increase in hematocrit with concurrent rapid decrease in platelet count, followed by abdominal pain, vomiting, or fluid accumulation are less likely to progress to severe dengue. More studies to evaluate vascular parameters to detect preclinical atherosclerosis in pediatric cases with a history of dengue infection may be warranted to obtain a more comprehensive picture.

SAMENVATTING PROEFSCHRIFT

Dengue is een belangrijk gezondheidsprobleem. De mondiale last van dengue is dramatisch gestegen over de laatste 60 jaar en treft meer dan de helft van de wereldpopulatie, met name in tropische en subtropische landen. Dengue is een muggen-overdraagbare RNA-virusinfectie en er zijn vier typen: DENV-1 tot DENV-4). Dengue infectie heeft een wijd spectrum van klinische verschijnselen, van asymptomatisch, mild, tot ernstige dengue ziekte. Het verloop van een dengue infectie bestaat doorgaans uit drie fases: een fase met koorts, een kritische fase en de herstelfase. De koortsfase kan 2-3 dagen duren, de kritische fase treedt over het algemeen 3 tot 6 dagen na de eerste verschijnselen op, en de herstelfase vindt vaak 2-3 dagen na de kritische fase plaats. In de kritische fase, kan plasmalekkage optreden, met hematologische verschijnselen met stijgende hematocrietwaarden (hemoconcentratie) en thrombocytopenie. Vroege herkenning van plasmalekkage is van groot belang, want tijdige en adequate toediening van vloeistoffen in deze kritische fase kan bijdragen aan het voorkomen van ernstige dengue of zelfs sterfte.

Dit proefschrift richt zich op de problemen bij het diagnosticeren van dengue bij kinderen en op belemmeringen voor tijdige diagnose. Met recent wetenschappelijk onderzoek en de bevindingen van de onderzoekingen gepresenteerd in dit proefschrift zullen verschillende relevante uitdagingen worden aangepakt om de behandeling van dengue verder te verbeteren, onder andere door voorlichting om het bewustzijn en de kennis van de gemeenschap en eerstelijnsartsen over vroege herkenning van de verschijnselen van dengue bij kinderen te vergroten.

Het eerste hoofdstuk van het proefschrift bevat een introductie over de belangrijkste onderwerpen die in het proefschrift worden behandeld. Vervolgens worden deze onderwerpen besproken in de context van de Indonesische dagelijkse klinische praktijk. In Hoofdstuk 2 wordt er een overzicht gegeven van de dengue-epidemiologie in Indonesië over een periode van 45 jaar. Er werd een stijging van incidentie aangetoond van 0,05/100.000 in 1968 tot ~35-40/100.000 in 2013 en deze stijging ging gepaard met een verschuiving in de leeftijd van de patiënten, van jonge kinderen naar oudere leeftijdsgroepen. Dit heeft consequenties voor gerichte surveillance en preventie. De oudere leeftijdsgroep

loopt risico op blootstelling aan de Aedes aegypti muggen, die overdag bijten, op school of in de werkomgeving. Vectorbestrijdingsprogramma's zouden gericht moeten zijn op het schoonmaken van de broedplaatsen van de muggen op de plaatsen waar de oudere leeftijdsgroepen zich vaak begeven.

In Hoofdstuk 3 werden de klinische verschijnselen en hematologische bevindingen bij kinderen met dengue-infectie bestudeerd. Dit onderzoek was een retrospectief cohortonderzoek met 611 vermoedelijke gevallen van dengue opgenomen in het Cipto Mangunkusumo Ziekenhuis tussen 2007 tot 2009. De klinische verschijnselen en laboratorium bevindingen werden beschreven en de dengue serotypes uit 81 dengue gevallen waren geïdentificeerd. Wij vonden een daadwerkelijke dengue-infectie in 415 (68%) gevallen, bestaande uit dengue-koorts in 23,4% van de gevallen, dengue-hemorragische koorts (DHF) zonder shock in 41,2% van de gevallen, en dengue-shock syndroom in 35,4% van de gevallen. Van de 81 gevallen, was 12,3% veroorzaakt door DENV-1, 35,8% door DENV-2, 48,2 door DENV-3, en 3,7% door DENV-4. De meest voorkomende verschijnselen waren koorts, petechiën, epistaxis, hepatomegalie, en trombocytopenie. Encefalopathie en gastro-intestinale bloeding werden uitsluitend bij het dengue-shock syndroom gezien. Leukopenie was meer prominent bij dengue-koorts dan in dengue-hemorragische koorts, terwijl de afwezigheid van leukopenie een teken kan zijn van ernstige dengue.

Hoofdstuk 4 beschrijft een vijfjarig cohortonderzoek naar de progressie van het klinische beloop en de behandeling van dengue in kinderen opgenomen in ons ziekenhuis, met uitgebreide informatie die artsen die te maken krijgen met dengue-infectie in dengue-endemische landen kunnen gebruiken in de dagelijkse praktijk. Dit onderzoek beschrijft het klinisch beloop en de prognostische uitkomsten bij 494 kinderen met een vermoeden van klinisch dengue en maakt een schatting de impact van opgenomen dengue-gevallen in de loop van tijd in een tertiair ziekenhuis van 2009 tot 2013. Zoals eerder aangegeven, vonden we encefalopathie en gastro-intestinale bloeding uitsluitend bij het dengue-shock syndroom. Dit onderzoek heeft aangetoond dat in onze tertiaire zorgomgeving, 52 (28%) kinderen opgenomen met dengue-koorts een dengue-shock syndroom ontwikkelden, en 9 (6%) kinderen opgenomen met DHF een dengue-shock syndroom ontwikkelden. Van alle gemeten variabelen was alleen de duur van de koorts significant geassocieerd

met klinische progressie. Dit benadrukt dat het klinisch verloop van dengue in kinderen moeilijk voorspelbaar is, maar ook het belang van nauwlettend klinisch toezicht van deze patiënten. Voor clinici die met de behandeling van opgenomen dengue patiënten te maken hebben, onderstrepen deze bevindingen dat nauwlettend toezicht van klinische verschijnselen en laboratorium bepalingen belangrijk is, omdat bijna een derde van dengue-gevallen kan overgaan naar dengue-shock syndroom. Koorts, bloedingsverschijnselen en trombocytopenie presenteerden zich vooral bij kinderen met dengue-koorts en DHF, terwijl verhoogde vasculaire permeabiliteit, dat als kenmerk van dengue wordt beschouwd, zich slechts bij DHF manifesteerde. Het aantal jaarlijkse dengue-opnames is tussen 2009 tot 2013 gedaald, terwijl de verdeling van de ernst van de ziekte stabiel bleef in ons ziekenhuis.

In de WHO dengue richtlijnen van 2011 worden zuigelingen beschouwd als een hoog-risico groep voor ernstige dengue, omdat zuigelingen gevoeliger zijn voor progressie naar dengue-shock syndroom vanwege hun capillaire fragiliteit die groter is dan bij oudere kinderen. Shock, plasma lekkage, en opvallende trombocytopenie kwamen meer voor bij zuigelingen dan bij oudere kinderen. Daarom hebben wij in Hoofdstuk 5 het profiel en de klinische symptomen bij 42 zuigelingen met bevestigde dengue bestudeerd, met behulp van patiënten data van een tertiair verwijzingsziekenhuis over een periode van 10 jaar. Dit onderzoek was uitgevoerd om de bewustwording van dengue te vergroten en om clinici die zuigelingen met dengue behandelen verdere inzichten te verschaffen over de klinische presentatie, ziektebeloop en behandeling. Bij de meeste zuigelingen is de dengue veroorzaakt door primaire infecties, maar bij sommigen kan de infectie zich tot ernstige dengue ontwikkelen, zoals vaker bij oudere kinderen met secundaire infecties wordt gezien. Diarree en hoest waren de atypische klinische verschijnselen die bij een derde van de zuigelingen met dengue aanwezig waren. Twee sterke indicatoren van dengue-shock syndroom in zuigelingen waren leververgroting en vochtophoping. De meeste zuigelingen met dengue waren anemisch bij ontslag, wat erop wijst dat de hematocriet-waarde geen betrouwbare indicator is voor hemoconcentratie om plasmalekkage vast te stellen, maar eerder het gevolg is van reeds bestaande anemie. Radiologisch onderzoek kan dan behulpzaam zijn als een alternatief om plasmalekkage te detecteren bij zuigelingen met vermoedelijke dengue.

Er waren problemen gemeld bij de wereldwijde toepassing van de 1997 WHO dengue-classificatie uit 1997 in dengue-endemische gebieden. Men was van mening dat de definitie van DHF te rigide was en te moeilijk toe te passen was in de eerstelijnszorg of settings met beperkte middelen. In de WHO classificatie van 1997 werd symptomatische dengue-infectie geclassificeerd als ongedifferentieerde koorts, dengue-koorts, en DHF. De ernst van DHF werd in vier niveaus geclassificeerd: DHF graad I en II (zonder shock) en DHF graad III en IV (shock/dengue-shock syndroom).

In 2009 zijn de WHO dengue richtlijnen aangepast door een consensusgroep van deskundigen. In de voorgestelde nieuwe richtlijnen werden denguegevallen geclassificeerd als niet-ernstige dengue of ernstige dengue. Om praktische redenen was niet-ernstige dengue onderverdeeld in 'dengue zonder waarschuwingstekens' en 'dengue met waarschuwingstekens.' De waarschuwingstekens omvatten buikpijn, persistent braken, klinische accumulatie van vloeistof, mucosabloeding, lethargie, leververgroting, en een verhoogde hematocriet waarde samen met een snelle daling in het aantal bloedplaatjes. De waarschuwingstekens in dengue zijn nuttig bij de vroege identificatie van dengue-shock syndroom. Vertraagde diagnose, vertraagde verwijzing naar gezondheidsinstellingen, en vertraagde rehydratie in de kritische fase van het ziektebeloop kunnen leiden tot een hogere sterfte bij ernstige gevallen van dengue.

Door de veranderingen in de richtlijnen, is het belangrijk om de diagnostische waarde van de waarschuwingstekens te onderzoeken in verschillende leeftijdsgroepen, vooral in kinderen. Dit wordt in Hoofdstuk 6 besproken. In zuigelingen zijn leververgroting (NPV (negatieve predictieve waarde) 80,8%) en klinische vochtophoping (NPV 75%) belangrijke kenmerken om ernstige dengue uit te sluiten; d.w.z. als beiden afwezig zijn, is ernstige dengue onwaarschijnlijk. In oudere kinderen en adolescenten is een verhoogde hematocriet waarde samen met snelle daling in het aantal bloedplaatjes het meest onderscheidend (NPV 76,6% bij kinderen en 91,9% bij adolescenten); in het geval van afwezigheid van deze tekens is ernstige dengue hoogst onwaarschijnlijk. Ook in deze leeftijdsgroepen is ernstige dengue onwaarschijnlijk als buikpijn, braken, of vochtophoping afwezig zijn.

In DHF kan er endotheliale dysfunctie optreden, wat tot plasmalekkage kan leiden, met verhogingen in biomarkers die indicatief zijn voor vasculaire schade. Het is echter

onbekend of deze vasculaire schade tijdelijk of blijvend is. Het onderzoek dat in Hoofdstuk 7 wordt gepresenteerd evalueerde of eerdere dengue-infectie in kinderen geassocieerd was met vasculaire parameters die indicatief zijn voor preklinische atherosclerose enkele jaren later. Intima-media dikte van de halsslagader (carotid intima-media thickness, cIMT) en arteriële stijfheid werden beoordeeld in 28 kinderen en adolescenten met eerdere DHF (het gemiddeld interval tussen DHF en de cardiovasculaire beoordeling was 8,4 jaar), en in 34 controles, in een setting met beperkte middelen. Deelnemers met eerdere DHF hadden een cIMT verhoging van 42,6 µm (95%CI 10,0 – 75,3, p=0,01), en 61,7 µm (95%CI 21,5 – 102,0, p<0,01) in een subgroep met dengue-shock syndroom. Er waren geen verschillen in de arteriële stijfheid. Studies naar de lange-termijn follow-up van kinderen die een dengue-infectie hebben doorgemaakt zijn nog steeds schaars.

In Hoofdstuk 8 werden de bevindingen en conclusies van dit proefschrift en worden aanbevelingen gegeven die gericht zijn op de versnelling in diagnosestelling van dengue-infectie bij kinderen. Ook wordeneen aantal uitdagingen besproken bij zo'n versnelling in de diagnosestelling, bij het voorkomen van het ontwikkelen van ernstige dengue, het versterken van de voorlichting over waarschuwingstekens van dengue bij de bevolking en huisartsen, en de behandeling van dengue in triage en vroege herkenning van ziekteprogressie.

Bewustzijn van de bevolking over waarschuwingstekens moet worden bevorderd in maatschappelijke campagnes, naast het voortzetten van vectorbestrijdings-programma's. Implementatie van educatie over de belangrijke waarschuwingstekens bij dengue voor huisartsen in de triage/gezondheidszorg faciliteiten in endemische landen is nodig om zo vaak als mogelijk de progressie naar ernstige dengue te voorkomen. In zuigelingen zijn leververgroting en klinische vochtophoping tekenen van waarschijnlijke progressie naar ernstige dengue. In kinderen en adolescenten wijzen de waarschuwingstekens van eerst een verhoogde hematocriet waarde samen met een snelle daling in het aantal bloedplaatjes, gevolgd door buikpijn, braken, of vochtophoping op progressie naar ernstige dengue. Om een alomvattend beeld te krijgen, zou verder onderzoek gericht moeten zijn op de vasculaire parameters om preklinische atherosclerose te herkennen in kinderen met een voorgeschiedenis van dengue-infectie.

About the author

Mulya Rahma Karyanti was born in Jakarta on November 9th, 1969. She completed her MD in 1994, and dedicated as general practioner in the primary healthcare in Cipayung district in 1996-1999. She then continued to work as pediatric residency and subspecialty training of infection and tropical diseases in the Medical Faculty Universitas Indonesia. In 2004, she started to work as general pediatrician and in 2005 she joined to become the staff member of the Division of Infection and Tropical Diseases, Department of Child Health, Cipto Mangunkusumo, a national tertiary referral hospital in Jakarta, Indonesia and since 2013 she holds position as the head of the Division of Infection and Tropical Diseases, Department of Child Health until currently. She has a training tropical Infectious disease on public health, WHO-SEARO, April 2009. She obtained her master of science degree in clinical epidemiology in the Julius Center for Health Sciences and Primary Care of the Utrecht Medical Center, Utrecht University, Netherland, from 2009 to 2011. Karyanti is married to Muhamad Ilhamy Setyahadi and has two sons name Muhamad Luthfi Prasetyo and Muhamad Ramadhan Nugroho.

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