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Review Article

Dengue Fever

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Dengue fever (DF) is the most common viral disease transmitted by the arthropods.[1] Dengue fever manifests as flu like illness affecting all age groups including infants, children and adults. Dengue hemorrhagic fever (DHF) is a life threatening complication of dengue characterized by high fever, hemorrhagic phenomena, thrombocytopenia and sometimes circulatory failure.[2] Early diagnosis is essential, based mainly on history, physical examination and laboratory investigations. There is mortality rate of 1-5% in untreated cases and in less than 1% with treatment. However with severe disease the mortality rate reaches to 26%.[3]

Dengue and DHF are fast emerging as a global health problems. [4] Dengue is endemic in more than 110 countries. It affects 50 to 100 million people worldwide a year with half a million hospitalizations and almost 12,500-25,000 deaths.[5] The incidence of dengue has increased 30 folds between 1960 and 2010. Factors responsible for dengue's spread include explosive population growth, unplanned urban overpopulation with inadequate public health systems and poor vector control.

Epidemiology in Pakistan

Dengue virus is now endemic in Pakistan with a peak incidence in the post monsoon period. Introduction of a new serotype (DEN-3) and a genotypic shift of endemic serotype (DEN-2) are the most probable factors for the recent out-break of DHF in this region.[6] First confirmed outbreak of DHF in Pakistan was reported from Karachi in 1994 and in the following year an epidemic of DF occurred in Baluchistan. [7,8] Later, a large outbreak occurred in 2006 in Karachi.[9] In 2011, more than 15,000 cases have been recorded in Lahore alone with a high mortality rate.

Transmission

Dengue fever virus (DENV) is an RNA virus of the family Flaviviridae; genus Flavivirus. Most are transmitted by arthropods (mosquitoes or ticks). There are four serotypes of the virus; these are referred to as DENV-1, DENV-2, DENV-3 and DENV-4. All four serotypes can cause the full spectrum of disease. Infection with one serotype is believed to produce lifelong immunity to that serotype but only short term protection against the others.[10] The severe complications on secondary infection occur particularly if someone previously exposed to one serotype then contracts another serotype.[11] In Pakistan the genotypes DENV-2 and DENV-3 were found to be prevalent.

The reservoirs for Dengue are both man and mosquito (Aedes aegypti). Man acts as a host. The viremia among humans builds up high titers two days before the onset of the fever and lasts 5–7 days after the onset of the fever. It is only during these two periods that the vector species get infected. Thereafter, the humans become dead-end for transmission. Aedes aegypti mosquito generally acquires the virus while feeding on the blood of an infected person and transmits disease to another non infected person. It is primarily a daytime feeder and lives around human habitation. [12] The mosquitoes breed in stored, exposed, water collection systems and other dark places.

Pathogenesis

The pathogenesis of DHF is poorly understood. Abnormal immune response has the major role in its pathogenesis.

Production of cytokines or chemokines: These include C3a, C5a, TNF-α, IL-2, IL-6, IL-10, Interferon-α and Histamine [13]

Activation of T lymphocytes: The level of T-cell activation is enhanced particularly in secondary dengue infection. Many dengue specific T cells have low affinity for other, probably previously encountered serotypes. Profound T cell activation may suppress or delay viral elimination leading to higher level of viral loads and thus enhanced symptoms seen in DHF.

Antibody mediated immune response: It has been observed that certain strains of dengue virus complex with non neutralizing antibodies and enter the mononuclear cells. Infected monocytes release vasoactive mediators resulting in increased vascular permeability and hemorrhagic manifestations that characterize DHF and dengue shock syndrome (DSS). Halstead described the antibody dependent enhancement upon the second infection with dengue virus.[14] Antibodies directed against dengue virus also cross react with human platelets and endothelial cells.[15] Increased platelet destruction, platelet dysfunction and endothelial cell dysfunction are also the result of cross reactivity between these antibodies to platelet gylcoproteins and endothelial cells.

Passively transferred Antibodies: Antibodies, predominantly of the IgG1 subclass, are the only

immunological substances known to be transferred from mother to fetus.[16] The fact that infants fail to develop clinical dengue illnesses until around 6 months of age accords with the presence of broadly reactive dengue neutralizing antibodies in their mothers' serum samples and the protection afforded by passively transferred dengue antibodies. [17]

Table 1: Clinical and Laboratory findings in Dengue fever

Course of illness	Febrile Phase	Critical Phase	Recovery Phase
Days of Illness	1-3	4-5	6-10
Clinical findings	Dehydration	Bleeding/Shock Organ impairment	Reabsorption
Hematocrit	N	↑↑	N
Platelet count	N	↓↓	N
Viremia	Present	Absent	Absent
Serology	Negative	Positive	Positive

Mechanism o various manifestations: Studies have shown that median age of dengue patients has decreased now and younger patients may be more susceptible. Severity of disease depends on virus strain, previous infection, host genetics and age. Epidemic potential is dependent on level of viremia, infectivity and virus serotype. DHF risk is the greatest for DEN-2; followed by DEN-3, DEN-4 and DEN-1, respectively.[18] Various factors underlie the clinical manifestations of DHF.

1. **Plasma leakage:** The plasma leakage is due to the increased vascular permeability induced by several mediators such as C3a and C5a during the acute febrile stage and this is more pronounced during toxic stage. The evidence of plasma leakage includes hemoconcentration, hypoproteinemia/hypoalbuminemia, pleural effusion, ascites and shock. Sometimes the rising hematocrit may not be evidenced because of either severe bleeding or early intravenous fluid administration.
2. **Bleeding tendency:** The bleeding diathesis is caused by:
   - Vasculopathy
   - Thrombocytopenia/ platelet dysfunction
   - Coagulopathy

a. **Vasculopathy:** A positive tourniquet test indicating the increased capillary fragility is found in the early febrile stage. It may be a direct effect of dengue virus as it appears in the first few days of illness during the viremic phase.

b. **Thrombocytopenia and platelet dysfunction:** Thrombocytopenia is almost always present in patients with dengue. Patients with DHF usually have platelet counts less than 100×10^9/l. Bone marrow suppression combined with increased peripheral destruction of platelets during the febrile and early convalescent phase of dengue disease are two important factors leading to profound thrombocytopenia.[19] Thrombocytopenia is most prominent during the toxic stage. However, during the recovery period, platelet count rises due to return of marrow cellularity. In the absence of substantial bleeding prophylactic platelet transfusions should be avoided to minimize risk of long-term complications. [20] Platelet dysfunction is another factor leading to bleeding. The platelet dysfunction might be the result of exhaustion from platelet activation triggered by immune complexes containing dengue antigen, absence of adenosine diphosphate (ADP) release and impaired platelet aggregation response to ADP. An increase in plasma-thromboglobulin and platelet factor4, indicating increased platelet secretary activity have also been observed. [21]

c. **Coagulopathy:** The coagulopathy associated with dengue infections is well described. Several groups have noted the presence of plasminogen cross-reactive antibodies and fibrinolytic activity during and after dengue infection. During the acute febrile stage, mild prolongation of the prothrombin time (PT) and partial thromboplastin time (APTT), as well as reduced fibrinogen levels, have been reported.[22] Variable reductions in the activities of several coagulation factors, including prothrombin, factors V, VII, VIII, IX and X, antithrombin and antiplasmin, have also been demonstrated. Fibrin degradation product or D-dimer may be elevated. Low levels of anticoagulant proteins C and S and antithrombin III were found to be associated with increasing severity of shock, presumably due to plasma leakage. Elevated levels of tissue factor, thrombomodulin and plasminogen activator inhibitor-1 reflect endothelial, platelet and/or monocyte activation and may be a secondary response to direct activation of fibrinolysis by the dengue virus. Release of heparin like substances e.g. heparan sulphate or chondroitin sulfate might also contribute to the overall picture.[23] Thrombocytopenia together with these abnormalities correlates with overall severity of disease. The coagulation abnormalities however are well compensated in the majority of patients without circulatory collapse.[24, 25]

Clinical Manifestations

Clinical course of dengue infection changes as the disease progresses. Dengue virus infection may be asymptomatic or may cause undifferentiated febrile illness (viral syndrome), dengue fever, or dengue hemorrhagic fever including dengue shock syndrome (DSS). (Figure1) Various diseases (viral/bacterial/parasitic) may mimic dengue fever and should be considered in differential diagnosis while evaluating a patient with suspected dengue. Incubation

period is 4–6 days (range 3–14 days). After an incubation period full spectrum of disease develops and is followed by 3 phases. (Table 1)

- Febrile phase
- Critical phase
- Recovery phase

Febrile Phase: After an incubation period, various non-specific, symptoms are seen. These include headache, backache and general malaise. Typically, the onset of DF is sudden with a sharp rise in temperature (usually lasting 5–7 days, occasionally accompanied by chills). Other manifestations include retro-orbital pain, photophobia, backache, and pain in the muscles and joints/bones, anorexia, constipation, colicky pain and abdominal tenderness, dragging pain in the inguinal region, sore throat and general depression. These symptoms usually persist from several days to a few weeks and vary markedly in frequency and severity. These clinical features are indistinguishable between DF and DHF. [26,27] Mild hemorrhagic manifestations like positive tourniquet test or petechiae and mucosal membrane bleeding may be seen in DF and DHF. Vaginal and gastrointestinal bleeding may also occur during this phase but are not common. [28] The findings of an enlarged and tender liver are more suggestive of DHF

The earliest abnormality in the complete blood count is a progressive decrease in total white cell count and this with previously mentioned clinical features should alert the physician to a high index of suspicion of dengue.

Critical Phase: The critical phase occurs towards the late febrile phase (usually between 3rd to 5th day of illness but may go up to 7th day) when a rapid drop in temperature may coincide with an increase in capillary permeability in some patients. In other viral infections, the patient's condition improves as the temperature subsides, but the contrary happens in DHF. At this point the patient will either become better if no or minimal plasma leak occurs, or worse if a critical volume of plasma is lost. The critical phase lasts about 24-48 hours. Varying circulatory disturbances can develop. In less severe cases, these changes are minimal and transient. Many of these patients recover spontaneously, or after a short period of fluid or electrolyte therapy. In more severe forms of plasma leakage, the patients may sweat, become restless, have cool extremities and prolonged capillary refill time. The pulse rate increases, diastolic blood pressure increases and the pulse pressure narrows. Abdominal pain, persistent vomiting, restlessness, altered conscious level, clinical fluid accumulation, mucosal bleed or tender enlarged liver are the clinical warning signs of severe dengue or high possibility of rapid progression to shock. [29,30]

The patient can progress rapidly to profound shock and death if prompt fluid resuscitation is not instituted. Thrombocytopenia and hemoconcentration (evidenced by a raised hematocrit (HCT) are usually detectable before the subsidence of fever and the onset of shock. The HCT level correlates well with plasma volume loss and disease severity. However, the levels of HCT may be equivocal when there is frank hemorrhage, early and excessive fluid replacement or untimely HCT determinations. Leucopenia with relative lymphocytosis, clotting abnormalities, elevation of transaminases [typically the level of AST is about 2-3 times the level of ALT], hypoproteinemia and hypoalbuminemia are usually observed in this phase.

Recovery (convalescent) Phase: After 24-48 hours of defervescence, plasma leakage stops and is followed by reabsorption of extravascular fluid. Patient's general condition improves, appetite returns, gastrointestinal symptoms disappear, hemodynamic status stabilizes and diuresis ensues. Some patients may have a rash or generalized pruritus. Bradycardia and electrocardiographic changes are not uncommon during this stage. During this phase, HCT level stabilizes or drops further due to hemodilution following reabsorption of extravascular fluid. The recovery of platelet count is typically preceded by recovery of white cell count. It is very important that every probable or confirmed case of dengue fever must be reported.

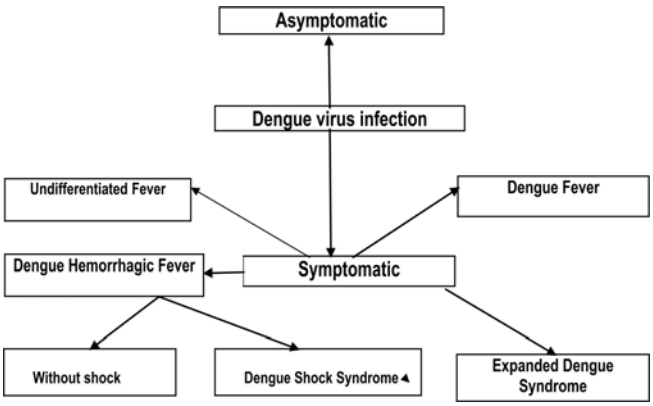

Figure 1: Clinical Course of Dengue virus Infection

Dengue hemorrhagic fever

The features of Dengue Hemorrhagic fever include:

•Fever: Acute onset of fever of 2-7 days duration

•Hemorrhagic manifestations, shown by any of the following:

- Petechiae , ecchymosis or purpura
- Bleeding from mucosa, gastrointestinal tract, injection sites, or other locations.
- Positive tourniquet test

• Thrombocytopenia

• Objective evidence of plasma leakage due to increased vascular permeability shown by any of the following:

- Rising hematocrit/hemoconcentration
- Evidence of plasma leakage such as pleural effusion, ascites or hypoproteinemia /hypoalbuminemia

DHF is further divided in four grades. Grade III and IV are classified as dengue shock syndrome (DSS)

Dengue shock syndrome

Criteria for dengue hemorrhagic fever as above with signs of shock: Tachycardia, cool extremities, delayed capillary refill, weak pulse, hypotension, lethargy or restlessness which may be a sign of reduced brain perfusion

Expanded Dengue Syndrome

In 2011 WHO guidelines the term of expanded dengue syndrome has been used to include unusual manifestations of dengue infection. These include neurological, hepatic, renal and other isolated organ involvement. These could be explained as a complication of severe profound shock or associated with underlying host conditions/diseases or co-infections.

Laboratory Diagnosis

Laboratory investigations of dengue fever include disease monitoring laboratory tests and diagnostic tests.

1. **Diagnostic Tests:** Laboratory confirmatory tests include:
   - Antibody detection (serology)
   - PCR
   - Detection of dengue virus protein (NS1 antigen)

Dengue Serology:

IgM Detection (by ELISA)

IgM detection by ELISA is the most widely used method for diagnosis of dengue fever. Once IgM is detectable, it rises quickly and peaks at about 2 weeks after onset of symptoms and it wanes to undetectable levels by 60 days. However in some patients it may persist for more 90 days. A positive result thus has to be interpreted and correlated cautiously with the clinical picture. If IgM dengue test is the only test available then establishing a negative IgM early in illness and demonstrating a positive serology later will be essential to exclude false negative results. In one study, IgM was detected in only 55% of patients with primary dengue infections between day 4-7 onset of fever, and it became positive in 100% of the patients after day 7. However, in secondary dengue infections, IgM was detected in only 78% of patients after day 7.[31] In another study, 28% of secondary dengue infections were undiagnosed when IgM was the only test performed. [32,33]

IgG detection by ELISA

In primary and secondary dengue infection, dengue IgG was detected in 100% of patients after day 7 of onset of fever. Therefore dengue IgG is recommended if dengue IgM is still negative after day 7 with the negative IgG in the initial test sample.

IgM/IgG ratio

The IgM/IgG ratio is used to distinguish primary infection from secondary dengue infection. A dengue virus infection is defined as primary if IgM/IgG ratio is greater than 1.2, or as secondary if the ratio is less than 1.2.

Rapid Diagnostic Tests (RDT)

Simple rapid tests such as the strip assays (immunochromatography test) are available for qualitative detection of dengue IgM and IgG. The yield of rapid tests was shown to be higher when samples were collected later in the convalescent phase of infection, with good specificity and could be used when ELISA test were not available.[34] However the result had to be interpreted in the clinical context because of false positive and negative results. [35,36] It is recommended that the dengue IgM be done by ELISA test after a rapid test, to confirm the status.

Other Tests:

Dengue viremia in patients is short, typically occurs 2–3 days prior to the onset of fever and lasts for four to seven days of illness. During this period the dengue virus, its nucleic acid and circulating viral antigen can be detected. During the early stages of the disease (up to six days of onset of illness), virus isolation, viral nucleic acid or antigen detection can be used to diagnose infection. At the end of acute phase of infection, immunological tests are the methods of choice for diagnosis.

Polymerase chain reaction (PCR)

Molecular tests such as the reverse transcriptase – polymerase chain reaction (RT- PCR) are useful for the diagnosis of dengue infection in the early phase (< 5 days of illness). It has a good sensitivity in the first 5 days of disease, but reduces after this, following the disappearance of the viremia. An additional advantage of RT- PCR is the ability to determine dengue serotypes. [37,38] It is useful in early phase of illness and its use is particularly considered for in-patients who present with diagnostic challenges in the early phase of illness.

Non-structural protein-1 (NS1 antigen)

The NS1 gene product is a glycoprotein produced by all flaviviruses and is essential for replication and viability of the virus. The protein is secreted by mammalian cells but not by insect cells. NS1 antigen appears as early as Day 1 after the onset of the fever and declines to undetectable levels by 5–6 days. Hence, tests based on this antigen can be used for early diagnosis. This antigen is present in high concentrations in the sera of dengue infected patients during the early phase of the disease. [39,40] The detection rate is much better in acute sera of primary infection (75%-97.3%) when compared to the acute sera of secondary infection (60% 70%). [41] The sensitivity of NS1 antigen detection drops from day 4-5 of illness onwards and usually becomes undetectable in the convalescence phase. [42]

Laboratory Findings in DF

The laboratory findings during an acute DF episode of illness are as follows:

• Total WBC is usually normal at the onset of fever; then leucopenia develops with decreasing neutrophil count and lasts throughout the febrile period.

• Platelet counts are usually normal, as are other components of the blood clotting mechanism. Mild thrombocytopenia (100,000 to 150 000 cells/mm3) is common and about half of all DF patients have platelet count below 100,000 cells/mm3, but severe thrombocytopenia (<50 000 cells/mm3) is rare. [43]

• Mild hematocrit rise (10%) may be found as a consequence of dehydration associated with high fever, vomiting, anorexia and poor oral intake.

• Serum biochemistry is usually normal but liver AST levels may be elevated.

Laboratory Findings in DHF:

• The WBC count may be normal or with predominant neutrophils in the early febrile phase. Thereafter, there is a drop in the total number of white blood cells and neutrophils, towards the end of the febrile phase. A relative lymphocytosis with increased atypical lymphocytes is commonly observed by the end of the febrile phase and into convalescence. These changes are also seen in DF.

• The platelet counts are normal during the early febrile phase. A mild decrease could be observed thereafter. A sudden drop in platelet count to below 100,000 occurs by the end of the febrile phase before the onset of shock or subsidence of fever. The level of platelet count is correlated with severity of DHF. In addition there is impairment of platelet function. These changes are of short duration and return to normal during convalescence.

• A rise in hematocrit occurs in all DHF cases, particularly in shock cases and is objective evidence of plasma leakage. It should be noted that the level of hematocrit may be affected by early volume replacement and by bleeding.

• Thrombocytopenia and hemoconcentration are constant findings in DHF. A drop in platelet count to below 100,000 cells/mm3 is usually found between the 3rd and 10th days of illness.

• Other common findings are hypoproteinemia/ hypoalbuminemia (as a consequence of plasma leakage), hyponatremia, and mildly elevated serum ALT (=200 U/L) with the ratio of AST: ALT > 2.

• A transient mild albuminuria is sometimes observed.

• Occult blood is often found in the stool.

• In most cases, assays of coagulation and fibrinolytic factors show reductions in fibrinogen, prothrombin, factor VIII, factor XII, and antithrombin III. A reduction in antiplasmin (plasmin inhibitor) has been noted in some cases. In severe cases with marked liver dysfunction, reduction is observed in the vitamin K-dependent factors such as factor V, VII, IX and X.

• Partial thromboplastin time and prothrombin time are prolonged in about half and one third of DHF cases respectively. Thrombin time is also prolonged in severe cases.

• Hyponatremia is frequently observed in DHF and is more severe in shock.

• Hypocalcemia (corrected for hypoalbuminemia) has been observed in all cases of DHF.

• Metabolic acidosis is frequently found in cases with prolonged shock. Blood urea nitrogen is elevated in prolonged shock.

2. Disease Monitoring Laboratory Tests:

Patients of dengue fever require continuous monitoring for proper management and following tests along with clinical judgment should be carried out serially to monitor the response of treatment ant determination of overall prognosis of these patients

- Complete Blood Count (CBC) with particular attention to;
  White blood cell count
  Hematocrit
  Platelet count
- Coagulation tests
- Liver Function Tests

Elevated liver enzymes is common and is characterized by greater elevation of the AST as compared to the ALT. [44] The frequency and degree of elevation of the liver enzymes are higher with DHF compared to DF.

Management:

There is no specific treatment for DHF. Therapy for DHF is wholly symptomatic and aims at controlling the clinical manifestations of shock and hemorrhage. Patients who do not receive a proper treatment usually die within 12–24 hours after shock ensues. The most important aspect of management of patients with DHF is close observation by the attending physicians and nurses with frequent clinical and laboratory monitoring.

Adequate fluid replacement to overcome the plasma leakage: It is recommended that patients must take full rest and plenty of fluids. During the febrile stage ingestion of adequate soft diet and drink is encouraged. For reducing fever, frequent sponging and paracetamol are provided. Aspirin and nonsteroidal anti-inflammatory drugs such as ibuprofen are contraindicated. The patient with suspected dengue infection should have daily follow-up at the outpatient clinic starting from the third day of fever to defervescence for 24 hours approaching the convalescent stage. The mortality and morbidity rates of patients with DHF can be reduced by early hospitalization and optimal supportive care. Prompt and adequate fluid replacement to overcome massive plasma leakage is a medical emergency. After proper management in the toxic stage for 24– 48 hours, the fluid in the extravascular space spontaneously

returns to the intravascular space. Patients uneventfully recover. Good prognostic signs are adequate urine output and regaining of appetite.

Control of bleeding: Some patients whose platelet count is reduced markedly may require platelet transfusion to avoid bleeding and shock. The risk factors for bleeding include the duration of shock, ingestion of aspirin or NSAID, administration of large amounts of plasma expanders and the improper management in the febrile and toxic stages. The Packed red blood cells may be required for patients who exhibit massive bleeding. Fresh frozen plasma is indicated for patients who have massive bleeding due to coagulopathy, or circulatory failure, which does not respond to intravenous crystalloid replacement. However, no evidence supports the benefit of preventive transfusion of platelet concentrate and FFP in patients with DHF, as the risk of bleeding is not solely based on the number of platelet counts or coagulopathy.

Immunization: No vaccine is yet available to provide protection against dengue fever.

Future scenario of Dengue in Pakistan

In Pakistan, the first confirmed outbreak occurred due to serotype DV-2 reported in 1994, thereafter, sporadic cases of DHF continued to be reported from different parts of the country. Analyzing the 2010 post monsoon situation, according to leading clinicians, by 2012, around 50 to 60 million people of Pakistan especially living in Lahore and Karachi might get infected with dengue virus. Thus urgent and efficient surveillance is required to prevent this catastrophe.[45]

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