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Original Article

Dengue hemorrhagic fever — A systemic literature review of current perspectives on pathogenesis, prevention and control

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Abstract *Background:* Dengue is an arboviral disease caused by dengue virus. Symptomatic dengue infection causes a wide range of clinical manifestations, from mild dengue fever (DF) to potentially fatal disease, such as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). We conducted a literature review to analyze the risks of DHF and current perspectives for DHF prevention and control.
Methods: According to the PRISMA guidelines, the references were selected from PubMed, Web of Science and Google Scholar database using search strings containing a combination of terms that included dengue hemorrhagic fever, pathogenesis, prevention and control. Quality of references were evaluated by independent reviewers.
Results: DHF was first reported in the Philippines in 1953 and further transmitted to the countries in the region of South-East Asia and Western Pacific. Plasma leakages is the main pathophysiological hallmark that distinguishes DHF from DF. Severe plasma leakage can result in

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hypovolemic shock. Various factors are thought to impact disease presentation and severity. Virus virulence, preexisting dengue antibodies, immune dysregulation, lipid change and host genetic susceptibility are factors reported to be correlated with the development of DHF. However, the exact reasons and mechanisms that triggers DHF remains controversial. Currently, no specific drugs and licensed vaccines are available to treat dengue disease in any of its clinical presentations.

Conclusion: This study concludes that antibody-dependent enhancement, cytokine dysregulation and variation of lipid profiles are correlated with DHF occurrence. Prompt diagnosis, appropriate treatment, active and continuous surveillance of cases and vectors are the essential determinants for dengue prevention and control.

Introduction

Dengue is one of the most common tropical diseases affecting humans. Dengue has become a major international problem in public health in recent decades. The World Health Organization (WHO) estimates that around 2.5—3 billion people are presently living in dengue transmitted zones. Dengue is an acute febrile disease triggered by an infection with dengue virus (DENV). DENVs are single positive-stranded RNA *flaviviruses*, members of the *Flaviviridae* family. This virus has four major serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Human become infected with dengue through the bite of DENV-carrying female *Aedes* mosquitoes, including *Aedes albopictus* and *Aedes aegypti*. Subsequent infection with distinctive serotype of DENVs has been associated with increase the risk of severe complications.

Clinically, the manifestations of DENV infection can range from mild-acute undifferentiated febrile illness to classical dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) according to WHO 1997 dengue guideline[1] (Fig. 1). DF is an acute febrile illness that presents symptoms such as bone or joint and muscular pains, headaches, leukopenia and rash. DHF has four major clinical manifestations: severe fever, hemorrhage, often with hepatomegaly and, in severe cases, circulatory failure.[2] Some of the infected individuals may develop hypovolemic shock which is a result of severe plasma leakage (Table 1). In clinical, some chronic disease were reported to trigger disease severity of dengue and DHF occurrence.[2—4] The geographical circulation of both vector and DENVs has led to the worldwide resurgence of epidemic DF and emergence of DHF in the past decades, leading to hyperendemic in several urban human populations of the tropics. WHO suggested a revised guideline which classified dengue disease into dengue and severe dengue.[5,6] To remain consistent with the reviewed publications, we use WHO 1997 criteria in this paper.

Dengue or dengue-like illness was firstly reported in 1780 in Madras, India, whereas the first virologically proven epidemic of DF in India occurred in 1963—1964 in Calcutta and Eastern Coast of India.[7] DHF, a severe syndrome developed from DF patients was first reported in the Philippines in 1953.[8] DHF was proposed to be caused by multiple DENVs infections owing to the isolation of different serotypes (DENV-2, 3 and 4) in patients in the Philippines in 1956. The multiple DENVs infections were also isolated from patients during an epidemic in Bangkok, Thailand in 1958.[9] After that, DHF was gradually identified in many countries such as Cambodia, China, India, Indonesia, Malaysia, Myanmar, Singapore and several Pacific Island. The mechanisms and pathogenesis of DHF are still yet fully understood. Currently proposed risk factors correlated with DHF include virus virulence,[10] immune enhancement,[11] cytokine storm,[12] change of lipid profile,[13] autoimmune responses,[14] host genetic factors,[15] bacteremia caused by *Staphylococcus aureus*[16,17] etc. (Fig. 2). Especially, immune enhancement was described from the trial report of the recombinant tetravalent dengue vaccine (CYD-TDV) in Asia and Latin America indicating that vaccinating children without prior infection (seronegative) may mimic an initial infection during the first step in developing antibody dependent enhancement (ADE). In addition, accurate and prompt diagnosis for DENVs infection as well as early detection of the potential DHF are urgently demanded clinically.[18—20]

Currently, there is still a lack of specific anti-viral drug as well as licensed vaccine for treatment and prophylactics against DENVs infection. DF and DHF remains a serious public health problem globally. DHF was recently reported in several dengue outbreaks and resulted in high mortality. Clinically, DHF is a serious threat, however the reasons for the occurrence of this disease are still unknown owing to its etiological complexity. In this review, we summarize and discuss findings of the mechanisms correlated with DHF development. We also demonstrated the current perspectives on prevention and control of DHF. This study provides important insights and viewpoints regarding the pathogenesis of DHF as well as prevention and treatment measures.

Methods

In this study, we summarize the updated information of pathogenesis, prevention and control of DHF. The study was

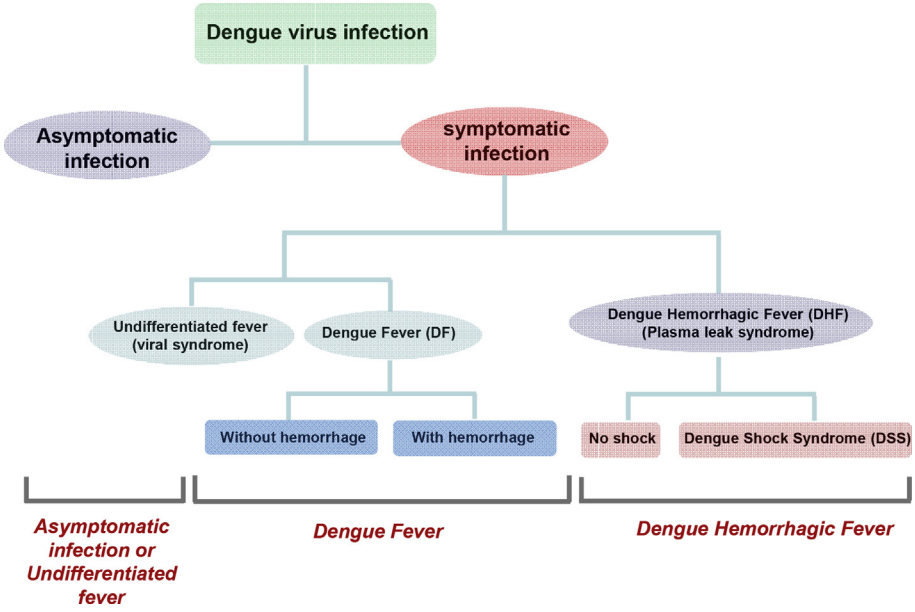

Figure 1. Flow chart of the classification of dengue infection and clinical presentation. The classification of DENVs infection were illustrated according to WHO 1997 Dengue hemorrhagic fever: Diagnosis, Treatment, Prevention and Control, 2nd edition.

according to the PRISMA guidelines to conduct a systemic literature review. Selected references were from PubMed, Web of Science and Google Scholar database using search strings containing a combination of terms that included dengue hemorrhagic fever, pathogenesis, prevention and control. Two independent reviewers evaluated the level of data quality from the selected literatures. Disagreements were resolved by joint discussion and consensus. Ethics approval and informed consent were not required for this study. The systemic search covered publication dates from 1987 to 2019. The systemic literature review of article selected flow chart was shown in Fig. 3.

Table 1 The clinical symptoms of dengue infection.

Category	Symptoms	Duration
Dengue fever (DF)	• "Flu-like" syndrome • Retro-orbital pain • Fever • Rash • Intense headache • Intense joint and muscle pain • Nausea	2—7 days
Dengue Hemorrhagic Fever (DHF)	• Plasma leakage • Pleural effusion, bleeding • Thrombocytopenia with $<$100,000 platelets/μL • Raise in hematocrit levels • Restlessness • Abdominal pain • Vomiting • Sudden drop in temperature	After 3—5 days of fever
Dengue Shock Syndrome (DSS)	• Temperature reaches 37.5 °C—38 °C • Hypotension • Decrease in platelet count leads to leakage of plasma subsequent shock • Fluid accumulation with respiratory distress • Critical bleeding • Organ impairment • Cardiorespiratory failure and cardiac arrest	After 3—5 days of fever

The disease classification was according to 1997 WHO dengue guideline.

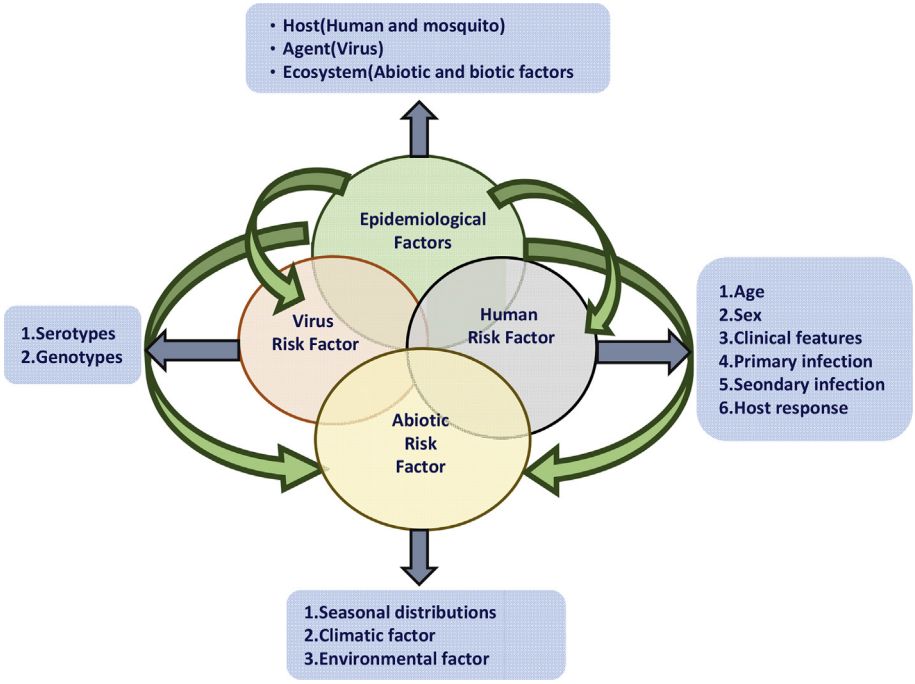

Figure 2. The risk factors correlate with dengue hemorrhagic fever. The risks factors including viral factors, epidemiological factors, human factors and abiotic factors correlated with DHF development were presented.

Results

Disease occurrence and pathogenesis

Several hypotheses are proposed to explain the reasons for DHF occurrence. These include changes of viral virulence, genetic susceptibility, cytokine storm, variation of lipid profile and immunological enhancement. Although some DHF patients have been reported without previous DENV-exposure, majority of the cases are seen in individuals infected with at least two different serotypes. Hemorrhage in dengue patients can be produced by multiple phenomena such as thrombocytopenia (abnormal low levels of platelets), coagulopathy (impaired coagulation), and disruption in the epithelial cells as well as disseminated intravascular coagulation (DIC). Here, we summarized the findings and perspectives correlated with DHF occurrence, mainly focusing on the effects of preexisting dengue antibodies enhancement, cytokine dysregulation and the changes of lipid profiles.

Antibody-dependent enhancement

Dengue generally produces a self-febrile illness that lasts between 2 and 7 days. Most of the patients are able to fully recover after the febrile period, however, some patients are unfortunate and enter the critical stages of dengue. The critical stage of dengue can lead to mortality if not treated properly in time. While the exact reason for the severity is still unknown, studies have linked the severity to Antibody dependent enhancement (ADE) (Fig. 4). This theory was first described in 1964, when it was observed that serious DENV infection was linked to secondary infection.[21] After a primary infection with a DENV serotype, the immune system produces antibodies that bind and neutralize secondary infection with the same serotype, however, a secondary heterotypic infection may lead to enhance severity. The antibodies produced from the primary infection have the ability to bind to the virus but lack the ability to neutralize. These cross-reactive antibodies form infectious virus-antibody complexes are able to bind and enter in cells that present the Fcγ-receptors such as, monocytes, macrophages, and dendritic cells, therefore, enhancing viral production leading to higher viral loads (Fig. 4).[22,23]

Studies utilizing plasma of DENV infected children who were hospitalized in Thailand linked the disease severity with higher viremia due to secondary DENV infection. They also reported that DENV-2 was associated with higher severity of disease during secondary infection compared to the secondary infection with any other DENV serotype[23,24] Furthermore, these observations were confirmed in studies done in Cuba which indicated that secondary DENV infection lead to higher viral load and to increase severity.[25–27] Dengue ADE can be validated *in vitro* using cells that express Fcγ and has demonstrated to produce higher viral loads in both mice and non-human primate models.[28] ADE has also been reported to enhance the severity of disease in infants with primary dengue infection during the first year of life when the levels of maternal dengue-specific antibodies reach the sub-neutralizing levels.[29–32] Overall, secondary heterotypic DENVs infection via ADE is considered a major risk factor that drives the disease severity. This phenomenon should be considered in development of an effective dengue vaccine, as the vaccine wishes to generate the protection against all four serotypes via giving four serotype antigens in a same shot.

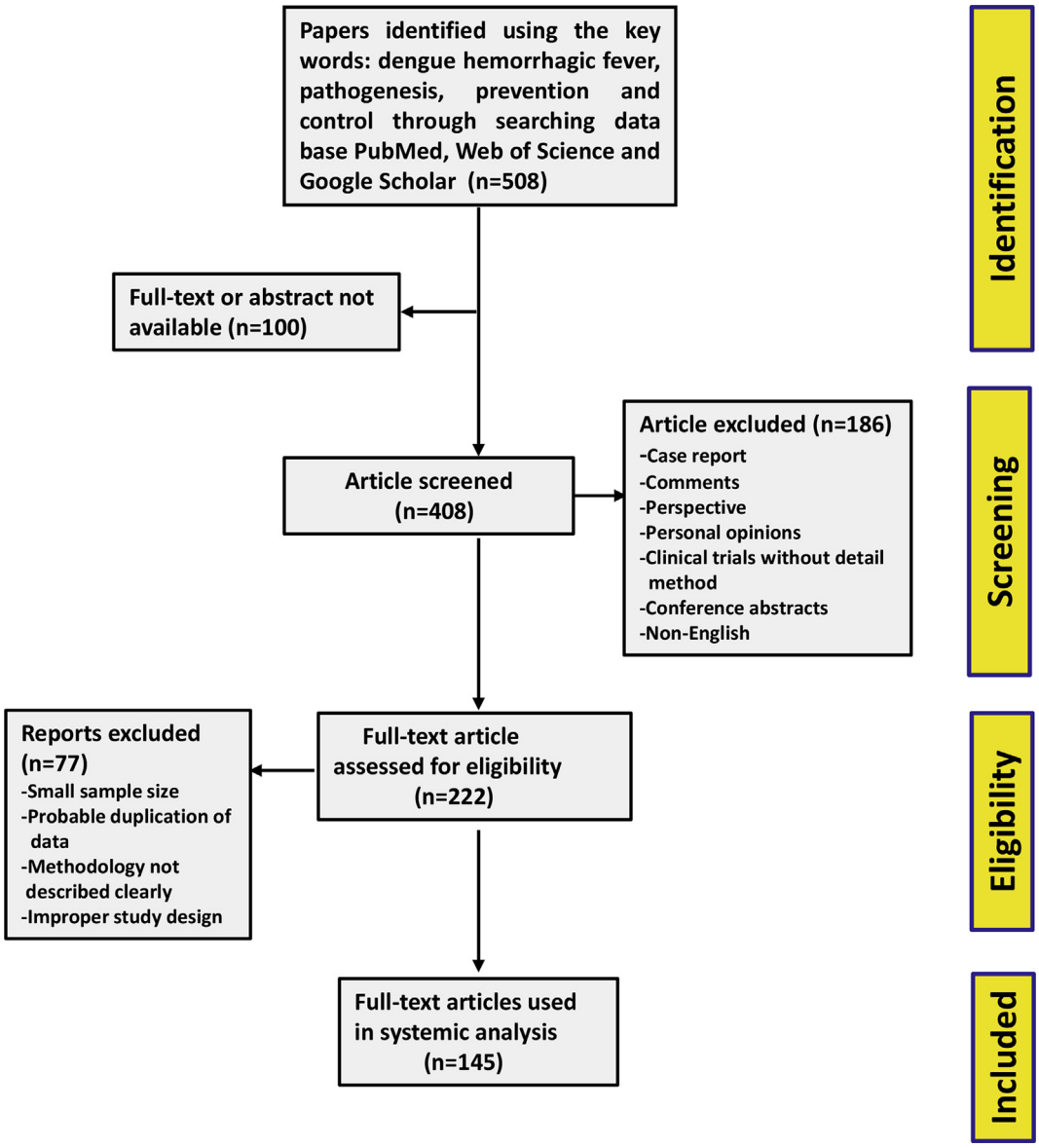

Figure 3. The flow chart of the criteria and selection guideline for included literature in this systemic review.

Cytokine dysregulation

In the development of DHF, DENV antibodies may be unable to neutralizing and alternatively promote the entrance of a second serotype of DENV into Fcγ-expressing cells, which results in amplified activation of complements and rapidly produces cytokine, especially pro-inflammatory type 1 cytokines, such as TNF-α and IFN-γ. These cytokines may have a direct effect on vascular endothelial cells leading plasma leakage. However, these cytokines produced in different phase of DENVs infection result in different outcomes. Previous reports indicated that in acute phase, DENV-specific T cell responses were greater in individuals with DF, in comparison to those with DHF. Additionally, early presence of DENV-specific T cell IFN-γ responses were significantly associated with milder clinical disease.[33] Several reports have shown the active interaction of cytokines during DENV infection from the acute phase leading to the presentation of DHF, suggesting a temporal association between cytokines and the plasma leakage severity. These immune mediators have various functions, including anti-inflammatory (IL-1RA), chemotactic (IP-10), growth factor (HGF), soluble receptor (sTNFRp75), adhesion (VCAM-1), and enzymatic (MMP-2) activities. A few have proven to be prognostic markers for disease severity or reported to be potent permeability enhancing cytokines: IFN-γ, TNF-α, IL-6, IL-8, VEGF-A, IL-2 and RANTES. However, besides these cytokines, immune mediators such as IL-1RA, MCP-1, TGF-β, HGF, IP-10, and sTNFRp75 are also associated with significant plasma leakage.[34] In addition, growth-regulated oncogene-alpha (GRO-α), a member of the CXC family, which plays an integral role in recruitment and activation of neutrophils in response to tissue injury and microbial infection, was reported to correlate with platelet counts and good prognosis. While higher Interferon induced

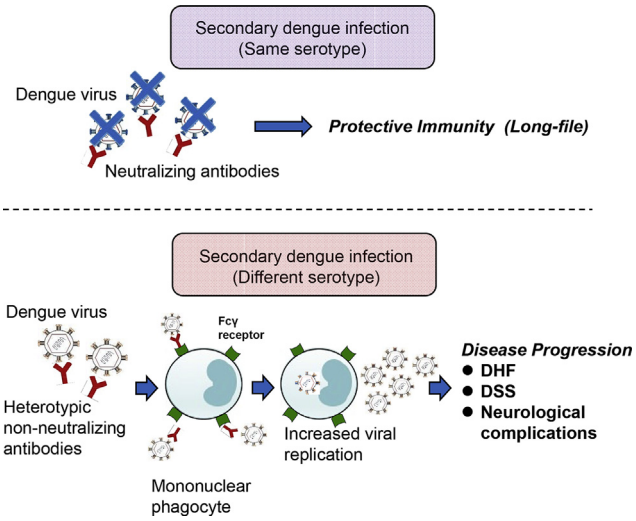

Figure 4. The mechanism of antibody-dependent enhancement of dengue virus infection. Primary DENVs infection induces long life protective antibody which can neutralize same serotype of DNEVs (upper). In secondary heterotypic DENVs infection, the previous anti-DENVs antibodies cross-react with the heterotypic DENVs and form immune-complex. The virus-antibody immune complex interacts with Fcγ receptors, which are mainly expressed on macrophage or phagocytes. The heterotypic DENVs propagate inside the Fcγ-expressing immune cells and further enhance the viral infectivity (lower).

Protein-10 (IP-10) was negatively correlated with total protein levels, contributing to the severity in DHF patients.[35]

Cytokine profiles were recently suggested as disease markers for DENV infection. A study performed in peripheral blood mononuclear cells (PBMC) from subclinical DENV patients showed significant differences in cytokine production based on the clinical manifestation. Secretion of IL-15, MCP-1 and IL-6 from PBMC of individuals that developed symptomatic DENV disease was noted. However, secretion of IL-12, IL-2R, macrophage inflammatory protein (MIP-1α), RANTES, Granulocyte-macrophage colony-stimulating factor (GM-CSF), and TNF-α was found in mild or non-dengue syndrome patients.[36,37] In addition, DENV non-structural Protein 1 (NS1) was reported to stimulate the necrosis of human endothelial cells via induction of migration inhibitory factor (MIF). MIF can enhance the secretion of heparanase-1 (HPA-1) and matrix metalloproteinase-9 (MMP-9) which resulting in endothelial glycocalyx degradation and hyper permeability.[38] Targeting MIF may represent a possible therapeutic approach for preventing dengue-induced vascular leakage. In addition, interleukin-33 (IL-33), a pleiotropic cytokine with pro-inflammatory effects was demonstrated to play a disease-exacerbating role in DENV-2 infection and increase pathology. Secretion of IL-33 is probably driven by CXCR2-expressing cells and reduction of IL-33 effects was observed by simultaneously treating with a CXCR2 receptor antagonist (DF2156A).[39]

Currently, several assays and models *in vitro* or *ex vivo* have been established for the evaluation of cytokine effects on DENV infections. It has been recommended an approach using cytokine-producing circulating cells (CPCCs) at single cell level in infection with DENV infection in clinical symptoms ranging from mild to severe disease. Studies using CPCCs indicated that TNF-α and IL-6 CPCCs were identified in primary or secondary acute DENV infection. Monocytes, B cells, and myeloid dendritic cells (mDCs) were the primary CPCCs, and the frequency of mDCs was significantly higher in CPCCs from DHF. Thus, CPCCs could be a new immune parameter with potential use to evaluate pathogenesis in this infection.[40] Regarding dengue *in vivo* model, utilizing animal models for studying dengue disease is challenging, as DENV is not known to infect non-human species naturally. Despite dengue viral replication and immune reaction being seen in non-human primates, they do not have a development of disease. However, certain humanized and immunodeficient mouse models, such as the AG129 mice, infected with mouse-adapted DENV strains showed symptom of severe disease similar to the symptoms observed in humans experiencing DHF. In addition, rhesus macaque model has been established for the use of DENV studies.[41,42] The macaques were infected intravenously with DENVs and in 3—5 days after infection developed classical dengue hemorrhage.[41] Increased MCP-1, IFN-γ and VEGF-A levels, and transiently decreased IL-8 levels were detected in rhesus macaques post infection and the cytokine profiles were similar with humans.[42]

Changes of lipid profile

During DENVs infection, lipoproteins are known to have a pathophysiological role in the immune response. Cytokine-induced alterations in the plasma lipid profile are a potential predictor of clinical outcome in DHF.[13] Changes of lipid profiles, such as total plasma cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were correlated with DHF.[13] Cholesterol as well as lipid rafts have been reported to require elements for promoting DENV entry and the signaling in many human cells.[43] The severity of dengue is associated with an increase of the inflammatory response characterized by the presence of pro-inflammatory cytokines and products of inflammasome activation such as IL-1β/IL-18. Additionally, it has been found alterations in the levels of low-density lipoproteins (LDL) and high-density lipoproteins (HDL). It is well known that HDL have immunomodulatory properties such as regulation of inflammasomes, thus these are expected to counteract hyperactivation of the inflammasome.[44] Moreover, significant positive correlations between the relative expression of IL-1β and IL-18, NLRP3, NLRC4 and LDL levels, suggest an association of LDL and HDL alteration with the imbalance in the inflammatory response, which may be correlated with the severity of dengue.[45] Lipid alterations have been observed mainly in patients developing the DHF. The increase of triglycerides (TG), very-low-density lipoproteins (VLDL) and HDL, and the decrease of total cholesterol (TC) and LDL have been observed in DHF.[46] The lowest cholesterol and VLDL levels have been found in DHF, and the mean cholesterol level is significantly lower in expired dengue patients, suggesting their correlation with severe bleeding and hepatic dysfunction.[47] In addition, low-density lipoproteins cholesterol (LDL-C) levels are associated with subsequent risk of developing DHF and severity of plasma leakage has been shown association with elevated

levels of lipopolysaccharides (LPS).[48,49] Another report indicates that polyunsaturated fatty acids (PUFA) might modulate inflammatory responses involved in the development of DHF/DSS.[50] Further, the mechanism of DENV-induced elevation of total cholesterol and lipid rafts formation was validated *in vitro* using Huh-7 cells and indicated that DENV infection increased cell surface quantity of low density lipoprotein receptor (LDLr) on infected cells as well as reduced phosphorylation level of the 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR).[43]

Other factors

Severe thrombocytopenia is a significant sign for DHF progression as it occurs in the latter phases of the febrile stage and early leakage phase (or even later) of DENVs infection, inducing a steady drop in platelet count which leaves the patient at significantly prone to spontaneous bleeding. It remains unclear the exact mechanism of this drop, however it is assumed to be immunological, such as DENV-2 infection triggering the activation of platelets have shown to be attacked and phagocytosed by immune cells.[51] Current evidences indicate that DENV NS1 and NS1 induced humoral immunity play important roles.[52] Antibodies against DENV NS1 was found to cross-react with human platelets and endothelial cells, further inducing endothelial cell damage and subsequent apoptosis.[53,54] DENV NS1 protein has been reported to cause a subsequent disruption of the endothelial monolayer integrity by activation of mouse macrophages and human peripheral blood mononuclear cells (PBMCs) via Toll-like receptor 4 (TLR4).[55,56] In addition, binding of DENV NS1 to Toll-like receptor4 (TLR4) on platelets can trigger platelets to be activated and further aggregate and attach to endothelium as well as being phagocytosed by macrophages, subsequently leads to thrombocytopenia and hemorrhage.[57,58]

Host susceptibility to DENV infection and DHF occurrence is complicated. Many studies focus on genetics of the host and its association in the pathogenesis of DF and DHF. Among these factors, human leucocyte antigen (HLA) has recently drawn attention. The human leucocyte antigen (HLA) system is the gene cluster situated on chromosome 6 in the human major histocompatibility complex (MHC), encoding the antigen-presenting proteins on the cell-surface. DENV infection has known to increase expression of HLA class I and II molecules on infected cells, suggesting that the level of the immune response generated from HLA presented peptides may be responsible for the immunopathology of DENV infection.[59] The HLA class I molecules loaded with viral antigen-derived peptides on CD8+ cytotoxic T lymphocytes are known to have a significant role in regulating the cells infected with virus. Regarding the correlation between HLA class I and DHF, the HLA-A1, HLA-B blank, HLA Cw1 and HLA-A29 antigens have been reported to show a significant difference in DHF/DSS when compared with the normal control group.[60] Further, a positive association has been found for HLA-A2 and HLA-B blank and a negative relationship for HLA-B13 in DHF patients.[61] A study of secondary DENV infections indicated that HLA-A*0207 is linked with being susceptible to severe DHF in patients.[62]

As to HLA class II molecules, they were mainly distributed on cells presenting antigens, such as B cells, dendritic cells, macrophages, and were in charge of activation of T helper cells a well as proposed to have contribution on the pathogenesis of DHF. Studies from Mexico indicated that patients carrying HLA-DR4 homozygous were less likely to develop DHF, suggesting that HLA-DR4 may be a protective factor against DHF.[63] These findings suggest that classical HLA class I and II alleles are linked with DHF development. However, further studies are needed to offer more evidences.

Prevention and control plans

Symptoms or signs for fast identify

Dengue infection may produce an asymptomatic or symptomatic infection, with about 20% resulting as symptomatic. In general DF is a self-febrile illness, which appears 3–10 days after an infected mosquito bites an individual. The earlier stages of dengue infection can be presented as a mild "flu-like" illness with similar symptoms to malaria, influenza, chikungunya and Zika.[64] The disease is characterized by retro-orbital pain, fever, intense headache, intense joint and muscle pain, and nausea.[65,66] Besides the self-febrile illness, dengue can lead to more severe disease manifestations such as hemorrhage and subsequent vascular leakage. During the severe presentation of the disease, patients can present with pleural effusion, bleeding, thrombocytopenia with $<$100,000 platelets/μL, raise in hematocrit levels, restlessness, abdominal pain, vomiting, and sudden drop in temperature.[67,68] In WHO guidelines of 1997, the more serious dengue illness manifestations were classified as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).[1] However, in 2009 WHO made some modifications of the guidelines for the classification and clinical management of dengue to Dengue and Severe dengue. This modification aimed to form a simple and uniform criterion to generate a standard approach to disease globally. Although the revised criteria are more sensitive to the diagnosis of severe dengue, its applicability in clinical remains an issue.

The beginning of the febrile phase is noted by rapid onset of severe fever which last from 2 to 7 days.[66] At this time, dengue can be distinguished from other similar diseases utilizing the tourniquet test.[69,70] The majority of DENV patients are able to fully recover after the febrile period without entering the critical phase of disease. Nevertheless, some individuals enter the critical phase, presenting warning signs, include severe abdominal pain, persistent vomiting, marked change in temperature, hemorrhagic manifestations, or change in mental status. Generally, patients get worst as their temperature reaches 37.5 °C–38 °C after which a drastic decrease in platelet count leads to leakage of plasma and subsequent shock and/or fluid accumulation with respiratory distress; critical bleeding, and organ impairment.[66] Warning signs are almost always observed in patients before the shock onset including restlessness, cold clammy skin, rapid weak pulse, and narrowing of the pulse pressure.[71] Patients that experience shock have likely lost large volume of plasma via vascular leakage. DSS patients must be monitored closely, as hypotensive shock can rapidly turn to cardiorespiratory failure and cardiac arrest.[72]

Disease management

In order to reduce dengue mortality and control the disease severity, early diagnosis is important for effective disease management. Currently, there is no antiviral drug or medication to get rid of the dengue virus, the medical physicians can simply relegate the symptoms. Some of the recommendations to manage dengue include, bed rest, antipyretics or sponging to control the fever, analgesics or mild sedatives to help with the pain, and fluid or electrolyte therapy to help with hydration. The major symptoms that distinguish DHF from DF are plasma leakage, irregular hemostasis and increased vascular permeability. Patients who developed the severe syndromes should be supplied with isotonic crystalloid solutions, such as 0.9% normal saline, Ringer's lactate, or Hartmann's solution in accordance with WHO guidelines.[1] Once patients pass the life-threatening period, recovery from the disease can be quick. The well-being of the patients are apparent as their appetites return and they begin to reabsorb extravascular fluids.[66]

Laboratory diagnosis

Due to dengue infection manifesting symptoms similar to other febrile illness, diagnosis of the viral infection has to further be confirmed with laboratory techniques. Usually in laboratory diagnosis, biomarkers have been targeted for detection of dengue. One of the more traditional diagnostic methods for the detection of DENV is the virus isolation from samples obtained from suspected DENV patients and cultured in multiple cell lines, such as mosquito cells (C6/36) and mammal cells (Vero, BHK-21 and LLC-MK2) or in live mosquitoes.[73,74] Despite being definitive, virus isolation for DENV detection is not practical, ass it can take several days to perform, in addition to being time consuming. This limitation led to the introduction of a molecular method by using Polymerase Chain Reaction (PCR) which was first described as a 2-step RT-PCR assay.[75] This method has been modified making it a single step Real Time-PCR assay. The PCR-based technique has some major advantages as the RNA of the virus can be identified from the beginning of illness, it is fast, specific and sensitive.[76] However, the utilization of PCR-based method may not always be an option, especially in developing countries or counties with lack of resources. Besides detection of the virus itself, viral proteins such as non-structural protein 1 (NS1) are ideal target biomarkers since they are secreted from infected cells, causing higher levels of NS1 to circulate in the blood of infected individuals. Another approach for DENV diagnosis, that was first described in 2000, is detecting NS1 in the blood of patients by utilizing an antigen-capture ELISA.[77] The development of the commercial NS1 tool has been beneficial as it is simple but offers high sensitivity and specificity therefore, allowing it to become the new standard for dengue diagnostics.[18,78–80]

Other than the virus or viral products, dengue can be confirmed based on the host immune response. Some serological diagnosis available include Western blotting, plaque reduction neutralization tests, indirect immunofluorescent antibody tests, IgM and IgG antibody-capture ELISAs and hemagglutination inhibition assay (HI) as the more useful diagnostic tests. Detection of IgM can be as early as 3–5 days after infection and remain at detectable levels for several months.[73] On the other hand, IgG appears later during the primary infection, and with a rapid response in a secondary infection. Moreover, detection of both IgM and IgG levels in the patients' serum assists to distinguish primary or secondary dengue infection.[81,82]

Using serology to detect DENV becomes complicated in areas of the world where other flaviviruses are circulating, such as Japanese encephalitis, yellow fever and Zika virus, due to similarities in epitopes on E protein of these flaviviruses, causing cross-reactivity of the antibody response. To minimize false-positive results, NS1 antigen capture should be combined with IgM and IgG serology. The combination of detecting NS1 along with detecting IgM and/or IgG has demonstrated a drastic improvement to diagnose dengue.[83] Currently, there are some available commercial kits that take advantage of this approach. Utilizing this combination method, the sensitivity of detection reaches close to 100%.[73]

Dengue vaccine

With high number of dengue cases through the world, the development of vaccines holds substantial potential in controlling the disease, particularly in protecting children from infection. Dengue has 4 distinct serotypes; after recovery form one serotype the infected individual has a long-term immunity against a subsequent infection with similar DENV serotype. However, subsequent DENV infection with a different serotype is linked antibody-dependent enhancement (ADE) which is a factor contributing to DHF manifestation.[84,85] Therefore, the development of a vaccine should induce long-lasting immunity and protect against all four DENV serotypes simultaneously.

The ideal vaccine for dengue should be administered as single doses, provide protection to fight all four DENV serotypes, display long-term protection and have no side effects.[86–88] Currently, there are only one vaccine approved for the prevention of dengue utilized in endemic populations. This vaccine is the recombinant tetravalent, live-attenuated, yellow-fever dengue virus vaccine known as Dengvaxia (CYD-TDV) which was developed by Sanofi Pasteur.[87,89–91] CYD-TDV is composed of the live-attenuated CYD vaccine virus serotypes expressing the structural genes encoding the membrane protein and envelope protein of each of the four dengue serotypes and the attenuated yellow fever (YF) 17D virus strain genetic backbone.[91–94] The strains used in CYD-TDV are genetically and phenotypically stable, non-hepatotropic and less neurovirulent than the strains used in YFV 17D.

Live attenuated vaccines such as CYD-TDV have some advantages over other prospective dengue vaccines: the live attenuated vaccines act as agents of RNA replication, inducing humoral and cellular immune responses; a single-dose vaccination regimen may induce immune responses; and vaccines can be produced at a relatively low cost.[95] In addition, CYD-TDV has been shown to induce controlled stimulation of human dendritic cells and other immune responses.[92] However, the level of efficiency varied by age, and serotype, and the anti-sera were two times higher in children who had a previous dengue exposure at the time of vaccination compared to those who had no previous exposure. This protection, however, was more apparent in those who had a prior history of dengue infection. Studies have

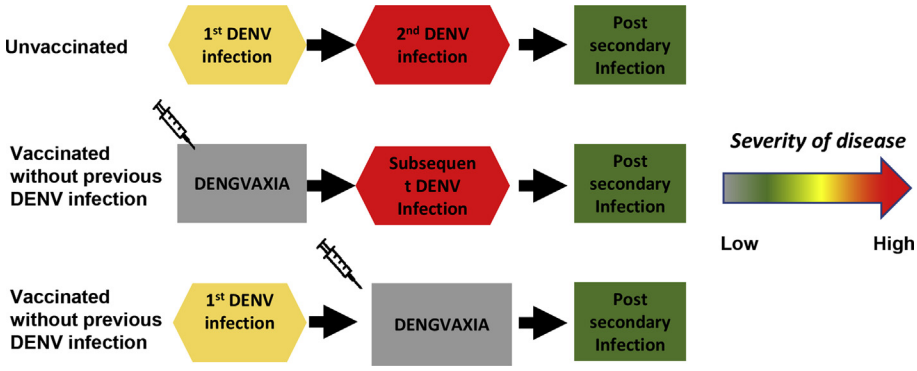

Figure 5. Potential outcome of Dengvaxia vaccine on Dengue infection. Unvaccinated (top row), an individual will experience a primary infection first, with mild symptoms (yellow) followed by a secondary infection with severe manifestation (red), and then postsecondary infections, with no manifestation (green). For individual receiving vaccine while being dengue naïve (middle row), their first natural infection behaves immunologically as a second natural infection would. Subsequent infections would immunologically behave as postsecondary infections. Vaccination for seropositive individuals (lower row) any subsequent infection would immunologically behave as a postsecondary infection indicating no clinical manifestation (green). Boxes are color-coded according to the level of disease risk thought to be associated with primary, secondary, and postsecondary infections. Green representing no clinical manifestation, yellow representing mild disease and red being DHF.

reported CYD-TDV protected against DENVs infection for a period of 5 years in individuals with previous exposure to DENVs before vaccination. Conversely there was a higher risk of severe manifestation in vaccinated individuals with no previous exposure (Fig. 5).[96,97] Thus, the utilization of the Dengvaxia vaccine is still under studies and there is room for further improvement.

Case surveillance and vector surveillance

Dengue morbidity can be lowered by the implementation of better prediction of outbreak and detection strategies through continuous monitoring at the status and distribution of cases and competent vectors; this following the principles of integrated vector management leading to enhanced vector control measures. More accurate estimates of the burden of dengue are essential to assess the progress of prevention measures. More efficient surveillance systems and dedicated studies are necessary for dengue prevention and control plans.

Currently, dengue has been reported in countries including the Eastern Mediterranean, Americas, Africa, Western Pacific and South-East Asia. In 2015, 2.35 million cases of dengue were reported in the Americas alone, with 10,200 cases diagnosed as DHF leading to 1181 deaths. In 2016, the cases throughout the Americas, Western Pacific and South-East Asia surpassed 3.34 million. The potential risk of a dengue outbreak now exists in Europe as local transmission was recorded for the first time in France and Croatia in 2010 with imported cases being detected in three other European countries.[98,99] An increase in cases have been noted in the West Pacific including countries like China, Cambodia, Malaysia, Singapore, Philippines, and Vietnam. In 2014, Taiwan experienced a large dengue outbreak, with a total of 15,732 DF cases reported, followed by an even larger outbreak with a total of 43,748 DF reported cases in 2015.[4,100–102]

In 2016, there were large outbreaks of dengue through the world. The Western Pacific Region reported over 375,000 suspected dengue cases by 2016. Out of these cases the Philippines reported 176,411 and Malaysia 100,028 cases.

It is noted that several large dengue outbreaks have been reported in 2016 worldwide but in 2017, the number of dengue cases reduced significantly. After a drop in the number of cases in 2017–18, a sharp increase in cases is being observed since 2019.[103] The dominant strains that cause dengue outbreaks are DENV-1 and DENV-2. Up to July 2019 dengue cases reports has been increased in countries of South East Asia such as Philippines (130,463), Lao People's Democratic Republic (15,657), Vietnam (115,186), Malaysia (75,913), Singapore (8,020), and Cambodia (4,532). The majorly circulating serotypes are DENV-1 and DENV-2 both in domestic and imported cases.[104] In the Americas region, up to June 2019, a total of 1,191,815 cases of dengue have been reported of which 546,589 (46%) were laboratory-confirmed and 5599 (0.47%) were classified as DHF. The reported case-fatality rate was 0.02%. Of the total number of reported cases 93% is from Brazil, Colombia and Honduras, with a trending to rise from the past year and circulation of the four DENV serotypes.[105]

In addition, GIS mapping for surveillance of dengue foci is currently suggested to validate to real-time realize dengue status. By localizing positive dengue cases within the study area, transmission of dengue can be controlled by identifying dengue foci, and then implying preventive strategies.[106] GIS mapping offers improved surveillance and community-based intervention programs for controlling dengue and aids in measuring the successful prevention rate in the mapped areas.

The rapid spread of DENV is likely due to changes in global climate, ineffective vector eradication, and expansion of human population.[107] The predominant vector responsible for the spread of the DENVs is the *Aedes* mosquito, mainly the *A. aegypti* and *A. albopictus*,[108] because of their susceptibility to the virus and their efficiency of transmission to humans.[109,110] Both *A. aegypti* and *A. albopictus* are located in sub-Saharan Africa, where the *A. aegypti* is native. Recently, *A. aegypti* has been introduced

in most of the tropical and subtropical regions owing to globalization and human activities.[111] The *A. albopictus* is native to South East Asia, however in the past 30—40 year they have expanded their distribution to all the five continents.[112,113] In the early 2000, the *A. albopictus* was first reported in Central Africa.[114,115]

In Asia, *A. aegypti* is consider the major vector of dengue spread and *A. albopictus* is the secondary vector. Geographically, *A. aegypti* spread in both tropical and subtropical areas and has living environment close to human. *A. albopictus* is more tolerant to cold temperature so that it has geographical spread larger to subtropical areas. *A. aegypti* live optimally in 26—30 °C and humidity of 70—80% along with the availability of breeding place and food sources. In the shortage of an effective vaccine or antiviral treatment against dengue infection, it is needed to consider the control and elimination of vector populations, mainly focused on the reproductive and growing stages, through management of environmental conditions, biological control and chemical inhibition. Thus, reducing the population of *Aedes* mosquito and the transmission of DENV.

Community-based control programs

Control and extermination of vector breeding sites are the most have been the aim of community-based control programs that divide the community in various groups considering their education level and knowledge about vectors and the disease.[116] These programs for mosquito elimination have shown significance in some countries as the population is proven to have high level of awareness of the vector.[116,117]

However, a successful community-based control is relied on the knowledge, education, and behavior of the people, as well as feasible strategies.[118] Training and distribution of information create a new level of education to generate abilities in the individuals to identify and implement preventive measures for extermination of vector and vector habitats, as well as bring awareness in the population.[119] It has been proved in a study in Thailand with the use of media to develop awareness.[120]

The biological control

The control of dengue disease is mainly relied on reduction or elimination of the vector population via conducting biological vector control plans. Conventional methods of vector control are mostly ineffective against this daytime biting mosquito, so new strategies are required. The genetically modified (GM) mosquitos are the major strategies for biological protection against dengue.[121] A traditional control measure known as sterile insect technique (SIT) has been used successfully for several years in many countries. SIT harms the health of male insects, reducing the ability to compete for wild-type female insects for breeding.[122]

The first control program focused on transgenic sterility have been approved for *A. aegypti*. The mosquitoes are genetically engineered to express a fluorescent marker and a late-acting conditional lethality trait, which means that most of those offspring do not growth up to adulthood.[123,124] Repeated releases of millions of genetically modified lethal gene-carried mosquitoes vastly exceed the wild *A. aegypti* population, intended to reduce the total adult population of mosquito. In 2008, the experimental transgenic mosquitos were released into the environment in the Cayman Islands, Malaysia, Panama and Brazil.[125] All of the releases experiments significantly reduced the population size of *A. aegypti* in the area. Eliminating female mosquitoes for the releasing of male *A. aegypti* into environment was performed by using the size difference to separate male and female pupae. It is not only intensive labor, time consuming and expensive, but also not 100% guaranteed, with a 0.02% contamination of females. One thing to consider is that releasing of genetic-modified male *Aedes* can suppress mosquito populations but demands a continuous and long-term release of large numbers of transgenic male mosquitoes.[126,127]

The other approach includes the release of transinfected mosquitoes which transmit the intracellular bacterium *Wolbachia*.[128,129] *Wolbachia* is safe and common present in up to 60% of insect species naturally, including butterflies, moths and some kinds of mosquitoes. *Wolbachia* can be used in control of mosquito population in two different strategies, reduction of vector reproductive capacity and suppress RNA virus replication. However, *Wolbachia* is not usually infects *A. aegypti*, the primary species of mosquito involved in the transmission of dengue and other flaviviruses.[130,131]

Wolbachia works by competing for iron and cholesterol against virus replication.[132,133] Both the viruses and *Wolbachia* need cholesterol to replication and growth inside the mosquito. When *Wolbachia* is present, it consumes these molecules and makes it harder for the viruses to grow.[134,135] In addition, scientists proposed another approach via the natural immune system of mosquitoes to make them stronger to resist virus infection of *Wolbachia* into mosquitos will induce several signaling pathways of the innate immune system, including Toll, immune deficiency (Imd) and JAK-STAT. Activation of those signaling pathways will lead to the increased resistance of various arboviruses.[136—138]

Although using *Wolbachia* as a feasible vector control tool, it is necessary to evaluate whether the invasion of *Wolbachia*-infected mosquitoes into the environment can cause displacement to wild-type populations, in field conditions.[139] Field releases are necessary to comprehend the invasion dynamics and to optimize release strategy using *Wolbachia*. Studies reported that *Wolbachia* infection is stable and persistent, and that continuous *Wolbachia* infection contributes to reduction of vector competence.[140] In January 2011, field release of *Wolbachia*-infected *A. aegypti* were performed near Australia, Cairns and Vietnam.[140] For all these releases, *Wolbachia*-infected *A. aegypti* is successfully invaded all the areas. But those studies focus on establishing the sustainability of *Wolbachia* in wild *A. aegypti* population. In order to understand the viability as a control measure for dengue, releases are now occurring in Indonesia, Brazil, Australia, and Colombia.

Chemical control

For many decades, control plans of vector populations have been through the use of insecticides from chemical origin or plant derivatives. Although they were the convenient strategies for vector control, some side effects by long-term using insecticides have been reported, including the

induction of insecticide-resistance in the target vectors as well as negative impacts on the environment.[141] However, scientists have developed several alternative ways to resolve these problems, such as introduction of plant-based insecticides prepared from plant derivatives.[142,143] These alternative reagents showed sustain effects and less toxicity to mosquitos and environments, respectively. Plant derivatives are not limited to insecticides only, moreover, potential repellents have also been proved their efficiency against *A. aegypti*. In addition, the other chemical compounds called insect growth regulators (IGRs), which were used for inhibition of the growth and development in insects by the inducing changes in the early stages of development and subsequently killing the insect preventing the formation of adult stage. Among a number of IGRs, diflubenzuron, cyromazine, pyriproxyfen, spinosad and methoprene have been reported to be used for larval control, thus reducing the populations of *A. aegypti*.[144,145]

Discussion

To date, the main risks which trigger DHF were still controversial owing to the complexity of disease progression caused by DENV-host interaction. In this study, we summarized the updated information regarding the risks of pathogenesis, prevention and control of DHF. In recent decades, several large dengue outbreaks were occurred in dengue endemic and epidemic countries, such as Taiwan, Philippine, China, Cambodia and Thailand. The high incidence of DHF with fatality has been reported, which resulted in this neglected disease being noticed.

Currently, DF and DHF remain to be viewed as a global issue. Understanding the pathogenesis and validating efficient prevention and control strategy are demanded against dengue invasions to humans. This study suggests that ADE, cytokine dysregulation and variation of lipid profiles are current recognized parameters correlated with DHF occurrence (Table 2). Other factors from both host and pathogen that may correlate or enhance directly or indirectly DHF are still needed further investigations. Among these factors, ADE is most acceptable risk correlated with DHF. DHF usually appeared following secondary dengue infections, but may sometimes appear following primary infections, especially in infants. In clinical, these patients display a higher level of DENV viral load and a slower rate of clearance of DENVs and virus—immune complexes than patients with classic DF syndromes. Clinically, DHF usually begins with a sudden rise in temperature and other

Table 2 The risk factors correlate with DHF development.

Risks	Description	Reference
Antibody Dependent Enhancement (ADE)	• This theory was first described in 1964.	21
	• The antibodies from primary DENV infection lack the ability to neutralize secondary heterotypic DENV infection.	22, 23
	• Cross-reactive antibodies form infectious virus-antibody complexes that enter cells, enhancing viral production.	84, 85
Cytokine dysregulation	• DENV antibodies promote the entrance of a second serotype of DENV, activating a rapid production of cytokine (especially pro-inflammatory type 1 cytokines)	33
	• Induced cytokines affect vascular endothelial cells leading plasma leakage.	34
	• Cytokines as prognostic markers for disease severity (IFN-γ, TNF-α, IL-6, IL-8, VEGF-A, IL-2 and RANTES L-1RA, MCP-1, TGF-β, HGF, IP-10, and sTNFRp75)	34
Changes of lipid profile	• Changes of lipid profiles correlated with DHF	13
	• Cholesterol and lipid rafts promoted DENV entry	43
	• LDL and HDL induce an imbalance in the inflammatory response correlated with the severity of dengue.	45
	• The increase TG, VLDL and HDL, and decrease of TC and LDL triggered DHF.	46
	• The lowest cholesterol and VLDL levels associated with severe bleeding and hepatic dysfunction.	47
	• LDL-C and LPS levels associated with risk of developing DHF and severity of plasma leakage	48, 49
Other factors	• Antibodies against DENV NS1 induced endothelial cell damage and subsequent apoptosis.	53, 54
	• DENV NS1 protein caused a subsequent disruption of the endothelial monolayer integrity	55, 56
	• Binding of DENV NS1 to Toll-like receptor4 (TLR4) led to thrombocytopenia and hemorrhage.	57, 58
	• DENV infection increased expression of HLA molecules on infected cells	59
	• HLA-A*0207 linked with severe DHF in patients	62

Abbreviation: dengue hemorrhagic fever (DHF); dengue virus (DENV); interferon gamma (IFN-γ); Tumor necrosis factor alpha (TNF-α); interleukin-6 (IL-6); interleukin-8 (IL-8); interleukin-2 (IL-2); Vascular endothelial growth factor A (VEGF-A); Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES); interleukin-1 receptor antagonist (L-1RA); Monocyte chemoattractant protein-1(MCP-1); Transforming Growth Factor Beta (TGF-β); Hepatocyte growth factor (HGF); interferon-inducible protein 10 (IP-10); Soluble tumor necrosis factor receptors p75 (sTNFRp75).

symptoms similar to those of DF. The body temperature of DHF patients remains high for 2—7 days. Hepatomegaly and splenomegaly were sometimes observed. Hemorrhagic tendency may apparent in many ways such as positive tourniquet test, petechiae, ecchymoses or purpura; mucosal bleeding, and hematemesis. The most common hemorrhagic features are petechiae, where the Epistaxis and gingival bleeding are uncommon and gastrointestinal bleeding may be observed in severe cases. In DHF, bleeding may not correlate with the platelet counts and usually occurs once the fever has developed, resulting in the difficulty for diagnosis and prediction of DHF development. Accordingly, prediction of the DHF development in the DF patient needs to check and consider more clinical manifestations and clinical laboratory diagnostic results.

Presently, several hypotheses regarding DHF pathogenesis were proposed. Most of these hypotheses are not mutually exclusive, and together they harbor multiple elements that collectively may explain most of the phenomena observed in the various presentations of DENV infection. The pathogenesis of DHF grades I/II is complicated and multifactorial, involving both viral and host factors. However, those necessary and sufficient factors have still not been clearly identified. It may be questioned whether factors that explain the pathogenesis of DHF in all patients do exist. As we mentioned before, the genetic predisposition may have a significant effect on disease outcome. Unfortunately, only a few studies have reported the host genetics with regard to the severity of DF/DHF. It is known that genetic variations consist of single-nucleotide polymorphisms (SNP) within genes that correlate with disease pathways. Many polymorphisms have small and independent effects on disease outcome and often act in concert with other polymorphisms and environmental risk factors. This eventually results in complex and variable disease outcomes. It is worthy conducting the studies involved in transcriptomics, proteomics, metabolomics, SNPs, and phenotypic disease characterization in well-defined DHF/DF cohorts to identify individual molecular markers for detection of potential DHF occurrence. In addition, accurate and prompt diagnosis, as well as appropriate treatment of DENVs infection are essential for reduction of dengue mortality and control the occurrence of DHF. Furthermore, active and continuous surveillance of cases and vectors are the determinants for dengue prevention and control. Widely conducting the dengue vaccination and biological control plans are currently not recommended, unless most safety and negative effects were extremely addressed.

Authors' contributions

WHW prepared the manuscript. MRCI and ASNU helped to collect the data and prepare the draft. WA, PLL and YHC revised the draft. SFW conceived the study and revised the draft.

Declaration of competing interest

The author reports no conflicts of interest in this work.

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