Von Willebrand Factor and Thrombocytopenia in Patients with Dengue Haemorrhagic Fever

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Abstract: The clinical manifestation of DHF includes mild or marked febrile syndromes with abrupt onset of headache, pain behind the eyes, muscle and bone pain, nausea, vomiting and rash. The pathophysiology of DHF in human is complex involving endothelial cell activation and the impaired endothelial barrier leading to plasma leakage triggering the activation of the haemostatic system. The objective of this study was to determine Von Willebrand Factor levels and platelet effects on the vascular endothelium in patients with dengue haemorrhagic fever. Fifty patients (males 34, females 16), were recruited, Grade 1 (n=41), Grade 2 (n=6), Grade 3 (n=2) and Grade 4 (n=1) DHF. Twenty patients at seventeen years and below (Grade 1) were compared with 21 adults (Grade 1) showed no statistical differences and grouped together for analysis. Blood sampling to determine haemoglobin, haematocrit, platelets and Von Willebrand Factor (VWF) at the febrile, defervescence and convalescent phases was performed. The patients were aged between 4 and 54 years old with Grade 2 patients being significantly older (P=0.03) than Grade 1 DHF. Comparisons between Grades 1 and 2 DHF showed no statistical differences in the parameters studied. Thrombocytopenia, elevated VWF levels from normal was evident in all phases suggesting endothelial activation. In Grades 3 and 4 DHF, thrombocytopenia, elevated VWF was also seen. No mortality was observed in the study. Endothelial activation was evident in dengue haemorrhage fever.

1 INTRODUCTION

Dengue fever is the most serious consequence of mosquito-borne infection worldwide. There are more than 2.5 billion persons at risk of infection and occur mainly in the sub-tropical regions of Asia, Africa and America (WHO 2008), the attacks have shifted mainly to adults (WHO 2011). In Indonesia, the overall incidence increased significantly from 0.05/100,000 in 1968 to 35-40/100,000 in 2013 (Karyanti et al 2014).

The actual numbers of dengue cases are underreported or misclassified (WHO 2017). Clinical manifestations of dengue include mild or marked febrile syndromes of abrupt onset with headache, pain behind the eyes muscle and bone pain, nausea, vomiting and rash which is life threatening. There is no specific treatment for dengue fever but maintaining patients' body fluid volume is critical. Dengue as defined by WHO (2009) as dengue with and without warning signs of plasma leakage and defined into four grades (Grades 1 to 4) The endothelium plays an important regulatory role in the circulation as a physical barrier and involved in the control of thrombosis and thrombolysis, vascular tone and growth of blood vessels (Verhamme & Hoylaerts 2006). Endothelial activation may be responsible for plasma leakage and shock (De Castro et al 2007) and endothelial injury is associated with elevated Von Willebrand Factor (VWF) (Connolly 1991, Mohle et al 1997). Thrombocytopenia is commonly observed in both mild and severe dengue syndrome and associated with clinical outcome (WHO 2009, Mourao et al 2007, Schexneiden & Reely 2005, Honda et al 2008). The level of platelet count correlates with severity of DHF and high haematocrit with marked thrombocytopenia support the diagnosis of dengue shock syndrome (DSS) (WHO 2011) and has been considered as an important factor responsible for bleeding events in DHF (Diaz-Quijano et al 2010). Platelet activation is significantly increased in

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dengue-patients especially with thrombocytopenia which exhibited signs of apoptosis pathway activation (Hottz et al 2013)

The objective of the study was to determine Von Willlebrand Factor levels and platelet effects on the vascular endothelium in patients with dengue haemorrhagic fever.

2 MATERIALS AND METHODS

The study received ethical approval from the Health Research Ethical Committee (No.438/TGL/KEPK FK USU-RSUP-HAM/2018), Faculty of Medicine, University of North Sumatera, Jakarta, Indonesia. The study was conducted at the Murni Teguh Memorial Hospital, Medan Indonesia. Subjects. Fifty patients (males 34, females 16) admitted to the hospital with fever were recruited and diagnosed according to WHO protocol (2009) to have Grade 1 (n=41), Grade 2 (n=6), Grade 3 (n=2) and Grade 4 (n=1) DHF. The Inclusion criteria: patients who met WHO criteria for dengue fever and willing to take part in the study and had one or more dengue serology positive for either IgM/IgG or NS1, Exclusion criteria: patients with other infections and systemic diseases and not willing to take part in the study. Normal Controls. Fifteen normal subjects (males n=14, female n=1) who are normotensive and not taken any medication, no history of health issues was recruited to serve as normal controls for the DHF study.

Blood Sampling and Laboratory Investigation. EDTA blood was used to determine Haemoglobin (Hb), Haematocrit (Hct) and platelets in the Siemens high volume haematological analyser (Advia 2120/1); Citrated plasma for Elisa analysis of Von Willebrand Factor (VWF) (USCN Life Sciences, Wuhan, China). Statistical Analysis. The Statistical Package for Social Sciences (SPSS 22 IBM Corp) was used to perform statistical analysis. The independent t-test for differences between groups at different phases of febrile, defervescence and convalescence and Analysis of Variance (ANOVA) was performed. A *P*-value of <0.05 was considered statistically significant.

3 RESULTS

Comparison of parameters (mean ± SD) studied in dengue haemorrhagic fever at febrile phase (Grade 1) between cohorts at age seventeen and below and above 17 years.

There were twenty cohorts (males n=13, females n= 7) at seventeen years and below and twenty-one cohorts (males n=14, females n=7) above 17 years old. Except for the significance in age (*P*=<0.001) there were no statistical significance in haemoglobin (Hb), haematocrit (Hct), platelets and VWF studied (Table 1). They were therefore combined (Grade 1) for further statistical analysis.

Table 1. Comparison of parameters (mean and SD) in dengue haemorrhage fever at febrile stage (Grade 1) between cohorts at age seventeen years and below and above.

	≤ 17 years	>17 years	*P*
N (male/female)	20 (13/7)	21 (14/7)	
Age years	10.9 (3.9)	29.8 (9.0)	**<0.001**
Range years	4 – 17	18 - 54	
Haemoglobin g//L	13.1 (1.3)	14.1 (2.1)	0.06
Haematocrit %	39.0 (4.4)	42.2 (6.2)	0.07
Platelets x10^9/L	111.7 (82.5)	73.1 (50.7)	0.05
VWF ng/mL	108.4 (29.0)	111.0 (30.7)	0.78

Normal values for VWF. Of the fifteen normal controls studied, the level of VWF were non-detectable in fourteen controls and one at 83.1 ng/mL (sensitivity of assay <0.94 ng/mL) mean 11.9 ± 31.4 ng/mL. The VWF levels of DHF at various phases are shown in Figure 1.

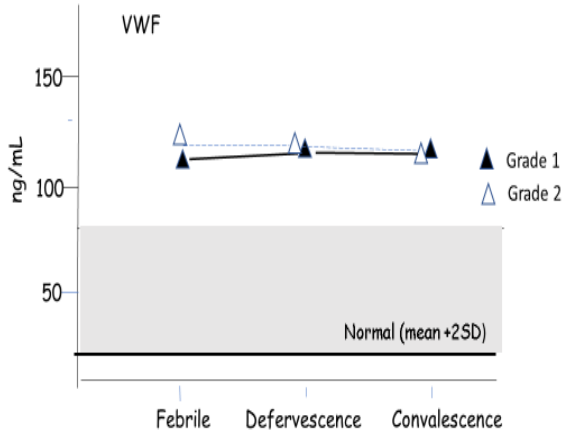

Figure 1.VWF in Grades 1 and 2 DHF at febrile, defervescence and conva;escence phases compared to normal controls (mean +2SD)

Dengue haemorrhagic fever: Comparison of Hb, Hct, platelets and VWF (mean ± SD) between Grades 1 and 2 at febrile, defervescence and convalescence phases and comparison to febrile phase. Thrombocytopenia (platelets <100 x10^9/L) was evident in both Grades 1 and 2 DHF at febrile, defervescence and convalescence phases with normal haematocrit and haemoglobin levels seen. Elevated VWF levels are seen but they were not significantly different between Grades 1 and 2. There were also no significant differences between the two groups of cohorts in the parameters studied. Similarly, no differences when compared to febrile phase (Table 2). Analysis of Variance (ANOVA) One-way ANOVA analysis for Hb, Hct, platelets and VWF. in either Grades 1 or 2 between different DHF phases showed no significant differences (not shown).

Table 2. Dengue haemorrhage fever: Comparison of parameters studied (mean and SD) between Grades 1 & 2 at febrile, defervescence and convalescence phases and compared to febrile phase.

	Grade 1	Grade1 – *P* vs *Febrile phase*	Grade 2	Grade 2- *P* vs *Febrile phase* (Gr1 vs Gr 2)	*P*
Febrile					
N (male/female)	41 (28/13)		6 (4/2)		**0.03**
Age years	20.6 (11.8)		30.8 (8.7)		0.46
Haemoglobin g//L	13.6 (1.8)		14.3 (2.1)		0.45
Haematocrit %	40.6 (5.6)		42.8 (6.1)		0.4
Platelets x10^9/L	94.8 (70.9)		70.5 (60.4)		0
VWF ng/mL	109.7 (29.6)		120.0 (29.1)		0.4
Defervescence					
Haemoglobin g//L	13.6 (1.7)	*0.93*	14.2 (2.0)	*0.91*	0.49
Haematocrit %	41.2 (6.6)	*0.64*	41.4 (8.3)	*0.75*	0.96
Platelets x10^9/L	78.4 (51.3)	*0.23*	66.5 (413)	*0.90*	0.54
VWF ng/mL	114.0 (24.9)	*0.48*	112.3 (26.1)	*0.37*	0.89
Convalescence					
Haemoglobin g//L	13.2 (1.7)	*0.35*	13.6 (1.8)	*0.65*	0.63
Haematocrit %	40.1 (5.3)	*0.68*	40.4 (5.6)	*0.50*	0.91
Platelets x10^9/L	101.8 (58.8)	*0.63*	97.0 (55.3)	*0.45*	0.85
VWF ng/mL	113.7 (24.8)	*0.51*	114.3 (13.0)	*0.68*	0.9

4 DISCUSSION

Dengue fever is the most serious consequence of mosquito-borne infection worldwide. Endothelial damage may also be caused by the virus itself. Thrombocytopenia is responsible for bleeding events in DHF (Diaz-Quijano et al 2010, Orsi et al 2013) but many factors can contribute to the onset of thrombocytopenia from a reactive immune response against platelets and decreased platelet production (Lin et al 2001, De-Castro et al 2007, Saito et al 2004), platelet activation and apoptosis (Hottz et al 2013). Bleeding manifestations and plasma leakage are complications seen in dengue and bleeding manifestation in adults may occur in the absence of plasma leakage (Wichmann et al 2004). In our study, thrombocytopenia was observed in all phases of DHF even though in convalescence phase the mean platelet numbers were higher than febrile and defervescence phases they did not reach statistical significance. Normal haemoglobin level and no haemoconcentration was observed indicating no bleeding episodes occurred despite the thrombocytopenia. Endothelial activation as evident by elevated VWF level suggest the possibility of plasma leakage in Grades 1 and 2 DHF. Other evidence like activation of the coagulation system and thrombocytopenia with haemoconcentration will support the cause of dengue shock syndrome (WHO 2011) and bleeding episodes (Diaz-Quijano et al 2010) which was not seen in our cohorts. Identifying the mechanisms affecting DHF would improve diagnosis and management therapy. No mortality was recorded in our study.

5 CONCLUSION

Thrombocytopenia and elevated VWF are seen in dengue haemorrhagic fever despite normal haemoglobin and haematocrit levels suggesting endothelial activation.in dengue haemorrhagic fever.

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CONFLICT OF INTEREST

The authors declared that they have no Conflict of Interest.

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