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Literature Review

CLINICAL MANIFESTATION APPROACH OF DENGUE VIRAL INFECTION

Ganis Tjahjono[1], Prihartini Widiyanti[1,3], Nasronudin[2a]
[1] Infectious and Tropical Disease Division - Department of Internal Medicine, Dr. Soetomo Hospital School of Medicine
[2] Institute of Tropical Disease - Universitas Airlangga
[3] Faculty of Science and Technology - Universitas Airlangga
[a] Corresponding author: nasronudindr@yahoo.com

ABSTRACT

Currently by an estimated 50-100 million dengue fever cases per year in worldwide, 500.000 were in the form of a disease is heavy Dengue Hemorraghic Fever (DHF) and Dengue Shock Syndrome (DSS). Survey serology in Indonesia show that DEN-1 and DEN-2 are the dominant serotipe virus until the end of the 1980s but the recent shift has occurred epizoötic where viruses DEN-3 dominant. (Dos Santos, 2004; Malavige, 2004; Stephenson, 2005). Dengue virus infection induces transient immune aberrant activation of CD4/CD8 ratio inversion and cytokine overproduction, and infection of endothelial cells and hepatocytes causes apoptosis and dysfunction of these cells. The aberrant immune responses not only impaire the immune response to clear the virus, but also result in overproduction of cytokines that affect monocytes, endothelial cells, and hepatocytes. Dengue-virus-induced vasculopathy and coagulopathy must be involved in the pathogenesis of hemorrhage, and the unbalace between coagulation and fibrinolysis activation, and prolonged duration of shock increase the likelihood of severe hemorrhage in DHF/DSS. Capillary leakage is triggered by the dengue virus itself or by antibodies to its antigen. To date, there are no effective strategies to prevent the progression of DHF/DSS. The control of dengue will be possible only after an efficient vaccine has been developed.

Key words: *aberrant immune, capillary leakage, hepatocytes, fibrinolysis, Dengue Shock Syndrome*

ABSTRAK

Saat ini diperkirakan sekitar 50-100 juta kasus demam berdarah per tahun di seluruh dunia, 500.000 adalah DBD. Survei menunjukkan bahwa serologi di indonesia dan yang dominan adalah DEN-1 DEN-2 serotipe virus hingga akhir tahun 1980-an tapi akhir-akhir ini telah terjadi pergeseran epizootic di mana virus DEN-3 dominan (Dos Santos, 2004; Malavige, 2004; Stephenson, 2005). Infeksi virus dengue yang aktif dapat menginduksi kekebalan tubuh dan rasio jumlah perbandingan CD4/CD8 dan kelebihan produksi cytokine, infeksi sel-sel endotel serta menyebabkan disfungsi hepatosit dan apoptosis sel-sel ini. Penyimpangan respon imun yang terkait tidak hanya respons kekebalan tubuh untuk membersihkan virus, tetapi juga mengakibatkan kelebihan produksi sitokin yang memengaruhi monosit, sel endotel, dan hepatosit. Virus dengue menginduksi vasculopathy coagulopathy dan terlibat dalam kegiatan patogenesis dari pendarahan, dan durasi shock sehingga dapat meningkatkan kemungkinan pendarahan yang parah pada DBD. Kebocoran kapiler terjadi karena virus dengue itu sendiri atau oleh antibodi terhadap antigen DBD. Tidak ada strategi efektif untuk mencegah perkembangan DBD, pengkontrolan DBD hanya mungkin setelah vaksin yang efisien telah berhasil dikembangkan.

Kata kunci: *Penyimpangan imun, kebocoran kapiler, hepatosit, fibrinolisis, Dengue Shock Syndrome*

INTRODUCTION

Dengue fever (DF) is an acute infectious disease, caused by dengue virus that have four type of serotipe (DEN-1, DEN-2, DEN-3 dan DEN-4). With traits that is spatially biphasic, fever myalgia, headache, pain in some part of the body, rash, limphadenopati and leucopenia. In most case, DF is self limited, but there is a risk of the development of being dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). DHF is a febrile disease with traits abnormality hemostasis and increase of vascular permeability as well as the development of progressive can be DSS. DSS is a condition shock hipovolemic, are clinically associated with hemoconcentration and can cause the death if adequate handling is not given. A mechanism that involved in pathogenesis hemorrhagic fever, a viral infection particularly regarding DHF/DSS unresolved.[1]

Currently, it has been estimated 50-100 million DF cases per year in worldwide, 500,000 were in the form of a disease is heavy DHF and DSS. Survey serology in Indonesia show that DEN-1 and DEN-2 are the dominant serotipe virus until the end of the 1980s but the recent shift has occurred epizoötic where viruses DEN-3 dominant.[2,3,4]

Based on data cases report in September 1998, cases DHF in adults (≥ 18) most widely is Jakarta (13,813), overtaken West Java (10,730), East Java (8,546), Central Java (6,879) and Jogjakarta (3,257).[5]

WHO is categorized dengue as one of the main international public health problem because wider geographical distribution both virus and its vector, the increased frequency of epidemics, co-circulation of various serotypes of the virus and the emergence of DHF in new places.[6]

Currently, there is no specific therapy against DHF. Provision of adequate fluid, can decrease mortality due to DHF. Control of the main vector (*Aedes aegypti*) costly and often ineffective is the only method of prevention is still available to this day. Therefore, the basic comprehension of the molecular pathogenesis dengue infection became very important for the development of diagnosis and therapeutic as well as facilitate the protective vaccine production, rather than aggravate the disease.[4,7]

The Body Response Against Infections Hemorrhagic Fever

After infected mosquito is bite human, replication of the virus in regional lymph gland and spreading to the lymphatic system, blood and other tissues. Replication in reticuloendotelial system and bark yield viremia.[6,8]

The immune system has a strong defense against bacteria or viruses invasion. The components that contribute to viral infections are the antibodies, phagocytes, IFN, NK cells and T cells. When a virus infects a cell, viral proteins are broken down into specific peptides were then expressed with the help of MHC-I molecules on the cell surface. Then the peptide will be known by Th1 cells which in turn activate effector cells or Tc CTC can destroy virus-infected cells by direct (lethal hit). NK cells have Fc receptors (Fcγ - R) plays a role in ADCC.[9]

Dendritic cells (DCs) (<1% of total blood cells in peripheral blood) derived from bone marrow, have a central role in the development of an immune response to both natural and adaptive. At least two subsets of myeloid DCs in humans, namely DCs (MDCS) and plasmacytoid DCs (PDCs). Both have strong antigen presentation capacity and capability stimulate Ag-specific T cell responses. PDCs are CD123+, with appropriate stimuli can migrate directly from the peripheral blood and lymphoid organs to limphonoduli. PDCs have the characteristics that the potential for a strong Ag presentation, lymphoid morphology and strongly stimulate the secretion of IFN-α -mediated stimulation of CD40 as a virus. So, MDCS and especially PDCs assist in anti - viral innate immunity and the formation of Th1 adaptive immune response against viral pathogens.[10]

Interferon response is the first line to inhibit viral infection. Interferon (IFN) is a cytokine that is secreted by the immune system is the first virus to infect target cells. IFN binds to the IFN receptor on the cell surface and activates the JAK target - STAT (transcription factors) signaling pathway to inhibit translation of viral RNA and protein synthesis, thus the virus will be eliminated. However, the dengue virus has its own strategy for tackling defense mediated by IFN. Dr. Eva Harris at the University of California, Berkeley, showed that treatment with IFN-α and IFN-γ before infection can reduce the replication of dengue virus in human cells. Same treatment when administered after infection have no effect at all, it shows that the dengue virus can suppress IFN signaling. Dr. Adolfo Garcia - Sastre, Mount Sinai School of Medicine, New York showed that the NS4B protein of dengue virus can inhibit the activation of STAT1 in cell culture systems, so this explains some of the mechanisms that can be suppressed IFN signaling through STAT1 barriers. Whether the same mechanism also occurs in vivo in patients with dengue, is still unclear. Although IFN and ribavirin combination therapy can be applied to HCV, which is classified with dengue virus (family Flaviviridae), is not effective when it comes to the treatment of dengue infection.[11,12]

Dengue virus infection causes lifelong imunity protective against the homologous serotype but only partial or temporary protection against subsequent infection by the other three serotypes. Already a generally accepted concept that secondary infection or multiple major risk factors occurrence of DHF/DSS as antibody - dependent enhancement. Other factors that play an important role in the pathogenesis of DHF have been formulated include the virulence of the virus, the genetic background host, activation of T cells and autoantibodies.[4,13]

CHARACTERISTIC OF DENGUE VIRUS

Dengue virus consists of a single strand of RNA included in the family Flaviviridae. The first discovered by Albert Sabin in 1944, there are four serotypes are classified according to biological and immunological criteria. The length of the viral genome is about 11 kb. Mature virion consists of three structural (core, premembrane and envelope) and seven non - structural proteins, namely NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS (Figure 1). Envelope protein required for various major biological functions for the virus, which binds to receptors on the surface of the host cell, allowing the virus to enter target cells. Envelop proteins are also associated with erythrocyte hemagglutination, induces the formation of antibodies and protective immune response. Non - structural proteins (NS1 - NS5) expressed as a membrane -associated and secreted forms also have an impact on the pathogenesis of severe disease. Unlike other viral glycoprotein, NS1 does not form a component of the virion but is expressed on the surface of infected cells. Levels of NS1 (sNS1) secreted in plasma associated with viral titers, being higher in patients with DHF compared with DF. Furthermore, increased levels of free sNS1 within 72 hours of the start of the illness, indicating the risk of patient become DHF. NS1 that very high levels of protein detected in the acute phase blood samples from patients with secondary dengue, rather than the primary infection. This suggests that NS1 has contributed to the formation of immune complexes in the circulation are likely to play an important role in the pathogenesis of severe dengue infection.[2,3,7,14]

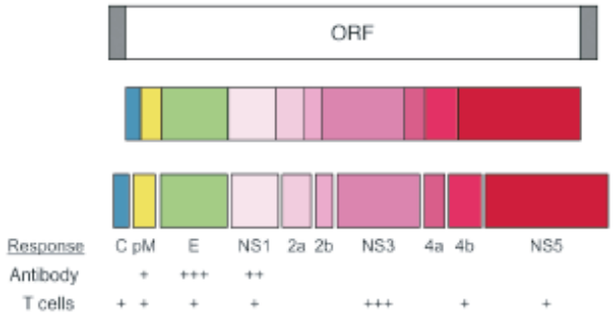

Figure 1. Dengue virus genome, a protein produced and the location of the main targets of the immune response.[7]

Latest Hypotheses About the Pathogenesis of Dengue Virus Infection

Several hypotheses on the pathogenesis of dengue virus infection have been proposed. Among these are the antibody-dependent enhancement (ADE) of infection, which has long been considered an important role. ADE hypothesis formulated to explain the finding that severe manifestations in DHF/DSS occurs when an infection to dengue virus with two different serotypes of dengue virus infection before. Antibodies are formed against a previous viral infection does not seem to be able to neutralize but rather aggravate the infection in vitro. Serum that taken from children before an infection which then progress to DHF/DSS further describe an ADE than just being DF. Epidemiological studies support an association between DHF/DSS with secondary dengue virus infections. Thus, ADE hypothesis is reinforced by the findings that: 1) Prospective cohort study of dengue epidemic, indicating that the majority of DHF/DSS occur during infection to two; 2) DHF/DSS in infants less classical than 1 year in the first dengue infection, the presence of antibodies to dengue virus factor of maternal circulation; 3) In vitro, non - neutralizing IgG antibodies can bind to dengue virus and attached to the Fc receptor and enhance dengue virus infection in monocytes or macrophages in the peripheral blood; 4) An increase in viremia was noted in vivo in animal models of rhesus macaques on secondary infection by DEN – 2; 5) In humans, high levels of viremia correlate with disease severity. It is not known how the addition of a viral infection caused by dengue enhancing antibodies can lead to DHF/DSS, is the proliferation of dengue virus infects or signal amplification through Fc receptors still require explanation. Due to the absence of animal model for DHF/DSS causal relationship between ADE with DHF/DSS is still no verification.[11,15,16,17]

Serotype crossreactive antibodies that resulting from previous infection binding with virions without neutralization and the increase of virus entry into monocytes, so that the number of virus-infected monocytes increased. As a result the number of activated T cells increased. This reflects the increased antigen presentation, increase in dengue - virus -specific T cells on subsequent infection and activation and proliferation faster than memory T cells. The T cells produce cytokines such as IFN-γ, IL - 2 and TNFα, as well as lysis of dengue virus -infected monocytes. TNFα is also produced by activated monocytes. Complement cascade is activated by a virus - antibody complexes and the release of several cytokines through C3a and C5a are also having a direct effect on vascular permeability. The synergistic effect of IFN-γ, TNFα and activation complement proteins trigger plasma leakage from endothelial cells in secondary dengue virus infections. However, some problems still can not be explained by this theory. Not all cases of DHF/DSS is secondary infection. Complement activation may be caused by severe disease and not the cause of DHF/DSS.[15,16]

Mongkolsapaya *et al* in 2003, did a study on the response of dengue virus specific T cells. In patients with DHF and DSS found some CD8+ T cells are virus - responsive most of undergoing apoptosis. This study is demonstrated the phenomenon of "original antigenic sin" that has been described a few years ago, the antibody responses to secondary viral infection is dominated by the activation of memory B cells cross reacting induced by primary infection. Activation of memory B cells will produce low affinity antibodies against the virus that causes secondary infections. So the levels of activated T cells with rapid death and domination of cellular immune responses by cells with low affinity to viral infections may inhibit viral clearance

and lead to high viral loads and increase imunopatologis (Mongkolsapaya, 2003; Stephenson, 2004). In late 2004, a study of 48 Vietnamese adults with secondary dengue virus infections showed that NS3 (epitopes targeted T cells) and T-cell responses cross-reactive responses do occur, but the magnitude was not significantly associated with clinical grading of disease.[18]

Virulence of Virus

The virulence of the virus, the ability to cause disease in the host, is an alternative hypothesis on the pathogenesis of DHF/DSS. Different manifestations of DF, DHF and DSS may be caused by dengue virus variants with different degrees of virulence. The risk for the occurrence of DHF/DSS higher in secondary infections with dengue virus serotype 2 when compared with other serotypes. Structural differences of the virus also found in isolates of DF and DHF patients. Furthermore, it has been reported that in viremia with high titers of dengue virus increases the severity of disease. Peak virus titer reached 100-1000 -fold higher in patients with DSS than in DF patients Thai children infected with dengue virus. Patients with secondary antibody response are likely to be DHF is twice than that has a primary antibody response. Evidence available to date do not all support the hypothesis of viral virulence.[15]

The analysis of individual genotypes in a single population showed little evidence that there are differences between the isolates DF patients with DHF/DSS, although exceptions have been reported. So the evidence of a link between viral genotype with clinical manifestations is still weak.[13]

Vulnerability Host

Some researchers have proposed the existence of predisposing DHF or DSS located on the human histocompatibility (HLA) haplotypes, but which locci that linked is still unclear or other genetic factors as the cause of a severe form of dengue is also unclear. Very few studies have been reported regarding the sensitivity of the host, especially regarding natural immunity. The latest scientific articles in Nature Genetics (Sakuntabhai, Nature Genetics, 2005) have reported the existence of genetic variants in the DC -SIGN promoter region is associated with severe forms of dengue infection. However, the molecular mechanism which in the genetic variants that affecting viral replication or trigger an excessive immune reaction on DHF and DSS still difficult to understand.[13,17]

Clinical and Pathological Manifestations of Dengue Virus Infection

DF symptoms include fever accompanied by headache, retroorbital pain, myalgia, arthralgia, race, mild leucopenia and thrombocytopenia. Pharyngeal hyperemia is almost in 97% of patients with DHF. Biphasic heat and the race was the most prominent characteristic of classical dengue fever. The symptoms will improve within 2–7 days. DHF is a syndrome of acute vascular permeability accompanied by abnormalities of hemostasis. Clinical features include plasma leakage, bleeding tendency and liver involvement. Capillary leak occurs rapidly in the period of a few hours, close to or at the time of the heat period ends when the classical DF symptoms subside. Pleural effusion, ascites, and hemoconcentration is indicative of a loss of intravascular volume. It can progress to shock if the patient did not receive fluid resuscitation. Manifestation hemorrhagic cranging from a positive torniquet test until spontaneous bleeding from the nose or gastrointestinal tract. Hemoconcentration and thrombocytopenia are the two main characteristic features of DHF/DSS. Liver involvement is common in dengue virus infection with a slight increase in serum transaminases. The liver is often enlarged, soft and slightly painful on palpation but usually no jaundice. Generalized lymphadenopathy obtained approximately in 50% of cases. Three organ systems (hematologic, vascular and hepatic) are involved in the pathological changes in DHF/DSS. Dengue virus infection causes dysfunction of the system either directly or indirectly, lead to the manifestation of DHF/DSS.[1,8,14]

Effects of Dengue Virus Infection on Blood Cells

Immune aberrant activation during dengue virus infection

In the analysis of blood samples collected during the outbreak of dengue virus serotype 3 from November to December 1998 in Southern Taiwan describe a disorder of immune status in patients with dengue. In the peripheral blood of uninfected persons, the number of CD4 + T cells more than CD8+. In the peripheral blood of patients with dengue, the number of CD8 + T cells more than CD4+, so the CD4/CD8 ratio decreased < 1. This phenomenon is not only found in patients with DHF/DSS alone, but also on the patient DF. Of the 21 patients with DF and 8 patients with DHF/DSS, found CD4/CD8 ratio is inverted in 10 cases. The frequency of CD4/CD8 ratio is inverted is higher in DHF/DSS (5/8) of the DF (5/21, $p < 0.05$). Kinetic Analysis imunofenotip CD4 + T cells and CD8+, indicating that the CD4/CD8 ratio reverse occurs during acute infection (6-14 days after the onset of heat). CD4/CD8 ratio is slowly returning to normal after a day to 15. It also found $CD4^{dim}$ monocytes and CD8dim, the percentage in peripheral blood mononuclear cells (PBMCs) were higher or highest on days 6–7, then down to the lower level healing moment later. Atypical lymphocytosis reached a peak on days 8-10, and then disappeared rapidly after day 12. Initial activation of mononuclear cells was confirmed by expression of the early activation marker, CD69, on day 4 after the onset of heat. CD69 stained on the surface of lymphocytes, as well as on monocytes, but more widely expressed in CD8+ T cells of the CD4+. With the description of atypical lymphocytosis and dynamic changes in CD4/CD8 ratio, an indication of the occurrence of immune activation during dengue virus infection aberan.[1,8]

Excessive Cytokine Production During Dengue Virus Infection

During acute dengue infection, mononuclear cells

activated excessively so expected that increased levels of cytokines can be found in the plasma. High levels of markers of T-cell activation such as soluble IL-2 receptor, soluble CD4, soluble CD8, IL-2 and IFN-γ, as well monokin eg TNFα, IFN-β and GM - CSF, all detected in children infected with dengue, and markers are higher in patients with DHF/DSS than in DF. High levels of cytokines in the serum inhibitors such as IL-10 or soluble receptors (soluble TNF receptor) sTNFRI and sTNFRII were also found in patients with DHF. In dengue patients in the study, cytokines such as RANTES, IL-8 and IL-6 levels are increased after infection with dengue virus. Levels of IL-6 and IL-8 in serum were higher in patients with DHF/DSS than in DF patients. IL - 6 has a dual role, as a mediator of pro-inflammatory or anti - inflammatory. In the kinetic analysis showed large variations at points different times and in different individuals, but increased serum levels of IL-6 high transient occurs either at day 7 or day 9-11 after the onset of heat. It is impressive when the host response to dengue virus infection through the formation of cytokines pro - inflammatory cytokines also formed simultaneously inhibitor against inflammation. The end result depends on the balance between the two.[1,19]

Thrombocytopenia and anti - platelet pntibodies

Thrombocytopenia is common in DF, and always found in DHF/DSS. Its pathogenesis is still poorly understood. Impressed that the dengue virus triggers bone marrow suppression that reduces platelet production and resulting in thrombocytopenia. One group of studies found that dengue virus-2 can bind to platelets in the presence of specific viral antibodies and this supports the role of immune-mediated clearance of platelets. Surprisingly, found IgM (not IgG) anti-platelet autoantibodies in patients with dengue. Titer was higher in DHF/DSS than in DF patients. The presence of these autoantibodies is not only triggered by complement lysis of platelets but also inhibit ADP-induced platelet aggregation. Cross-reactions of antibodies against dengue virus proteins, particularly NS1 and platelets, suggesting a role for the pathogenesis of anti-platelet autoantibody during dengue virus infection.[1,15]

Immune Deviation Caused Dengue Virus Infection

Patients infected with dengue usually experienced leucopenia for a few days during the acute infection, with the characteristics of a decrease in the absolute number of neutrophils and monocytes. Impaired T cell responses associated with PHA-stimulated monocytes $CD4^{dim}$ or $CD8^{dim}$ deficiency. Detection of early activation marker, CD69 on $CD8^{+}$ T cells, NK cells and monocytes, and lymphocytes are atypical shapes showed activation of lymphocytes by dengue virus infection. Dengue virus can infect Langerhans cells or immature dendritic cells and can replicate more efficiently in these cells than in monocytes or macrophages. Infected dendritic cells stimulate maturation cytokine production and TNFα and IFNα, but not IL-6 or IL-12. Levels of IL-12 in patients with higher DF than in DHF. Blunted blood PDC response to dengue virus infection associated with higher levels of viremia and is part of the natural immune response and changes cascade causes severe disease pathogenesis. In patients with DHF stage III and IV, not detected the presence of IL-12. Production deficiency of IL-12 can cause a shift to a Th2 response and mismatch generation of cytotoxic T cells. Dengue virus infection appears to strongly influence the immune response such as, monocytosis $CD4^{dim}$ or $CD8^{dim}$ early, inversion of CD4/CD8 ratio were temporary, atypical lymphocytosis with a large percentage and depressed T-cell proliferation. Immune deviation is not only slow viral clearance, but also triggers excessive production of cytokines and anti- platelet autoantibodies that started the sequence of the pathogenesis of dengue virus infection.[1,3,10]

Effects of Dengue Virus Infection of Endothelial Cells

Vasculopathy due to dengue virus

Overview of the most distinctive and best indicator of disease severity is plasma leakage. Plasma leakage due to increased capillary permeability in a diffuse manner and manifest as hemoconcentration, pleura effusion or ascites. It usually occurs on days 3-7, during the current easing of dengue fever. Plasma leakage occurs systemically, developing progressive, but it will get better in 1-2 days in patients receiving fluid resuscitation adequately. No tissue or organ function abnormalities occur. Although edema perivaskuler seems obvious, but no evidence of damage to vascular endothelial cells. Dengue virus can infect endothelial cells in vitro led to the release of cytokines and chemokines IL-6, IL-8 and RANTES. Dengue virus infection can cause endothelial cell apoptosis in vitro, but the effect is directly dependent on the dengue virus isolates were used. Endothelial cells were infected with dengue virus can activate complement and induce the expression of adhesion molecules such as ICAM-1. The expression of ICAM-1 along with the production of IL-8 and RANTES would invite polymorphonuclear cells, mononuclear cells, and then finally freed vasopermeability and trombomodulin, a marker of endothelial damage. Increased levels of circulating trombomodulin in the acute stage of DHF/DSS, indicating that the structural failure of endothelial cells in vivo. Apparently, direct viral cytopathic effects and immune -mediated leukocyte recruitment and anti - dengue virus antibodies, both of which cause structural damage to endothelial cells. Vascular leakage can be caused by infection with dengue virus, both directly and indirectly. Endothelial cells play an important role in maintaining hemostasis, therefore, endothelial cell damage due to dengue virus infection can affect either the balance of procoagulant or anticoagulant endothelium that increase the risk of bleeding. Recruitment of platelets by activated endothelial cells may also cause thrombocytopenia.[1,3]

Coagulopathy Due to Dengue Virus

Hemorrhagic manifestations caused by the dengue virus, which is more common is vascular - platelet abnormalities, but when severe bleeding can occur with

DIC. Hemostasis is maintained by a balance between coagulation and fibrinolysis. Coagulation system can be activated by the intrinsic and extrinsic pathways to form thrombin converts fibrinogen to fibrin. Fibrinolytic system on the other hand can break down fibrin. Fibrinolytic system consists of plasminogen, a proenzyme that can be turned into an active enzyme plasmin by plasminogen activators several kinds. Plasminogen activator is a major endogenous tissue -type plasminogen activator (tPA). Plasminogen activator inhibitor (PAI-1), which is produced by platelets, and endothelial liver, on the other hand is a major inhibitor of tPA. In general, secondary coagulation activation trigger fibrinolysis activation that inhibits the release of large amounts of PAI-1 rapidly.

During acute dengue virus infection, coagulation parameters such as platelet count, activated partial thromboplastin time (APTT), as well as fibrinolytic parameters tPA and PAI-1 changes. APTT elongated, while tPA increased. Both coagulation and fibrinolysis are both activated and this activation is more severe in patients with DHF/DSS than in DF. After healing increased levels of PAI-1 and the platelet count in line with decreased levels of tPA and APTT returned to normal. The ratio of tPA/PAI-1 was higher in patients with DHF/DSS than in DF patients. Elongation of APTT and increased the ratio of tPA/PAI-1 in the acute phase of dengue virus infection associated with disease severity and can be used as an early indicator of DHF/DSS. APTT and prothrombin time is an indicator of intrinsic and extrinsic pathways of coagulation. Only APTT, not prothrombin time that extending to dengue virus infection, suggesting that abnormalities in the intrinsic pathway. This can be caused by a decrease in the synthesis of specific factors or the increased use of specific factors. Disorder of liver function is responsible for the reduction in the synthesis of specific factors in the intrinsic pathway. Increased use of these factors as indicated by high levels of tPA is also associated with prolongation of the APTT but less meaningful. Thus, both a decrease in the synthesis or increased consumption of coagulation factors, both involved in the lengthening of APTT.

Hiperfibrinolisis in the acute phase of DHF/DSS, due to the increased production of tPA. Correlation and linear regression analysis showed a significant association between IL-6 serum with tPA in DHF, but not in the dengue fever. Dengue virus infection stimulates endothelial tPA and to produce IL-6. Synthesis of tPA could be blocked by anti-IL-6 antibody, suggesting that tPA production by endothelial cells is dependent IL-6. Furthermore, antibodies against dengue virus E protein, can bind to human plasminogen. It can inhibit the action of plasmin or plasminogen activation strengthens. So, both coagulation and fibrinolysis are experienced hyperactivation in the acute phase of dengue virus infection. Imbalance between coagulation and fibrinolysis may cause bleeding in DHF/DSS.

Bleeding that occurs in DHF/DSS is not directly due to the prolongation of the prothrombin time and partial thromboplastin time, nor is it just because of thrombocytopenia. The most powerful risk factor causes bleeding is the presence of prolonged shock. It shows that patient with a long shock, there has been a leakage of plasma and bleeding.[20]

Effects of Dengue Virus Infection in Liver Cells

Dengue virus is hepatotrophic. Dengue virus antigen was detected in the liver cells and virus particles found in liver biopsy specimens from patients with DHF. Dengue virus can infect the liver and cause hepatitis. Increased levels of serum transaminases in patients with dengue and degree of elevated levels of *aspartate aminotransferase* (AST) associated with bleeding events. In hepatitis due to dengue virus, the AST level higher than *alanin aminotransferase* (ALT) with a ratio of about 1-1.5, while hepatitis due to other viruses, ALT is higher than AST. By using hepatoma cell lines, dengue virus can lead to apoptosis and chemokine RANTES production through oxidative stress and activation of NF-κB. Furthermore, RANTES is typically caused by the dengue virus but not by enteroviruses in the liver cells. Patients with dengue virus infection had serum levels of RANTES were higher compared with other viral infections. RANTES is a chemokine that can lead to the recruitment of lymphocytes and NK cells to sites of inflammation. Liver is damage caused by dengue virus was due to a direct effect of viral replication or indirect effects of inflammation mediated by RANTES, still require further investigation. The balance between virus elimination and tissue damage may result in disease severity. It is known that the liver is where most of the production of coagulation factors, the decreased levels of these factors is attributed to an increase in consumption, as well as impaired synthesis. The last thing is more likely due to injury to the liver. IL-6 can lead to down regulation of the synthesis of factor XII, is the first factor that initiate clotting through the intrinsic pathway. Elongation of *activated partial thromboplastin time* (APTT) in patients with DHF are caused by a deficiency in the intrinsic pathway is likely due to impaired synthesis in the liver factor XII.[1,3,14]

SUMMARY

Dengue virus infections causes dengue fever (DF), dengue hemorrhagic fever (DHF) dan dengue shock syndrome (DSS). Current hypotheses antibody-dependent enhancement, virus virulence, and IFN-γ/TNFα –mediated immunopathogenesis are insufficient to explain clinical manifestations of DHF/DSS such as thrombocytopenia and hemoconcentration. Dengue virus infection induces transient immune aberrant activation of CD4/CD8 ratio inversion and cytokine overproduction, and infection of endothelial cells and hepatocytes causes apoptosis and dysfunction of these cells. The coagulation and fibrinolysis systems are also activated after dengue virus infection. The aberrant immune responses not only impaire the immune

response to clear the virus, but also result in overproduction of cytokines that affect monocytes, endothelial cells, and hepatocytes. Platelets are destroyed by crossreactive anti-platelets antibodies. Dengue-virus-induced vasculopathy and coagulopathy must be involved in the pathogesis of hemorrhage, and the unbalance between coagulation and fibrinolysis activation, and prolonged duration of shock increase the likelihood of severe hemorrhage in DHF/DSS. The overproduced IL-6 might play a crucial role in the enhanced production of anti-platelet or anti-endothelial cell autoantibodies, elevated levels of tPA, as well as a deficiency in coagulation. Capillary leakage is triggered by the dengue virus itself or by antibodies to its antigen. To date, there are no effective strategies to prevent the progression of DHF/DSS. The control of dengue will be possible only after an efficient vaccine has been developed.

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