Review Article
Pathogenesis of Dengue Haemorrhagic Fever and Its Impact on Case Management

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Plasma leakage and intrinsic coagulopathy are the pathological hallmarks in dengue haemorrhagic fever (DHF). Viral virulence, infection enhancing antibodies, cytokines and chemical mediators in the setting of intense immune activation are the key players implicated in the pathogenesis of DHF; the exact nature of which is yet to be fully understood. The pathophysiological changes attendant clinical features of plasma leakage necessitate recognition of changing physiological parameters for the early recognition of plasma leakage and appropriate fluid therapy. On the other hand, the changes in the haematological indices resulting from coagulopathy can tempt the clinician to initiate other modalities of therapy. A clearer understanding of the pathogenesis of DHF and the appreciation that both of these fundamental pathological changes share common pathogenic mechanisms would facilitate the appropriateness of management decisions and the early recognition of severe disease. Thus, thrombocytopenia, reduced fibrinogen, and prolonged partial thromboplastin time early in the disease course connoted severe disease and attended plasma leakage rather than clinical bleeding. The detection of plasma cytokine profile by a multiple bead immunoassay could also complement clinical parameters in predicting severe disease early in the disease course. Thus, MIP-β indicates good prognosis while IFN-γ portends severe disease.

1. Introduction

Infection by any one of the four serotypes of dengue virus (DENV) remains asymptomatic in the vast majority. Clinical spectrum among symptomatic infection ranges from undifferentiated fever (viral syndrome), dengue fever (DF), and dengue haemorrhagic fever (DHF) to the expanded dengue syndrome with isolated organopathy (unusual manifestations). DF can be without haemorrhage or have unusual haemorrhage, while DHF can be without shock or with shock, that is, dengue shock syndrome [1].

The WHO criteria for the clinical diagnosis of DHF requires the presence of acute and continuous fever of 2 to 7 days, haemorrhagic manifestations associated with thrombocytopenia (100,000 cells/c.mm or less) and haemocencentration (haematocrit >20% from baseline of patient or population of same age). Haemorrhagic manifestations could be mucosal and or skin or even a positive tourniquet test which is the commonest. Hepatomegaly occurs at some stage of DHF and often precedes plasma leakage and hence a valuable early predictor of plasma leakage [1].

DHF is most commonly seen in children with secondary dengue infection but has been documented in primary infection with DENV-1 and DENV-3, as well as in infants. These infants had acquired maternal dengue antibody and subsequently experienced a dengue infection [2]. Greater baseline vascular permeability among children could also be a contributor for more severe disease among children than among adults [3]. Epidemiological and serological studies done both in Thailand and Cuba support the importance of secondary dengue infections as a risk factor for DHF. Since the first observations by Halstead et al. in 1970, DHF has been present in situations where more than one serotype circulates [4, 5]. The disease burden and a resurgence of recurrent epidemics of DHF are attributable to social dynamics and a variety of epidemiological factors such as a high vector density, a high virus circulation, and a population at risk of secondary infection by virtue of previous exposure.
Besides secondary infection, chronic diseases such as bronchial asthma and diabetes have been suggested as risk factors for DHF. Also, whites have higher risk of developing DHF than blacks. DENV-2 virus is known to replicate to higher concentration in the peripheral blood cells of whites compared with those of blacks [6]. Abnormal haemostasis and plasma leakage are the main pathophysiological hallmarks in DHF. Even though more than half a century has elapsed since plasma leakage was first identified its precise mechanism remains elusive. The main factor implicated in the development of DHF rather than the relatively innocuous DF in dengue infection is secondary dengue infection but other factors like viral virulence and host characteristics are also important. Severe disease is the result of a complex interaction between the virus and the immune response evoked by the host with secondary infection [7].

2. Plasma Leakage in DHF

2.1. Pathophysiology. Plasma leakage is specific to the pleural and peritoneal surfaces. In DHF there is no vasculitis and hence no injury to the vessel walls, and plasma leakage results from cytokine mediated increase in vascular permeability. The ensuing movement of albumin and the resultant reduction of intravascular oncotic pressure facilitate further loss of fluid from the intravascular compartment. The basic Starling principle still holds true in explaining microvascular ultrafiltration based on the balance of the oncotic and hydrostatic pressures. However the glycocalyx, which is a gelatinous layer lining the vascular endothelium is also implicated in controlling fluid movement by the adherence of albumin molecules in to its matrix, damage of which, leads to loss of albumin into the extravascular compartment [8–11].

2.2. Immunopathogenesis. The immune system is implicated in the pathogenesis of DHF owing to the increased propensity to develop DHF with secondary dengue infection. The innate immune mechanisms comprising the complement pathway and NK cells as well as humoral and cell-mediated immune mechanisms launched in response to antigenic stimulation are involved in the clinical manifestations. Complement activation as well as vascular permeability may be influenced by viral products like NS1. Different immune mechanisms in the form of antibody enhanced viral replication leading to an exaggerated cytokine response impacts vascular permeability [12–14].

Infection with one dengue serotype elicits immunity to that serotype but does not provide long-term cross-protective immunity to the remaining serotypes. Subsequent infection with a different serotype results in the binding of the new virus to cross reactive nonneutralising antibody from the previous infection facilitating the uptake by mononuclear phagocytes enabling amplified viral replication. The resulting increase in viral load then drives an immunopathogenic cascade and the resultant exaggerated cytokine response leads to a transient increase in microvascular permeability. The precise way in which microvascular permeability is altered is not clear but is more likely to be a functional change rather than structural damage, as dengue shock is rapidly recoverable, and no inflammation is evident in the leaking surfaces [15–19]. Adding to the complexity of the underlying immunopathogenic mechanisms resulting in changes in vascular permeability is the proposal of an alternative mechanism whereby the rapid mobilisation of serotype cross-reactive memory T cells trigger the release of biological mediators. Some of the other factors implicated in this orchestration include viral virulence, molecular mimicry, and immune complex and/or complement mediated dysregulation, and genetic predisposition, all of which have been shown to correlate with disease severity. However, as yet no mechanism has been identified that links any of these established immunological derangements with a definitive effect on microvascular structure or function consistent with the observed alteration in permeability. In addition, most of the immunological abnormalities so far identified do not differ substantially from those seen in other infections without an apparent effect on permeability.

Neutralising antibodies are key factors in the aetio-pathogenesis of the disease. However, the cellular immune response is also important. It has been demonstrated that memory dengue T lymphocyte response after a primary infection includes both serotype-specific and serotype-cross-reactive T lymphocytes [20]. NS3 protein seems to be the major target for CD4+ and CD8+ T cells. Cytokines that may induce plasma leakage such as interferon γ, interleukin (IL) 2, and tumour necrosis factor (TNF) α are increased in DHF cases [20, 21]. Also, interferon γ enhances uptake of dengue particles by target cells through increasing Fc cell receptors [22]. Other cytokines such as IL-6, IL-8, and IL-10 are also increased. A protein of 22–25 kDa has been associated with the pathogenesis of DHF. This cytotoxic factor able to induce increased capillary permeability in mice is capable of reproducing in mice all the pathological lesions that are seen in human beings, and has been detected in sera of DHF patients [23].

A recent study has demonstrated the plasma cytokine profile in dengue fever from a Brazilian population which was detected by a multiplex bead immunoassay. MIP-β was indicated as a good prognostic marker which is in contrast to IFN-γ that was associated with severe disease. Both cytokines serve to discriminate mild from severe cases. It has also been shown that during the course of dengue different cytokine profiles may be present and vary according to determined clinical manifestations. The cytokine profiles identified by bead array multiplex system may favour an early identification of patients with the worst prognosis and may contribute to the establishment of more directed therapeutic procedures than the present ones [24].

Complement activation as a result of immune complexes (virus-antibody) or immune activation and cytokine production could also be involved in the mechanism of plasma leakage. Certain complement fragments such as C3a and C5a are known to enhance permeability. NS1 antigen in dengue virus has been shown to regulate complement activation and hence could play a role in the pathogenesis of DHF [12, 13, 25–27]. Clearly immunopathogenic mechanisms are involved in plasma leakage and coagulopathy.
However alternate immune pathways are also implicated in a protective role adding to the complexity and intricacy of the pathogenesis of DHF. Activated NK cells release granzyme A, which has cytolytic functions. MIP-1β produced by human monocytes and dendritic cells as well as activated NK cells and lymphocytes is chemotactant for NK cells, recruiting them to inflammatory sites. These mechanisms could play a protective role in the immunopathology of DHF by the early and efficient clearance of DENV by direct or indirect NK functions thereby limiting viral replication and its attendant cascading cytokine mediated plasma leakage. NK cells have been associated with mild dengue [28, 29].

In summary monocytes, macrophages, and dendritic cells are the major targets for DENV.

During secondary infection with a different DENV serotype cross-reactive nonneutralising antibodies bind to DENV and facilitate uptake via Fc receptors resulting in enhanced viral replication. The resultant higher viral antigen load leads to an exaggerated activation of cross-reactive dengue specific T cells. Biological mediators released by the activated T cells as well as virus infected cells along with complement activation by viral proteins, and immune complexes are implicated in increasing vascular permeability and coagulopathy.

These biological mediators influence clinical outcomes to a variable extent. Thus IL-1β, IFN-γ, IL-4, IL-6, IL-13, IL-7, and GM-CSF are associated with severe clinical manifestations while MIP-1β is elevated in patients with mild dengue. Marked thrombocytopenia is evident in patients with elevated IL-1β, IL-8, TNF-α, and MIP-1, while increased levels of MIP-1 and GM-CSF correlated with hypotension [24].

2.3. Haemorrhagic Manifestations in DHF. The pathogenesis of bleeding in DHF is unclear even though well-recognised coagulation disturbances do exist. The clinical haemorrhagic manifestations range from a mere positive tourniquet test, skin petechiae and ecchymoses to epistaxis, and gum bleeding to severe gastrointestinal haemorrhages. Thrombocytopenia is a consistent finding, while prolonged partial thromboplastin time and reduced fibrinogen concentration are the other abnormal haemostatic indices evident from early in the disease course. These haematological abnormalities seem to correlate better with the timing and severity of plasma leakage rather than the clinical haemorrhagic manifestations [30].

These recent findings raise the possibility for common pathogenic mechanisms responsible for both plasma leakage and abnormalities in the haemostatic indices. The true nature of the intrinsic coagulopathy evident early in the disease course and in mild forms of dengue can be confounded by the advent of hypovolemic shock and hypoxia in DHF with severe plasma leakage with less than optimal correction.

Thrombocytopenia is initially due to bone marrow suppression during the febrile viraemic phase of the illness. Progressive thrombocytopenia with defervescence result from immune mediated platelet destruction. Virus-antibody complexes have been detected on the platelet surface of DHF patients suggesting a role for immune-mediated destruction of platelets [31, 32]. Augmented platelet adhesiveness to vascular endothelial cells resulting from the release of high levels of platelet-activating factor by monocytes with heterologous secondary infection also contributes to the thrombocytopenia [33]. Thrombocytopenia however correlates poorly with bleeding manifestations. Spontaneous bleeding been uncommon even with counts below 100,000 cells/c.mm. It is strongly associated with the severity of vascular leakage. Counts below 100,000 cells/c.mm or a rapid drop in the platelet count was associated with severe disease.

The role of the glycocalyx rather than the endothelial cells per se in controlling ultrafiltration in the microvasculature is increasingly recognised and in vivo animal studies have shown the permeation of fibrinogen to the endothelial surface similar to albumin [11].

The low plasma fibrinogen detected in DHF could thus be a reflection of loss into the interstitial spaces in the setting of increased vascular permeability. Heparan sulphate forms an integral part of the glycocalyx which when damaged by the initial cytokine response in DHF gets liberated to the circulation and acts like an anticoagulant which could explain the prolonged APTT [34]. The disturbance in both these important haemostatic indices are unlikely to cause spontaneous bleeding. Haemorrhages are triggered by trauma in this setting of coagulopathy.

Development of antibodies potentially cross-reactive to plasminogen could have a role in causing haemorrhage in DHF [35]. However different studies have shown conflicting results as some have demonstrated an activation of fibrinolysis while others have shown an inhibition of the fibrinolytic pathway in DHF [30].

2.4. Endothelial Cells in DHF. Precise knowledge on the extent to which DENV infects endothelial cells is lacking as few studies have addressed the issue in the viraemic phase of the illness. Even though DENV has infected endothelial cells in vitro it is doubtful whether it reflects the effect in human infection as limited human autopsy studies have detected only the dengue antigen but not the genome in various cell types ranging from monocytes, liver sinusoidal cells, alveolar macrophages, peripheral blood, and splenic lymphocytes. How important these findings are in the pathogenesis of clinical features are uncertain as some studies have shown swelling of endothelial cells but not cell death or vasculitis [36], while others have detected apoptosis of endothelial cells in lungs and intestinal mucosa in fatal DHF cases, but the extent of apoptosis has not been documented [36]. DENV alters the endothelial cell surface protein production, its expression, and transcriptional activity.

Expression of ICAM-1 (intercellular adhesion molecule-1) and beta-integrin on micro vascular endothelium by DENV has been reported. DENV also affects the expression of cytokine receptors. These may contribute to the mechanisms involved in plasma leakage in DHF.

The role of DENV infected endothelial cells in the pathogenesis of coagulopathy in DHF is equally intriguing. There is upregulation of tissue plasminogen, thrombomodulin, protease activated receptor-1, and tissue factor receptor,
while there is downregulation of tissue factor inhibitor and activated protein C.

3. Clinical Implications

DHF cases have increased in the recent past and will continue to increase in numbers in time to come as DHF is commoner in secondary dengue infection. The probability of secondary dengue infection in a given population is expected to increase owing to the presumed high prevalence of previous exposure to clinical or asymptomatic dengue infection based on epidemiological data particularly in dengue endemic regions in the world. Despite the complexity of the immunopathogenic mechanism involved in severe disease, what is inexorable is that all patients with DHF have plasma leakage, the magnitude and progression of which will impact outcome. Dengue infection must be diagnosed early and in all such patients clinicians need to be alert and vigilant to identify DHF patients early at the inception of plasma leakage before shock sets in. Appropriate interventions with judicious fluid therapy at this stage could offset adverse outcomes and ensure a favourable outcome. Immunopathogenic mechanisms implicated in DHF could serve to meet the challenges of identifying in the febrile phase patients who could behave as DHF during the disease course. In this context assay of specific biomarkers identified in dengue could be useful. Thus while MIP-1 $\beta$ indicates good prognosis, IFN-$\gamma$ portends severe disease. Clinicians should also appreciate that both plasma leakage and disturbances of haemostatic indices share common immunopathogenic mechanisms. Disturbances in the haemostatic indices should thus be correlated to the severity of plasma leakage rather than the tendency for spontaneous clinical bleeding manifestations. Such considerations would serve to complement the accuracy of the prediction and identification of patients with severe disease. Intelligent application of such knowledge in relation to the temporal relation of the disease course will also facilitate interventional decision making and improve its accuracy and appropriateness. Thus, low plasma fibrinogen and prolonged APTT in the absence of shock early in the disease is to be expected in DHF and interpreted as heralding plasma leakage and not DIC, and its magnitude gives an idea of the severity of leakage. On the contrary the same indices of coagulopathy should have a different interpretation in the setting of shock owing to the confounding effects of hypovolemia and hypoxia and even the probability of associated DIC in such a setting.

Similarly thrombocytopenia is best used as a marker of severe disease particularly when it is <100,000 cells/c.mm or when there is a rapid drop. Its usefulness is as an indicator of prognosis during the disease course rather than a parameter for therapeutic interventions. Recognising the poor correlation of thrombocytopenia with bleeding should caution the clinician against the futility albeit danger of prophylactic platelet transfusions.

Clinicians should also bear in mind that cytokines play different roles in the pathogenesis of DHF. Some been stimulatory while others tend to downregulate the immunological network.

Critical alterations in the cytokine balance with attended adverse, rather than beneficial outcomes could be expected if corticosteroids are used for immunosuppression when such management decisions are based on the superficial consideration of immunological mechanisms as the underlying basis of DHF pathogenesis. Even though cytokines are implicated in the pathogenesis of increased vascular permeability absence of inflammation and the transient nature of altered permeability with a tendency for spontaneous cessation of plasma leakage also raises the irrationality of using steroids and other anti-inflammatory agents.

4. Concluding Remarks

Plasma leakage and coagulopathy are the fundamental pathological changes responsible for clinical manifestations, morbidity, and mortality in DHF. A complex interplay between immunological mechanisms with viral and host factors are implicated in the pathogenesis. Both humoral and cell-mediated immune mechanisms eventually result in the release of cytokines responsible for changes in the selective microvascular permeability and the resultant plasma leakage. Plasma leakage progresses either rapidly or slowly to cease completely and predictably after 24 to 48 hours of onset, raising the possibility of existence of underlying functional change rather than structural damage and inflammation in the vasculature. The influence of DENV on endothelial cells may be direct or indirect via release of mediators from infected or activated immune cells. Changes in the expression of adhesion molecules, enzymes, and cytokine receptors on endothelial cells are implicated in increasing the vascular permeability as well as activation of the coagulating system.

The two fundamental pathological attributes in DHF are plasma leakage and intrinsic coagulopathy.

The balance of hydrostatic and oncotic pressures is important in plasma leakage. However the glyocalyx also plays a crucial role in fluid fluxes. The permeation of fibrinogen apart from albumin into its matrix, as well as the release of heparan sulphate from its brush surface impacts both plasma leakage and intrinsic coagulopathy. The recognition of the role of biological markers on these pathogenic mechanisms can have far reaching diagnostic and therapeutic implications.

Clinicians should strive to predict severe disease before the advent of shock. Clinical predictors such as tender hepatomegaly and tachycardia after defervescence are exceedingly useful to suspect incipient plasma leakage. Technological advances and the availability of multiplex cytokine profile would facilitate these efforts. It could also open up new vistas in developing interventions targeting specific cytokines to reduce plasma leakage. However the importance of diligent and accurate monitoring of heart rate, pulse pressure, urine output, and haematocrit for the early detection of plasma leakage and adjustments to fluid therapy should not be overlooked and constitute an essential and integral part of case management. Our understanding of the pathogenesis of DHF and the availability of biological markers could serve to complement the clinician’s efforts. Prevention of immune enhanced viral replication is another area to focus specific
therapeutic interventions. Use of fresh frozen plasma for this purpose is an exciting area of research [37].

References


