LABORATORY PREDICTORS OF DENGUE SHOCK SYNDROME DURING THE FEBRILE STAGE

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Abstract. The clinical manifestations of dengue hemorrhagic fever (DHF) consist of three successive stages: febrile, toxic and convalescent. The toxic stage is the critical period, which may manifest as circulatory disturbance or even profound shock in some patients. We attempted to determine predictors for the risk of dengue shock syndrome (DSS) during the febrile stage. One hundred one children with acute febrile illness were enrolled in the study, with a mean age of 11 years old. The diagnosis included dengue fever (DF) 21 cases, DHF grade I 30 cases, DHF grade II 33 cases, DHF grades III and IV 10 cases; children with other febrile illnesses (OFI) 7 cases were used as controls. Complete blood counts, coagulation tests, von Willebrand factor antigens (VWF:Ag) and ristocetin cofactor activity (VWF:Rcof) were determined daily during hospitalization and 2-4 weeks after discharge from the hospital. The results revealed any one of the following abnormal laboratory findings during the febrile stage served as a predictor for risk of DSS: increase in hematocrit >25%, a platelet count <40,000/μl, an activated partial thromboplastin time >44 seconds, a prothrombin time >14 seconds, a thrombin time >16 seconds or a VWF:Ag or VWF:Rcof >210%. The relative risk ranged from 4.8 to 10.9. Simple laboratory investigations with complete blood count, coagulation test or the more sophisticated von Willebrand factor, are helpful in predicting the risk for DSS during the febrile stage.

Key words: dengue shock syndrome, dengue hemorrhagic fever, predictors

INTRODUCTION

Symptomatic dengue virus infection, which includes dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), is a major communicable diseases in many countries throughout the world. The worldwide incidence of dengue virus infection is estimated at over 100 million cases annually (Monath, 1994; Gibbons, 2002). Thailand has been an endemic area with multiple outbreaks since 1958. The most prevalent age group is children 5-9 years old,

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followed by children 10-14 years old. However, more recently young adults have also been infected. As a result of appropriate clinical management, the case-fatality rate has decreased from 10% (296/2,706) in 1958 to 0.12% over the past five decades (Kalayanaroj, 1999). The fatal cases were those with DHF (38%) and DSS (62%) while none of the patients with DF died (Ungchusak *et al*, 2007).

The three stages of DHF are febrile, toxic and convalescent. The febrile stage 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, varying degrees of circulatory disturbance develop due to plasma leakage from increased vascular permeability (Suwanik *et al*, 1967).

Our study aimed to establish predictors for DSS using simple laboratory tests, which were a complete blood counts and coagulation tests, and more sophisticated tests: von Willebrand factor analysis.

MATERIALS AND METHODS

Subjects

One hundred one patients 5-15 years old, suspected of having dengue virus infection admitted at the Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, were recruited into the study. The study was approved by the Faculty Ethics Committee and informed consent was obtained from parents or caregivers. The patients had blood drawn daily during hospitalization and at 2-4 weeks follow-up after discharge from the hospital. The patients' demographic data are shown in Table 1.

The diagnostic criteria for dengue infection and severity of DHF were based on WHO criteria (WHO, 1977). All patients with DF and DHF had serological confirmation using dengue specific IgM and IgG by enzyme-linked immunosorbent assay (ELISA), while those with other febrile illnesses (OFI) had negative results. They were expected to have other self-limited infections.

D0 was designated as the day of defervescence when the temperature dropped below 37^{o}C without subsequent elevation. D-1 and D-2 were designated as one and two days before defervescence, respectively, and D+1 and D+2 were designated as one and two days after defervescence, respectively.

Laboratory testing

From the daily blood sample, 3.0 ml was placed in EDTA, 1.8 ml in citrated buffer and 1 ml was allowed to clot. The complete blood counts were analyzed within 4 hours of blood collection using a technicon H* 3 Hematology Analyzer. The activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) were examined within 4 hours of blood collection using a semi-automated blood coagulation analyzer, Systemex CA50. Von Willebrand factors were examined on the initially thawed citrated plasma kept at -70^{o}C. VWF antigen (VWF:Ag) was assayed by ELISA (Bartlett *et al*, 1976; Voller *et al*, 1976; Cejka, 1982) using anti-human VWF rabbit IgG and anti-human VWF rabbit IgG conjugated with peroxidase purchased from Dade Corp, USA. Ristocetin cofactor activity (VWF:Rcof) was measured for Behring Coagulation Time using an automated coagulation analyzer with a commercial von Willebrand factor reagent kit purchased from Dade Behring OUBD2, Germany.

Table 1
Demographic data of studied patients.

Diagnosis	Number of patients	Age (years) Mean (range)	Sex	
			Male	Female
Dengue fever	21	10.0 (5-15)	12	9
DHF grade I	30	10.0 (4-16)	18	12
DHF grade II	33	11.0 (6-14)	21	12
DSS	10	10.0 (4-14)	4	6
OFI	7	12.0 (6-15)	3	4
Total	101	12.0 (4-16)	58	43

Statistical analysis

Chi-square and Fisher's exact tests were used for discrete data where appropriate. The Mann-Whitney *U* and Wilcoxon signed ranks tests were used for continuous data. A p-value < 0.05 was considered statistically significant.

RESULTS

The complete blood counts revealed patients with DF and OFI had mean hematocrits (Hct) significantly lower than those of patients with DHF. The mean Hct in patients with DHF was highest on the day of defervescence, then the Hct began to decline, as shown in Fig 1. The mean Hct was highest in patients with DSS. The mean incremental change in Hct in patients with DF was 6.0% and in patients with OFI was 8.0%, which were significantly lower than those patients with DHF (grade I 20.1%, grade II 21.1%, DSS 29.3%).

Patients with DF and DHF had mean platelet counts below 100,000/μl on D-2, D-1 and D0, then these began to rise on D+1 and D+2. Patients with DF had a mean platelet count higher than those with DHF but lower than those with OFI. Patients with DSS had the lowest mean platelet counts, ranging from 25,000-40,000/μl for D-1 to D+2, as shown in Fig 2. The platelet counts were normal at follow-up.

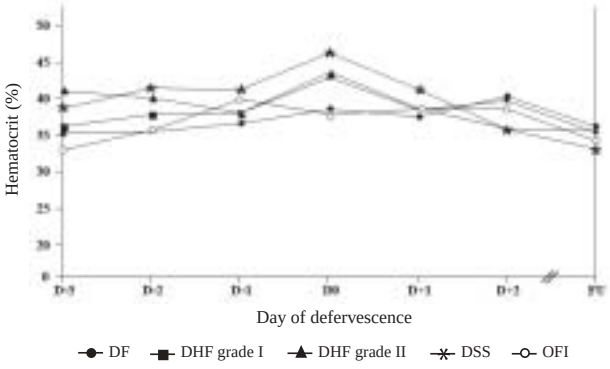

Fig 1–Mean hematocrits of patients with dengue fever (DF), dengue hemorrhagic fever (DHF) grade I, II, dengue shock syndrome (DSS) and other febrile illnesses (OFI) by day related to defervescence and at 2-4 weeks follow-up (FU).

The mean APTT, PT and TT values in patients with DF, DHF grades I and II and OFI were slightly longer than those at the 2-4 week follow-up visit, except for those with DSS, who had values significantly more prolonged for APTT, PT and TT on D-1, D0 and D+1 than at the 2-4 week follow-up visit, as shown in Fig 3. The mean levels for VWF:Ag and VWF:Rcof in patients with DF, DHF grades I and II, and

Table 2
Comparison of laboratory findings in dengue shock syndrome patients.

Variables	DSS/DHF			DSS/All febrile patients		
	Case (%)	RR	95% CI	Case (%)	RR	95% CI
Complete blood counts						
Rise in Hct >25%	7/73 (0.09)	7.7	2.2-26.5	7/101 (0.07)	11.5	3.3-40.1
Platelets <40,000 µl	8/73 (0.11)	4.8	1.1-21.3	8/101 (0.08)	8.2	1.9-36.7
Rise in Hct >25% and platelets <40,000 µl	6/73 (0.08)	6.9	2.3-21.1	6/101 (0.06)	10.2	3.3-31.2
Coagulation test						
APTT >44 sec	7/73 (0.09)	4.7	1.3-16.8	7/101 (0.07)	6.7	1.9-24.1
PT >14 sec	7/73 (0.09)	7.1	2.1-24.7	7/101 (0.07)	8.9	2.5-31.5
TT >16 sec	8/73 (0.11)	9.9	2.3-42.8	8/101 (0.08)	13.6	3.1-59.5
Prolonged APTT, PT and TT	6/73 (0.08)	10.7	3.7-30.6	6/101 (0.06)	15.3	5.3-44.4
Von Willebrand factor						
VWF:Ag >210%	9/73 (0.12)	10.9	1.5-81.7	9/101 (0.09)	15.6	2.1-118.1
VWF:Rcof >210%	9/73 (0.12)	7.0	0.9-52.6[a]	9/101 (0.09)	12.6	1.7-96.0
VWF:Ag & VWF:Rcof >210%	9/73 (0.12)	16.3	2.2-121.4	9/101 (0.09)	24.7	3.3-185.7

RR, relative risk; CI, confidence interval; DHF, dengue hemorrhagic fever grades I to IV; DSS, dengue shock syndrome; all febrile patients, patients with DF, DHF and other febrile illnesses
[a]Relative risk was not significant.

OFI were not significantly different during the course of the acute illness, but were significantly higher than those at the 2-4 week follow-up visit. Patients with DSS had significantly higher levels on D-1, D0, D+1 and D+2 as shown in Figs 4 and 5.

The assessment of relative risk to develop DSS included simple laboratory results of hematocrit, platelet count, coagulation tests and more sophisticated results of von Willebrand factor on D-1, D0 and D+1. The relative risk ranged from 4.8 to 16.3, as shown in Table 2. The relative risk dramatically increased (8.2-24.7) when all patients with acute febrile illness were included in the statistical analysis.

DISCUSSION

This study evaluated complete blood counts, coagulation tests and VWF during the febrile, toxic and convalescent stages of patients with DF, DHF and DSS. The categorization of blood samples related to defervescence was essential for including patients with the same pathophysiological stage in the same group since the febrile stage lasts 2-7 days.

The endothelial cell injury resulting in increasing vascular permeability and plasma leakage induces hemoconcentration in patients with DHF. A hematocrit rise of more than 20% from baseline for age is one of the laboratory diagnostic criteria for DHF. The hematocrit rise was more prominent in DSS one day before defervescence, the day of defervescence and one day after defervescence. In our study, the incremental increase in hemat-

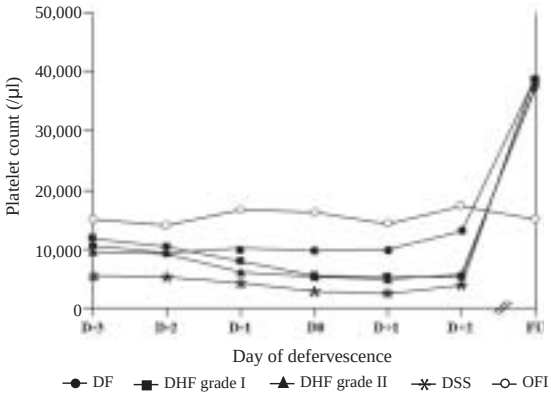

Fig 2–Mean platelet counts in patients with dengue fever (DF), dengue hemorrhagic fever (DHF) grade I, II, dengue shock syndrome (DSS) and other febrile illnesses (OFI) by day related to defervescence and at 2-4 weeks follow-up (FU).

ocrit in patients with DSS was 29.3%. The platelet count reached its lowest level of 25,000/µl in patients with DSS. Therefore, an increase in Hct of >25% and a platelet count less than 40,000/µl may be predictors for DSS during the febrile stage, with relative risks of 7.7 (95% CI 2.2-26.5) and 4.8 (95% CI 1.1-21.3), respectively. The combination gave a relative risk of 6.9 (95% CI 2.3-21.1).

With endothelial cell injury, there is a release of procoagulant components, activation of the coagulation cascade with thrombin generation, increased antifibrinolytic factors and consumption of natural anticoagulants. These consequences will lead to the prolongation of APTT, PT and TT (Wills *et al*, 2002). Prolongation of APTT, PT and TT are predictors for DSS during the febrile stage with a relative risk of 4.7-10.7.

Endothelial cell injury is demonstrated by elevation of soluble thrombomodulin (sTM) (Buttep *et al*, 2006; Sosothikul *et al*, 2007). The elevated sTM

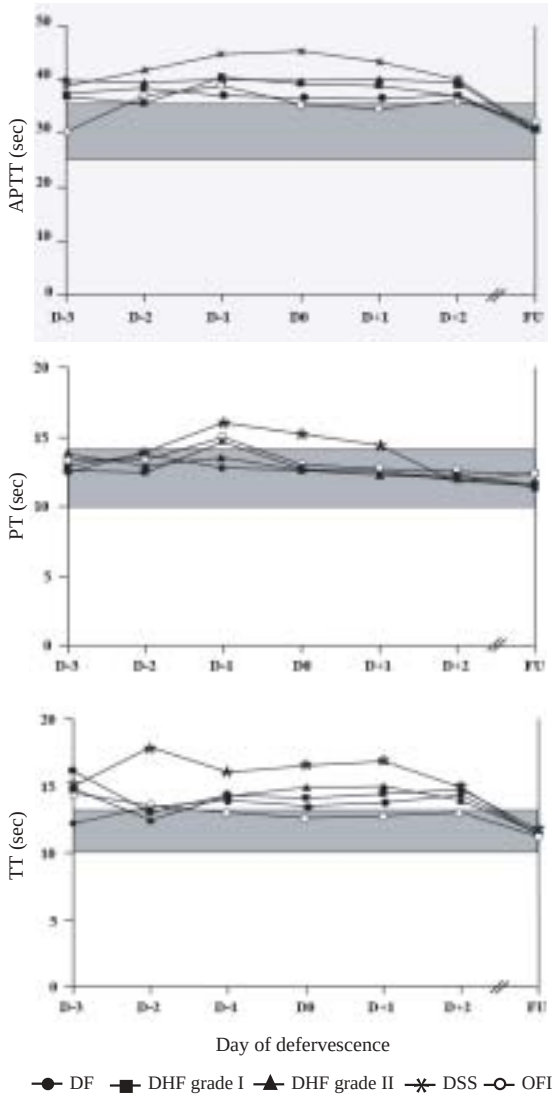

Fig 3–Mean values for activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) in patients with dengue fever (DF), dengue hemorrhagic fever (DHF) grade I, grade II, dengue shock syndrome (DSS) and other febrile illnesses (OFI) by day related to defervescence and at 2-4 weeks follow-up(FU). The normal range of APTT was 26-36 seconds, PT 10-14 seconds and TT 10-13 seconds represented by shaded area.

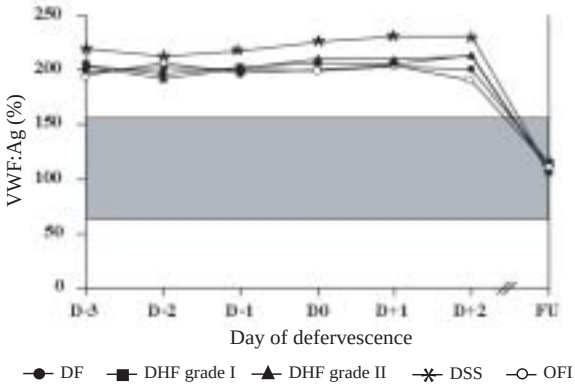

Fig 4–Mean levels of von Willebrand factor activity (VWF:Ag) in patients with dengue fever (DF), dengue hemorrhagic fever (DHF) grade I, II, dengue shock syndrome (DSS) and other febrile illnesses (OFI) by day related to defervescence and at 2-4 weeks follow-up (FU). The normal range of VWF:Ag was 60-156% represented by the shaded area.

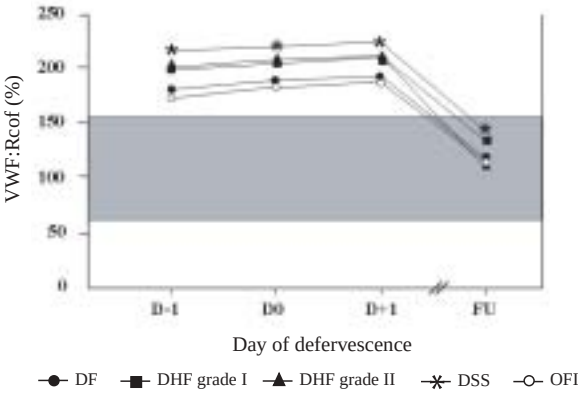

Fig 5–Mean levels of ristocetin cofactor activity (VWF:Rcof) in patients with dengue fever (DF), dengue hemorrhagic fever (DHF) grade I, II, dengue shock syndrome (DSS) and other febrile illnesses (OFI) by day related to defervescence and at 2-4 weeks follow-up (FU). The normal range of VWF:Rcof was 58-172%, represented by the shaded area.

to >10 ng/ml during the febrile stage is a predictor for DSS. However, the assay of sTM is not routinely available. VWF, another marker of endothelial cell injury, was studied. Our results are in agreement with a previous study by Basuki (2003). A high level of VWF is followed by a low platelet count, as a result of platelet aggregation due to the interaction between platelets and dengue virus infected endothelial cells (Funahara *et al*, 1987) or an immune complex process (Srichaikul and Nimmanitya, 2000). Levels of VWF:Ag and VWF:Rcof >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 0.9-52.6), respectively. The combination provided a relative risk of 16.3 (95% CI 2.2-121.4). VWF assay is more commonly available in the hospital since the prevalence of von Willebrand's disease occurs in 0.1 to 1.3% of the population.

A limitation of this study was the lack of VWF multimer analysis. However, a small number of patients with dengue infection have been previously studied (Sosothikul *et al*, 2007). A shift from high-molecular-weight to lower-molecular weight multimer or supranormal multimers were detected. As a result of endothelial cell injury by dengue virus, an elevated VWF may be found.

Laboratory tests reveal a high hematocrit, a low platelet count and prolongation of coagulation tests. Significantly abnormal laboratory findings are seen in DHF grades III and IV or DSS. These laboratory tests available in hospitals may be helpful as predictors for DSS during the febrile stage. The more sophisticate assays of VWF:Ag and VWF:Rcof are also predictors of DSS during the febrile stage. Early diagnosis should be helpful for optimal management to achieve a favorable outcome.

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