Dengue Virus Infection: Predictors for Severe Dengue

Jeanne A. Pawitan

Department of Histology, Faculty of Medicine, University of Indonesia. Jl. Salemba 6, Jakarta, Indonesia.

Correspondence mail to: *jeanneadiwip@fk.ui.ac.id*

ABSTRACT

Dengue virus (DENV) infection is a mosquito born disease that is endemic in all WHO regions, except European region, and may present a broad range of severity. It may appear as an asymptomatic condition, dengue fever (DF), or life threatening forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), or the currently defined severe dengue. Currently there are means to diagnose DENV infection, but there is no accurate means to early predict the progress into severe manifestations. Therefore, this article addresses the factors that might be used to predict the progress into severe dengue.

Predictors for severe dengue are the previously established warning signs, and coexisting conditions, as is recommended by the WHO, in addition to Caucasian race, and people with AB blood group. In the future, viral load assessment, viral serotype testing, NS1, cytokine, elastase, hyaluronan, soluble thrombomodulin, and NO level, and circulating endothelial cell detection test are promising to be studied and developed as early predictors of severe dengue.

Key words: *NS1, antibody, cytokines, elastase, hyaluronan, endothelial cells.*

INTRODUCTION

Dengue virus (DENV) infection is a mosquito-born disease that has spread very fast in the last 50 years, and is endemic in all WHO regions, except European region,[1] and in Indonesia, it is a major public health problem.[2,3]

Dengue virus infection may present a broad range of severity, and may appear as an asymptomatic condition, dengue fever (DF), or life threatening forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue fever is the mild form of DENV infection. In 1997 WHO classification, DHF is classified into 4 grades of severity, with the most severe grade III and IV being defined as DSS. However, difficulties in applying the grading in clinical situation lead to the suggestion of a new and more practical classification in 2008, though previous classification is still widely used. The new classification is currently being compared to the previous classification in 18 countries, and the study will end in 2010. In the new classification, DENV infection is classified into severe and non-severe dengue that is again divided into non-severe dengue with and without warning signs.[1]

Currently there are means to diagnose DENV infection, 4-7 but there is no accurate means to early predict the severity of disease, as cases of non-severe dengue without any warning signs may later develop into severe dengue.[1] Therefore, search for other factors other than the consensus warning signs is very important to help in early predicting the cases that might progress into severe dengue. Early prediction is very important to avoid unnecessary hospitalization, or to give more attention and hospitalization to those with non severe dengue that are predicted to progress into severe dengue.

Accumulating evidence showed that the severity of the manifestation of DENV infection depended on some factors. Therefore, this article addresses those

factors, i.e. viral related factors such as dengue virus virulence, viral load, and viral components and various host conditions such as age, genetics, nutritional status, host immune reaction, coexisting conditions, physical and laboratory findings, and plasma levels of various substances.

DENGUE VIRUS

Dengue viruses are single stranded RNA viruses that belong to the family *Flaviviridae*. There are four serotypes, i.e. DENV-1, DENV-2, DENV-3 and DENV-4. The four serotypes are transmitted to humans by mosquitoes, principally the *Aedes aegypti*, and other species, i.e. *Aedes albopictus*, *Aedes polynesiensis* and several species of the *Aedes scutellaris* complex.[1]

Virulence and Sequence of Infection

In vitro and animal studies showed that the severity of the manifestation of dengue virus infection is supposed to be influenced by the intrinsic properties and virulence of the infecting serotype and strain,[8-11] and observations in human suggested that sequence of infection is important for the severity of the disease.[12] This supposition is supported by the fact that secondary heterotypic infections with the Asian genotype strain of DENV-2 serotype were associated with DHF and DSS in Southeast Asia and America.[8,13] On the contrary, American genotype strain of DENV-2 serotype showed reduced pathogenicity, was associated with mild disease, and supposed to be less able to replicate in *Aedes aegypti*, and thus less transmissible. Those two strains were genetically different at the envelope amino-acid 390, and in the sequence of the 3' untranslated region, which both are virulence determinants.[8]

In secondary dengue infection, the virus can be detected at the early stage (febrile phase),[7.14] but the antibodies begin to increase four days after the onset of symptoms.[7] To know the sequence of infection, both virus and antibody testing are needed, while in the early stage, only the virus serotype can be tested. Therefore, only virus serotype can be used as an early predictor. However, further studies are needed to get a final conclusion about the cost effectiveness of the test, as unnecessary additional test is a burden to the patient.

Viral Components

The viral genome encodes three structural proteins i.e. nucleocapsid or core protein (C), membrane associated protein (prM), and envelope protein (E), and at least seven nonstructural proteins, i.e. NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5.[6, 11] Among the nonstructural proteins, NS1 is highly conserved and supposed to play a major role in the severity of the disease, as increased level of soluble NS1 (free sNS1) in serum correlates with viremia levels,[15] and disease severity.[9]

Further, free sNS1 level of > or = 600 ng/mL within 72 hours of illness onset might be used as predictor of DHF.[15]

Currently, NS1 detection kit is commercially available, and can be used in a lab with limited equipment to confirm dengue infection within a few hours.[13] However, studies are needed to evaluate the ability, the cost effectiveness, and when necessary to upgrade this test kit to measure NS1 semi-quantitatively to early predict severe dengue.

Viral Load

A study in mouse model for DHF showed that intra-dermal injection of 3x109 PFU of DENV-2 strain16681 caused systemic hemorrhage in all of the mice at the third day of injection, while injection of 4x107 to 8x107 PFU only caused subcutaneous hemorrhage in one of the three injected mice. This study showed that higher viral load is important in causing DHF. 10 However, viral load is difficult to be assessed in clinical setting.

AGE

Several studies showed that young and old ages were associated with severe dengue.[16-18] Age is a readily available data and indeed, in the WHO recommendation for treatment, infant and elderly are included in group B patients that should be referred to a hospital.[14]

GENETICS

A study on Cuban DHF/DSS outbreaks showed that people of Negroid race had lower risk for DHF/DSS compared to Caucasoid race. This finding is in line with those reported in African and Black Caribbean populations.[19] Moreover, in secondary infection, people with AB blood group have higher risk to get grade-3 DHF than grade-1 and -2, or dengue fever. 20 Further, three studies on genetic polymorphism of transporters associated with antigen presentation (TAP) genes showed that certain types of polymorphism were associated with reduced or increased risk to develop DHF and DSS. [21,23] Race and blood group are simple data that can be obtained from anamnesis of the patient, so they may be used as predictors, but test for polymorphism might not be cost effective to be developed as a predictor for severe dengue.

NUTRITIONAL STATUS

A study on 245 Vietnamese infants with predominantly primary dengue virus infections showed that infants with low height and weight for age were fewer in number among DHF/DSS cases compared to 533 healthy baby controls.[24] However, another study on 4,532 children confirmed of dengue infection compared to control showed that children with under nutrition had a greater risk of DSS.[25] Nutritional status is easy to assess, but further studies are needed before we can use it as a predictor for severe dengue.

HOST IMMUNE REACTION

Studies in the pathogenesis of DHF and DSS in dengue virus infection are accumulating. A lot of studies showed that host immune reaction may play a role in the development of DHF and DSS, including cell mediated immune response, cytokine production by various cells of the innate and specific immune responses, and humoral immune response.

Cells of The Innate Immune Response

In innate immune response, natural killer (NK) cells are important effector cells, as they increase in number after viral infection. The NK cells have both activation and inhibitory receptors. Ligand binding on activation receptors cause exocytosis of perforin and grandzymes in their granules, which lead to virus laden cell lysis, and cytokine release **(Table 1)**, including several cytokine with chemoattractant activity (chemokine).[26]

Further, chemokines may activate specific cellular immune response, including Th1 and Th2 and other T cells, NK cells themselves, and monocytes to restrain the virus. However, virus may develop a machinery to evade the restriction, and may use the chemokine pathway for viral replication and attacking neighboring cells.[11]

A study in 55 patients with dengue infection showed that NK (CD56++ CD3-) cell number was increased during the acute phase. Thus, increased number of NK cells in acute phase was suggested as a marker of mild disease. [26] Further, a study in mouse model showed that in DHF there were no significant numbers of NK cells, dendritic cells, Langerhans cells or neutrophils found in the hemorrhage tissues.[10] However, another study showed that infants with DSS (DHF grade III) had more CD69+ NK cells compared to those with non-shock DHF.[27] Therefore, further studies should be done, before we can use NK cell number as a predictor for severe disease.

Cells of The Specific Immune Response

In dengue virus infection, T and B lymphocytes were shown to be the target of dengue virus infection. [28, 29] However, further study showed that T and B cells were not the principal targets, 30 and they acted as effector cells that were activated upon exposure to dengue virus antigens that were presented by the infected antigen presenting cells. Activation of T lymphocytes leads to complement activation and the production of various cytokines, 10 and excessive T lymphocyte activation may lead to a pathologic cytokine response that are correlated with severe disease **(Table 1)**.[9]

Further, decrease in CD4 and CD8 T cells was observed in patients with severe manifestations.[26] However, another study showed that infants with DSS (DHF grade III) had more CD69+ NK cells and CD8+ and CD4+ T lymphocytes compared to those with non-shock DHF.[27] Therefore, CD4 and CD8 T cell number is inconclusive, and using them as predictors for severe DHF and DSS need further studies.

Cytokines

Cytokines are produced by cells of both the innate and specific immune response. Monocytes,[31]and splenic macrophages [30] are the principal direct targets in dengue virus infection. Other cells of the innate immune response such as dendritic,[11] mast cells,[32] and other cells such as endothelial cells, 10, 11 and hepatocytes,[33] may also be targeted. Those infected cells secrete cytokines upon dengue virus infection. The cytokines and their soluble receptor levels in DHF patients are higher compared to those with dengue fever, thus suggest the role of certain cytokines in disease severity.[34] Therefore, increase in certain cytokine levels may be used as predictors of severe dengue **(Table 1)**. However, vast epidemiological studies in severe dengue patients are needed to get a whole picture of the phases where increased cytokine level occurred, and also cost effective studies are needed to make a conclusion, whether cytokine levels can be used as early predictor.

Humoral Immune Response

One of the hypotheses for the pathogenesis of DHF and DSS was proposed 3 decades ago, and titled the antibody dependent enhancement (ADE). This hypothesis proposes that neutralizing antibodies will confer life-long protection to secondary infection with homologous virus; while upon secondary infection with heterotypic dengue virus serotype, the pre-existing antibody is non-neutralizing and may be harmful.[30, 37,38] This ADE phenomenon might be due to virus-antibody immune complex-cell attachment through

Table1. Altered cytokine level as candidate predictor of severe dengue

Cytokine	Source	Predictor
IFN-α [9]	T lymphocyte [9]	Severe disease[9]**
IFN-γ [26, 33]	NK cells[26], monocytes, T cells and hepatocytes[33]	(In vitro study)[33]**
TNF-α[9, 10]	T lymphocyte [9, 10]	Severe disease [9] endothelial cell apoptosis1[0]**
MIP-1α[11, 32]	Dendritic cells,[11] mast cells[32]	(In vitro study)[11, 32]**
MIP-1β[11, 32]	Dendritic cells, [11] mast cells[32]	(In vitro study)[11, 32]**
RANTES1[1, 32]	Dendritic cells, [11] mast cells[32]	(In vitro study)[11, 32]**
IL-2[33]	monocytes, T cells and hepatocytes[33]	(In vitro study)[33]**
IL-6[33]	monocytes, T cells and hepatocytes[33]	(In vitro study) [33]**
IL-8,[35]	Dendritic cells, 11endothelial cells[35]	Endothelial damage[35]**
IL-10[9]	T lymphocyte[9]	Severe disease9**
VEGF A[34]	T cells[34]	Plasma leakage, DHF[34]*
sVEGFR2 (inverse)[34]	endothelial cells[34]	Plasma leakage, DHF[34]*
Cytotoxic factor[36]	Thy-l .2$^+$, Ly 1$^+$23$^-$ T lymphocytes of mouse spleen[36]	Plasma leakage, thrombocytopenia,[10, 36] hemorrhage 3[6] **

IFN= interferon, TNF= tumor necrosis factor, GM-CSF= granulocyte-macrophage colony-stimulating factor, MIP= macrophage inflammatory protein, RANTES= regulated on activation normal T cell expressed and secreted, IL=interleukin, VEGF= vascular endothelial growth factor, sVEGFR2= soluble VEGF receptor 2, *= strong predictor candidate, **= further studies are needed

surface Fcγ receptor.[39] Moreover, various cited studies on dengue patients showed that peak viremia was higher in secondary dengue infection,[9] and preexisting non-neutralizing heterologous antibodies to dengue virus was reported to be one of the risk factors for DHF.[10]

Recently, an ADE assay to detect the presence of ADE in serum was developed. The assay uses Fc gamma RIIA bearing cell lines, and finally virus growth is measured by a standard plaque assay procedure. 40 However, a nested case-control study in infants using DENV3 ADE assay showed that ADE activity in DHF cases were not significantly higher compared to non DHF dengue infection.[41]

Besides antibody against the virus, antibody might be produced against NS1. Specific antibody to endothelial-cell-bound NS1 is supposed to contribute in the vascular leakage syndrome through antibody-dependent activation of the complement cascade that mediates cytolysis that is followed by subsequent increase in vascular permeability. In addition, NS1 induces auto-antibodies to extracellular matrix proteins and platelet,[9] which leads to thrombocytopenia.

Another hypothesis is that NS1 antibody cross reacts with the endothelial cells and induces damage through inflammatory activation of the endothelial cells via the transcription factor NF-kB regulated pathway. Inflammatory activation leads to cytokine and chemokine production.[42] Therefore, NS1 antibody level is a predictor candidate for disease severity, but cannot be used as early predictor as antibodies begin to rise later in the course of the disease.

COEXISTING CONDITIONS

A study during an outbreak in Taiwan on 644 patients with dengue virus infection showed that preexisting diabetes mellitus, hypertension, and uremia were significant risk factor for severe DHF and DSS.[17] Indeed patients with coexisting condition, such as diabetes mellitus, hypertension, and renal failure are included in group B patients that should be referred to a hospital, according to WHO guidelines.[14]

CLINICAL AND LABORATORY FINDINGS

Clinical and laboratory findings are established predictors for DSS, and recent studies reconfirmed these predictors. Studies to find out the predictors for DSS in children showed that the clinical findings that can be used as predictors of DSS are abdominal tenderness, hepatomegaly, lethargy, cold extremity,[18] and bleeding.[43] Further, laboratory findings that were associated with DSS were platelet cell count of ≤ 75,000/mm3 and haematocrit value of 50%, 18 or a rise of more than 22% from baseline haematocrit.[43]

PLASMA/SERUM LEVELS OF VARIOUS SUBSTANCES AND CIRCULATING CELLS

Increase in plasma level of various substances was found in dengue infection, including elastase,[35] hyaluronan,[44] soluble thrombomodulin,[45] nitric oxide, [46] soluble adhesion molecules, and circulating endothelial cells.[47]

Elastase

Elastase is found in azurophilic granules of neutrophils together with other substances including oxygen radicals, and cathepsin G. Activation and degranulation of neutrophils due to IL8 cause

the release of elastase into the plasma. Elastase is of high importance as it may cause endothelial damage and inactivates major inhibitors of the complement, coagulation and fibrinolytic system that lead to the activation of the system. Therefore, elastase may activate the complement that contribute to vasodilatation and increased capillary permeability, which together with endothelial damage lead to plasma leakage.[36]

Moreover, a study on 186 children with dengue infection showed that the level of elastase was increased in 70% of the patients, and the levels of elastase were significantly correlated to IL8 levels. Further, elastase level that was measured on admission was higher in patients with shock, ascites and pleural effusion compared to normotensive patients.[35] Therefore, high elastase level may be used as a predictor for severe dengue in children.

Hyaluronan

Hyaluronan (HA) is found in connective tissues. Circulating HA is degraded by sinusoidal endothelial cells in the liver. As sinusoidal endothelial cells may be targeted by the virus, impairment in the HA clearance may occur. A study showed that serum HA level, which was measured by ELISA based method, was significantly increased in patients with DSS. The level decreased during convalescent state, but was still higher compared to those of healthy controls. The increase in serum HA level in patient with DSS was significantly higher compared to those of acute hepatitis A patients.[44] Therefore, serum HA level may be used as a predictor of severe dengue.

Soluble Thrombomodulin

Soluble thrombomodulin (sTM) is a marker of vascular endothelial cell injuries and dysfunction. A study showed that sTM level, which was measured by ELISA, was higher in patients with DSS (DHF grades III and IV) compared to those with dengue fever and DHF grade I, II from day -3 until day +2. Therefore, sTM level may be used as an early predictor in DSS. [45]

Nitric Oxide

Nitric oxide (NO) is produced by endothelial cells, and endothelial cell damage and dysfunction that happened in dengue infection may cause a decrease in serum NO level. In a study on 110 patients with dengue infection, their serum NO levels were significantly lower compared to those of controls. However, patients with DSS had significantly higher NO levels than those with DHF I/II. [46] Therefore, high NO level in dengue positive patient may be used as predictor of severe dengue.

Soluble Adhesion Molecules and Circulating Endothelial Cells

A study showed that there was a significant increase in soluble intercellular adhesion molecules (sICAM) and soluble vascular cell adhesion molecules (sVCAM) plasma level in most of DHF patients compared to healthy controls, and 4 out of 13 patients with DHF had circulating endothelial cells (CEC) in their peripheral blood.[47] Another study showed that increased CEC was found in DSS patients from day 0 until day +2 as compared to those with dengue fever and non severe DHF (grade I, II).[45]

CLINICAL RELEVANCE OF VARIOUS POSSIBLE EARLY PREDICTORS

Predictors for severe dengue are the previously established warning signs according to WHO guidelines, i.e. clinical findings: abdominal tenderness, hepatomegaly, lethargy, cold extremity, and bleeding, and laboratory findings: platelet cell count of ≤ 75,000/mm3 and hematocrit value of 50%, or a rise of more than 22% from baseline hematocrit. Further, coexisting condition, and young and old ages may be used as early predictors of severe dengue, as is recommended by the WHO, 14 in addition to Caucasian race, and people with AB blood group.

In the future, simple methods for viral load assessment, viral serotype testing, NS1 level semi-quantitative measurement, and easy tests to measure plasma/serum cytokine, elastase, hyaluronan, soluble thrombomodulin, and NO level, and a simple method to detect circulating endothelial cells are promising methods to be developed and tested for their cost-effectiveness as early predictors of severe dengue.

CONCLUSION

Early predictors of severe dengue are the previously established warning signs, and coexisting conditions, as is recommended by the WHO, in addition to Caucasian race, and people with AB blood group. In the future, some additional tests are promising to be studied and developed into easy and cost effective test to be used as early predictors of severe dengue.

REFERENCES

1 Nathan MB, Dayal-Drager R, Guzman M. Epidemiology, burden of disease and transmission. WHO. Dengue guidelines for diagnosis, treatment, prevention and control. New edition. Geneva: WHO;2009. p.1-21.

2 Pohan HT, Lie KC, Suhendro, SantosoWD, Eppy. An open pilot study of the efficacy and safety of Polygeline in adult subjects with dengue haemorrhagic fever. Acta Med Indones. 2009;41(2):47-53.

3 Nelwan RHH. Polygeline for adult dengue haemorrhagic fever and spectrum of unusual manifestations. Editorial. Acta Med Indones. 2009;41(2):45-6.

4 Lam SK, Ew CL, Mitchell JL, Cuzzubbo AJ, Devine PL. Evaluation of a capture screening enzyme-linked immunosorbent assay for combined determination of immunoglobulin M and G antibodies produced during dengue infection. Clin Diagn Lab Immunol. 2000;7(5):850-2.

5 Dussart P, Petit L, Labeau B, Bremand L, Leduc A, Moua D, et al. Evaluation of two new commercial tests for the diagnosis of acute dengue virus infection using NS1 antigen detection in human serum. PLoS Negl Trop Dis. 2008; 2(8): e280. www.plosntds.org.

6 Kumarasamy V, Chua S K, Hassan Z, Wahab A H A, Chem Y K, Mohamad M, et al. Evaluating the sensitivity of a commercial dengue NS1 antigen capture ELISA for early diagnosis of acute dengue virus infection. Singapore Med J. 2007;48(7):669-73.

7 Buchy P, Peeling R. Laboratory diagnosis and diagnostic tests. WHO. Dengue guidelines for diagnosis, treatment, prevention and control. New edition. Geneva: WHO; 2009. p. 91-107.

8. Bernardo L, Izquierdo A, Prado I, Rosario D, Alvarez M, Santana E, et al. Primary and secondary infections of Macaca fascicularis monkeys with Asian and American genotypes of Dengue virus 2. Clin Vaccine Imunol. 2008;15(3):439–46. doi:10.1128/CVI.00208-07.

9. Avirutnan P, Zhang L, Punyadee N, Manuyakorn A, Puttikhunt C, Kasinrerk W, et al. Secreted NS1 of dengue virus attaches to the surface of cells via interactions with heparan sulfate and chondroitin sulfate E. PLoS Pathog. 2007;3(11):e183. doi:10.1371/journal.ppat.0030183.

10. Chen HC, Hofman FM, Kung JT, Lin YD, Wu-Hsieh BA. Both virus and tumor necrosis factor alpha are critical for endothelium damage in a mouse model of dengue virus-induced hemorrhage. J Virol. 2007;81(11):5518-26.

11. Medin CL, Fitzgerald KA, Rothman AL. Dengue virus nonstructural protein NS5 induces Interleukin-8 Transcription and Secretion. J Virol. 2005;79(17):11053–61.

12. Halstead SB. Observation related to pathogenesis of dengue hemorrhagic fever. VI. Hypothesis and discussion. Yale J Biol and Med.1970;42:350-62.

13. Ocazionez RE, Gómez SY, Cortés FM. [Dengue hemorrhagic fever serotype and infection pattern in a Colombian endemic area] [Article in Spanish]. Rev Salud Publica (Bogota). 2007;9(2):262-74. (abstract).

14. Deen J, Lum L, Martinez E, Tan LH. Clinical management and delivery of clinical services. WHO. Dengue guidelines for diagnosis, treatment, prevention and control. New edition. Geneva: WHO;2009. p. 23-55.

15. Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. J Infect Dis. 2002;186(8):1165-8.

16. Guzman MG, KouriG, Bravo J, Valdes L, Vazquez S, Halstead SB. Effect of age on outcome of secondary dengue 2 infections. Int J Infect Dis.2002;6:118–24.

17. Lee MS, Hwang KP, Chen TC, Lu PL, Chen TP. Clinical characteristics of dengue and dengue hemorrhagic fever in a medical center of southern Taiwan during the 2002 epidemic. J Microbiol Immunol Infect. 2006;39(2):121-9.

18. Pham TB, Nguyen TH, Vu TQ, Nguyen TL, Malvy D. Predictive factors of dengue shock syndrome at the children Hospital No. 1, Ho-chi-Minh City, Vietnam. [Article in French]. Bull Soc Pathol Exot. 2007;100(1):43-7. (Abstract).

19. Sierra B, Kourí G, Guzmán MG. Race: a risk factor for dengue hemorrhagic fever. Arch Virol. 2007;152(3):533-42.

20. Kalayanarooj S, Gibbons RV, Vaughn D, Green S, Nisalak A, Jarman RG, et al. Blood group AB is associated with increased risk for severe dengue disease in secondary infections. J Infect Dis. 2007;195(7):1014-7.

21. Soundravally R, Hoti SL. Polymorphisms of the TAP 1 and 2 gene may influence clinical outcome of primary dengue viral infection. Scand J Immunol. 2008;67(6):618-25.

22. Soundravally R, Hoti SL. Immunopathogenesis of dengue hemorrhagic fever and shock syndrome: role of TAP and HPA gene polymorphism. Hum Immunol. 2007;68(12):973-9.

23. Soundravally R, Hoti SL. Significance of transporter associated with antigen processing 2 (TAP2) gene polymorphisms in susceptibility to dengue viral infection. J Clin Immunol. 2008;28(3):256-62.

24. Nguyen TH, Nguyen TL, Lei HY, Lin YS, Le BL, Huang KJ, et al. Association between sex, nutritional status, severity of dengue hemorrhagic fever, and immune status in infants with dengue hemorrhagic fever. Am J Trop Med Hyg. 2005;72(4):370-4.

25. Kalayanarooj S, Nimmannitya S. Is dengue severity related to nutritional status? Southeast Asian J Trop Med Public Health. 2005;36(2):378-84.

26. Azeredo EL, De Oliveira-Pinto LM, Zagne SM, Cerqueira DIS, Nogueira RMR, Kubelka CF. NK cells, displaying early activation, cytotoxicity and adhesion molecules, are associated with mild dengue disease. Clin Exp Immunol. 2005;143: 345–56.

27. Chau TN, Quyen NT, Thuy TT, Tuan NM, Hoang DM, Dung NT, et al. Dengue in Vietnamese infants-results of infection-enhancement assays correlate with age-related disease epidemiology, and cellular immune responses correlate with disease severity. J Infect Dis. 2008;198(4):516-24.

28. Lin Y, Wang KJ, Lei HY, Lin YS, Yeh TM, Liu HS, et al. Virus replication and cytokine production in dengue virus-infected human B lymphocytes. J Virol. 2002;76:12242-9.

29. Mentor NA, Kurane I. Dengue virus infection of human T lymphocytes. Acta Virol. 1997;41:175–6.

30. Blackley S, Kou Z, Chen H, Quinn M, Rose RC, Schlesinger JJ, et al. Primary human splenic macrophages, but not T or B cells, are the principal target cells for dengue virus infection in vitro. J Virol. 2007;81 (24):13325–34.

31. Halstead SB. Antibody, macrophages, dengue virus infection, shock, and hemorrhage: a pathogenetic cascade. Rev Infect Dis. 1989;11:830–9.

32. King CA, Anderson R, Marshall JS. Dengue virus selectively induces human mast cell chemokine production. J Virol.2002;76(16):8408-19.

33. Chen JC, Ng MML, Chu JJH. Molecular profiling of T-helper immune genes during dengue virus Infection. Virol J. 2008; 5:165. (11pages). http://www.virologyj.com/content/5/1/165

34. Juffrie M, van der Meer GM, Hack CE, Haasnoot K, Sutaryo, Veerman AJP, et al. Inflammatory mediators in dengue virus infection in children: Interleukin-8 and its relationship to neutrophil degranulation. Infect Immun. 2000;68(2):702–7.
35. Srikiatkhachorn A, Ajariyakhajorn C, Endy TP, Kalayanarooj S, Libraty DH, Green S, et al. Virus-induced decline in soluble vascular endothelial growth receptor 2 is associated with plasma leakage in dengue hemorrhagic fever. J Virol. 2007;81(4):1592–600.
36. Khanna M, Chaturvedi UC, Sharma MC, Pandey VC, Mathur A. Increased capillary permeability mediated by a dengue virus-induced lymphokine. Immunol. 1990;69:449-53.
37. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med. 1977;146:201–17.
38. Kliks SC, Nimmanitya S, Nisalak A, Burke DS. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. Am J Trop Med Hyg. 1988;38:411–9.
39. Rodrigo WWSI, Jin X, Blackley SD, Rose RC, Schlesinger JJ. Differential enhancement of dengue virus immune complex infectivity mediated by signaling-competent and signaling-incompetent human Fc_RIA (CD64) or Fc_RIIA (CD32). J Virol. 2006;80:10128–38.
40. Moi ML, Lim CK, Kotaki A, Takasaki T, Kurane I. Development of an antibody-dependent enhancement assay for dengue virus using stable BHK-21 cell lines expressing Fc gammaRIIA. J Virol Methods. 2010;163(2):205-9.
41. Libraty DH, Acosta LP, Tallo V, Segubre-Mercado E, Bautista A, Potts JA, et al. A prospective nested case-control study of dengue in infants: rethinking and refining the antibody-dependent enhancement dengue hemorrhagic fever model. PLoS Med. 2009;6(10):e1000171.
42. Lin CF, Chiu SC, Hsiao YL, Wan SW, Lei HY, Shiau AL, et al. Expression of cytokine, chemokine, and adhesion molecules during endothelial cell activation Induced by antibodies against dengue virus nonstructural protein 1. J Immunol. 2005; 174: 395-403.
43. Tantracheewathorn T, Tantracheewathorn S. Risk factors of dengue shock syndrome in children. J Med Assoc Thai. 2007;90(2):272-7.
44. Honsawek S, Kongtawelert P, Pothacharoen P, Khongphatthanayothin A, Chongsrisawat V, Poovorawan Y. Increased levels of serum hyaluronan in patients with dengue infection. J Infect. 2007;54(3):225-9.
45. Butthep P, Chunhakan S, Tangnararatchakit K, Yoksan S, Pattanapanyasat K, Chuansumrit A. Elevated soluble thrombomodulin in the febrile stage related to patients at risk for dengue shock syndrome. Pediatr Infect Dis J. 2006 ;25 (10):894-7.
46. Trairatvorakul P, Chongsrisawat V, Ngamvasinont D, Asawarachun D, Nantasook J, Poovorawan Y. Serum nitric oxide in children with dengue infection. Asian Pac J Allergy Immunol. 2005;23(2-3):115-9.
47. Cardier JE, Rivas B, Romano E, Rothman AL, Perez-Perez C, Ochoa M, et al. Evidence of vascular damage in dengue disease: demonstration of high levels of soluble cell adhesion molecules and circulating endothelial cells. Endothelium 2006;13(5):335-40.