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Original Article

Effect of dengue hemorrhagic fever on thrombomodulin level

Hendra Widjaja, Max F. J. Mantik

Abstract

Background Thrombocyte and endothelial cells play an important role in dengue hemorrhagic fever pathogenesis. Thrombomodulin is a part of glycoprotein membrane in endothelial cells. Therefore, thrombomodulin level will increase if endothelial cells disruption occurs.

Objective To acknowledge the correlation between the degree of dengue hemorrhagic fever and thrombomodulin level.

Methods This was a cross-sectional study. Subjects were hospitalized pediatric patients with age ranging from one to 13 year old in pediatric ward at Prof. Dr. R.D. Kandou Hospital, Manado, who had fever. Three milliliters of blood were taken from vein, and were divided for two tests which were routine blood analysis and thrombomodulin analysis. Different data resulted from the dengue hemorrhagic fever group were processed, and analyzed statistically using F Test and LSD (least significant difference) test. The relation between dengue hemorrhagic fever and thrombomodulin was analyzed with Spearman correlation coefficient.

Results There was a significant result in the difference of thrombomodulin level on four dengue hemorrhagic fever groups which were classified according to the severity of dengue hemorrhagic fever. There was a very significant positive correlation between the severity of dengue hemorrhagic fever and thrombomodulin level in detecting endothelial cells impairment.

Conclusion Thrombomodulin level can be used as a marker to detect endothelial cells impairment in dengue hemorrhagic fever. Higher grade of dengue hemorrhagic fever will have higher thrombomodulin level. **[Paediatr Indones. 2009;49:259-63]**.

Keywords: *dengue hemorrhagic fever, thrombomodulin, endothelial cells*

Infection of dengue virus in human may manifest as dengue fever or dengue hemorrhagic fever. Dengue hemorrhagic fever (DHF) has several clinical features such as fever, rash, hemorrhage, liver enlargement, ascites, and encephalopathy.[1-6] DHF grade III and IV are the severe manifestation of this disease. Until now, there are several theories regarding the pathogenesis of DHF, i.e. viral virulence, immunopathology, antigen-antibody complex, endothelial cells and thrombocyte, also apoptosis.[1-10]

Thrombocyte and endothelial cells are predicted to have an important role in DHF pathogenesis. Manifestations such as thrombocytopenia and increasing capillary permeability in DHF suggest that there is impairment in integrity of endothelial cells. These two components have been known as having united function in maintaining homeostasis. If one of the components is damaged, it will affect the other. Thrombocyte has another function as secretory cell which has granules containing various mediators. Endothelial cells have numerous receptors, besides producing vasoactive products such as prostacyclin,

From the Department of Child Health, Medical School, Sam Ratulangi University, Prof. R. D. Kandou General Hospital, Manado, Indonesia.

Reprint request to: Hendra Widjaja, MD, Department of Child Health, Medical School, Sam Ratulangi University, Prof. R. D. Kandou General Hospital, Jl. Raya Tanawangko, Manado 95115, Indonesia. Telp. +62-431-821652. Fax. +62-431-859091. E-mail: *hw_230670@yahoo.co.id, h3nw1@doctor.com*

platelet activating factor (PAF), plasminogen factor, interleukin-1, and thrombomodulin. Endothelial cells disruption results in thrombocyte aggregation and activates coagulation.[1,3,5-8, 11-18]

Thrombomodulin concentration on endothelial activation is related to shock, while PAI-1 concentration is related to massive bleeding. Dengue virus will immediately activate primary pathogenesis, direct degradation of fibrinogen; or secondary pathogenesis, by activating procoagulant hemostatis mechanism. Endothelial protein C receptor (EPCR) has an important role in controlling the activity of C protein. By adding monoclonal antibody, it will block the C protein binding in EPR which will result in higher risk of death.[17-22]

Methods

This research used analytical observation method with cross-sectional approach on all DHF cases in pediatric ward (*Irina E*), Prof. Dr. R.D. Kandou Hospital, Manado, from June until November 2008. Subjects were all hospitalized pediatric patient with age ranging from one to 13 years old in pediatric ward (*Irina E*) Prof. Dr. R.D. Kandou Hospital, Manado, who had fever. The data were obtained from anamnesis, physical examination, routine blood analysis, and thrombomodulin examination using ELISA method.

The inclusion criteria were children aged one to 13 years old, who were diagnosed with DHF based on 1997 WHO criteria, and whose parents agreed to participate in this study by filling the research form. Exclusion criteria were children with malaria, disseminated intravascular coagulation, diabetes mellitus, Kawasaki disease, acute glomerulonephritis, and systemic lupus erythematosus.

There were two types of variable in this research, i.e. non-dependent variable (the severity of DHF) and dependent variable (the level of thrombomodulin). Descriptive analysis and Spearman's Rho correlation analysis were used in this research. All of the obtained data were processed by using SPSS version 15. Data results had a 95% confidence interval (CI) and $P<0.0001$.

Results

Subjects were 47 hospitalized patients with DHF, consisted of 14 subjects in intensive care room (ICU) and 33 subjects in pediatric ward (*Irina E*), Prof. Dr. R.D. Kandou Hospital, Manado. From 47 subjects, 25 subjects were diagnosed with DHF grade I, eight with DHF grade II, eight with DHF grade III, and six with DHF grade IV. On daily observation, one patient in DHF grade III group had melena and one patient with DHF group IV died.

The lowest thrombomodulin level was 3.04 ng/ml, while the highest was 22.61 ng/ml. The mean value of thrombomodulin level was 8.4323 ng/ml. The level of thrombomodulin based on the severity of DHF is shown in **Table 1.**

The mean level of thrombomodulin in four groups (from grade I to IV) showed that there was a vast endothelial disruption. Higher severity of DHF resulted in higher level of thrombomodulin. Thrombomodulin level is being used as a probe to detect endothelial cells disruption, which can be seen on **Figure 1.**

The difference of thrombomodulin level in the four groups (grade I to IV) had a significant difference with F test ($P<0.0001$), as shown in **Table 2**.

The result of thrombomodulin level was used to detect endothelial cells disruption, as shown in **Table 3.**

The correlation between thrombomodulin level and severity of DHF was analyzed using Spearman's Rho coefficient analysis. Based on this analysis, the correlation coefficients were $r_s=0.755$, $P<0.0001$.

From these results, there was a very significant positive correlation between the increasing severity

Table I. Trombomodulin level in DHF patient

	N	Mean	SD	95% for CI Mean		Lowest level (min)	Highest level (max)
				LB	UB		
DHF I	25	6.0092	2.15445	5.2099	6.9885	3.04	8.93
DHF II	8	9.5513	0.95511	8.7528	10.3497	7.29	10.42
DHF III	8	10.1925	1.82806	8.6642	11.7208	7.91	12.61
DHF IV	6	14.3150	5.80181	8.2264	20.4036	6.86	22.61
Total	47	8.4323	3.86738	7.2968	9.5678	3.04	22.61

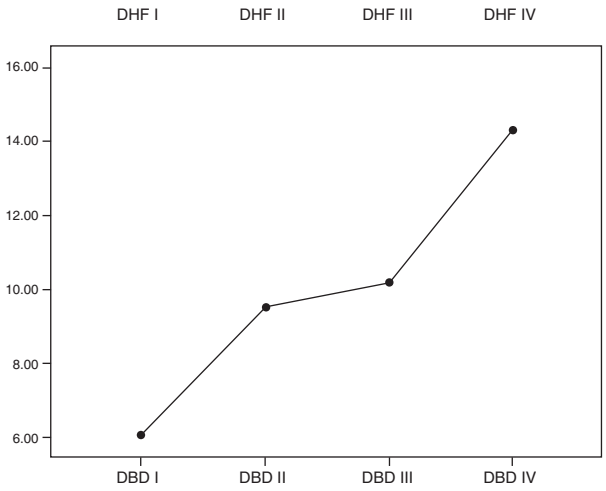

Figure 1. Thrombomodulin level on DHF

Table 2. The ANOVA Using F Test

	db	Sum of Squares	Variance	F	P
Between Groups	3	378.5	126.2	17.531	< 0.0001
Within Group	43	309.5	7.2		
Total	46	688.0	3.86738		

Table 3. Least Significant Difference test result

Control Group DHF Grade		Difference	p
I	II	3.45	0.003
	III	4.09	0.001
	IV	8.22	<0.0001
II	III	0.64	0.635
	IV	4.76	0.002
III	IV	4.12	0.007

of DHF and thrombomodulin level in detecting endothelial cells disruption. This correlation is shown by graphic in **Figure 2.**

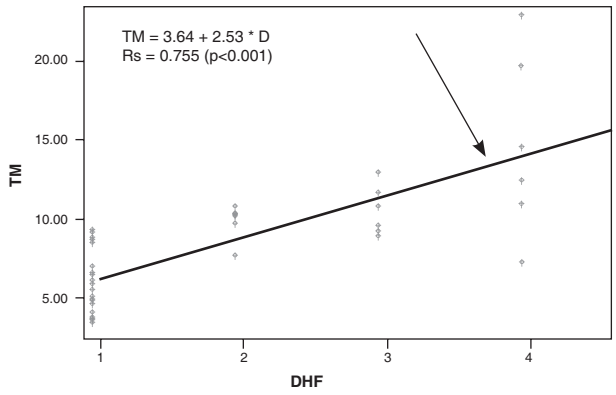

Figure 2. The correlation between thrombomodulin level and DHF severity

Discussion

In this study, we tried to find the correlation between thrombomodulin level and severity of DHF. Butthep et al[23] performed a study on thrombomodulin level by using ELISA method with Diaclone brand. The values of thrombomodulin were 6.55 (SD 1.7) in DHF grade I, 8.27 (SD 4.7) in DHF grade II, and 10.89 in dengue shock syndrome. A study by Wills et al[24], in Vietnam, found that on all cases of dengue shock syndrome, the lowest level of thrombomodulin was 2.8 ng/ml, while the highest was 17.2 ng/ml, with median value of 9 ng/ml.

In this study, the mean level of thrombomodulin in the four groups from grade I to IV showed that there was a vast endothelial cells disruption. Higher grade of DHF had higher level of thrombomodulin. The difference of thrombomodulin level from the four groups had a significant different with F test ($P<0.0001$).

Butthep et al[23] showed that the value of thrombomodulin on dengue shock syndrome increased from day –3 until day +2, and there was a significant difference ($P<0.005$) with Wilcoxon test. [23]

The mean value of thrombomodulin level to detect endothelial cells impairment ($P<0.0001$) in DHF grade I compared with DHF grade II was 3.45, compared with DHF grade III was 4.09, and compared with DHF grade IV was 8.22. Those values had a significant difference ($P<0.0001$) using LSD test analysis. While, comparison between DHF grade II with grade III DHF was 0.64, and there was a significant difference ($P<0.0001$) by using T test. The comparison between DHF grade II and grade IV was 4.76, and there was also a significant difference ($P<0.0001$) by using T test. The comparison between DHF grade III with grade IV was 4.12, and there was also a significant difference ($P<0.0001$) by using T test.

The level of thrombomodulin will rise on dengue shock syndrome as reported by Butthep et al.[23] They found that there was increasing level of thrombomodulin 2-3 days before fever subsided, that it could be use as a sign for endothelial cells impairment led to plasma leakage.[23]

Based on WHO classification, DHF is classified into four grades. Higher grade of DHF results in more vast leakage of plasma, as seen in higher

level of thrombomodulin level. The correlation between thrombomodulin level and endothelial cells impairment was analyzed by using Spearman's Rho test, and the result was $r_s = 0.755$ with $P < 0.001$.

This study also found significant positive correlation between increasing severity of DHF and thrombomodulin level in detecting endothelial cells disruption.

Endothelial cells have an important role in DHF pathogenesis. Increasing capillary permeability in DHF suggests that there is impairment in integrity of the endothelial cells. This component has been known for a long time for its function in maintaining homeostasis. Endothelial cells have many receptors, besides producing vasoactive products such as prostacyclin, platelet activating factor (PAF), plasminogen factor, interleukin-1 and thrombomodulin. Disruption in endothelial cells will release vasoactive agents.[1,3,5-8, 11-18]

In conclusion, thrombomodulin level can be used as a marker to detect endothelial cells impairment in DHF. Higher grade of DHF will have higher thrombomodulin level. High thrombomodulin level in DHF grade IV suggests that there is vast endothelial cells impairment with poor result. Therefore, thrombomodulin level can be considered as an early marker to detect endothelial cells impairment in DHF patients. Furthermore, future research on vascular endothelial cells related to other condition in DHF patient is needed.

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