DENGUE: AN OVERVIEW

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ABSTRACT

Dengue is one of the highest occurring vector-borne diseases. It is caused by dengue viruses 1- 4. Currently, the disease is classified into dengue with or without warning signs and severe dengue based on WHO 2009 dengue classification. As of today, neither specific drugs nor commercial vaccine exist for dengue. The best treatment yet would be support, management and proper medical care. With no pathognomonic features that could differentiate it from other febrile illnesses, clinical diagnosis alone is insufficient. Yet, despite the current advances and existence of various laboratory diagnostic methods of dengue, a consensus singular method has not been established. There are several hypotheses or theories regarding the vaguely understood immunopathogenesis of dengue. Amongst these are the viral factors, host-immune factors and host-genetic factors. In addition to these, the occurrence of asymptomatic dengue has further complicated the disease. However, these individuals provide opportunities in the search for protective factors against dengue.

Keywords: *asymptomatic, dengue, diagnosis, immunopathogenesis*

Overview of Dengue

Dengue virus (DENV) infection is undoubtedly one of the most rapidly spread mosquito-borne viral diseases causing major health problems worldwide. The incidences of dengue has grown drastically around the world in recent decades with unprecedented geographic expansion as a result of increased global movement of humans, plants and hematophagous arthropods via shipping and air traffic (1). It is estimated that around 390 million people are infected each year which is more than triple the current estimate by WHO (2). This figure includes 96 million severe cases and 300 million mild or asymptomatic episodes. As a resulting implication, this suggests that the reservoir of the disease is far larger than expected. Before the 1970s, only nine countries had experienced severe dengue epidemics (3). However, at status quo, not only is the number of cases increasing as the disease spreads to new areas, explosive outbreaks are also occurring. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. The American, South-east Asia and the Western Pacific regions are the most seriously affected areas (3). This review provides our understanding of dengue in terms of the virus per se, its clinical manifestations, pathogenesis of the disease, tests that are used to diagnose it and management of the disease.

Dengue Virus

The DENV is a positive-sense single stranded RNA virus belonging to the genus *Flavivirus*, from the family of *Flaviviridae*, sharing the same category with other 70 different viruses (4). The genome of DENV is approximately 11 kb long and the mature virions are composed of three structural protein genes that encode the nucleocapsid or core protein (C), membrane-associated protein (M), envelope protein (E), and seven nonstructural (NS) protein genes. The gene order is 5'-C'prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'(5). There are four serotypes of DENV, DENV-1 through DENV-4 and all four serotypes are closely related but antigenically distinct. It is known that E protein is not only a functional protein molecule that binds to receptors on the host cell membrane but also a major antigen, which can induce neutralizing antibody and host specific protective immunity (6-8). As enveloped viruses, DENVs enter the cells through receptor mediated endocytosis (DC-SIGN, heparin-sulfate, clathrin-mediated) (9-12) where the plasma membrane invaginates to form an endocytotic vesicle around the enveloped virus (13-14). The deposition of the nucleocapsid into the cytoplasm occurs right after the virion envelope fuses with the plasma membrane and internal cell membranes rearrange to establish specific sites of replication (15-17). The positive sense dengue virus RNA will be first

translated to make RNA polymerase, which is required for transcription of positive-strand RNA into negative-strand RNA. The negative-strand RNA will serve as template for the replication of positive-strand RNA. During the first 12 to 16 hours after infection, there is no encapsidation of RNA but the RNA serves as the template for replication and translation for the formation of dengue viruses. During the late eclipse period, the positive-strand RNA must be diverted to viral assembly (18). During late replication process, the affinity of RNA polymerase complexes towards positive-strand and negative-strand RNA changes, resulting in the predominance of positive-strand RNA later in infection (19). The assembly of nucleocapsids starts with the increasing concentration of C protein (20). The nucleocapsids first assemble from C protein and followed by envelopment through "budding" of nucleocapsids via membrane containing integral E and prM proteins (18). The virus is then released from the infected cells via secretory exocytosis when the virus-containing secretory vesicles fuse with the plasma membrane (21). At the end of the viral replication, cleavage of prM occur before or during the release of virus from infected cells, and this process is accompanied by reorganization of the virion envelope and also viral maturation (22).

The most common vectors that transmit DENV are *Aedes aegypti and Aedes albopictus*. However, DENV transmission are not limited to those with different species of *Aedes* mosquitoes such as *Aedes polynesiensis, Aedes scutellaris,* and*Aedes stegomyia* may act as vector for the disease as well (23). There are three types of transmission cycles for dengue virus, the primitive enzootic transmission cycle, epidemic transmission cycle and also urban endemic/ epidemic transmission cycle (24). Most of the time, multiple virus serotypes are co-circulating in the same city (23, 25). Nevertheless, in recent years, human activities had enhanced the mosquito breeding activities, which has led to increased interaction between mosquitoes and humans, and eventually increased rate of viral dispersal between mosquitoes and humans (26-27). Slight movement will disrupt the feeding of the female mosquitoes, and only later it will return to the same or another person to continue feeding (24). Because of this feeding behavior, the female mosquitoes can feed on many persons in the same household in a short time. Therefore, it is common that several members of the same household might be infected, and usually the virus is being transmitted from the same mosquito to different people in the same household (25, 28-30). To this effect, many individuals are susceptible to being infected by DENV of which some may show clinical symptoms while others do not. Previous studies of asymptomatic infections have been reported to show varying ratios of symptomatic to asymptomatic dengue infection. This is clearly exemplified by different surveys such as in Thailand, during a prospective survey carried out in 1980–1981 in Bangkok amongst school children; it was estimated the ratio of asymptomatic/symptomatic cases to be 6.1:1. In addition, the DENV-4 infections that were detected during this survey were entirely asymptomatic (31). In contrast, another prospective study was conducted in Kamphaeng Phet, Northern Thailand, between 1998 and 2000 and the results were quite different since the ratio was only 1.1:1 (32). In Singapore, authorities assumed a ratio of asymptomatic/symptomatic infections between 2:1 and 10:1 (33). It had been shown in clinical-based study, asymptomatic dengue is common in primary DENV-2 or DENV-4 infections in children (34-35) while over 95% of primary infections of circulating Southeast Asian genotype DENV-2 in adults were asymptomatic during an outbreak in Santiago de Cuba in 1997 (36). Based on our preliminary findings from three different hospitals around Klang Valley as well, we observed that about 40% of the household members of the dengue patients are tested positive with dengue. These household members are not sick, but are actually having asymptomatic dengue infections. These differences might be explained by individual variations in susceptibility or variability in the virulence of DENV strains. The epidemiology of dengue may differ according to the region and country. The spread of the virus is imminent not only among the infected individuals but also among the healthy ones that are predisposed to the bites of the infected mosquito. Based on the data and reports mentioned above, it is undeniable that the asymptomatic DENV cases should not be taken lightly as they play a key role in helping researchers to decipher the immunological aspect of the human host in fighting against the DENV infection. To date, it is still unknown as to whether asymptomatic cases may be the reservoir of infection which will indeed spur the increase of DENV incidence rates. Besides that, this will also greatly increase the risk of occurrence of severe dengue in the future, as the previous DENV infection had gone undetected. However, these asymptomatic individuals provide avenues for researchers to look into the protective factors that protect them from developing symptoms during DENV infection.

Manifestation of Disease

DENV infection are mostly asymptomatic, however, a wide variety of clinical manifestations may occur, ranging from mild febrile illness to severe and fatal disease (37). The differential diagnosis is broad and varies as the disease evolves. Other diseases that should be considered as part of the differential diagnosis, depending on the clinical picture and local disease prevalence, include typhoid, malaria, leptospirosis, viral hepatitis, rickettsial diseases, and bacterial sepsis (38). Previously, patients were classified as having either dengue fever or dengue hemorrhagic fever, with the latter classified as grade 1, 2, 3, or 4. Over a number of years, there was increasing concern regarding the complexity and usefulness of this classification system (39). With the recent revision of the World Health Organization (WHO) dengue classification, patients are now classified as having either dengue or severe dengue (37, 39). Patients who recover without major complications are classified as having dengue, whereas those who have any of the following conditions are designated as having severe dengue: plasma leakage resulting in shock, accumulation of serosal fluid sufficient

to cause respiratory distress, or both; severe bleeding; and severe organ impairment.

The sudden onset of the symptoms starts after an incubation period of 3 to 7 days. Patient will undergo an initial febrile phase, a critical phase around the time of defervescence, and a spontaneous recovery (convalescence) phase. The initial phase is typically characterized by high temperature (≥38.5°C) which may be accompanied by headache, vomiting, myalgia, arthralgia and sometimes with a transient macular rash. This phase lasts for 3 to 7 days, after which most patients recover without complications. However, during the transition from the febrile to the critical phase, between days 4 and 7 of the illness, vascular leakage may develop in some patients. Signs of impending deterioration include persistent vomiting, increasingly severe abdominal pain, tender hepatomegaly, a high or increasing hematocrit level that is concurrent with a rapid decrease in the platelet count, serosal effusions, mucosal bleeding, and lethargy or restlessness (38). During this critical period, hemorrhagic manifestations are also most common. With proper management after approximately 48 to 72 hours, altered vascular permeability may be short-lived, spontaneously reverting patient to a normal level and concurrent rapid improvement in the patient's symptoms may be seen. The convalescence phase may be abrupt but prolonged with some having profound fatigue for several weeks after recovery.

Diagnosing dengue via laboratory assays

Clinical diagnosis alone for dengue is not sufficient because dengue has no pathognomonic clinical features from other febrile illnesses (40). This is where laboratory confirmation methods are vital to find out the etiological agent and to allow proper management and treatment of disease. Ideally, a laboratory assay for dengue detection should be rapid, simple, with high sensitivity and specificity, able to detect dengue at any phase of illness, preferably able to distinguish primary and secondary infections as well as the different serotypes. However, this ideal test has not been materialized yet due to (i) the complexity of dengue pathogenesis; (ii) hyperendemicity and multiple sequential infections and (iii) clinical conditions of patients including viremia and antibody response. Currently, at different phases of illness, different laboratory methods are being used to diagnose dengue. Virus isolation, genome and antigen detection is conducted during the early or febrile phase of illness; whereas at later phases, where antibodies have been formed serological-based assays are applied (Figure 1). The serological-based assays such IgM capture ELISA are favorable in hospitals of dengue endemic countries (41) due to its inexpensiveness, simplicity and (sometimes) rapid turnover as well as the narrow time frame of viremia. Nonetheless, serological assays can be a challenge in hyperendemic areas where pre-existing antibodies complicates diagnosis (42). These

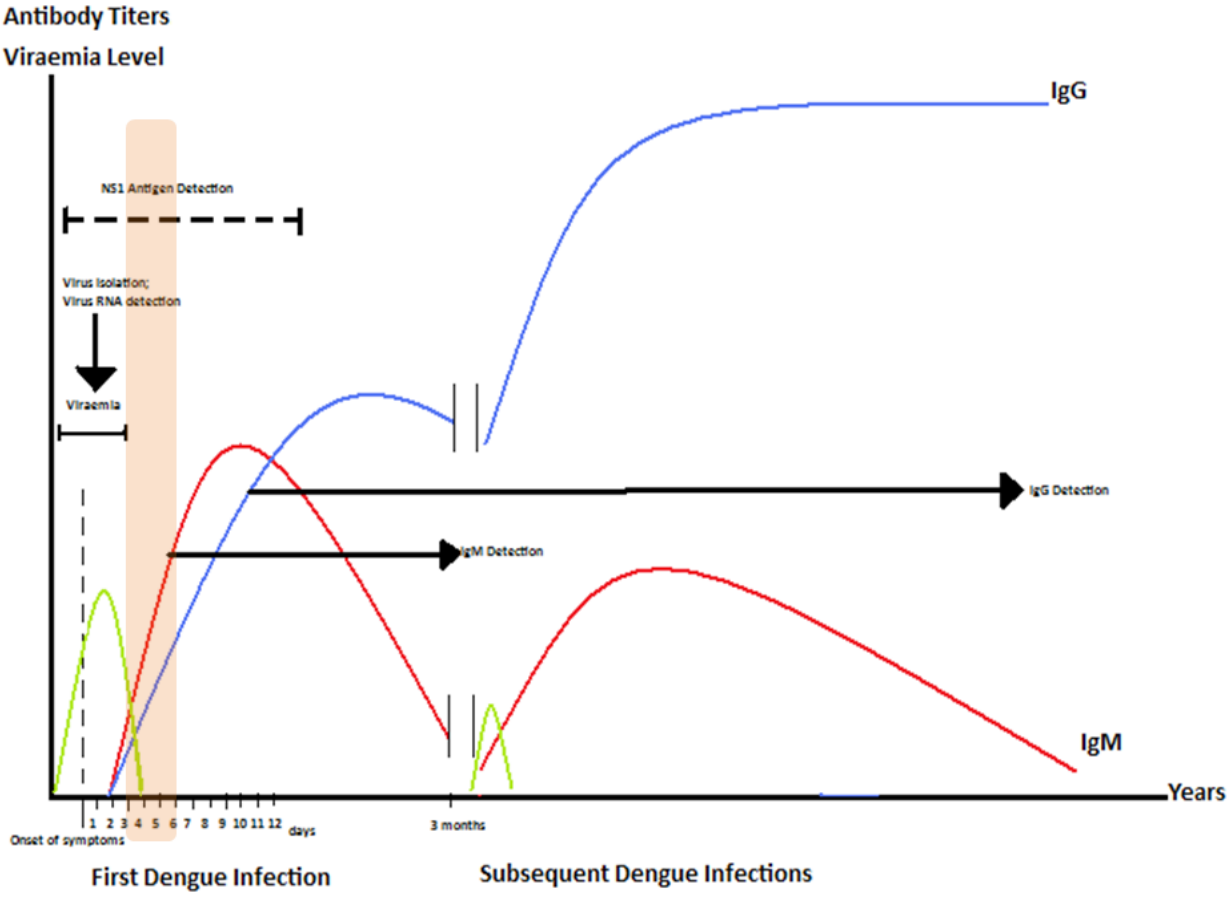

Figure 1: *Time course of primary/secondary dengue infections and the suitability of dengue diagnostics at different phases of illness.*

assays also require 2 samples (acute /defervescence and convalescence) to be able to confirm a patient via seroconversion as a single antibody positive sample may indicate an infection that has occurred in the past 3 months (43). Many serological assays have commercialized test kits in various formats including microplate, strips and cassettes. These kits have variable sensitivity and specificity, and may not have been evaluated against referenced serum panels (44). The sensitivity of the commercialized IgM ELISA kits ranged from 61.5 -- 99.0% with specificities ranging from 79.9 -- 97.8% when analyzed worldwide. The rapid diagnostics tests ideal for bedside diagnosis, had lower sensitivities ranging from 20.5 -- 97.7% and specificities of 76.6 -- 90.6%. To facilitate early diagnosis and confirmation of the etiologic agent, various polymerase chain reaction (PCR)-based techniques have been developed, optimized, innovated and simplified to detect dengue RNA including multiplex reverse-transcription (RT)-PCR (45-46), real time multiplex RT-PCR (47-51), nucleic acid sequence-based amplification (NASBA) (52), RT-Loop mediated isothermal amplification (LAMP) (53) and transcription-mediated amplification (TMA) (54). This method has minimized hazardous contact with live DENV and reduced the result turnaround time, enabling physicians to take early course of action in managing dengue patients. Despite many genomic detection methods for dengue detection, not all have been evaluated (42) and in one such external assurance study, only 10.9% of participating laboratories with different molecular-based method met all criteria for optimal performance (55). In this quality assurance study, 80.4% of participating laboratories needed to improve their DENV detection diagnosis procedures because (i) laboratories applying the same protocols had different reproducibility rates; (ii) false negative rates were high and (iii) false positive was detected in some laboratories (55). Furthermore, this technique is not favorable in poor endemic areas because the method is expensive with need of specialized equipment and skilled personnel. Ever since a decade ago, DENV NS1 detection has been pursued diligently with the invention of NS1 ELISA and rapid test kits. The NS1 antigen has been detected in serum and plasma of dengue infected patients from the onset of fever up to early convalescence (56-58). This antigen detection method is useful for low-resource settings as the kits are often lower priced and simple to use (59). Nevertheless, the commercialized ELISA and rapid NS1 kits had shown poor sensitivity ranging from 52 - 94% and 48- 91%, respectively when evaluated in different endemic countries. With the complicated immune status of dengue patients and assays that require more standardizations, combinations of different methods such as detection of both NS1 and IgM/IgG have proved to increase the overall performance (60). Hence, it seems that without the ideal diagnostic test for dengue, the best way currently to diagnose and confirm dengue is to run different assays or to obtain paired sera.

Dengue pathogenesis

Principally, disease manifestations often involve complex interactions between invading pathogen and the host immune response. Dengue infections undoubtedly fall in this category, where the clinical outcomes of dengue have been postulated to be intrinsically caused by both viral-induced and immune-mediated pathogenesis. Traditionally, it has been believed that secondary infections in dengue are more severe. This was first recorded in the Philippines in 1953/54, where a more severe form of dengue-associated disease was noted accompanied by bleeding, leakage, shock and death (61). Subsequently, various retrospective and prospective clinical studies showed evidence of dengue severity in secondary infections supported by appearance of DHF/DSS in infants with passively transferred maternal antibodies to dengue (36, 62-66). However, there are also cases where in DENV hyperendemic areas or in people with pre-existing antibodies, severe dengue had not occurred (67-68). Furthermore, severe dengue manifestations during primary infections have also been observed in children and adults (34, 69).

Immune cells such as macrophages and dendritic cells (DCs) are often recruited to sites of infection upon stimulation by chemokines. These are phagocytic cells which are supposed to kill DENV-infected cells. However, DENV preferentially targets these cells for infection and replication. As the cells circulate through the body, more DENV is released and viremia sets in. In a secondary infection, this phenomenon is further enhanced by non-neutralizing antibodies of the previous infection, a scenario termed as antibody-dependent enhancement. The non-neutralizing antibodies bind to DENV and via Fc receptors, viral entry into cells is increased (70-72), directly influencing the viremic state of the host (34). The innate immune system also responds to DENV infections by recognition of viral pathogen-associated molecular pattern (PAMPs) via pattern recognition receptors (PRRs) such as TLR-5, RIG-1 and MDA5 (73); consequently causing the secretion of type I interferons which have strong antiviral action (74). Nevertheless DENV have been shown to evade and modulate this pathway to inhibit/ delay the innate response. DENV can avoid interaction with cellular PRRs (75) or by expression of antagonist proteins to disrupt the type I interferon activation pathway including IRF-3 inhibition (76) as well as STAT1 and STAT2 downregulation(77-78). Subsequently, antiviral actions against DENV can be delayed, and furthermore this may ultimately impair the ability to evoke the adaptive immune response.

The DENV-infected tissue-resident DCs, working as an antigen presenting cells, will travel up to the lymph node to activate cells involved in the cell-mediated immunity. The bone marrow derived cell (B cells) will produce dengue specific antibodies to neutralize DENV. With a low-fidelity RNA-dependent RNA polymerase, DENV may be creating quasispecies to avoid recognition by the immune system (79). This was observed by sequence variation and epitope changes in DENV proteins, hence increasing viral fitness and decreasing neutralization abilities of dengue specific antibodies (80-81). Recently, memory B cells from previous DENV infections dominate the secondary infections and

produces the non-neutralizing antibodies in antibody-dependant enhancement (ADE) (82-83). The cell-mediated immune system involves activation of $CD4^+$ and $CD8^+$ T cells for viral clearance. T cells are activated when the T cell receptor and a co-stimulatory molecule binds to the major histocompatibility complex (MHC) molecule bearing the antigenic peptides on APCs. The activated cytotoxic T cells can recognize and kill DENV-infected cells. Antigenic variability and sequence variation by DENV may allow the virus to avoid/delay T cell detection. In secondary infections, the cross reactive memory T cells are postulated to be activated rapidly and in greater quantities (84). These cells are also hypothesized to have lower affinity and avidity towards the subsequent heterologous serotypes and are hence less efficient in viral clearance (85). Subsequently, these phenomena may trigger a cascade of events including skewed/overproduction of cytokines leading to increased vascular permeability. Cytokine irregularities have been observed via blood sera analyses where various cytokines have been implied as risk factors for increased dengue severity including IL-8, IL-10, IL-13, IL-18, IFN-γ, TNF-α, MCP-1, RANTES and MIP-1β (86-91). Many of these cytokines have been implied as modulators in vascular leakage and have influential impact on endothelium permeability. Obviously, these postulated hypotheses, one way or another can alter the physiological state of the host body which includes regulation of endothelium permeability, nevertheless less emphasis has been placed on the endothelium as a cause of DENV pathogenesis. DENV have been shown to infect endothelial cells both *in vivo* and *in vitro* (92-95). Morphological damage in the endothelium related to vascular leakage was seldom observed, despite the presence or increase in circulating endothelial cell markers, von Willebrand factor, pro-coagulants in severe dengue patients. In patients, the kinetic of DENV infection on the endothelial cells is obscure whereas *in vitro*, increase permeability in endothelial cells is believed to be contributed by the secretion of various factors triggered by immune response such as cytokines, chemokines, and also the complement activating factors, and not directly linked to the infection of DENV per se. However, the DENV infected endothelial cells (ECs) have shown to trigger various chemokine and cytokine responses and these may contribute to vascular permeability by modulating tight junctional changes (96-98). Moreover, oxidative stress in imminent in endothelial function and dysfunction where in dengue infections, oxidative stress markers such as glutathione peroxidase,glutathione,malo ndialdehyde, VCAM-1, high-sensitivity C-reactive protein and platelet-activating factor–acetylhydrolase have been found to be modulated as dengue severity increased (99)s. Nevertheless, endothelial permeability in dengue infection seems to be a transient effect when no extensive cell death and damage was observed upon DENV infection *in vitro* (96, 98, 100).

The modes whereby DENV evades our immune system are many; this combined with flaring up of the immune system are believed to cause pathological disease. But this may be the incomplete picture of dengue pathogenesis because various other factors have also been postulated as severity risk in dengue infections such as age (101), sex (102), nutritional status (103), immune status, co-morbidities (104), autoimmunity (105), genetic background (HLA and non-HLA molecules) and DENV fitness (or virulence).

A great deal of effort has been devoted to understanding the immunopathogenesis of clinical dengue infections. Nevertheless, there remains a lack of specific ground rules to define the effect of viral virulence in dengue pathogenesis, as host susceptibility and also certain other factors might affect the pathogenicity of DENV infections. The factors conferring protection against clinical dengue infection have seldom been investigated. Therefore, in our recent study (unpublished data), we postulated that the molecular mechanisms underlying the asymptomatic DENV infection may confer protection towards the manifestation to clinical dengue infection. We found that the asymptomatic individuals elicited pre-existing neutralizing antibodies and certain immune response genes expressed in these individuals to be in contrast with severe dengue patients when gene expression studies were done. Our findings may highlight the potential association of certain host genes conferring protection against clinical dengue to better understand the immunopathogenesis of dengue infection.

Vaccine

There are currently no licensed dengue vaccines available; however, several vaccine candidates are under development. These include live attenuated virus vaccines, live chimeric virus vaccines, inactivated virus vaccines, and live recombinant, DNA and subunit vaccines (106). Live viral vaccines have advanced to clinical trials, but have shown problems, such as unequal immunogenicity of the four serotypes and viral interference among the four serotypes in tetravalent formulations (107). This includes subunit vaccines that mostly focused on the E protein or its derivatives. However, the difficulty of eliciting balanced levels of neutralizing antibodies to each of the four serotypes remains a major concern. The E protein is the major component on the surface of DENV virion and is a dominant target of Ab responses against DENV. Passive immunization with anti-E antibodies provides protection against DENV infection in mice (108). In addition, monoclonal antibodies against prM/M have been shown to provide protection against DENV challenge (109). However, this is contradictory to a study done by Dejnirattisai *et al.* 2010, which showed that prM antibodies do not neutralize infection but potently promote ADE instead (110). Besides that, in our unpublished data, the antibody titers towards prM and E antigens were seen to be higher than the neutralizing antibodies in dengue patients who showed heterotypic infection, indicating that there seems to be cross reacting antibodies that may hinder the neutralization of DENV infection. These observations may serve as a point to note that the development of dengue vaccine is yet a long way to go with much consideration on cross reactivity of antibodies and host immune response towards heterotypic infections.

Management

There is currently no effective antiviral agent to treat DENV infection, and treatment remains supportive, with particular emphasis on careful fluid management (37). In cases where patients who do not have complications and are able to tolerate oral fluids, they remain at home unless bleeding or warning signs suggestive of vascular leakage develop (38). Development of any warning signs indicates the need for hospitalization and close observation. Prompt fluid resuscitation to restore plasma volume is given if the condition progresses to dengue shock syndrome followed by ongoing fluid therapy to support the circulation at a level just sufficient to maintain critical organ perfusion. Blood transfusion can be life saving for patients with severe bleeding that compromises cardiovascular function, but it should be undertaken with care because of the risk of fluid overload. There are recent developments of establishments in therapeutics and design of randomized, controlled trials of drugs targeting the virus or the immune response (111). However, currently, there is no evidence in favor of the use of any specific therapeutic agent for dengue (38).

Conclusion

Over the past decades, the field of dengue research has been growing with the realization of the burden of disease coupled with the prospect of various antiviral therapeutics and vaccines. However, dengue has been problematic in an overall manner mainly because of its complexity. Being a disease with complicated features, it is of utmost importance that there should be more improved overall understanding of the disease to combat this global public health challenge. Besides that, no vaccine can be of immediate global curative, and efforts to improve treatment through application of existing best practices in triage and fluid management, along with efforts to develop new antiviral or other therapeutic drugs should be continued.

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