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Review Article

Hemostatic derangement in dengue hemorrhagic fever

Ampaiwan Chuansumrit [a,*], Wathanee Chaiyaratana [b]

[a] Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[b] Research Center, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

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ABSTRACT

Dengue hemorrhagic fever (DHF) is a more severe manifestation of dengue virus infection. Patients with DHF exhibit abnormal hematological indices, including high hematocrit, low white blood cells, low neutrophils, high lymphocytes, increased atypical lymphocytes, low platelets, slightly prolonged activated partial thromboplastin time, prothrombin time, and thrombin time. Abnormal platelet functions manifest as impaired platelet aggregation to ADP, and concurrent increases in plasma thromboglobulin and platelet factor 4 levels are also seen. Variable reductions in the activities of coagulation factors including prothrombin, V, VII, VIII, IX, and X may be present. The plasma level of antithrombin is typically normal, but protein C and protein S are modestly reduced. Within the fibrinolytic system, slightly increased levels of tissue-plasminogen activator accompanied by slightly increased plasminogen activator inhibitor-1 and decreased thrombin activatable fibrinolysis inhibitor have been demonstrated. These derangements are prominent in patients with DHF grades III and IV, collectively known as dengue shock syndrome. Moreover, patients with excessive depletion of intravascular volume from plasma leakage and/or massive bleeding from endothelial dysfunction, thrombocytopenia, platelet dysfunction, and coagulopathy may exhibit shock, prolonged shock and repeated shock. DIC is also commonly found in these complicated patients. However, most patients recover spontaneously with normalization of abnormal laboratory profiles during the convalescent stage or within one to two weeks after defervescence.

Contents

Introduction

Dengue is one of the most serious consequences of mosquito-borne viral infection worldwide. The dengue virus consists of four subtypes, numbered 1 to 4. Infection with any of the four subtypes causes similar clinical symptoms that vary from a mild degree of dengue fever (DF) to more severe manifestations of dengue hemorrhagic fever (DHF). During the past five decades, the incidence has increased 30-fold with geographic expansion to new countries, and in the present decade, from urban to rural settings [1]. It is estimated that 50–100 million cases are reported annually and more than 2.5 billion people are at risk of infection in tropical and subtropical regions of Asia, Africa and America [2].

Clinical manifestations

The clinical manifestations of DF include a mild or marked febrile syndrome of abrupt onset, with headache, pain behind the eyes, muscle

* Corresponding author at: Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rama VI Road, Bangkok 10400, Thailand. Tel.: +66 2 201 1749; fax: +66 2 201 1748.
E-mail address: ampaiwan.jua@mahidol.ac.th (A. Chuansumrit).

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pain, bone pain, joint pain, nausea, vomiting and rash. Apart from petechiae, bleeding manifestations are not common. Patients with DHF have similar signs and symptoms followed by plasma leakage. Typical cases of DHF are characterized by four major clinical manifestations defined by the World Health Organization (WHO) in 1997 [3]: (i) sustained high fever for 2–7 days; (ii) a hemorrhagic tendency, such as a positive tourniquet test, or clinical bleeding; (iii) thrombocytopenia (platelets $\leq 100 \times 10^9$/L); and (iv) evidence of plasma leakage manifested by hemoconcentration (>20% increase in hematocrit) or pleural effusion which can be demonstrated by a right lateral decubitus chest radiograph taken on the day after defervescence. The severity of DHF is categorized into four grades according to the WHO in 1997 [3]: grade I, without overt bleeding but a positive tourniquet test; grade II, with a clinical bleeding diathesis such as petechiae, ecchymosis, epistaxis, gastrointestinal hemorrhage, menorrhagia, gum bleeding, hematuria, or rarely, serious bleeding into the central nervous system, heart or lungs; (iii) grade III, circulatory failure manifest by a rapid, weak pulse and narrowing pulse pressure or hypotension with cold, clammy skin and restlessness; and grade IV, profound shock in which pulse and blood pressure are not detected. According to the recent WHO classification in 2009 [1], dengue virus infection is defined as dengue with and without warning signs, and severe dengue. Therefore, DF is defined as dengue without warning signs of plasma leakage; DHF grade I is dengue with warning signs of plasma leakage but no bleeding except for a positive tourniquet test; DHF grade II is dengue with warning signs of plasma leakage and bleeding; DHF grade III is severe dengue with threatened shock; and DHF grade IV is severe dengue with profound shock. DHF grades III and IV are known as dengue shock syndrome (DSS).

The clinical manifestations of DHF are defined into three stages [3] including the febrile stage, which lasts 2–7 days followed by an abrupt fall to normal or subnormal temperature; the toxic stage, which lasts 24–48 hours; and finally, a rapid clinical recovery without sequelae in the convalescent stage. The toxic stage is the most critical period shortly after a rapid drop in temperature, and a varying degree of circulatory disturbance develops due to plasma leakage from increased vascular permeability. Therefore, patients with the following warning symptoms and signs [1,4] should be hospitalized: severe abdominal pain, severe nausea and vomiting, poor appetite, thirst, irritability, restlessness, sleepiness, behavioral change, cold clammy skin, clinical deterioration, oliguria, any bleeding episodes apart from petechiae and ecchymosis, and abnormal laboratory findings either of hematocrit >42% or rising to >10-15% above baseline for age and sex, or platelets $<100 \times 10^9$/L. However, the diagnosis of dengue virus infection should be confirmed by viral isolation, the presence of dengue nonstructural protein 1 antigen and/or dengue-specific IgM and IgG antibodies determined by capture enzyme-linked immunosorbent assay (ELISA) in acute and convalescent sera. Primary dengue infection is defined by an IgM to IgG ratio of ≥ 1.8 while the remainder are defined as secondary dengue infections. Both DF and DHF are caused by primary and secondary dengue infection.

Pathophysiology of DHF

The pathophysiology of DHF in humans is complex. Several studies have demonstrated that the clinical symptoms of DHF are due to an immune response involving production of cytokines/chemokines, as well as activation of endothelial cells, T-lymphocytes, monocytes and platelets [5–16]. The elevated mediators include C3a, C5a, tumor necrosis factor (TNF)-α, interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, interferon (INF)-γ, monocyte chemotactic protein (MCP)-1 and histamines. A recent study of cytokines/chemokines in sequential daily blood samples drawn from 164 hospitalized DHF patients revealed two patterns of cytokines/chemokines [16]. IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-γ, and MCP-1 were increased, while IL-1β, IL-2, vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) were decreased during the febrile and toxic stages. It has been postulated that dengue virus infects monocytes/macrophages initially where is replicates. TNF-α is the first cytokine to be released. Simultaneously, dengue virus induces CD4 + T cells to produce the T helper (Th)1 cytokines IFN-γ, IL-1β and IL-2 into the circulation. These cytokines can inhibit virus replication, induce B-cells to produce specific antibody against the virus, promote inflammatory cell infiltration, upregulate major histocompatibility complex antigens, and increase macrophage-mediated killing and NK cell activity. TNF-α also induces MCP-1 production to recruit leucocytes to inflammatory sites, activating endothelial cells, and upregulating adhesion molecules on the endothelial cells, resulting in leucopenia and increased vascular permeability. TNF-α also induces IL-6 release, and the synergistic effect of IL-6 and IL-1β promotes high fever in the febrile stage. MCP-1 stimulates the production of IL-4 that induces the differentiation of Th-1 to Th-2. The Th-2 response will exacerbate the clinical symptoms of DHF. IL-4 also activates IL-10 that inhibits Th-1 cytokine production of TNF-α, IFN-γ, IL-1β and IL-2. On the contrary, IL-10 activates Th-2 cytokine production of IL-4, IL-6 and IL-8. Ultimately, the consecutive action of these cytokines/chemokines leads to leucopenia, increased vascular permeability, thrombocytopenia, and activation of coagulation and fibrinolysis found in patients with DHF and more prominently in patients with DSS.

Hematological derangement

The laboratory findings in patients with DHF reflect the pathophysiology that evolves over a period of several days. In order to clarify the exact day of illness, the day of defervescence in the toxic stage is designated as D0. D-1 to D-3 are designated as one to three days before defervescence in the febrile stage and D + 1 to D + 2 are designated as one to two days after defervescence in the convalescent stage. The abnormal laboratory findings include those found in basic hematological tests of high hematocrit, low white blood cells, low neutrophils, high lymphocytes, increased atypical lymphocytes, low platelets, slightly prolonged activated partial thromboplastin time (APTT) and prothrombin time (PT), and slightly prolonged or normal thrombin time (TT) as shown in Table 1. Most patients recover spontaneously, and all abnormal tests normalize during the convalescent stage or within one to two weeks after defervescence. However, shock, prolonged shock and repeated shock are found in patients with excessive depletion of intravascular volume from plasma leakage and/or massive bleeding from endothelial dysfunction, thrombocytopenia, platelet dysfunction, and coagulopathy. Additional DIC is commonly found in patients with shock. Patients with uncontrolled massive bleeding and shock have a high mortality rate.

Rising hematocrit

The prominent high hematocrit is due to plasma leakage from the intravascular compartment due to increased vascular permeability beginning in the febrile stage and further pronounced during the toxic stage [17]. The highest hematocrit is found on D0, following which it begins to decline. Importantly, patients with DSS have a significantly higher hematocrit (47.2% ± 6.0) compared to those with DHF grade II (42.8% ± 3.7), DHF grade I (42.2% ± 4.5), and DF (40.0% ± 5.4) [18]. However, patients with hemolytic anemia such as thalassemia, G6PD deficiency and other hereditary hemolytic anemia often present with anemia rather than high hematocrit due to the ongoing hemolytic process during the acute febrile stage [19]. Moreover, patients with congenital bleeding disorders such as hemophilia often present with anemia due to the bleeding risk aggravated by endothelial dysfunction, thrombocytopenia, mild platelet dysfunction and coagulopathy [19,20] starting from the early febrile stage through to the toxic stage.

The impaired endothelial barrier leads to the leakage of plasma components into the perivascular space and to the triggering of the coagulation system. Endothelial damage may be caused by the dengue virus itself, cytokine secretion from monocytes/macrophages, and/or complement activation. Reduced levels of complement factors

Table 1
Hematological tests among patients with dengue fever (DF), dengue hemorrhagic fever grades I and II (DHF I & II), dengue shock syndrome (DSS) and other febrile illnesses (OFI).

	Control	Febrile Stage				Toxic Stage				Convalescent Stage			
		DF	DHF I & II	DSS	OFI	DF	DHF I & II	DSS	OFI	DF	DHF I & II	DSS	OFI
Hct (%)	36.9 ± 2.1	35.8 ± 2.7	38.6 ± 3.6	40.5 ± 2.9	36.2 ± 11.3	39.4 ± 3.0	39.3 ± 3.6	41.8 ± 3.7	37.9 ± 8.2	39.0 ± 9.9	38.2 ± 4.0	38.6 ± 4.4	38.6 ± 7.4
(n)	(59)	(23)	(56)	(6)	(5)	(18)	(52)	(6)	(6)	(19)	(71)	(16)	(5)
Platelet ($x10^9$/L)	338 ± 120	100 ± 30	92 ± 51	50 ± 11	152 ± 46	97 ± 30	54 ± 27	28 ± 22	162 ± 58	115 ± 39	53 ± 29	32 ± 14	158 ± 57
(n)	(59)	(23)	(56)	(6)	(5)	(18)	(52)	(6)	(6)	(19)	(71)	(16)	(5)
WBC ($x10^9$/L)	8.3 ± 2.1	3.1 ± 2.1	2.2 ± 0.9	3.0 ± 1.6	3.5 ± 1.3	4.1 ± 2.5	3.7 ± 1.4	4.2 ± 1.5	3.4 ± 1.0	5.1 ± 1.9	5.4 ± 2.1	7.2 ± 3.1	3.2 ± 0.5
(n)	(69)	(40)	(47)	(6)	(5)	(29)	(47)	(8)	(7)	(29)	(67)	(23)	(5)
Neutrophils (%)	47.2 ± 8.8	49.6 ± 14.5	49.2 ± 15.2	43.4 ± 27.0	52.5 ± 12.2	34.3 ± 15.7	33.4 ± 13.3	35.9 ± 12.8	44.2 ± 15.2	29.6 ± 13.3	30.8 ± 11.6	32.0 ± 6.7	40.0 ± 17.1
(n)	(64)	(40)	(47)	(6)	(5)	(29)	(47)	(8)	(7)	(29)	(67)	(23)	(5)
Absolute neutrophil ($x10^9$/L)	4.0 ± 1.5	1.5 ± 1.1	1.1 ± 0.5	1.2 ± 0.6	1.8 ± 0.6	1.4 ± 1.4	1.2 ± 0.5	1.6 ± 0.8	1.6 ± 0.8	1.5 ± 0.8	1.6 ± 0.8	2.2 ± 1.0	1.3 ± 0.7
	(64)	(40)	(47)	(6)	(5)	(29)	(47)	(8)	(7)	(29)	(67)	(23)	(5)
Lymphocyte (%)	40.4 ± 7.9	33.2 ± 9.7	35.9 ± 11.9	34.7 ± 16.5	31.8 ± 9.1	39.6 ± 10.7	40.4 ± 12.3	39.3 ± 10.1	42.7 ± 12.8	42.8 ± 9.5	41.0 ± 9.3	43.2 ± 7.5	46.0 ± 12.2
(n)	(64)	(40)	(47)	(6)	(5)	(29)	(47)	(8)	(7)	(29)	(67)	(23)	(5)
Absolute lymphocyte ($x10^9$/L)	3.2 ± 1.0	1.0 ± 0.7	0.8 ± 0.5	1.2 ± 1.3	1.1 ± 0.4	1.6 ± 1.0	1.5 ± 0.7	1.6 ± 0.6	1.4 ± 0.5	2.1 ± 0.9	2.2 ± 1.1	3.2 ± 1.5	1.5 ± 0.4
	(64)	(40)	(47)	(6)	(5)	(29)	(47)	(8)	(7)	(29)	(67)	(23)	(5)
Atypical lymphocyte (%)	1.1 ± 1.4	4.7 ± 5.8	2.8 ± 3.4	8.3 ± 6.0	0.7 ± 1.6	9.1 ± 7.3	11.4 ± 7.4	7.9 ± 5.4	3.0 ± 3.2	12.3 ± 6.0	12.8 ± 5.9	10.2 ± 5.0	5.8 ± 6.6
(n)	(64)	(40)	(47)	(6)	(5)	(29)	(47)	(8)	(7)	(29)	(67)	(23)	(5)
Absolute atypical lymphocyte ($x10^9$/L)	0.1 ± 0.1	0.2 ± 0.4	0.1 ± 0.1	0.2 ± 0.2	0.03 ± 0.0	0.4 ± 0.5	0.5 ± 0.5	0.3 ± 0.3	0.1 ± 0.1	0.6 ± 0.5	0.7 ± 0.5	0.7 ± 0.6	0.2 ± 0.1
	(64)	(40)	(47)	(6)	(5)	(29)	(27)	(8)	(7)	(29)	(67)	(23)	(5)
APTT (sec)	30.8 ± 1.9	37.6 ± 4.3	38.7 ± 6.2	41.7 ± 5.5	35.3 ± 5.7	36.6 ± 4.1	39.6 ± 5.2	45.3 ± 4.4	35.4 ± 5.0	36.7 ± 5.0	38.8 ± 5.5	41.7 ± 1.4	35.4 ± 6.4
(n)	(59)	(23)	(56)	(13)	(5)	(18)	(52)	(9)	(6)	(19)	(71)	(15)	(5)
PT (sec)	11.8 ± 0.7	14.1 ± 0.9	13.3 ± 2.7	14.1 ± 3.2	14.8 ± 1.8	12.6 ± 0.8	12.6 ± 1.4	15.2 ± 2.8	13.0 ± 1.8	12.2 ± 1.2	12.2 ± 1.7	13.0 ± 1.6	12.6 ± 1.8
(n)	(59)	(23)	(56)	(13)	(5)	(18)	(52)	(9)	(6)	(19)	(71)	(15)	(5)
TT (sec)	11.4 ± 0.6	13.2 ± 1.5	14.1 ± 3.3	16.3 ± 4.2	13.7 ± 1.6	13.5 ± 1.5	14.5 ± 2.0	16.5 ± 3.1	12.6 ± 0.5	14.0 ± 1.8	14.5 ± 2.2	15.8 ± 2.9	12.9 ± 1.0
(n)	(59)	(23)	(56)	(13)	(5)	(18)	(52)	(9)	(6)	(19)	(71)	(15)	(5)

n, number of patients tested.

C3, C3 proactivator, C4 and C5 have been postulated to be a result of C3 consumption through the activation of both the classical and alternative pathways. The consequence of complement activation -- possibly by immune complexes -- leads to the release of anaphylatoxins C3a and C5a and subsequent release of histamine, a potent mediator of vascular permeability [5,6,12,14,15]. Recent studies of cytokines/chemokines revealed that increased MCP-1 levels are present from D-2 [9,16]. MCP-1 can trigger chemotaxis and transendothelial migration of monocytes. Other mediators such as IL-4, IL-6, IL-8, Il-10, IFN-γ, and TNF-α were also elevated and may contribute to increased endothelial permeability. Evidence of endothelial injury has been shown by elevated plasma levels of von Willebrand factor (vWF) during acute illness. The mean levels of vWF antigen and ristocetin cofactor activity in patients with DSS on the D-1, D0, D + 1 and D + 2 were significantly higher than those of other groups of patients [21,22]. VWF is released from endothelial cells in the form of ultra-large multimers that will be cleaved by ADAMTS 13 (a disintegrin-like and metalloprotease with thrombospondin type 1 domain 13). Levels of vWF antigen and ristocetin cofactor activity >210% may be used as predictors for DSS during the febrile stage with relative risks of 10.9 (95% CI 1.5-81.7) and 7.0 (95% CI 2.2-121.4), respectively [22]. Multimers of vWF have been studied in a small number of patients with DHF, revealing a shift from high molecular weight multimers to low molecular multimers with larger than normal multimers during the toxic stage [23]. Additionally, the levels of ADAMTS 13 were low in all three stages of patients with DHF, [23] possibly corresponding to the process of DIC, especially in patients with DSS. Recent studies have assessed the number of circulating endothelial cells (CECs) and several other endothelial markers. The number of CECs was not significantly increased on D-2 to D + 2 except for patients with DSS. Plasma levels of soluble thrombomodulin (sTM) on D-1 and D0 were significantly higher than that those of the convalescent stage in patients with DHF, and patients with DSS had a significantly higher level compared to other groups of patients [18] as shown in Table 2. Levels of soluble thrombomodulin (sTM) >10 ng/mL during the febrile stage can be used as one of the predictors for DSS in the subsequent toxic stage [18]. These levels reflect the prominent increased endothelial permeability leading to massive plasma leakage and shock in patients with DSS. In addition, the levels of soluble vascular adhesion molecule-1 (sVCAM-1) on D-1 and D0 were significantly higher than those in the convalescent stage but there was no significant difference between different grades of DHF. However, the levels of soluble intercellular adhesion molecule-1 (sICAM-1), and soluble E-selectin were slightly higher than those in the convalescent stage although this was not statistically significant.

Low platelets

Platelet counts begin to fall during the febrile stage and reach their nadir during the toxic stage. Thrombocytopenia can be a result of three processes. First, depression in bone marrow function resulting in decreased production [24]. Second, the increase in megakaryocytes in the bone marrow combined with shortened platelet survival are indicative of increased platelet destruction [24,25]. Third, increased platelet consumption from the interaction between platelets and endothelial cells infected with dengue virus was demonstrated *in vitro* and suggested that some dengue-injured endothelial cells might promote platelet adherence and lysis [26]. Immune complexes containing dengue antigen have been found on platelets [27]. Finally, increased binding of platelets and neutrophils to dengue-infected endothelium has been demonstrated *in vitro* [28]. Recently, it was shown that increased IL-8 levels correlate with low platelet counts on D0 and D + 1, at which time platelet counts reach their nadir [16]. Subsequently, the number of platelets rapidly increases in the convalescent stage reaching baseline levels within 7–10 days after defervescence. Additionally, abnormal platelet functions during the acute phase of disease, manifest as impaired platelet aggregation to ADP, and concurrent increases in plasma thromboglobulin and platelet factor 4 levels, indicate that platelet secretion is increased [29].

Low white blood cells but high lymphocytes

White blood cell, absolute neutrophil and lymphocyte counts are at their lowest level on D-3 to D-1, and begin to rise on D0. A recent study demonstrated a significant correlation between the increased MCP-1 level and leucopenia in DHF during the febrile stage from D-2 to D-1, and between increased MCP-1 and monocytopenia during the febrile stage on D-1 to D0 in the toxic stage [16]. In addition, increased IL-6 was correlated with low neutrophil counts on D0 of the toxic stage, and with the degree of neutropenia during the convalescent stage on D + 1 and D + 2 [16].

Atypical lymphocytes increase on D-2 to D-1 reaching their highest level on D + 1 to D + 2. In clinical practice, these atypical or transformed lymphocytes can be found in a significant number (more than 10%) as early as the febrile stage from D-3 onward, [30] which may be used as a diagnostic aid to differentiate DHF from other viral and bacterial infection with reliable accuracy, especially in secondary dengue infection [31]. These transformed lymphocytes might be antibody-producing B-lymphocytes. A recent study demonstrated that few dengue responsive CD8 + T-cells were recovered during the acute febrile stage. Profound T-cell activation and death may contribute to the systemic manifestations of DHF, and the original 'antigenic sin' in the T-cell responses may suppress or delay viral elimination, leading to higher viral load and increased immunopathology [32].

Activation of coagulation and fibrinolysis

The aPTT, PT and TT [23,33–36] during the acute febrile illness are typically slightly longer than at the convalescent stage except in those with DSS, where the values are significantly more prolonged on D-1, D0 and D + 1 compared to the convalescent stage. These changes have been considered to be partly due to liver damage since liver enzymes may be elevated. The abnormal screening studies may also be in part due to coagulation activation. Variable reductions in the activities of coagulation factors including prothrombin, V, VII, VIII, IX, X, antithrombin and α-2 antiplasmin have been demonstrated during the acute phase of DHF [23,33,36] with a gradual increase during the convalescent stage. Importantly, tissue factor (TF) was significantly increased during the febrile stage especially in patients with DSS with gradual normalization during the convalescent stage. However, fibrin degradation products such as D-dimer were not significantly elevated to a degree consistent with DIC in most patients, with the exception of those subjects with profound shock.

The changes in the fibrinolytic system have been recently described. Slightly increased levels of tissue-plasminogen activator (t-PA) accompanied by slightly increased levels of plasminogen activator inhibitor −1 (PAI-1) and decreased thrombin activatable fibrinolysis inhibitor (TAFI) during the febrile and toxic stages were demonstrated. However, the levels of PAI-1 were significantly increased in patients with DSS. The markedly elevated PAI-1 in these patients has been shown to correlate with poor clinical outcome. The balance of coagulation and fibrinolysis is essential for a favorable outcome. The most profound coagulation and fibrinolysis derangements are found in patients with severe manifestations of DSS who may suffer high mortality and morbidity rates.

Finally, plasma levels of antithrombin are commonly normal although levels of protein C and protein S are slightly reduced during the febrile and toxic stages in patients with DHF. Interestingly, these natural anticoagulants were significantly reduced in patients with DSS. Protein C and S may be low due to consumption during DHF accompanied by direct liver damage from the dengue virus itself. However, these levels also spontaneously normalize during the convalescent stage.

Table 2
Tests for endothelium, coagulation, fibrinolysis and anticoagulants among patients with dengue fever (DF), dengue hemorrhagic fever grade I and II (DHF I & II), dengue shock syndrome (DSS) and other febrile illnesses (OFI).

	Control	Febrile Stage				Toxic Stage				Convalescent Stage			
		DF	DHF I & II	DSS	OFI	DF	DHF I & II	DSS	OFI	DF	DHF I & II	DSS	OFI
Endothelium													
vWF:Ag (%)	110.1 ± 23.7	200.0 ± 8.2	200.0 ± 12.4	216.6 ± 10.4	199.3 ± 5.7	199.3 ± 7.9	208.2 ± 14.4	225.9 ± 12.1	200.3 ± 9.3	201.3 ± 9.2	210.2 ± 8.2	230.2 ± 10.3	197.1 ± 7.2
(n)	(55)	(23)	(60)	(13)	(9)	(18)	(57)	(10)	(7)	(19)	(77)	(15)	(6)
vWF:Ricof (%)	121.1 ± 13.6	187.2 ± 9.9	202.0 ± 18.6	212.5 ± 10.9	181.1 ± 11.6	190.7 ± 11.2	206.4 ± 16.4	226.4 ± 9.5	189.7 ± 17.3	193.2 ± 17.0	208.3 ± 17.6	231.3 ± 9.7	186.2 ± 22.4
(n)	(55)	(14)	(40)	(9)	(6)	(9)	(57)	(10)	(7)	(16)	(56)	(9)	(5)
CEC (cells/mL)	1.8 ± 1.5	2.3 ± 1.8	2.1 ± 1.2	3.7 ± 1.6	1.7 ± 2.2	2.8 ± 1.5	4.2 ± 1.7	9.5 ± 2.3	2.9 ± 1.6	2.8 ± 1.7	4.4 ± 1.8	14.6 ± 1.9	3.8 ± 1.0
(n)	(61)	(26)	(55)	(7)	(5)	(20)	(44)	(8)	(7)	(22)	(66)	(20)	(61)
sTM (ng/mL)	3.2 ± 1.5	6.0 ± 3.0	6.5 ± 3.2	10.9 ± 1.6	3.9 ± 2.6	6.6 ± 3.2	5.9 ± 2.7	16.2 ± 8.1	4.7 ± 3.3	5.3 ± 2.7	6.2 ± 3.1	11.5 ± 3.3	1.9 ± 3.4
(n)	(65)	(27)	(58)	(8)	(5)	(22)	(53)	(9)	(7)	(22)	(73)	(24)	(5)
Coagulation													
TF (pg/ml)	214.2 ± 70.0	254.1 ± 84.3	238.6 ± 63.3	231.3 ± 90.3	nd	237.3 ± 91.6	238.3 ± 95.3	239.3 ± 95.3	nd	209.4 ± 80.4	224.0 ± 63.8	268.1 ± 165.9	nd
(n)	(9)	(17)	(18)	(17)		(17)	(18)	(11)		(17)	(18)	(19)	
TAT (μg/L)	20.9 ± 6.2	26.7 ± 4.4	19.5 ± 8.0	18.9 ± 6.3	16.0 ± 5.0	23.8 ± 8.9	19.8 ± 7.5	23.5 ± 13.0	16.5 ± 4.4	25.4 ± 6.4	20.1 ± 8.7	17.9 ± 8.1	17.9 ± 3.4
(n)	(63)	(14)	(41)	(13)	(6)	(15)	(34)	(10)	(4)	(9)	(28)	(24)	(3)
Fibrinolysis													
t-PA (ng/ml)	1.4 ± 0.9	2.3 ± 1.5	2.2 ± 1.2	4.0 ± 2.4	2.6 ± 1.4	2.2 ± 1.6	2.2 ± 1.4	3.8 ± 2.4	2.0 ± 1.0	2.0 ± 1.8	1.9 ± 1.4	2.5 ± 1.7	1.2 ± 1.4
(n)	(96)	(27)	(57)	(17)	(22)	(27)	(47)	(11)	(14)	(31)	(42)	(29)	(5)
PAI-1 (ng/ml)	18.9 ± 17.2	19.9 ± 10.6	21.6 ± 16.4	77.1 ± 95.2	18.7 ± 7.5	17.2 ± 8.8	18.3 ± 9.4	64.9 ± 114.8	21.9 ± 6.8	15.9 ± 6.9	15.7 ± 6.4	51.8 ± 49.2	14.6 ± 7.2
(n)	(102)	(31)	(65)	(17)	(20)	(31)	(51)	(12)	(16)	(32)	(47)	(32)	(5)
Anticoagulants													
Antithrombin (%)	114.9 ± 11.5	110.8 ± 11.3	111.7 ± 17.9	91.8 ± 14.9	83.8 ± 24.8	131.7 ± 21.0	106.2 ± 19.6	89.0 ± 29.5	97.6 ± 14.4	134.2 ± 12.6	119.3 ± 20.9	105.7 ± 29.8	102.4 ± 28.7
(n)	(21)	(10)	(10)	(14)	(5)	(10)	(10)	(6)	(5)	(5)	(6)	(10)	(5)
Protein C (%)	102.7 ± 18.8	90.5 ± 18.9	87.5 ± 30.0	65.1 ± 16.4	81.8 ± 26.4	94.1 ± 20.2	79.7 ± 21.1	65.5 ± 25.2	76.2 ± 25.3	97.0 ± 20.4	82.3 ± 25.4	77.1 ± 21.6	87.0 ± 25.1
(n)	(21)	(10)	(10)	(14)	(5)	(10)	(10)	(6)	(5)	(5)	(6)	(10)	(5)
Protein S (%)	85.8 ± 15.2	67.0 ± 20.7	66.8 ± 19.3	46.1 ± 13.0	67.2 ± 22.3	70.5 ± 17.9	62.2 ± 22.4	36.5 ± 19.4	73.6 ± 21.0	71.8 ± 14.4	80.0 ± 32.5	55.4 ± 17.4	66.8 ± 27.5
(n)	(21)	(10)	(10)	(14)	(5)	(10)	(10)	(6)	(5)	(5)	(6)	(10)	(5)
Free protein S (%)	78.0 ± 14.3	62.6 ± 7.6	57.3 ± 16.4	57.9 ± 17.9	64.2 ± 12.4	61.9 ± 12.8	56.7 ± 15.3	41.2 ± 15.9	76.8 ± 22.9	63.4 ± 9.9	58.8 ± 16.1	50.6 ± 15.1	58.4 ± 10.9
(n)	(21)	(10)	(10)	(14)	(5)	(10)	(10)	(6)	(5)	(5)	(6)	(10)	(5)

n, number of patients tested; nd, no data; vWF:Ag, von Willebrand factor antigen; vWF:Ricof, von Willebrand factor activity of ristocetin cofactor; CEC, circulating endothelial cells; sTM, soluble thrombomodulin; TAT, thrombin-antithrombin complex; TF, tissue factor; t-PA, tissue plasminogen; PAI-1, plasminogen activator inhibitor-1.

Treatment

At present, there is no specific treatment for patients with DHF. The awareness of dengue virus infection with early recognition of hemoconcentration and bleeding is essential for a favorable outcome. Prompt and accurate diagnosis, appropriate fluid replacement and cessation of bleeding are essential. In cases of underlying hematologic-oncologic diseases, patients may present with unusual manifestations of anemia and/or profound bleeding episodes.

Local measures including anterior nasal packing with gelfoam is recommended for patients with epistaxis. Hormonal therapy such as premarin, primulute N or oral contraceptive pills is suggested for females exhibiting excessive menstrual bleeding (pictorial blood loss assessment chart (PBAC) scores approaching 30) [37,38]. Blood component therapy is recommended in patients with massive bleeding: red blood cells for volume replacement, fresh frozen plasma (FFP) and cryoprecipitate for restoring hemostasis, and platelet concentrates for raising the number of platelet counts in patients with thrombocytopenia. Importantly, patients should be maintained at a body temperature $\geq$37 °C and pH $\geq$7.2. Hypothermia, acidosis and coagulopathy are often referred to as 'the lethal triad'. In cases of massive bleeding unresponsive to FFP 20 ml/kg, cryoprecipitate 0.2-0.4 unit/kg and platelet concentrates 0.2-0.4 unit/kg, the use of recombinant activated factor VII (rFVIIa) 100 μg/kg at 30 min to 4 hour interval for 1–3 doses has been reported, with an effective response rate of 65.8% (25/38) [39]. Platelet concentrate requirement in the rFVIIa group was lower than the placebo group (6.3% vs. 33.3%) in a randomized, double-blind, placebo-controlled study of rFVIIa to control bleeding in children with DHF [40].

Conclusion

Dengue viral infection is one of the most prevalent emerging worldwide health problems. The hemostatic derangement in patients with serious manifestations of DHF is complicated. Despite comprehensive management, patients still face high rates of morbidity and mortality. An effective vaccination strategy for dengue is badly needed.

Conflict of interest statement

The authors stated that they had no interest that might be perceived as posing a conflict or bias.

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