



RESEARCH ARTICLE

Planning social mobilization and communication for Dengue Hemorrhagic fever Diagnosis, Treatment, Prevention and Control: A step- by- step guide¹Rohit Udhav Bharti*, ²Dr. Sudha Garudkar¹Department of Pharmaceutics, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India-431606²Maharashtra University of Health Science, Nashik, Maharashtra, India.**Received 15 November 2014; Accepted 25 November 2014****ABSTRACT**

Dengue fever and dengue haemorrhagic fever are important arthropod-borne viral diseases. Each year, there are ~50 million dengue infections and ~500,000 individuals are hospitalized with dengue haemorrhagic fever, mainly in Southeast Asia, the Pacific and the Americas. Illness is produced by any of the four dengue virus serotypes. A global strategy aimed at increasing the capacity for surveillance and outbreak response, changing behaviours and reducing the disease burden using integrated vector management in conjunction with early and accurate diagnosis has been advocated. Antiviral drugs and vaccines that are currently under development could also make an important contribution to dengue control in the future. So in this review we are going to discuss about Pathophysiology of Dengue, Clinical sign, laboratory diagnosis of infection- in vivo & in-vitro, treatment of dengue by medicinal plants. Also study on development of vaccine on dengue.

Key words: Dengue virus, NS3 protease, Diagnosis, Medicinal plants, Prevalence**INTRODUCTION:**

Dengue is one of the most important mosquito-borne viral diseases affecting humans, with over half of the world's population living in areas at risk. Originally, dengue virus infections occurred mainly as epidemics in tropical and subtropical countries. But over time, with increasing globalization and the geographic spread of inhabitants of *Aedes aegypti* and *Aedes albopictus* mosquitoes, the dominant vectors for dengue virus transmission, dengue virus infection has steadily penetrated every corner of the world^{1,2}. Dengue virus has four serotypes, and each of them can cause a spectrum of diseases ranging from asymptomatic, mild febrile (dengue fever, DF) to a life-threatening illness, dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). Approximately 50 to 100 million people contract dengue fever annually, and about 200,000 to 500,000 of these are DHF/DSS, which has a mortality rate about 1%-5%, mainly in children under 15 years of age³. Dengue is the most important arthropod-borne viral infection of humans. Worldwide, an estimated 2.5 billion people are at risk of infection, approximately 975 million of whom live in urban areas in tropical and sub-tropical countries in Southeast Asia, the Pacific and the Americas⁴. Transmission also occurs in Africa and the Eastern Mediterranean, and rural communities are increasingly

being affected. It is estimated that more than 50 million infections occur each year, including 500,000 hospitalizations for dengue haemorrhagic fever, mainly among children, with the case fatality rate exceeding 5% in some areas⁴⁻⁸.

The annual average number of dengue fever/dengue haemorrhagic fever (DF/DHF) cases reported to the World Health Organization (WHO) has increased dramatically in recent years. For the period 2000-2004, the annual average was 925,896 cases, almost double the figure of 479,848 cases that was reported for the period 1990-1999. In 2001, a record 69 countries reported dengue activity to WHO and in 2002, the Region of the Americas alone reported more than 1 million cases. Although there is poor surveillance and no official reporting of dengue to WHO from countries in the African and Eastern Mediterranean regions, in 2005-2006 outbreaks of suspected dengue were recorded in Pakistan, Saudi Arabia, Yemen, Sudan and Madagascar⁴⁻⁸, and a large outbreak of dengue involving >17,000 cases was documented in the Cape Verde islands in 2009⁹. Travellers from endemic areas might serve as vehicles for further spread¹⁰⁻¹³. Dengue epidemics can have a significant economic and health toll. In endemic countries in Asia and the Americas, the burden of dengue is approximately 1,300 disability-adjusted life years

(DALYs) per million populations, which is similar to the disease burden of other childhood and tropical diseases, including tuberculosis, in these regions¹⁴.

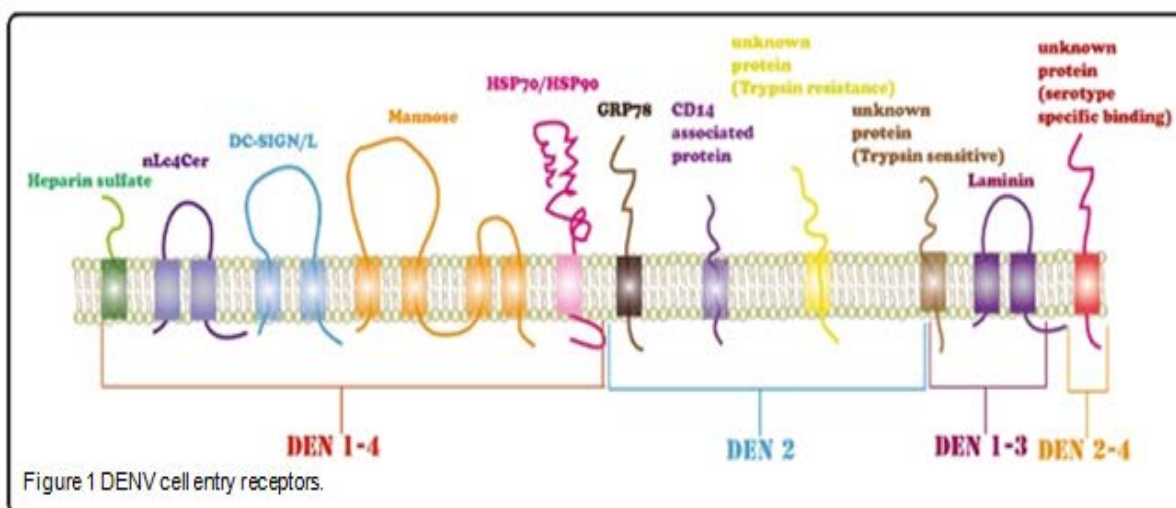
The geographical areas in which dengue transmission occurs have expanded in recent years and all four dengue virus serotypes (DENV-1-4) are now circulating in Asia, Africa and the Americas, a dramatically different scenario from that which prevailed 20 or 30 years ago. The molecular epidemiology of these sero-types has been studied in an attempt to understand their evolutionary relationships¹¹. This Review will provide an update on our understanding of the pathogenesis of this successful pathogen, how we diagnose and control infection and the progress that has been made in vaccine development.

DENGUE VIRUS PATHOGENESIS

Dengue viruses belong to the genus flavivirus within the Flaviviridae family. DENV-1-4 evolved in non-human primates from a common ancestor and each entered the urban cycle independently an estimated 500-1,000 years

ago¹⁵. The virion comprises a spherical particle, 40-50 nm in diameter, with a lipopolysaccharide envelope. The positive single-strand RNA genome, which is approximately 11 kb in length, has a single open reading frame that encodes three structural proteins — the capsid (C), membrane (M) and envelope (E) glycoproteins and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Important biological properties of dengue viruses, including receptor binding, haemagglutination of erythrocytes and the induction of neutralizing antibodies and the protective immune response, are associated with the E glycoprotein.

Each DENV shares around 65% of the genome, which is approximately the same degree of genetic relatedness as West Nile virus shares with Japanese encephalitis virus. Despite these differences, each serotype causes nearly identical syndromes in humans and circulates in the same ecological niche¹⁶.



The mosquito vectors, principally *Aedes aegypti*, become infected when they feed on humans during the usual five-day period of viraemia. The virus passes from the mosquito intestinal tract to the salivary glands after an extrinsic incubation period, a process that takes approximately 10 days and is most rapid at high ambient temperatures¹⁷. Mosquito bites after the extrinsic incubation period result in infection, which might be promoted by mosquito salivary proteins¹⁸. In the skin, dengue viruses infect immature dendritic cells through the non-specific receptor dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN¹⁹). Infected dendritic cells mature and migrate to local or regional lymph nodes where they present viral antigens to T cells, initiating the cellular and humoral immune responses. There is also

evidence of abundant replication of DENVs in liver parenchymal cells and in macrophages in lymph nodes, liver and spleen, as well as in peripheral blood monocytes²⁰. Both in vitro and in vivo, macrophages and monocytes participate in antibody-dependent enhancement (ADE)²¹⁻²³. ADE occurs when mononuclear phagocytes are infected through their Fc receptors by immune complexes that form between DENVs and non-neutralizing antibodies. These non-neutralizing antibodies result from previous heterotypic dengue infections or from low concentrations of dengue antibodies of maternal origin in infant sera²⁴. The co-circulation of four DENV serotypes in a given population might be augmented by the ADE phenomenon²⁵.

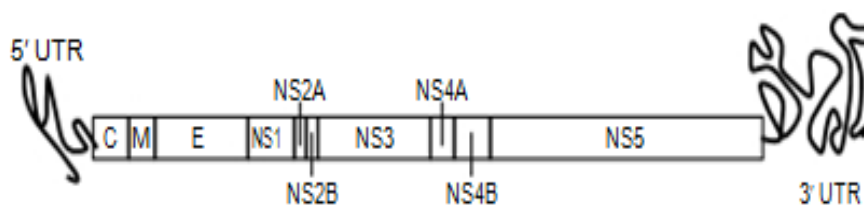


Figure 1: The dengue virus genome. The single open reading frame encodes three structural proteins (the capsid (C), membrane (M) and envelope (E) glycoproteins) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5).

CLINICAL SIGNS AND IMMUNOLOGICAL RESPONSE

Dengue-associated deaths are usually linked to DHF/ DSS. Even though no vaccines or drugs are available, severe disease can be successfully managed by careful monitoring of the warning signs and early initiation of aggressive intravenous rehydration therapy. During the early febrile stage (the symptoms of which include fever, malaise, headache, body pains and rash), clinicians cannot predict which patients will progress to severe disease.

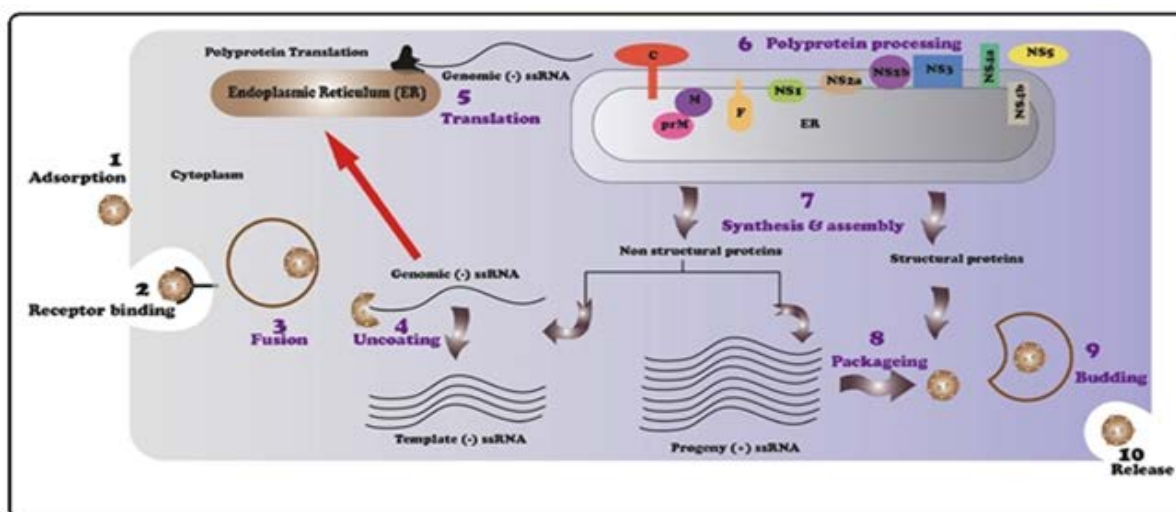


Figure 2: Dengue virus replication cycle.

Later, during defervescence, symptoms such as bleeding, thrombocytopenia of $<100,000$ platelets mm^{-3} , ascites, pleural effusion, haematocrit $>20\%$ and clinical warning signs, such as severe and continuous abdominal pain, restlessness and/or somnolence, persistent vomiting and a sudden reduction in temperature (from fever to sub-normal temperature) associated with profuse perspiration, adynamia (loss of strength or vigor) and sometimes fainting, can be indicative of plasma extravasation and the imminence of shock. At this point, patients should receive fluid replacement (crystalloids) to avoid haemodynamic instability, narrowness of blood pressure and hypotension. Early resuscitation can prevent other complications, such as massive haemorrhage, disseminated intravascular coagulation, multiple organ failure, and respiratory failure due to non-cardiogenic pulmonary oedema²⁶⁻²⁹. Treatment of uncomplicated dengue cases is only supportive, including plenty of oral fluids during the febrile period and

paracetamol (acetaminophen), the daily dosage of which should not be exceeded to prevent intoxication mainly related to liver function. When dengue shock becomes prolonged or recurrent, intravenous fluids should be given carefully according to age and dosage to prevent fluid overload as this can result in pulmonary oedema. The acquired immune response to dengue infection consists of the production of antibodies that are primarily directed against the virus envelope proteins. The response varies depending on whether it is a primary or secondary infection^{30,31}. A primary antibody response is seen in individuals who are not immune to dengue and a secondary immune response is observed in patients who have had a previous dengue infection (FIG. 3). A primary infection is characterized by a slow and low-titre antibody response. Immunoglobulin (Ig)M antibodies are the first isotype to appear, by day 3-5 of illness in 50% of hospitalized patients and by day 6-10 of illness in 93-99% of cases. The IgM levels peak ~ 2 weeks after the onset of

fever and then generally decline to undetectable levels over the next 2-3 months³². Dengue-specific IgG is detectable at low titre at the end of the first week of illness and slowly increases.

By contrast, during a secondary infection, high levels of IgG antibodies that crossreact with many flaviviruses are detectable even in the acute phase and rise dramatically over the following 2 weeks³². The kinetics of the IgM response are more variable; as IgM levels are significantly lower in secondary dengue infections, false-negative test results for dengue-specific IgM have been reported during secondary infections^{33,34}. Following a dengue infection, IgG can be lifelong, which complicates the

serodiagnosis of past, recent and current infections^{32,34}. IgA and IgE responses have also been documented but the utility of detecting these immunoglobulins as markers for dengue serodiagnosis requires further study³⁵.

In areas where two or more flaviviruses are circulating, multiple and sequential flavivirus infections make differential diagnosis difficult owing to the presence of pre-existing antibodies and the phenomenon of original antigenic sin (during sequential flavivirus infections, B-cell clones responding to the first infection synthesize antibodies with higher affinity for the first infecting virus than for the second infecting virus)³⁶.

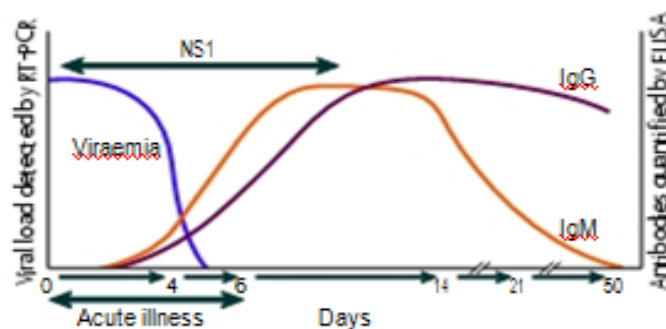


Figure 3: Dengue virus, antigen and antibody responses used in diagnosis. Ig, immunoglobulin; NS, non-structural.

LABORATORY DIAGNOSIS OF DENGUE INFECTION

Laboratory confirmation of dengue infection is crucial as the broad spectrum of clinical presentations, ranging from mild febrile illness to several severe syndromes, can make accurate diagnosis difficult. Among the methods available for dengue diagnosis, virus isolation provides the most specific test result. However, facilities that can support viral culture are not always available. The detection of the viral genome or viral antigens also provides evidence of infection. Seroconversion of IgM or IgG antibodies is the standard for serologically confirming a dengue infection. The presence of IgM or high levels of IgG in acute serum collected from a suspected dengue case suggests a probable dengue infection^{37,38}. BOX 1 shows the laboratory criteria for confirmed and probable dengue infections.

Box 1 | Laboratory diagnosis of a dengue virus infection

Confirmed dengue infection/Probable dengue infection

- Virus isolation / •IgM positive
- Genome detection / • Elevated IgG titre (that is, 1,280 or greater
- Antigen detection / by haemagglutination inhibition test)
- IgM or IgG seroconversion

IN VIVO DENGUE PATIENTS

Studies over the years with specimens collected from the peripheral blood of dengue patients reveal that virus can be recovered or detected in a variety of cells. However, a

general consensus concerning which cell lineages are involved in dengue viremia has never been conclusive, partly due to the variation of timing in specimen collection. Upon admission to the hospital with clinical symptoms, patients are always several days after the infection and frequently at the peak or downturn in viremia. By that time, a complex network of immune responses initiated and is in the action of viral clearance. Perhaps, this may explain why immune cells are commonly associated with the detection and/or isolation of virus in dengue patients³⁹. Thus, the cells that are infected early, before the peak in viremia, and accounting for dengue viremia are still unknown.

PLATELETS IN DENGUE

One of the important clinical hallmarks in dengue virus infection in patients is platelet dysfunction, which occurs throughout the acute phase, and/or thrombocytopenia, which frequently occurs at the defebri stage, thus this is a subject of interest, especially in understanding the possible mechanisms leading to the observed phenomena. There are a few proposed mechanisms that may explain platelet dysfunction and/or thrombocytopenia:

(i) decreased production, (ii) direct infection by virus, (iii) increased consumption, or (iv) immune-complex lysis. The first mechanism has been observed. Early in

infection of dengue virus, it exerts a transient depressive effect on megakaryocytes in the bone marrow^{40, 43}, which subsequently becomes normocellular or hypercellular a few days after onset of fever^{42,43}. In vitro and in vivo, dengue virus has been demonstrated to have toxic effects on platelets in the presence and absence of acute and convalescent patient serum, lending some support for the second mechanism^{44, 46}. In addition, dengue viral RNA has been isolated from or detected in platelets isolated from secondary dengue virus infected patients⁴⁷. However, the precise mechanisms for the development of dysfunctional platelets and thrombocytopenia in dengue patients remain unknown. Also, the interactions of dengue virus with platelets, including entry and possible virus production, have not been investigated.

We have proposed that platelets may be a critical element in early dengue virus infection^{48,50}, which may partially account for the dysfunction of platelets. Subsequent systematic investigations, with biological assays and electron microscopy, reveal that dengue viral RNA, either the positive stranded genome or negative stranded template, and the presence of mature virus-like particles, are consistently observed in platelets isolated from dengue confirmed patients during the acute phase of infection [47-51]. A micrograph of dengue virus-like particles within platelets isolated from confirmed dengue patients is depicted. Typical clustering of dengue virus-like particles surrounded by a vesicle was observed in platelets and occasionally single or isolated dengue virus-like particles were observed⁵¹. Infrequently, dengue virus-like particle with a fuzzy morphology were observed associated with or released from platelets.

However, we could not rule out the possibility that these dengue virus-like particles containing platelets are in the category of megakaryocyte-derived microparticles⁵². In addition, immunofluorescent staining of platelets isolated from confirmed dengue patients reveals that viral antigens can be observed not only in platelets, but also in cells with the similar morphology as proplatelets, while some dengue viral antigens were observed in presumably the micromegakaryocytes. This observation is consistent with early reports by Nelson et al.^{38,47}, who originally observed the presence of immature and nonplatelet forming megakaryocytes circulating in dengue patients and by Bhamarapravati and Boonyapaknavik⁵³, who noted that positive staining for dengue viral antigen in human tissues was demonstrated only in the lymphoid like cells. Interestingly, the nucleated micromegakaryocytes, which are similar in size and morphology to lymphocytes, have been well documented^{54,57}. The presence of micromegakaryo-

cytes, as opposed to megakaryocytes, suggests that production of platelets from bone marrow increases in response to dysfunctional or low numbers of platelets in the circulation of acute dengue patients.

Although platelets do not have a nucleus, they possess functional spliceosomes that are able to process pre-mRNAs into mature mRNA, from which proteins can be translated and processed^{55,56}. In vitro experiments were set up to investigate the susceptibility of platelets to support dengue virus production, which may directly contribute to the platelet dysfunction. A low level of dengue virus production could occur in infected platelets with the peak occurring at 18 hours post infection, suggesting that dengue virus is capable of replicating in platelets and dengue viral antigens may be expressed on the surface of platelets. Alternatively, the moderate viremia changes may result from the transient ability of platelets reproduction in culture conditions⁵⁸, which may have the capacity of capturing and releasing dengue viruses in later hours. Perhaps, this may account for the rise of platelet-associated antibodies (PAIgM/IgG) during acute dengue virus infection⁴⁹ and the increased incidence of phagocytosis of platelets from patients with secondary infections by human macrophages⁵⁹. In addition, administration of intravenous immunoglobulin, which saturates phagocytosis and impedes antibody production, lacked efficacy when used to treat severe thrombocytopenic patients with secondary dengue virus infection⁶⁰. As a whole, these evidences suggest that dengue virus may take a ride and experience ongoing maturation within platelets produced from infected progenitor megakaryocytes. Platelets are anucleate cells that have hemostatic and inflammatory functions^{61,62} and are composed of a concentrate of megakaryocyte membrane, cytoplasm, granules, and organelles⁶³. Platelets circulate throughout blood vessels during which they monitor the integrity of the vascular system. All functional platelet responses must be tightly regulated to ensure that the formation of blood clots is of sufficient size to seal off the damaged area, while not disrupting blood flow to vital organs by causing vessel occlusion^{64,66}. With the observation that dengue viral antigens are associated with proplatelets or micromegakaryocytes⁵⁸⁻⁶⁹ in blood during acute dengue virus infection, it is likely that a platelet lineage parental cell, megakaryocytes, may be involved in the production of dengue virus during acute infection. In addition, platelets contain several key elements related to dengue virus infection, such as DC-SIGN⁷⁰ as well as complement and Fc receptor, which have been implicated in virus uptake^{71,72}. It is also possible that a unique receptor or coreceptor is required

for viral binding and entry into platelets. However, this particular receptor or coreceptor may not be evenly distributed or allocated in platelets since platelets are demarcated from the membrane of megakaryocytes, which may result in heterogeneous populations of platelets. This heterogeneity of platelet alloantigen referred to as human platelet alloantigen (HPA) polymorphism in the literature, and how it contributes to dengue virus infection and dengue disease severity warrants further investigation.

VIRUS ISOLATION

The *Aedes albopictus* mosquito C6/36 cell line is the method of choice for DENV isolation, although other mosquito (such as *Aedes pseudoscutellaris* AP61) and mammalian (including Vero cells, LLC-MK2 cells and BHK21 cells) cell lines can also be used^{73,74}. Sera that have been collected from suspected dengue cases in the first 3-5 days of fever (the viraemic phase) can be used for virus isolation. After an incubation period permitting virus replication, viral identification is performed using dengue-specific monoclonal antibodies in Immunofluorescence and PCR assays^{75,76}. Serum is often used for virus isolation but plasma, leukocytes, whole blood and tissues obtained at autopsy can also be used^{77,78}.

DENGUE CONTROL AND PREVENTION STRATEGIES

A global strategy for dengue prevention and control was promulgated more than 10 years ago and comprises five major elements (BOX 2). Efforts have since been made to focus on three fundamental aspects: surveillance for planning and response, reducing the disease burden and changing behaviours to improve vector control⁷⁹. The 2002 World Health Assembly Resolution urged greater commitment among Member States and WHO to implement this strategy⁸⁰. Of particular significance is the 2005 revision of the International Health Regulations⁸¹, which includes mention of DF (and yellow fever) as an example of a health 'event that may constitute a public health emergency of international concern' and which, under such circumstances, should be notified to WHO.

In recent years several new, improved or validated tools and strategies for dengue control and prevention have been developed and are available to public health practitioners and clinicians. Vector control. To reduce or prevent dengue virus transmission there is currently no alternative to vector control. Most endemic countries have a vector control component in their dengue control and prevention programmes but its delivery by public health practitioners is frequently insufficient, ineffective or both. Given its behaviour and generally close association with humans, the principal vector *A. aegypti* requires the use of a combination of vector-control

methods, notably environmental management methods and chemical control methods based on the application of larvicides and adulticide space sprays⁸². Chemical controls typically must be added to water stored for domestic use, including drinking water. The active ingredients of four larvicides have been assessed by the International Programme on Chemical Safety (IPCS) to determine their safety for use as mosquito larvicides in drinking water at dosages that are effective against *Aedes* spp. larvae. Since the early 1970s the organophosphate temephos has been widely used, but increasing levels of resistance^{83,84}, householders' rejection of the treatment of their drinking water, and difficulties in achieving high and regular levels of coverage are important technical and operational constraints.

Biological control agents, including larvivorous fish and copepods, have had a demonstrable role in controlling *A. Aegypti*^{85,86}, but operational difficulties particularly the lack of facilities and expertise in mass rearing, and the need to frequently re-introduce these agents into some container habitats have largely precluded their widespread use.

Box 2: The global strategy for dengue prevention and control

- Vector control, based on the principles of integrated vector management
- Active disease surveillance based on a comprehensive health information system
- Emergency preparedness
- Capacity building and training
- Vector control research

MEDICINAL PLANTS AS DENGUE VIRUS INHIBITORS

Plants have been traditionally used to cure a number of human diseases. To date, few plants derivatives due to their medicinal properties have successfully been tested against viral diseases. The first step of DENV cycle is to attach with host via host receptors. Envelop protein (E) is involved in viral and host attachment. Thus, dengue infection can be inhibited by targeting envelop protein/ inhibiting host-viral interactions. NS2-NS3 protease and NS5 also serve as important antiviral drug targets due to their role in viral replication and other cellular processes. To date, many medicinal plants have been tested against DENV and some of them showed significant inhibition effects in the DENV replication cycle. Antiviral effects of methanolic extracts of *Andrographis paniculata*, *Citrus limon*, *Cymbopogon citrates*, *Momordica charantia*, *Ocimum sanctum* and *Pelargonium citrosum* on dengue virus serotype-1 (DENV-1) were investigated by Tang and colleagues. *A. paniculata* showed the most antiviral inhibitory effect followed by *M. charantia* in in-vitro

assays. Both these plants can be advantageous in developing novel antiviral compounds⁸⁷. *Carica papaya* contains two important biologically active compounds, chymopapain and papain, which are used in digestive disorders⁸⁸. *C. papaya* leaves extract prepared in water has been tested against dengue fever. After the administration of aqueous extract in dengue infected patient, the platelet count increased from $55 \times 10^3/\mu\text{L}$ to $168 \times 10^3/\mu\text{L}$, White blood cells from $3.7 \times 10^3/\mu\text{L}$ to $7.7 \times 10^3/\mu\text{L}$ and neutrophils from 46% to 78%. Thus *Carica papaya* can be used to target dengue fever⁸⁹. Extracts of neem leaves and pure neem compound (Azadirachtin) were tested against DENV-2 replication. In-vitro activity was assessed in $C_{6/36}$ (cloned cells of larvae of *Aedes albopictus*) cells. Aqueous neem extracts at its maximum nontoxic concentration of 1.897 mg/ml, completely inhibited 100-10,000 TCID₅₀ of virus as indicated by the absence of cytopathic effects⁹⁰. Talarico and colleagues investigated lambda- and iota-carrageenans, sulfated polysaccharides containing linear chains of galactopyranosyl residues against DEN-2, DEN-3 using Vero and HepG2 cells and found that these compounds are potent dengue inhibitors. The inhibitory action was exerted by dual interference with virus adsorption and internalization of nucleocapsid into the cytoplasm. Carrageenans did not interfere with viral protein synthesis and virus multiplication. Thus, carrageenans can be used in developing new therapies by interfering with virus adsorption in host cell⁹¹. Rehman and colleagues investigated *Quercus lusitanica* extract against DEN-2 replication. At a concentration of 180 $\mu\text{g}/\text{ml}$, *Q. lusitanica* was found to completely inhibit dengue virus infection. Furthermore, methyl gallate from fractionalized crude extracts showed 96% inhibition at the maximum non-toxic dose (MNTD) of 100 $\mu\text{g}/\text{mL}$ ⁹². DEN-2 infection was inhibited by WSS45 (Sulfate derivative of an alpha-D-glucan) derived from *Gastrodia B1* in BHK (Baby hamster kidney fibroblast cells) cells with an EC (50) value of $0.68 \pm 0.17 \mu\text{g}/\text{mL}$. WSS45 interfered with virus adsorption but showed no veridical effect. Thus, WSS45 can be used to increase virus detaching from the host cell surface⁹³. Replication of DEN-2 was significantly reduced by two compounds; 1-beta-d-ribofuranosyl-3-ethynyl-[1, 2, 4]triazole (ETAR) and 1-beta-d-ribofuranosyl-4-ethynyl-[1, 3]imidazole (IM18). ETAR also reduced replication of DEN-1, DEN-3 and DEN-4. Therefore, ETAR can be used as a potential therapeutic drug against dengue viruses⁹⁴. Kiat and colleagues investigated two cyclohexenyl chalcone derivatives of *Boesenbergia rotunda* (L.), 4-hydroxypanduratin A and panduratin A and showed inhibitory activity of both

compounds against DEN-2 NS3 protease with the Ki values of 21 and 25 μM , respectively⁹³. A series of new mono- and dialkylated flavanones having NS5 RNA-dependent RNA polymerase (RdRp) inhibiting activity named chartaceones A-F (1-6) along with pinoembrin was identified from *Cryptocarya chartacea*. Chartaceones C-F (3-6) which are dialkylated flavanones showed significant NS5 RdRp inhibiting activity, with IC(50) ranging from 1.8 to 4.2 μM ⁹². Zandi and colleagues investigated four bioflavonoids (quercetin, daidzein, naringin and hesperetin) against DEN-2 using Vero cells and proved that only quercetin had significant anti-DEN-2 inhibitory activity. Thus, there is a need to further investigate these compounds to develop novel inhibitors against DENV⁹¹. Three flavonoids; fisetin, naringenin and rutin were tested against DENV-2 serotype using foci forming unit reduction assay (FFURA) and quantitative real-time polymerase chain amplification (qRT-PCR). After viral adsorption, Fisetin was added that lead to DENV-2 replication inhibition with a half maximal inhibition concentration (IC₅₀) value of 55 $\mu\text{g}/\text{mL}$ and selectivity index (SI) of 4.49. In Vero cells, rutin and naringenin did not inhibit DENV-2 replication⁸⁹. Recently, Tang and colleagues investigated methanolic extracts of *Andrographis paniculata*, *Citrus limon*, *Cymbopogon citratus*, *Momordica charantia*, *Ocimum sanctum* and *Pelargonium citrosum* on DEN-1. Among all the six medicinal plants, *A. paniculata* showed the most antiviral inhibitory effects followed by *M. charantia*. Thus, these two plants need further investigations to develop potential dengue treatment⁸⁷.

With the increased political recognition of dengue as a public health problem and commitment to prevention and control, better organized control services using new tools and partnership strategies, based on the principles of integrated vector management, are likely to have a major impact on dengue transmission⁹².

VACCINE DEVELOPMENT

As a result of the failure of vector control, the continuing spread and increasing intensity of dengue has renewed interest and investment in dengue vaccine development, making a safe, effective and affordable tetravalent dengue vaccine a global public health priority⁹³. Dengue vaccine development has been in progress for several decades, however the complex pathology of the illness, the need to control four virus serotypes simultaneously and insufficient investment by vaccine developers have hampered progress⁹⁴. The observation that DHF/DSS is associated with DENV secondary infection poses a special challenge to the development of a dengue vaccine,

leading to a requirement that such vaccines should induce a robust immune response against the four serotypes in naive as well as previously immune individuals. Animal models are only partially useful for vaccine evaluation. The poor understanding of the

mechanisms involved in inducing protective immunity against dengue infection poses additional challenges^{93,94}. Finally, cases of DHF/DSS have recently been documented 20 or more years after primary dengue infection, which adds a new dimension to the problem.

Table 1: Selected dengue vaccine candidates

| Vaccine approach | Developer | Status |
|---|-------------------------|-------------|
| Live attenuated tetravalent chimeric YF-DEN vaccine | Sanofi Pasteur | Phase II |
| Live attenuated tetravalent viral isolate vaccine | WRAIR and GSK | Phase II |
| Live attenuated chimeric DEN2-DEN vaccine | CDC and Inviragen | Phase I |
| Recombinant E subunit vaccine | Merck | Phase I |
| Live attenuated tetravalent vaccine comprising 3' deletion mutations and DEN-DEN chimeras | US NIH LID and NIAID | Phase I |
| Subunit recombinant antigen (domain III) vaccine | IPK/CIGB | Preclinical |
| Live attenuated chimeric YF-DEN vaccine | Oswaldo Cruz Foundation | Preclinical |
| Tetravalent DNA vaccine | US NMRC and GenPhar | Preclinical |
| Purified inactivated tetravalent vaccine | WRAIR and GSK | Preclinical |

CDC, Centers for Disease Control and Prevention; CIGB, Center for Genetic Engineering and Biotechnology; GSK, GlaxoSmithKline; IPK, Pedro Kouri Tropical Medicine Institute; NIAID, National Institute for Allergy and Infectious Diseases; US NIH LID, United States National Institutes of Health Laboratory of Infectious Diseases; US NMRC, United States Naval Medical Research Center; WRAIR, Walter Reed Army Institute of Research; YF, yellow fever.

CONCLUSION:

Dengue infection has emerged as a major health concern in Southeast Asia, the Pacific and America. Developing tetravalent vaccine against all four dengue serotypes is quite challenging. To date, there is no licensed vaccine available for dengue virus. Therefore, there is an urgent need to develop an alternative solution to combat this endemic infection. Dengue virus enveloped protein is involved in virus cell entry; NS3 and NS5 are involved in viral replication and other cellular processes; therefore, these can serve as an important drug target to combat this life-threatening disease. Several medicinal plants have been tested against dengue virus entry and replication; many of them showed significant inhibitory effects. Above all the final winning goal of fight will be the discovery of DF vaccine or specific antiviral agents.

Nevertheless, it will be very exciting to see these medicinal plants as potential DENV inhibitors to progress through clinical developments and hopefully, provide dengue patients with much needed, more effective therapies. Otherwise dengue will grow up and soon take an epidemic proportion in our country.

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