A Note On Dengue

Brought to you by:
Pgb Slater India

Like us at:
www.facebook.com/Pgb SlaterIndia

Visit our website:
http://pgblaster.wordpress.com
Created and Edited By:
Prithwiraj Maiti
Admin
Pgblaster India
Disclaimer

It is to be notified that all data and pictures used in this document are from external sources which may be subjected to copyright.

So all the users of this document are requested to use it in their personal purpose only.

This document and all the documents of Pgblaster India website are distributed only for the purpose of learning, not for republication.

All rights of this complete document go to the site and creator mentioned above.

ALL RIGHTS RESERVED.
Dedicated to,

All the fans of Pgblaster India family.
Index

Introduction and structure of dengue virus (Page no. 6)

Life cycle (Page no. 7)

Molecular pathogenesis of Dengue (Page no. 8)

Clinical symptoms of dengue (Page no. 12)

Differential diagnosis (Page no. 15)

Special issues (Page no. 15)

Antibody response to dengue infection (Page no. 19)

Laboratory diagnosis (Page no. 20)

Treatment guideline (Page no. 27)
DENGUE

1. Introduction to Dengue Virus:
   - **Genus**: Flavivirus
   - **Family**: Flaviviridae
   - **Serotypes**: 4 antigenically distinct serotypes occur in nature: DEN1, DEN2, DEN3, DEN4.
   - **Vector**: Each of the above serotypes of Dengue virus emerges independently into an epidemic cycle of transmission through *Aedes aegypti*.

2. Structure of the infective virus:

   **OUTER STRUCTURE AND STRUCTURAL PROTEINS**

   - Flavivirus particles are spherical in shape, with a lipid envelope; the particles are approximately 50 nm in diameter.
   - The lipid envelope is derived from host cell membranes.
   - **Virions contain three structural proteins**:
     1. The small basic **capsid protein** (C), which surrounds the genome of the virus.
     2. The envelope contains two proteins known as the **envelope** (E) and the **membrane** (M).
   - Two types of virions are recognized:
     1. **Mature** extracellular virions contain **M protein**.
     2. **Immature** intracellular virions contain **precursor M** (prM) protein, which is proteolytically cleaved during maturation to yield M protein.
   - Study of X-ray crystallographic structure of **mature virions** suggested that 90 E protein dimers are closely packed on the virion surface in a “herringbone” arrangement. **The dimers lay parallel to the lipid membrane, giving the virion a smooth surface**.
   - In contrast, a cryo-EM structure of the **immature virion** revealed a dramatically different arrangement, with **spikes composed of trimeric prM/E protein heterodimers projecting from the virion surface**. As a result of this arrangement, the **immature virion was slightly larger** (~ 60 nm diameter) than the mature particle.
STRUCTURE OF DENGUE VIRUS GENOME

• The dengue virus genome is **one single-stranded, positive-sense RNA molecule** which is **infectious**.
• The genome encodes a single large open reading frame of approximately 10,200 nucleotides that encodes **10 proteins**, **3 structural proteins** encoded by the 5’ quarter of the genome and **7 nonstructural (NS) proteins** encoded by the remainder of the coding region.
• The gene order is **C- prM- E- NS1- NS2A- NS2B- NS3- NS4A- NS4B- NS5**.
• The 5’ and 3’ end contains **2 non-coding regions (NCR)** which may play a role in the **initiation of RNA replication** by serving as **attachment sites for a replicase complex** (formed by viral NS3, NS5 and host proteins).

A special overview: The NS1 antigen

• This antigen has shown it to be **associated with double-stranded replicative form (RF) RNA**, suggesting **involvement in viral RNA replication**.
• Furthermore, mutations within NS1, including mutations resulting in loss of either or both glycosylation motifs, have been shown to significantly inhibit or abolish viral replication and RNA accumulation, and affect virulence property.
• **Inhibition of replication is most prominent during the initial phases of replication, suggesting an important role for NS1 in early replication events.**
• NS1 may also play some role in virion assembly/maturation, as it has been found to be associated with immature E protein in the lumen of the ER.
• NS1 is cotranslationally inserted into the lumen of the endoplasmic reticulum, where it is glycosylated and forms dimers.

LIFE CYCLE OF DENGUE VIRUS AND ITS VECTOR (Aedes aegyptii)

Transmission and infection with dengue virus:

• The primitive enzootic transmission cycle of dengue virus involves canopy-dwelling Aedes species mosquitoes and lower primates in the rain forests of Asia and Africa.
• **The viruses are maintained in an Ae. aegypti-human-Ae. aegypti cycle, with periodic epidemic occurring at 3 to 5 year intervals.**
Humans are infected with dengue virus by the bite of an infective Aedes mosquito.

- Adult Ae. aegypti mosquitoes are unobtrusive, prefer to rest indoors and feed on humans during daylight hours.
- The female mosquitoes often disrupt the feeding process at slightest movement and return to the same or a different person to continue feeding moments later. The Ae. Aegypti females may thus feed on several persons during a single blood meal and transmit dengue virus to multiple persons within a short period of time.

-----This behavior makes Ae. Aegypti an efficient epidemic vector.

Molecular pathogenesis of Dengue virus

1. Entry into the skin and consecutive events

- During the feeding of mosquitoes on humans, dengue virus is injected into the bloodstream, with spillover in the epidermis and dermis, resulting in infection of immature Langerhans cells and keratinocytes.
- Infected cells then migrate from the site of infection to the lymph nodes, where monocytes and macrophages are recruited, which are the major targets of dengue virus.
- Consequently, infection is amplified and virus is disseminated through the lymphatic system and results in primary viraemia.
- As a result of primary viraemia, several cells of mononuclear lineages (monocytes, myeloid lineages, liver and splenic macrophages) have been infected.

2. Entry of dengue virus into the target cell

- Monocytes and macrophages (mΦ) are the principal target cells for dengue viruses.
- The virus E-protein mediates the attachment to cells, E-protein is both the target as well as the modulator of host immune response.
- The receptor molecules for E protein currently being explored include:
  1. Proteins,
  2. Fc receptors,
  3. Glycosaminoglycans (GAGS) and
  4. Lipopolysaccharide binding CD14-associated molecules.
The entry of virus into the target cells includes 2 events:
1. Adsorption and,
2. Penetration.
Studies using electron microscopy have indicated that adsorption is a temperature independent process that occurs at both 40°C as well as at 37°C, whereas viral penetration is a temperature dependant process, occurring only at 37°C.

3. Fusion of dengue virus to the target cell

The establishment of infection requires the entry of DEN virus into cells, followed by release of nucleocapsid, which is achieved by the fusion of viral membrane with a cellular membrane.
At first the virus is taken inside through receptor mediated endocytosis as discussed above.
The conformational changes due to plasma membrane receptor-virus binding/ by the acidic pH in the endosomes lead to exposure of the ‘fusion peptide’, which interacts with the target membrane and initiates the fusion event.

(***Note 1: Antibody dependant enhancement reaction:

- In the presence of subneutralizing amounts of antibody, Fc receptors also mediate attachment and uptake of dengue viruses into certain target cells such as monocytes and macrophages.
- This entry mechanism, termed as antibody dependent enhancement (ADE), may play a role in development of Dengue haemorrhagic fever (DHF) and the Dengue Shock Syndrome (DSS) which occur as the consequence of sequential infections with different dengue serotypes.

***Note 2: Importance of E protein and prM protein interaction:

- A main function of E protein, membrane fusion, is regulated by interaction with a second viral protein, prM.
- It should be recapitulated that prM protein is found mainly in the immature virions.
- The association of prM with E-protein stabilizes certain pH sensitive epitopes present on E-protein, thereby preventing the conformational
changes which occur at acidic pH and are essential for the activation of fusogenic activity of E protein.

• Besides its role in viral assembly, the prM protein has also been included in novel recombinant formulations in which it is generally co-expressed with E protein. The resultant E-prM complexes have been shown to be immunogenic and protective, when used in vaccine formulations against challenge with Dengue viruses.

4. Effects of dengue virus invasion

• The 3 major systems which are attacked by the dengue viral infection are:
  1. Immune system,
  2. Liver and,
  3. Endothelial linings of blood vessels.

   **Immune system**

• Following infection, mononuclear cells predominantly die by apoptosis.
• Abortively infected mononuclear cells are stimulated to produce the bulk of mediators which are involved in the inflammatory and homeostatic responses against the dengue virus.
• These mediators include:
  1. IL-8,
  2. IL-10,
  3. IL-12,
  4. IFN-ϒ,
  5. IFN-α,
  6. Elastase,
  7. TNF-α,
  8. Soluble TNF and IL-2 receptors.
• It is also hypothesized that complement system plays a major role in the pathogenesis of dengue as it has been shown that the serum concentration of C3a and C5a have been increased in dengue patients, whereas in DSS patients, there is a marked reduction of the complement components.
Liver

- Liver is commonly involved in dengue infection.
- There is limited inflammation in liver, which is observed by **prevalence of apoptosis over necrosis**.
- Hepatocyte apoptosis causes an injury to the liver, which causes an elevated hepatic enzyme levels.
- This injury to the liver is also responsible for decreased synthesis of coagulation factors and development of coagulopathy.

Endothelial linings of blood vessels

- It has been speculated that dengue virus infection of endothelial cells plays a major role in the *increased peripheral microvascular permeability* seen in dengue.
- **It should be remembered that, in contrast to mononuclear cells, endothelial cells do not carry Fc receptors and thus can’t take up the immune complexed virus. Therefore, mere presence of viral antigen in endothelial cells is no proof of viral replication.**
- It is worth mentioning that, the major non-structural protein (NS-1) has been shown to bind preferably to *liver and lung tissue*, which may be responsible for **selective hepatic damage and selective pulmonary vascular leakage** found in dengue.

5. Progression to DHF and DSS

- Dengue virus infects macrophages, lymphocytes, and endothelial cells.
- In dengue hemorrhagic fever, the lymphoreticular system shows loss of lymphocytes in T-cell-dependent zones.
- An Arthus-like reaction with monocytic and lymphocytic perivascular infiltration without necrosis may be seen in skin.
- **Monocyte infection is believed to mediate rapid activation of complement via the classical pathway and perhaps by the alternative pathway as well, ultimately resulting in increased vascular permeability and a cascade of coagulation defects, including thrombocytopenia and clotting abnormalities.**
- The process develops quickly and, if not managed effectively, can lead to **systemic hypotension** and progress to shock (DSS) and death in just a few hours.
CLINICAL SYMPTOMS OF DENGUE

DENGUE FEVER

Children:

- Primary infections with dengue virus types 2 and 4 are thought to be largely inapparent, regardless of age.
- Primary infections with dengue types 1 and 3 are more often overt.
- In children, the progression of the illness is characteristic. A relatively mild first phase with abrupt onset of fever, malaise, pharyngeal inflammation, rhinitis, vomiting, headache, anorexia and cough may be followed after 2–5 days by rapid deterioration and physical collapse. Infants and children with high fevers may experience febrile convulsions.

Adult:

- In classic DF, after an incubation period of 2–7 days, there is a sudden onset of fever, accompanied by a sensation of chilliness, which rapidly rises to 103–106°F, usually accompanied by frontal or retro-orbital headache.
- Occasionally, back pain precedes the fever.
- A transient, macular, generalized rash that blanches under pressure may be seen during the first 24–48 hours of fever.
- The pulse rate may be slow in proportion to the degree of fever.
- Myalgia or bone pain occurs soon after onset and increases in severity.
- During the second to the sixth day of fever, nausea and vomiting are apt to occur; during this phase, generalized lymphadenopathy, cutaneous hyperesthesia or hyperalgesia, taste aberrations and pronounced anorexia may develop.
- Coincident with or 1 or 2 days after defervescence, a generalized maculopapular rash may appear, sparing the palms and soles. It disappears in 1–5 days.
- About the time of appearance of this second rash, the body temperature, which has fallen to normal, may become elevated slightly and establish the biphasic temperature curve. (***)
- Epistaxis, petechiae and purpuric lesions, although uncommon, may occur at any stage of the disease.
Swallowed blood from epistaxis may be passed by the rectum or vomited, and can be interpreted as bleeding of gastrointestinal origin. In some cases, patients may be in danger of exsanguinating, but without abnormal vascular permeability. This syndrome can be confused with DSS.

**DHF/ DSS**

- The DHF syndrome is differentiated from DF by its association with thrombocytopenia and capillary leakage.

- **Dengue haemorrhagic fever (DFH grades I and II)** is a severe form of dengue illness and is characterized by haemoconcentration, thrombocytopenia and coagulation abnormalities.

- When accompanied by narrow pulse pressure or hypotension, the illness is designated as **dengue shock syndrome (DHF grades III and IV).**

**DSS**

- Patients, particularly those in clinical shock, may show symptoms of mental obtundation with **varying neurological reflex changes suggestive of encephalopathy.**

- There frequently are scattered petechiae on the forehead and extremities, spontaneous ecchymoses may appear, and **easy bruisability** and bleeding at sites of venipuncture are common. **Application of a tourniquet to an extremity (tourniquet test) demonstrates easy bruising.**

- There may be **circumoral and peripheral cyanosis.**

- **Respiration is rapid.**

- **The pulse is weak, rapid and thready.**

- **The heart sounds are faint.**

- **The pulse pressure is frequently narrow (20 mm Hg).**

- The **liver** may become **palpable** two or three finger breadths below the costal margin and usually is **firm and non-tender.**

- A chest radiograph shows **unilateral (right) or bilateral pleural effusions.**
• Approximately 10% of patients manifest gross ecchymosis or gastrointestinal bleeding. After a 24- or 36-hour period of crisis, convalescence is fairly rapid in children who recover.
• The temperature may return to normal before or during the stage of shock.

Children and infants:
• Clinically, infants often present with failure to feed, coryza, hepatomegaly, drowsiness and vomiting.
• **Shock and bleeding are more common in infants than in children.**

Adult:
• DHF and DSS have been generally thought to occur only in children. This impression derives from the extensive clinical experience in South-east Asia where, because of high transmission rates, the disease was typically restricted to childhood.
• By contrast, the early literature on DF mostly centred on adults.
• With the global spread of dengue it is likely that there will be an increase in the number of adults presenting with severe dengue.
DEFFERENTIAL DIAGNOSIS

- Classical dengue illness can be an easy diagnosis to make in endemic regions with experienced clinical staff and a high prior probability that a febrile illness with rash and thrombocytopenia is caused by dengue.
- Most of the symptoms and signs accompanying dengue infection are common to many febrile illnesses, with few features that reliably discriminate dengue especially early. The differential diagnosis inevitably is very large. It includes:
  - Measles,
  - Rubella,
  - Enterovirus,
  - Influenza,
  - Typhoid,
  - Chikungunya,
  - Scarlet fever,
  - Malaria,
  - Leptospirosis,
  - Hepatitis A,
  - Rickettsiosis,
  - Bacterial sepsis,
  - Viral haemorrhagic fevers (including Ebola, Lassa fever, etc.),
  - West Nile fever,
  - O’nyong-nyong fever and
  - Rift Valley fever (usually without a rash).

Special issue: 1. Plasma leakage

It has been suggested that two separate mechanisms may exist to account for the elevated vascular permeability in patients with DHF.

1. Some level of vascular permeability that lasts for at least several days occurs in all patients with clinically overt dengue.
2. The second event is a rapid and marked but self-limiting increase in microvascular permeability, superimposed upon the first, which allows plasma water to flood out of the intravascular compartment and leads to sudden hypovolemic shock as the patients’ compensatory mechanisms (increased lymphatic drainage, reabsorptive capacity) fail to cope.
• **This higher microvascular permeability in childhood probably results from the greater density and surface area of growing microvessels in children than in adults and may help explain why children are more prone to develop DSS than adults.**

Special issue: 2. WHO definitions of DHF and DSS

WHO Case Definitions

Case definition for dengue haemorrhagic fever:

The following must all be present:

- **Fever, or history of acute fever, lasting 2–7 days.**
- **Haemorrhagic tendencies**, evidenced by at least one of the following:
  1. A positive tourniquet test; (Discussed later)
  2. Petechiae, ecchymosis or purpura;
  3. Bleeding from the mucosa, gastrointestinal tract, injection sites or other locations;
  4. Haematemesis or melaena.
- **Thrombocytopenia** (100,000 cells per mm3 or less).†
- **Evidence of plasma leakage** due to increased vascular permeability, manifested by at least one of the following:
  1. A rise in the haematocrit equal to or greater than 20% above the average for age, sex and population;
  2. A drop in the haematocrit following volume replacement treatment equal to or greater than 20% of baseline;
  3. Signs of plasma leakage such as pleural effusion, ascites and hypoproteinemia.

Case definition for dengue shock syndrome:

*All of the above four criteria for DHF must be present plus evidence of circulatory failure manifested by:*

- **Rapid and weak pulse**, and,
- Narrow pulse pressure [< 20 mm Hg] or manifested by:
  1. **Hypotension** for age, and
  2. **Cold, clammy skin and restlessness.**
Special issue: 3. The tourniquet test

- The tourniquet test is performed by **inflating a blood pressure cuff on the upper arm** of the patient to **a point midway between systolic and diastolic pressure for 5 minutes**, and **the number of resulting petechiae counted in a 2.5 cm square on the volar aspect of the forearm** (Volar aspect: on the same side of palmar aspect) just distal to the antecubital fossa.
- **A test is considered positive when 20 or more petechiae are observed in the 2.5 cm square.**
- The World Health Organization recommends use of the tourniquet test to document “**haemorrhagic tendencies**, as one of the four elements in the clinical case definition of DHF.

Special issue: 4. Coagulopathy

- Thrombocytopenia (platelet count <100,000/μl) forms one of the four criteria in the WHO case definition of DHF.
- **In DHF, platelet function is abnormal and transfused platelets have a markedly shortened survival time.**

<table>
<thead>
<tr>
<th>Platelet function tests</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time</td>
<td>Mildly prolonged.</td>
</tr>
<tr>
<td>Partial prothrombin rime</td>
<td>Mildly prolonged.</td>
</tr>
<tr>
<td>Serum fibrinogen level</td>
<td>Slightly reduced.</td>
</tr>
<tr>
<td>Coagulation factors (II, V, VII, VIII, IX, X)</td>
<td>Reduced.</td>
</tr>
<tr>
<td>Levels of protein C, protein S and antithrombin III</td>
<td>Normal or mildly reduced.</td>
</tr>
</tbody>
</table>

- There are abnormalities in all the major pathways of the coagulation cascade confirmed by **low levels of the natural anticoagulant proteins, together with increased levels of the major procoagulants tissue factor**.
- These findings are compatible with the possibility that **dengue infection activates fibrinolysis in the absence of a thrombotic stimulus**, degrading
fibrinogen directly, and prompting a marked and effective secondary activation of various procoagulants’ homeostatic pathways.

• **Bleeding in dengue probably results from a combination of thrombocytopenia, poor platelet function and increased fibrinolysis.**

**Special issue: 5. Neurological manifestations**

• The commonest neurological manifestations associated with dengue infection are:
  1. Headache,
  2. Restlessness and
• More severe neurological features include:
  1. Altered consciousness,
  2. Generalized convulsions
  3. Coma
• But a milder alteration of consciousness, lethargy, drowsiness and occasionally agitation are more common.
• CSF findings:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF pressure</td>
<td>Moderately elevated.</td>
</tr>
<tr>
<td>Protein content</td>
<td>Mildly raised.</td>
</tr>
<tr>
<td>Cells</td>
<td>No cells in the CSF or a mild lymphocyte pleocytosis (&lt; 500 cells/μl).</td>
</tr>
</tbody>
</table>

**Special issue: 6. Myalgia**

• Myalgia or back pain is a presenting feature in 10–50% of patients.
• The **creatinine phosphokinase is usually normal or moderately elevated.**
• Muscle biopsy shows a mild perivascular mononuclear infiltrate and lipid accumulation.
• Anecdotally, myalgia and back pain can be debilitating and can continue for many weeks after the end of the febrile period.
The skin is involved at some stage in the majority of patients with dengue.

During the first few days of illness there may be a generalized macular blush and the skin is sensitive to touch.

Later, spontaneous petechiae develop in up to 80% of patients and bleeding at injection sites and into the conjunctival and mucous membranes become common.

An irritating haemorrhagic erythema of the palms and soles of the hands and feet, which may desquamate, has been described in adults.

During the recovery period a macular, discrete and occasionally itchy rash can develop. This has a characteristic appearance with extensive erythematous areas surrounding discrete islands of apparently normal skin.

ANTIBODY RESPONSE TO PRIMARY AND SECONDARY DENGUE INFECTION

Response to primary dengue infection:

- When dengue infection occurs in persons who have not previously been infected with a flavivirus or immunized with a flavivirus vaccine (e.g. for yellow fever, Japanese encephalitis, tick-borne encephalitis), the patients develop a primary antibody response characterized by a slow increase of specific antibodies.
- IgM antibodies are the first immunoglobulin isotype to appear. These antibodies are detectable in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10.
- IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months.
- Anti-dengue serum IgG is generally detectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life.

Response to secondary dengue infection:

- During a secondary dengue infection (a dengue infection in a host that has previously been infected by a dengue virus, or sometimes after non-
dengue flavivirus vaccination or infection), antibody titres rise rapidly and react broadly against many flaviviruses.

- The dominant immunoglobulin isotype is IgG which is detectable at high levels, even in the acute phase, and persists for periods lasting from 10 months to life.
- Early convalescent stage IgM levels are significantly lower in secondary infections than in primary ones and may be undetectable in some cases, depending on the test used.

***Note: To distinguish primary and secondary dengue infections, IgM/IgG antibody ratios are now more commonly used than the haemagglutination-inhibition test (HI).

LABORATORY DIAGNOSIS OF DENGUE

Introduction:
Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques.

- After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. During this early
stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection.

- At the end of the acute phase of infection, serology is the method of choice for diagnosis.

**VIRUS ISOLATION (CELL CULTURE)**

- Specimens for virus isolation should be collected early in the course of the infection, during the period of viraemia (usually before day 5).
- Virus may be recovered from serum, plasma and peripheral blood mononuclear cells and attempts may be made from tissues collected at autopsy (e.g. liver, lung, lymph nodes, thymus, bone marrow).
- Because dengue virus is heat-labile, specimens awaiting transport to the laboratory should be kept in a refrigerator or packed in wet ice.
- For storage up to 24 hours, specimens should be kept at between +4 °C and +8 °C. For longer storage, specimens should be frozen at -70 °C in a deep-freezer or stored in a liquid nitrogen container. Storage even for short periods at –20 °C is not recommended.
- **Cell culture** is the most widely used method for dengue virus isolation. The mosquito cell line C6/36 (cloned from Ae. albopictus) or AP61 (cell line from Ae. pseudoscutellaris) are the host cells of choice for routine isolation of dengue virus.
- Since not all wild type dengue viruses induce a cytopathic effect in mosquito cell lines, cell cultures must be screened for specific evidence of infection by an antigen detection Immunofluorescence assay using serotype-specific monoclonal antibodies and flavivirus group-reactive or dengue complex-reactive monoclonal antibodies.

**ANIMAL INOCULATION**

- When no other methods are available, clinical specimens may also be inoculated by intracranial route in suckling mice or intrathoracic inoculation of mosquitoes.
- Newborn animals can develop encephalitis symptoms but with some dengue strains mice may exhibit no signs of illness.
- Virus antigen is detected in mouse brain or mosquito head squashes by staining with anti-dengue antibodies.
NUCLEIC ACID DETECTION

RNA is heat-labile and therefore specimens for nucleic acid detection must be handled and stored according to the procedures described for virus isolation. The following methods are used for nucleic acid detection:

1. RT-PCR
   - They offer better sensitivity compared to virus isolation with a much more rapid turnaround time.
   - All nucleic acid detection assays involve three basic steps:
     1. Nucleic acid **extraction and purification**.
     2. **Amplification** of the nucleic acid, and
     3. **Detection and characterization** of the amplified product.
   - Extraction and purification of viral RNA from the specimen can be done by traditional liquid phase separation methods (e.g. phenol, chloroform) but has been gradually replaced by silica-based commercial kits (beads or columns) that are more reproducible and faster.
   - Laboratories use mainly 2 types of techniques:
     1. **Nested RT-PCR** and,
     2. **One-step-multiplex-RT-PCR**.
   - The products of these reactions are separated by **electrophoresis on an agarose gel**, and the amplification products are visualized as bands of different molecular weights in the agarose gel using **ethidium bromide dye**, and compared with standard molecular weight markers. In this assay design, dengue serotypes are identified by the size of their bands.

2. REAL TIME PCR
   - The real-time RT-PCR assay is a one step assay system used to quantitate viral RNA and using primer pairs and probes that are specific to each dengue serotype.
   - **The use of a fluorescent probe enables the detection of the reaction products in real time**, in a specialized PCR machine, without the need for electrophoresis.
   - Real-time RT-PCR assays are of 2 types:
     1. **Singleplex** (i.e. detecting only one serotype at a time) or
     2. **Multiplex** (i.e. able to identify all four serotypes from a single sample).
The multiplex assays have the advantage that a single reaction can determine all four serotypes without the potential for introduction of contamination during manipulation of the sample.

However the multiplex real-time RT-PCR assays, although faster, are currently less sensitive than nested RT-PCR assays.

3. Isothermal amplification methods

- The NASBA (nucleic acid sequence based amplification) assay is an isothermal RNA specific amplification assay that does not require thermal cycling instrumentation.
- The initial stage is a reverse transcription in which the single-stranded RNA target is copied into a double-stranded DNA molecule that serves as a template for RNA transcription.
- Detection of the amplified RNA is accomplished either by electrochemiluminescence or in real-time with fluorescent-labelled molecular probes.

ANTIGEN DETECTION

NS1 ANTIGEN TEST

- New developments in ELISA and dot blot assays directed to the non-structural protein 1 (NS1) demonstrated that high concentrations of this antigens in the form of immune complexes could be detected in patients with both primary and secondary dengue infections up to nine days after the onset of illness.
- The NS1 glycoprotein is produced by all flaviviruses and is secreted from mammalian cells. NS1 produces a very strong humoral response.
- Commercial kits for the detection of NS1 antigen are now available, though they do not differentiate between dengue serotypes.

ANTIBODY DETECTION (SEROLOGICAL TESTS)

MAC-ELISA (IgM Antibody-Capture Enzyme-Linked Immunosorbent Assay)

- For the (MAC-ELISA) total IgM in patients’ sera is captured by anti-µ chain specific antibodies (specific to human IgM) coated onto a microplate.
Dengue-specific antigens, from one to four serotypes (DEN-1, -2, -3, and -4), are bound to the captured anti-dengue IgM antibodies and are detected by monoclonal or polyclonal dengue antibodies directly or indirectly conjugated with an enzyme that will transform a non-coloured substrate into coloured products.

The optical density is measured by spectrophotometer.

Serum, blood on filter paper and saliva, but not urine, can be used for detection of IgM if samples are taken within the appropriate time frame (five days or more after the onset of fever).

Most of the antigens used for this assay are derived from the dengue virus envelope protein (usually virus-infected cell culture supernatants or suckling mouse brain preparations).

MAC-ELISA has good sensitivity and specificity but only when used five or more days after the onset of fever.

IgG-ELISA

The IgG ELISA is used for the detection of recent or past dengue infections.

This assay uses the same antigens as the MAC-ELISA.

The use of E/M (Envelop/ Membrane) -specific capture IgG ELISA (GAC) allows detection of IgG antibodies over a period of 10 months after the infection.
IgG antibodies are lifelong as measured by E/M antigen-coated indirect IgG ELISA, but a fourfold or greater increase in IgG antibodies in acute and convalescent paired sera can be used to document recent infections. This method can be used to detect IgG antibodies in serum or plasma and filter-paper stored blood samples and permits identification of a case as a primary or secondary dengue infection.

IgG/IgG ratio

A dengue virus E/M protein-specific IgM/IgG ratio can be used to distinguish primary from secondary dengue virus infections. IgM capture and IgG capture ELISAs are the most common assays for this purpose. Dengue infection is defined as primary if the IgM/IgG OD ratio is:

1. Greater than 1.2 (using patient’s sera at 1/100 dilution) or
2. Greater than 1.4 (using patient’s sera at 1/20 dilutions).

The infection is secondary if the ratio is less than 1.2 or 1.4.

Haemagglutination-Inhibition Test

The HI test is based on the ability of dengue antigens to agglutinate trypsinized human O RBC. Anti-dengue antibodies in sera can inhibit this agglutination and the potency of this inhibition is measured in an HI test. At first the serum samples are treated with acetone or kaolin to remove non-specific inhibitors of haemagglutination. Then they are adsorbed with trypsinized type O human RBC to remove non-specific agglutinins.
Optimally the HI test requires paired sera obtained upon hospital admission (acute) and discharge (convalescent) or paired sera with an interval of more than seven days.

The response to a primary infection is characterized by the low level of antibodies in the acute-phase serum drawn before day 5 and a slow elevation of HI antibody titres thereafter.

During secondary dengue infections HI antibody titres rise rapidly, usually exceeding 1:1280.

TESTS USED IN VACCINATION TRIALS:
PLAQUE REDUCTION AND NEUTRALIZATION TEST (PRNT)

Vaccine trials are performed in order to measure vaccine safety and efficacy in vaccinated persons.

This assay is the most reliable means of measuring the titre of neutralizing antibodies in the serum of an infected individual as a measure of the level of protection against an infecting virus. (***)

The assay is based on the principle that neutralizing antibodies inactivate the virus so that it is no longer able to infect and replicate in target cells.

After a second dengue virus infection, high-titre neutralizing antibodies are produced against at least two, and often all four, dengue viruses as well as against non-dengue flaviviruses.
This cross reactivity results from memory B-cells which produce antibodies directed at virion epitopes shared by dengue viruses. This phenomenon is known as “original antigenic sin”.

TREATMENT GUIDELINE: MANAGEMENT OF A SUSPECTED CASE OF DENGUE

Step I—Overall assessment

History

The history should include:

- Date of onset of fever/illness
- Quantity of oral intake
- Assessment for warning signs
- Diarrhoea
- Change in mental state/seizure/dizziness
- Urine output (frequency, volume and time of last voiding)
- Family or neighbourhood dengue
- Travel to dengue endemic areas
- Swimming in waterfall (consider leptospirosis, typhus, malaria)
- Recent unprotected sex or drug abuse (consider acute HIV seroconversion illness)
- Co-existing conditions (e.g. infancy, pregnancy, obesity, diabetes mellitus, hypertension)
Physical examination

The physical examination should include:

- Assessment of mental state
- Assessment of hydration status
- Assessment of haemodynamic status
- Checking for tachypnoea/acidotic breathing/pleural effusion
- Checking for abdominal tenderness/hepatomegaly/ascites
- Examination for rash and bleeding manifestations
- Tourniquet test (repeat if previously negative or if there is no bleeding manifestation).

- **A full blood count** should be done at the first visit.
- A haematocrit test in the early febrile phase establishes the patient’s own baseline haematocrit.
- **A decreasing white blood cell count makes dengue very likely.**
- **A rapid decrease in platelet count in parallel with a rising haematocrit compared to the baseline is suggestive of progress to the plasma leakage/critical phase of the disease.**
- In the absence of the patient’s baseline, age-specific population haematocrit levels could be used as a surrogate during the critical phase.
- Laboratory tests should be performed to confirm the diagnosis.
- However, it is not necessary for the acute management of patients, except in cases with unusual manifestations.
Step II—Diagnosis, assessment of disease phase and severity

On the basis of evaluations of the history, physical examination and/or full blood count and haematocrit, clinicians should be able to determine whether the disease is dengue, which phase it is in (febrile, critical or recovery), whether there are warning signs, the hydration and haemodynamic status of the patient, and whether the patient requires admission or not.

Step III—Management

*Disease notification*

- In dengue-endemic countries, cases of suspected, probable and confirmed dengue should be notified as soon as possible so that appropriate public health measures can be initiated.
- Laboratory confirmation is not necessary before notification, but should be obtained.
- In non-endemic countries, usually only confirmed cases will be notified.
- Suggested criteria for early notification of suspected cases are that:
  1. The patient lives in or has travelled to a dengue-endemic area,
  2. Has fever for three days or more,
  3. Has low or decreasing white cell counts, and/or
  4. Has thrombocytopenia ± positive tourniquet test.
- In dengue-endemic countries, the later the notification, the more difficult it is to prevent dengue transmission.

*Management decisions*

Depending on the clinical manifestations and other circumstances, patients may be grouped as followed:

<table>
<thead>
<tr>
<th>Group</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>May be sent home.</td>
</tr>
<tr>
<td>Group B</td>
<td>Referred for in-hospital management.</td>
</tr>
<tr>
<td>Group C</td>
<td>Require emergency treatment and urgent referral.</td>
</tr>
</tbody>
</table>
Treatment according to groups A–C

Group A – Patients who may be sent home

These are patients who are able to tolerate adequate volumes of oral fluids and pass urine at least once every six hours, and do not have any of the warning signs, particularly when fever subsides.

- Ambulatory patients should be reviewed daily for disease progression (decreasing white blood cell count, defervescence and warning signs) until they are out of the critical period.
- Those with stable haematocrit can be sent home after being advised to return to the hospital immediately if they develop any of the warning signs and to adhere to the following action plan:

  **Encourage oral intake of oral rehydration solution (ORS), fruit juice and other fluids containing electrolytes and sugar to replace losses from fever and vomiting.**

  Adequate oral fluid intake may be able to reduce the number of hospitalizations.

  Give paracetamol for high fever if the patient is uncomfortable. The interval of paracetamol dosing should not be less than six hours.

  Do not give aspirin, ibuprofen or other NSAIDs as these drugs may aggravate gastritis or bleeding.

Instruct the care-givers that the patient should be brought to hospital immediately if any of the following occur:

1. No clinical improvement,
2. Deterioration around the time of defervescence,
3. Severe abdominal pain,
4. Persistent vomiting,
5. Cold and clammy extremities,
6. Lethargy or irritability/restlessness,
7. Bleeding (e.g. black stools or coffee-ground vomiting),
8. Not passing urine for more than 4–6 hours.

Patients who are sent home should be monitored daily by health care providers for:
1. Temperature pattern,
2. Volume of fluid intake and losses,
3. Urine output (volume and frequency),
4. Warning signs,
5. Signs of plasma leakage and bleeding,
6. Haematocrit, WBC and platelet counts.

**Group B – Patients who should be referred for in-hospital management**

If the patient has dengue with warning signs, the action plan should be as follows:

- Obtain a reference haematocrit before fluid therapy.
- Give only isotonic solutions such as 0.9% saline, Ringer’s lactate, or Hartmann’s solution.
- Start with 5–7 ml/kg/hour for 1–2 hours, then reduce to 3–5 ml/kg/hr for 2–4 hours, and then reduce to 2–3 ml/kg/hr or less according to the clinical response.
- Reassess the clinical status and repeat the haematocrit. If the haematocrit remains the same or rises only minimally, continue with the same rate (2–3 ml/kg/hr) for another 2–4 hours.
- If the vital signs are worsening and haematocrit is rising rapidly, increase the rate to 5–10 ml/kg/hour for 1–2 hours.
- Reassess the clinical status, repeat the haematocrit and review fluid infusion rates accordingly.
- Give the minimum intravenous fluid volume required to maintain good perfusion and urine output of about 0.5 ml/kg/hr. Intravenous fluids are usually needed for only 24–48 hours.
- Reduce intravenous fluids gradually when the rate of plasma leakage decreases towards the end of the critical phase.
Patients with warning signs should be monitored by health care providers until the period of risk is over. A detailed fluid balance should be maintained.

Parameters that should be monitored include:
1. **Vital signs and peripheral perfusion** (1–4 hourly until the patient is out of the critical phase),
2. **Urine output** (4–6 hourly),
3. **Haematocrit** (before and after fluid replacement, then 6–12 hourly),
4. **Blood glucose**, and
5. **Other organ functions** (such as renal profile, liver profile, coagulation profile, as indicated).

*If the patient has dengue without warning signs, the action plan should be as follows:*

- Encourage oral fluids.
  If not tolerated, start intravenous fluid therapy of 0.9% saline or Ringer’s lactate with or without dextrose at maintenance rate.

- Give the minimum volume required to maintain good perfusion and urine output.

- Intravenous fluids are usually needed only for 24–48 hours.

- Patients should be monitored by health care providers for:
  1. Temperature pattern,
  2. Volume of fluid intake and losses,
  3. Urine output (volume and frequency),
  4. Warning signs,
  5. Haematocrit, WBC and platelet counts.
  6. Other laboratory tests (such as liver and renal functions tests) can be done, depending on the clinical picture and the facilities of the hospital or health centre.
Group C – Patients who require emergency treatment and urgent referral when they have severe dengue

Patients require emergency treatment and urgent referral when they are in the critical phase of disease, i.e. when they have:

- **Severe plasma leakage** leading to dengue shock and/or fluid accumulation with respiratory distress;
- **Severe haemorrhages**;
- **Severe organ impairment** (hepatic damage, renal impairment, cardiomyopathy, encephalopathy or encephalitis).

All patients with severe dengue should be admitted to a hospital with access to intensive care facilities and blood transfusion.

Judicious intravenous fluid resuscitation is the essential and usually sole intervention required.

Plasma losses should be replaced immediately and rapidly with isotonic crystalloid solution or, in the case of hypotensive shock, colloid solutions.

The crystalloid solution should be isotonic and the volume just sufficient to maintain an effective circulation during the period of plasma leakage.

There should be continued replacement of further plasma losses to maintain an effective circulation for 24–48 hours.

Blood transfusion should be given only in cases with suspected/severe bleeding.

This is a strategy in which larger volumes of fluids (e.g. 10–20 ml boluses) are administered for a limited period of time under close monitoring to evaluate the patient’s response and to avoid the development of pulmonary oedema.
Figure 3 – Care of adult patients with dengue with warning signs and severe dengue.
The goals of fluid resuscitation include:

- **Improving central and peripheral circulation** (decreasing tachycardia, improving blood pressure, pulse volume, warm and pink extremities, and capillary refill time <2 seconds),
- **Improving end-organ perfusion**,
- **Maintain a stable conscious level** (more alert or less restless),
- **Maintain an urine output ≥ 0.5 ml/kg/hour**,
- **Decreasing metabolic acidosis**.

**Treatment of Haemorrhagic Complications**

Severe bleeding can be recognized by:

- **Persistent and/or severe overt bleeding** in the presence of unstable haemodynamic status, regardless of the haematocrit level;
- **A decrease in haematocrit after fluid resuscitation** together with unstable haemodynamic status;
- **Refractory shock that fails to respond to consecutive fluid resuscitation** of 40-60 ml/kg;
- **Hypotensive shock with low/normal haematocrit** before fluid resuscitation;
- **Persistent or worsening metabolic acidosis ± a well-maintained systolic blood pressure**, especially in those with severe abdominal tenderness and distension.

The action plan for the treatment of haemorrhagic complications is as follows:

- Give **5–10ml/kg of fresh-packed red cells** or **10–20 ml/kg of fresh whole blood** at an appropriate rate and observe the clinical response.
- **It is important that fresh whole blood or fresh red cells are given.** Oxygen delivery at tissue level is optimal with high levels of 2,3 di-phosphoglycerate (2,3 DPG). Stored blood loses 2,3 DPG, low levels of which impede the oxygen-releasing capacity of haemoglobin, resulting in functional tissue hypoxia.
- A good clinical response includes improving haemodynamic status and acid-base balance.
- **Consider repeating the blood transfusion** if there is further blood loss or **no appropriate rise in haematocrit after blood transfusion**.
There is little evidence to support the practice of **transfusing platelet concentrates** and/or **fresh-frozen plasma** for severe bleeding. It is being practised **only when massive bleeding can’t be managed with just fresh whole blood/fresh-packed cells**, but it may exacerbate the fluid overload.

Great care should be taken when inserting a naso-gastric tube which may cause severe haemorrhage and may block the airway. A lubricated oro-gastric tube may minimize the trauma during insertion.

Insertion of central venous catheters should be done with ultra-sound guidance or by a very experienced person.

**Special care: Fluid overload**

**Causes of fluid overload are:**
- Excessive and/or too rapid intravenous fluids;
- Incorrect use of hypotonic rather than isotonic crystalloid solutions;
- Inappropriate use of large volumes of intravenous fluids in patients with unrecognized severe bleeding;
- Inappropriate transfusion of fresh-frozen plasma, platelet concentrates and cryoprecipitates;
- Continuation of intravenous fluids after plasma leakage has resolved (24–48 hours from defervescence);
- Co-morbid conditions such as congenital or ischaemic heart disease, chronic lung and renal diseases.

**Early clinical features of fluid overload are:**
- Respiratory distress, difficulty in breathing;
- Rapid breathing;
- Chest wall in-drawing;
- Wheezing (rather than crepitations);
- Large pleural effusions;
- Tense ascites;
- Increased jugular venous pressure (JVP).

**Late clinical features are:**
- Pulmonary oedema (cough with pink or frothy sputum ± crepitations, cyanosis);
- Irreversible shock (heart failure, often in combination with ongoing hypovolaemia).
The action plan for the treatment of fluid overload is as follows:

- **Oxygen therapy** should be given immediately.
- **Stopping intravenous fluid therapy** during the recovery phase will allow fluid in the pleural and peritoneal cavities to return to the intravascular compartment. This results in diuresis and resolution of pleural effusion and ascites. **Recognizing when to decrease or stop intravenous fluids is key to preventing fluid overload.**
- When the following signs are present, intravenous fluids should be discontinued or reduced to the minimum rate necessary to maintain euglycaemia:

  **Signs of cessation of plasma leakage:**
  - Stable blood pressure, pulse and peripheral perfusion;
  - Haematocrit decreases in the presence of a good pulse volume;
  - Afebrile for more than 24–48 days (without the use of antipyretics);
  - Resolving bowel/abdominal symptoms;
  - Improving urine output.

Management of fluid overload

- The management of fluid overload varies according to the phase of the disease and the patient’s haemodynamic status.
- If the patient has stable haemodynamic status and is out of the critical phase (more than 24–48 hours of defervescence), stop intravenous fluids but continue close monitoring.

*If necessary, give oral or intravenous furosemide 0.1–0.5 mg/kg/dose once or twice daily, or a continuous infusion of furosemide 0.1 mg/kg/hour. Monitor serum potassium and correct the ensuing hypokalaemia.*

- If the patient has stable haemodynamic status but is still within the critical phase, reduce the intravenous fluid accordingly.
- **Avoid diuretics during the plasma leakage phase because they may lead to intravascular volume depletion.**
- **Patients who remain in shock** with low or normal haematocrit levels but show signs of fluid overload may have occult haemorrhage. Further infusion of large volumes of intravenous fluids will lead only to a poor outcome. **Careful fresh whole blood transfusion** should be initiated as soon as possible. If the patient remains in shock and the haematocrit is elevated, **repeated small boluses of a colloid solution** may help.
Supportive care and adjuvant therapy may be necessary in severe dengue. This may include:

1. **Renal replacement therapy**, with a preference to continuous veno-venous haemodialysis (CVVH), since peritoneal dialysis has a risk of bleeding.

2. **Vasopressor and inotropic therapies** as temporary measures to prevent life threatening hypotension in dengue shock and during induction for intubation, while correction of intravascular volume is being vigorously carried out;

3. Further **treatment of organ impairment**, such as severe hepatic involvement or encephalopathy or encephalitis;

4. Further **treatment of cardiac abnormalities**, such as conduction abnormalities may occur (the latter usually not requiring interventions).
For more helpful documents like that, please visit our website: http://pgblaster.wordpress.com.

Like us at: http://www.facebook.com/PgblasterIndia.

Thank you once again to be a part of Pgblaster India family and supporting us.

All the documents provided by that above site are subject to copyright. See website creative commons license for getting more details.

©Pgblaster India, 2012. All rights reserved.