



Advancement in Dengue Treatment: A review

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ABSTRACT

This review describes and commentates on recent advances in the understanding of dengue diagnosis and immunity, plus clinical research on vaccines and therapeutics. We expand specifically on the role of the dermis in dengue virus infection, the contribution of cellular and humoral immune responses to pathogenesis and immunity, NS1 and mechanisms of virus immune evasion. Additionally we review a series of therapeutic intervention trials for dengue, as well as recent clinical research aimed at improving clinical diagnosis, risk prediction and new mechanism to inhibit dengue virus infection

Keywords: Dengue; Vaccine; Diagnosis; Immunology; Clinical research, new mechanism to inhibit dengue virus infection

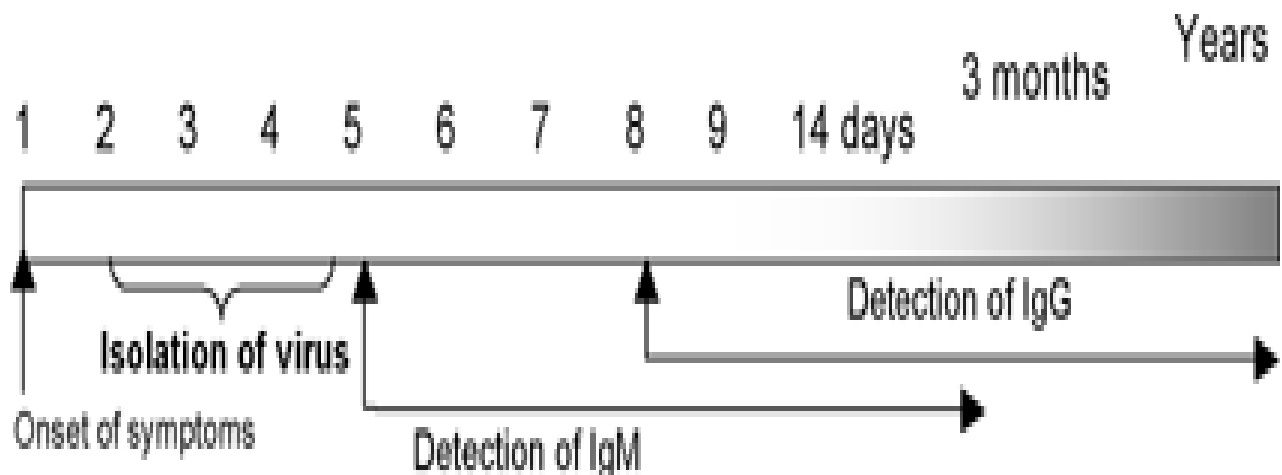


A blood sample to attempt virus isolation and serology. If an autopsy is performed, blood from the heart should be collected. In addition to blood, the following tissue samples should be obtained: heart, liver, kidney, lung, intestines, spleen, lymph nodes, brain, and skin from areas where the rash occurred. If fresh tissues are available, they will be tested for virus isolation. If an autopsy has been performed and no fresh tissues are available, tissues fixed in formalin should be submitted for immunohistochemical studies.

Lab diagnosis:

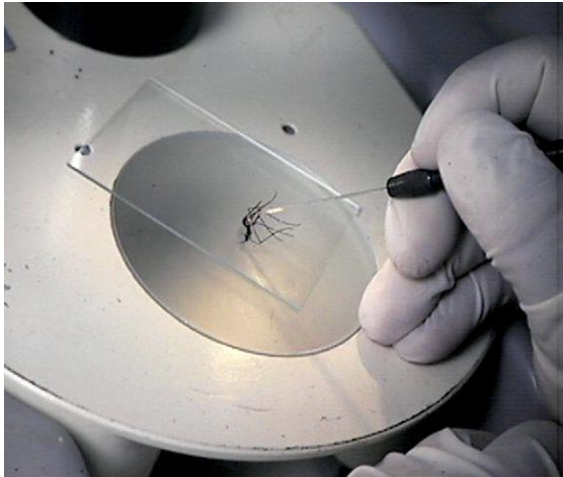
Virus isolation
Antibody detection
Antigen detection
Viral nucleic acid detection

General time line of a primary infection from virus isolation to detection of IgM & IgG



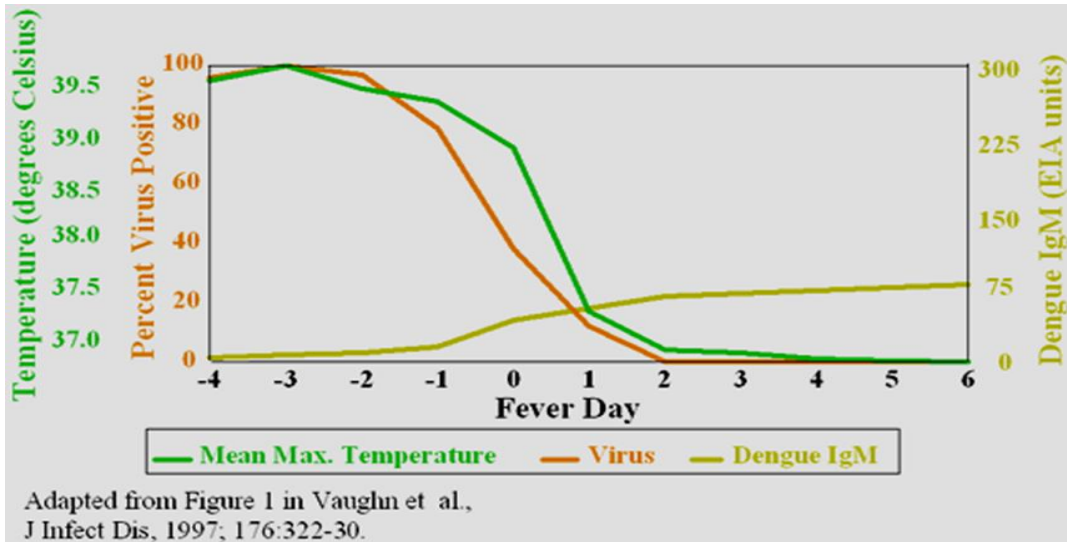
Isolation of virus:

I/C inoculation of adult Toxorhynchites:



Isolation of virus using mosquitoes- During the early febrile phase of the illness most sensitive and relatively rapid method .Virus can be isolated within 2 days I/C inoc. of adult or larval Toxorhynchites mosquitoes 5 to 20 mosquitoes per specimen. Method of detection-detection of antigen in head squash by IF-serotype specific MoCl DEN antibodies.cell lines Mammalian – Vero, PS, BHK-21 Mosquito- C6-36, AP-61,TRA-284(most sensitive), no cytopathic effect. Detection of antigen by IF Suckling mice-I/C Very insensitive method for Dengue.

Antibody detection: IgM – ELISA (MAC-ELISA), Haemagglutination inhibition test (HI), Complement fixation test (CFT), Plaque reduction neutralization test (PRNT). IgM antibodies appear by day 5.



Adapted from Figure 1 in Vaughn et al., J Infect Dis, 1997; 176:322-30.

IgM ELISA- IgM antibodies appear by day 5 after onset of illness and appear to peak by about 2 weeks and then decline by about 2 to 3 months.
IgM ELISA Dengue

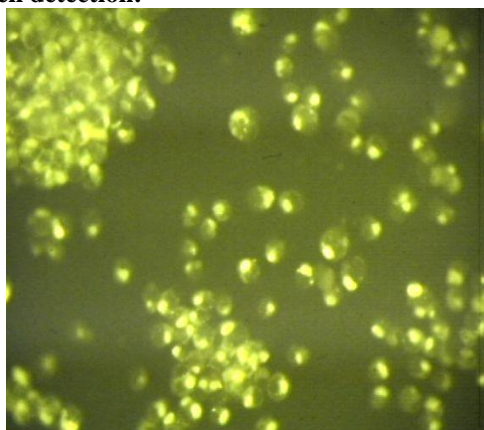


To distinguish primary from secondary infection of Dengue: (Haemagglutination inhibition test) - Secondary inf.-HI antibodies in

acute phase sample and by high titres (>1:1280) in convalescent serum Ratio of IgM and IgG antibodies.Western blotting of serum - Primary infection - Antibodies against E glycoprotein and the NS3 and NS5 proteins .Secondary inf- reveal a broader array of viral protein bands

Viral nucleic acid detection: Reverse transcriptase-polymerase chain reaction (RT-PCR) for DEN Extraction of RNA from serum, viremic host, host tissues, infected cell culture fluids Synthesis of cDNA using serotype specific primers.Identification of the amplified product.Result within 3-4 hrs

Antigen detection:



Immunocytochemical staining of peripheral blood mononuclear cells-acute phase IF staining of antigen in – Infected cell cultures, field collected arthropod vectors Direct detection of dengue viral antigen in human sera by CCIE and by Monoclonal RIA.

Innovation in the development of DEN diagnostics:

NSI assay, Rapid diagnostic kit (Dengue duo kit), NS1 antigen & IgM & IgG in strips.

NS1 antigen detected up to 5 days.Result within 20mts.For early case detection & outbreak investigation NS1 antigen capture ELISA.

Rapid test for NS1 antigen,IgM & IgG:



Advantage Dengue NS1 Ag card test is based on immunochromatographic test principle for the detection of Dengue NS1 antigen. When a sample is added to the device, Dengue NS1 Antigen if present in the sample will bind to the anti-dengue NS1 gold colloidal conjugate making antigen antibodies complex. This complex migrates along the membrane to the test region and forms the visible pink line at “T” as antibody-antigen-antibody gold colloid forms. The intensity of the test bands in the device will vary depending upon the amount of antigen present in the sample. The

appearance of any pink. red colour in a specific test region should be considered as positive for that particular antigen. A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

RECENT ADVANCEMENT IN DENGUE TREATMENT:

Important advances in the field of anti-dengue virus research (2011): These review focuses on Recent development in the AG129 mouse model& disease compound that have been able to be active in available cell & animal model system within these past year.

Designing cyclopentapeptide inhibitor as potential antiviral drug for dengue virus ns5 methyltransferase: Received April 11, 2012; Accepted April 16, 2012; published April 30, 2012

Homeopathic treatment on prevention of dengue: ⁽²⁸⁾

The formula is as follows: RHUS TOX./ EUPATORIUM PERF./ CHINA OFF./ LEDUM PALUSTRE/ GELSEMIUM/ 5CH/ aã. Just take this formula to a homeopathic pharmacy and they will know what to do. The formula can be made in globules (sacarose), tablets (lactose), or drops (alcohol at 30%) at ANY HOMEOPATHIC PHARMACY. Include next to the components of the above formula your choice of:
globules 12g, or
tablets 12g, or
drops 15ml.

This formula should be used for dengue prevention in the following manner:

Take 3 globules or tablets or drops once a day while the epidemic season lasts. This formula can be taken by adults as well as children of any age, except that children should not use the alcohol-based drops. The globules or tablets should be slowly dissolved in the mouth.

For babies, the mother can dissolve 2 globules in a teaspoon of water to facilitate administration of the medicine. If the baby is UNDER 3 months old and is being breast-fed, the mother can take 6 globules before nursing, since the effect will pass to the child through the mother’s milk.

Pregnant women can and should use this formula. this formula can be given to babies of any age that are not being breast-fed. it should be used for the treatment of dengue or even a suspected case of dengue, as follows:

Take 3 globules (or tablets or drops) every two hours and then, as the symptoms improve, begin spreading out the administration of the medicine to once every three hours and then once every four

hours, etc., until complete remission of the symptoms.

Siddha remedies for dengue, viral fever By Sheela Rani Chunkath 23rd December 2012 12:00 AM INDIAN EXPRESS

The Government of Tamil Nadu is one of the few state governments that has been aggressively promoting the use of herbal and Siddha medicines for the control of dengue and other viral fevers. This initiative is being personally reviewed by the chief minister herself and hence is being implemented very well in the state.

The government has recommended the extracts of papaya leaves, mountain neem leaves and *Andrographis paniculata* (Nilavembu in Tamil) for the prevention and treatment of dengue fever. The last one is available along with other herbs as Nilavembu Kudineer Chooranam in almost all Siddha hospitals, dispensaries, Siddha section of primary herbal centres and other hospitals in the state. Siddha vaidyars are also dispensing freshly prepared extracts of all herbals. The programme has caught on well as the government's approval has helped people believe in the prescribed herbals. In her earlier articles, she wrote about the efficacy of Sudarshana Chooranam and Dhanvantaram tablets in combating viral fevers. According to her ayurvedic vaidyar, Sudarshana Chooranam tablets are also very effective in combating dengue fevers. Sudarshana Chooranam tablets are made from 50 herbals, and one of the main ingredients is *Andrographis paniculata*—the same herb which is found in Nilavembu Kudineer. Siddha practitioners normally prescribe Nilavembu Kudineer, while ayurvedic practitioners prescribe Sudarshana Chooranam. Since Sudarshana Chooranam is available in the tablet form, it is easier to administer and distribute.

The dosage prescribed by ayurvedic vaidyars for chikungunya, dengue and other viral fevers is one tablet of Sudarshana Chooranam and one tablet of Dhanvantaram, once in four hours, for children between the ages of three and five. For children between six and eight, it is one-and-a-half tablets each; for children between nine and twelve, it is two tablets each, once in four hours. For adults, the dosage is as much as four tablets of Sudarshana and three tablets of Dhanvantaram, once in four hours. The tablets are to be taken three to four times a day, usually in empty stomach. Ayurvedic vaidyars caution that even though Sudarshana Chooranam and Dhanvantaram tablets are extremely effective, one should consult a vaidyar if the fever persists, especially in the children. Sudarshana Chooranam tablets are extremely effective because of the synergistic effect of the 50 herbals they contain.

Andrographis paniculata has antipyretic, antifungal, antibiotic, anti-inflammatory, antimalarial and liver-protective properties. As the composition of Sudarshana Chooranam is found in the Sarangadhara Samhita—written in the early part of the 14th century—and has continued to remain popular, one can clearly say that this herbal composition has stood the test of time.

If the fever is accompanied with a cold, Taleesadi Chooranam is usually administered. For children, a quarter teaspoon can be given with honey, three to four times a day. For adults, up to one teaspoon can be given with honey. As you may know, honey is a necessary adjuvant, as it reduces kapha and phlegm. Sudarshana Chooranam, Dhanvantaram and Taleesadi form part of the village health nurse's medicine kit that was introduced almost 10 years ago by the present chief minister of Tamil Nadu. My house is always well-stocked with these herbals, and they are my first line of defence against cold, cough and fever.

While suffering from fever, one should pay attention to one's diet. Easy to digest parboiled kanji rice will help to manage fever, as it provides energy while at the same time it does not trouble the stomach. Malayalis love kanji, but not so in case of the Tamilians and others. I used to hate it, but it is so efficacious that I am now a convert.

The writer was earlier Health Secretary, Tamil Nadu, and is currently Additional Chief Secretary, and Chairman and MD, Tamil Nadu Handicrafts Development Corporation.

Public health officers releasing *P. reticulata* fry into an artificial lake in the Lago Norte district of Brasília, Brazil, as part of a vector control effort.

Research efforts to prevent and treat dengue include various means of vector control, vaccine development, and antiviral drugs. With regards to vector control, a number of novel methods have been used to reduce mosquito numbers with some success including the placement of the guppy (*Poecilia reticulata*) or copepods in standing water to eat the mosquito larvae.

Attempts are ongoing to infect the mosquito population with bacteria of the *Wolbachia* genus, which makes the mosquitoes partially resistant to dengue virus.

There are also trials with genetically modified male *A. aegypti* that after release into the wild mate with females, and their offspring live through the larval stage but die as pupae, before reaching sexual maturity. There are ongoing programs working on a dengue vaccine to cover all four serotypes. One of the concerns is that a vaccine

could increase the risk of severe disease through antibody-dependent enhancement (ADE). The ideal vaccine is safe, effective after one or two injections, covers all serotypes, does not contribute to ADE, is easily transported and stored, and is both affordable and cost-effective. As of 2012, a number of vaccines were undergoing testing.

The most developed is based on a weakened combination of the yellow fever virus and each of the four dengue serotypes. It is hoped that the first products will be commercially available by 2015.

Recently added Drugs (DSS) -

- Fosamprenavir Calcium
- Moexipril Hydrochloride
- Telmisartan and Hydrochlorothiazide
- Olmесartan Medoxomil and Hydrochlorothiazide
- Mipomersen Injection

- Alogliptin and Pioglitazone
- Sumatriptan Transdermal System (TDS)
- Alogliptin
- Bedaquiline

The preferable new treatment for dengue would be an antiviral drug. At present, a specific antiviral drug is not available; however, there have been a lot of attempts to discover one. In phytomedicine, several sulfated polysaccharides extracted from seaweeds have been studied and high antiviral activity against dengue virus has been observed. In modern medicine, ribavirin, glycyrrhizin and 6-azauridine are reported to have cytostatic and inhibitory effects on the dengue virus. An adenosine analog is another promising drug currently being studied. The chemical 'NITD008' is the best example.⁽³⁰⁾

VACCINE:

Currently, five types of dengue vaccines are in development:

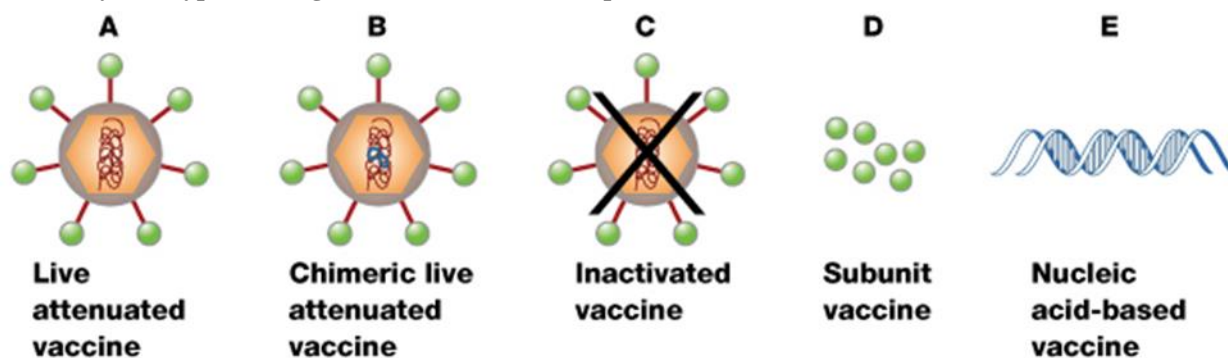


Figure 1: Types of dengue virus vaccines

Scientists are currently developing five types of vaccines to protect people from dengue infections: (a) live attenuated vaccines, (b) chimeric live attenuated vaccines, (c) inactivated vaccines, (d) subunit vaccines, and (e) nucleic acid-based vaccines.

Live attenuated vaccines are made of weakened versions of the dengue virus (Figure 1A). An ideal live attenuated vaccine would produce a robust immune response similar to that of natural infection — but without the disease or symptoms of natural infection. Because the weakened virus does not replicate well, an ideal live attenuated vaccine would produce low levels of the virus in the blood and only minimal symptoms of infection. The low levels of virus in the blood would make it unlikely that mosquitoes could transmit the attenuated virus. Live attenuated vaccines are generally inexpensive to produce, which makes them widely available. In addition, because there are four dengue viruses, the ideal vaccine should provide balanced immunity to all four dengue serotypes.

Chimeric Live Attenuated Vaccines: Chimeric live attenuated vaccines are designed by combining

genes from different sources to create a live attenuated virus (Figure 1B). Scientists are currently studying vaccines in which dengue viral genes have been genetically engineered into either a live attenuated vaccine for yellow fever or an attenuated dengue virus from a single serotype. Ideal chimeric live attenuated vaccines should have the same characteristics that are described above for live attenuated vaccines.

Inactivated and Subunit Vaccines: Two additional vaccines are the inactivated and the subunit vaccines. How do scientists make these vaccines? Inactivated vaccines are made of virus particles that have been destroyed (Figure 1C), and subunit vaccines are made of dengue proteins (Figure 1D). In both vaccines, the dengue antigens are able to produce an immune response. The inactivated and subunit vaccines generally have a high level of

safety because the virus does not replicate. They may, however, require booster vaccinations to provide long-term immunity, and they may be more expensive to produce than live attenuated vaccines.

Nucleic Acid-based Vaccines: What are the characteristics of the nucleic acid-based vaccines? Nucleic acid-based vaccines are designed by introducing DNA copies of specific dengue viral genes into cells (Figure 1E). The dengue genes are expressed as dengue proteins, which produce an immune response. This type of vaccine is relatively simple to produce, but may require multiple doses to provide immunity. Therefore, this option may not be practical for widespread vaccinations.

Researchers continue to make progress toward developing safe, effective vaccines against dengue. In preclinical and clinical trials, a large number of vaccine candidates are currently being evaluated for their safety and effectiveness. It is possible that a safe and economical dengue vaccine will be commercially available in the next few years.

Vaccine: Development and Therapy, Advances in the development of vaccines for dengue fever 2 May 2012. In this review, we discuss the current lead vaccine candidates in clinical trials, as well as some second-generation vaccine candidates undergoing preclinical evaluation. In addition, we discuss DENV epidemiology, clinical disease and strategies used for Flavivirus antivirals in the past, the development of new DENV therapeutics, and their potential usefulness for prophylaxis and treatment

World health organisation Phase IIb study of CYD-TDV September 2012.

Neem-papaya juice passes dengue test : times of India Chennai: Pushpa Narayan, TNN(Dec 20, 2012,) 05.48AM IST” juice of papaya leaf, common neem and hill neem being given to dengue patients in government hospitals in Tamil Nadu has been found to have anti-viral properties. Tests conducted at the King Institute of Preventive Medicine found that the Siddha preparation brought down symptoms and speeded up the recovery of patients. Scientists dropped the four strains of the dengue virus on the cell lines (drawn

from the gut cells of dengue causing albopictus mosquito) and added the herbal preparation. Normally, the virus would have entered the cells. "After two days, we found the cell-lines were unaffected. The virus was neutralized by the herbal formulation. Further tests showed it blocked the virus from entering the cell. In cells where the virus had already entered, it prevented multiplication," said King Institute director Dr P Gunasekaran.

New mechanism to inhibit dengue virus infection: Researchers have identified a protein that is essential for the spread of dengue infection in mosquitoes. Blocking this protein could be the strategy for developing dengue vaccines.

There is currently no approved specific treatment or vaccine for dengue fever, and an estimated two billion people are at risk for being bitten by Aedes mosquitoes and infected with the dengue virus (DENV).

"These results further our understanding of DENV pathogenesis in the mosquito vector and highlight a potential target protein for the creation of a DENV transmission-blocking vaccine to break the host-vector transmission cycle," the researchers said.

Having previously identified a number of genes, the researchers from University of South Carolina and Central Michigan University now focused on one of them -- which they termed as CRVP379 -- that codes for a putative cysteine-rich venom protein.

The researchers reported that CRVP379 is required during DENV infection in mosquito cells and in live mosquitoes, and that there is a direct correlation between the amount of CRVP379 expressed in the mosquito gut (where infection initiates) and the level of DENV infection in the gut and in whole mosquitoes.

They showed that CRVP379 interacts with a protein called prohibitin that is a putative DENV receptor in mosquitoes.

When the researchers fed Aedes mosquitoes antibodies able to recognize CRVP379, potentially blocking the interaction of the protein with either DENV or prohibitin, they found that this inhibits DENV infection of the mosquitoes.

Moreover, the scientists have been able to detect antibodies against CRVP379 in human blood samples, demonstrating that the protein is able to elicit an immune response in humans. 26 October 2015 by Gayatri Mahajan.

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