The Correlation Between Thrombopoietin and Platelet Count in Adult Dengue Viral Infection Patients

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ABSTRACT

Aim: to investigate alteration in humoral regulation during the course of dengue viral infection

Methods: a prospective analytic study had been conducted involving 40 subjects with dengue viral infection. Subjects were recruited according to consecutive non-probability sampling. Subjects were categorized according to days of illness, platelet counts and serum thrombopoietin (TPO) levels. The plasma TPO levels examinations were done once daily until the platelet counts reached more than 100,000/mm3.

Results : statistical analysis showed the mean serum TPO levels were increased during thrombocytopenia phase of the disease, and differ significantly from the convalescent phase (mean value 428pg/ml vs 220.1 pg/ml, p = 0.00). There was also a statistically significant inverse correlation between serum TPO levels and platelet counts (p = 0.00).

Conclusion: TPO levels were significantly increased in adult patients with dengue infection in which platelets in circulation were markedly reduced, and the TPO levels were inversely related to the platelet counts.

Key words: TPO levels, platelet count, dengue fever infection.

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INTRODUCTION

Dengue hemorrhagic fever (DHF) defined as an acute fever caused by dengue viral infection. DHF has significant morbidity and mortality in many southeast Asian countries. The prominent features of DHF were thrombocytopenia and plasma leakage. These conditions were the key diagnostic tools to differentiate dengue fever (DF) and dengue hemorrhagic fever (DHF).

The cause of thrombocytopenia in DHF is still controversial. Some experts presumed it might be due to derangements in thrombopoiesis and increased platelets destruction in circulation. Other studies also found the presence of platelet dysfunction. [2-4] Bone marrow has an important role in inducing thrombocytopenia. Suppression on the hematopoietic system occurred during dengue viral infection. Platelet production originating from megacaryocytes in bone marrow was a steady ongoing process. From published clinical studies, TPO has been known as the main regulator substance of megacaryocytes and thrombopoiesis to maintain adequate platelet counts in circulation. Significant increased of TPO levels is found in patient with low circulating level of megacaryocytes and platelets. [5,6] Based on these results, it was considered important to know the profile of TPO in dengue viral infection. Aim of the study was to investigate the alteration in humoral regulation of thrombopoiesis during course of dengue viral infection in adults and find the correlation between platelet counts and plasma TPO levels in dengue viral infection.

METHODS

This research was a prospective analytic observational study, conducted from January to June 2000 in Internal Medicine ward in Medical Faculty University of Indonesia, Dr. Cipto Mangunkusumo National General Hospital, Jakarta and Persahabatan hospital.

Subjects were recruited based on consecutive non

probability sampling methods. Patients with dengue viral infection who met the inclusion criteria and did not have any exclusion criteria were recruited in this study. The inclusion criteria were male or female patients whose age between 14 and 60 years old. Patients were diagnosed DHF and DF based on WHO diagnostic criteria 1997 with platelet counts below 100,000/mm³. The exclusion criteria were patients with abnormal liver function, renal insufficiency, tuberculous infection, pregnancy, splenomegaly, and mixed infection proven by clinical and laboratory examination. The independent variables that would be observed were etiologic virus of infection, and platelet counts. The dependent variable was plasma TPO levels.

Each patient who met the inclusion criteria underwent anamnesis, physical and laboratory examination to find the presence of exclusion criteria previously mentioned. Subjects were included in analytical observational study. During hospitalization subjects were checked for Hb levels, hematocryt, platelet counts and TPO levels every 24 hours until the platelet counts reached 100,000/mm3. Duration of fever was calculated from the first day which the fever occurred based on anamnesis. Early phase of dengue infection began at the first occurrence of fever until 7th day of illness. Convalescent phase of dengue infection was after 7th day of illness. From each patient 5 ml venous blood sample was taken for TPO levels examination using ELISA methods. Normal value was considered below 50 pg/ml.

Minimal sample size required was 21 subjects based on calculation correlation coefficient test with power of 90 % and 0.05 significant level.

Descriptive data would be presented in narration, table and figures. Univariat analysis to obtain mean value, median and standard deviation; bivariat analysis to calculate the relation between dependent variables and independent variables. Comparison of mean was done using t-test for continuous data and nonparametric test (Mann-Whitney) for abnormal data distribution. Spearman correlation test to calculate any relation between two variables. Data was processed using personal computer and SPSS 10 software program.

RESULTS

There were 40 subjects recruited in this prospective analysis study with mean of age 22.4 years old (SD 6 years). There were more female subjects (52.5 %) than male ones. DHF group consist of 60 % of cases. Most of them were with secondary infection (83.3%) and only

16.7 % of cases with primary infection. Subject characteristics are shown in table 1.

Tabel 1. Subject Characteristics

Characteristics	Number of pts	Percentage
Age group (years)		
- 14 – 19	11	27,5%
- 20 – 25	19	47,5%
- 26 – 31	7	17,5%
- > 31	3	7,5%
mean 22.4 (SD 6)		
Sex		
- male	19	47,5%
- female	21	52,5%
Number of pts		
- DD	16	40%
- DBD	24	60%

In kinetics description of DHF infection, mean peripheral platelet counts had already decreased on 3rd day of illness with mean platelet counts of 42.333 (SD 30.350), and remain low until 7th day with mean platelet counts of 77.692 (SD 52.431). It reached normal level on 8th day of illness with mean platelet counts of 104.871 (SD 53.695). From statistical analysis, the platelet counts between DHF and DF infection showed only significant difference on 4th day of illness (p = 0.02). Profile of platelet counts was shown in figure 1.
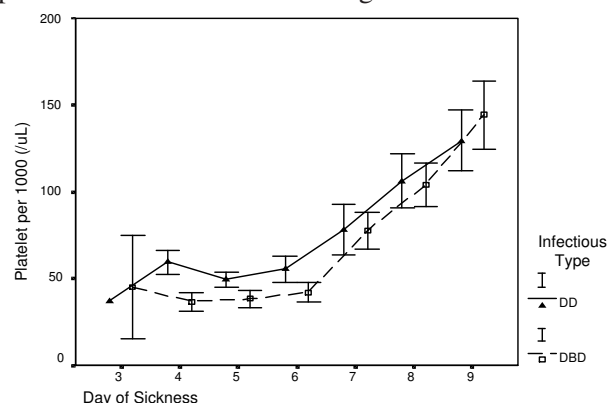

Figure 1. Profile of Platelet Counts on 3rd Day Until 9th Day of Observation

Profile of TPO Activity in Early and Late Phase of Dengue Infection

TPO plasma levels in group of 3rd day until 7th day of illness was ranged from 128.7 to 1,500 pg/ml with mean value of 428 pg/ml (median value of 383.2 pg/ml). In group of 8th day and 9th day of illness, the TPO plasma levels ranged between 0 and 1.543 pg/ml with mean value of 220.1 pg/ml (median value 144,7 pg/ml). From statistical analysis it was shown that mean TPO level in group of 3rd day to 7th day of illness was significantly different with mean TPO level of 8th day and 9th day of illness (p = 0.00).

Profile of Platelet Count in Early and Late Phase of Dengue Infection

The platelet counts of 3rd day until 7th day of illness ranged between 17.500 and 120.500/mm3 with mean value of 55,701/mm3. In group of 8th day and 9th day of illness this ranged between 71,500 and 260,000/mm3 with mean value of 133,750/mm3 (median value of 129,500/mm3). Statistical analysis showed significant difference of platelet counts between these two groups.

Profile of TPO levels and platelet counts changes in dengue infection is shown in figure 2.
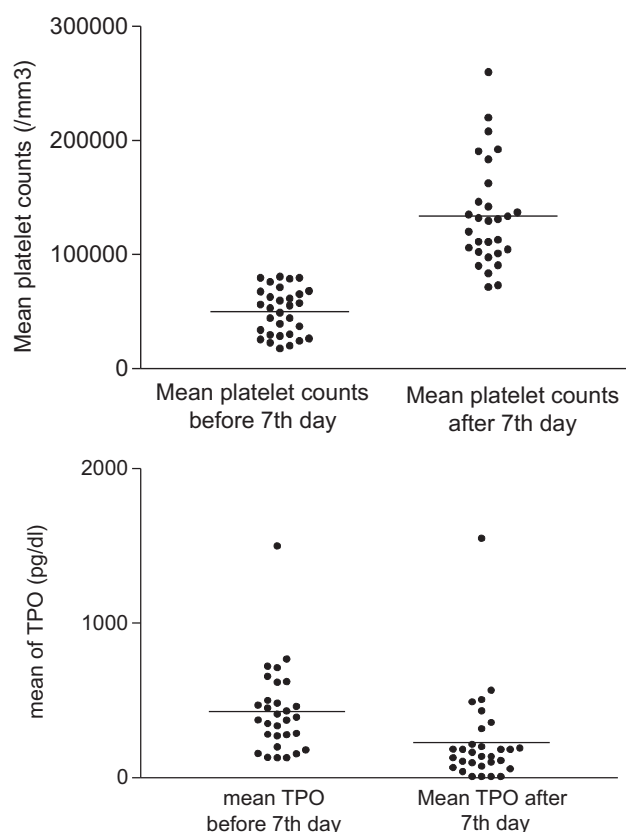

Figure 2. Profile of TPO Activity and Dynamics of Platelet Counts in Dengue Infection

Profile of TPO Plasma Levels in Accordance to Platelet Counts

Plasma TPO levels at platelet counts under 100,000/ul ranged between 60 and 1,187 pg/ml with mean value of 395 pg/ml (median value of 348.3 pg/ml). On the other hand, TPO plasma levels at platelet counts above 100,000/ul was ranged between 0 and 530 pg/ml with mean value of 153.6 pg/ml (median value of 109.8 pg/ml). Statistical analysis revealed significant different of mean TPO levels between these two groups (p=0.00).

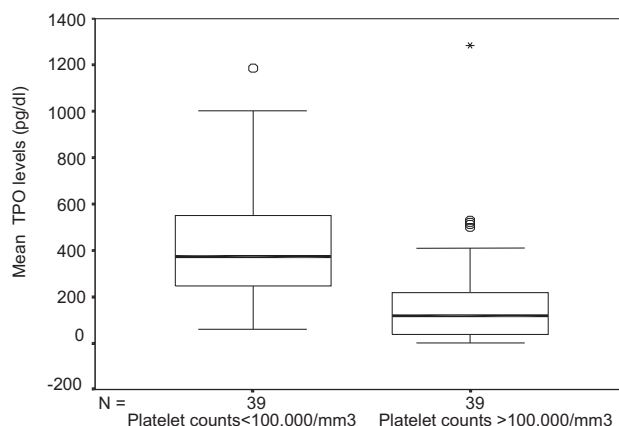Profile of mean TPO levels is shown in figure 3.

Figure 3. Profile of Mean Trombopoietin Level in Accordance to Platelet Counts. Mean TPO Levels at Platelet Counts Below <100.000/mm3 were Decreased Compared At Time When Platelet Counts Reached 100.000/mm3. Diagrams Showed Mean Value with Sd, $P = 0,00$ (Uji Wilcoxon)

Profile of TPO Levels in The Clinical Course of DHF and DF Infection

TPO plasma levels increased on 3rd day and reach maximum on 4th day of illness. It started to decrease after 7th day of illness. Profile of TPO levels is shown in figure 4. Statistical analysis found abnormal data distribution of TPO mean value. TPO plasma levels in DHF group were not different significantly with ones in DF group. Statistical analysis of mean TPO plasma levels is shown in table 2.

Correlation Between Platelet Counts and TPO Plasma Levels in Dengue Infection

Statistical analysis using Spearmann correlation test found that platelet counts had inverse correlation with plasma TPO levels. It means that while platelet counts was lowering, the TPO levels tended to increase and it went the other way around. (figure 5)

By statistical analysis, significant correlation was found with p value = 0.00 is shown in figure 6.

Severity of Thrombocytopenia Profile

Severity of thrombocytopenia in DHF group was ranged between 6,000 and 54,000/mm3 with mean value of 25,041/mm3 (median value of 21,500/mm3). In DF group it ranged between 12,000 and 67,000/mm3 with mean value of 30,725/mm3 (median value of 28,500 pg/ml). From statistical analysis it was found that severity of thrombocytopenia profile was significantly different between these two groups (p= 0.00). Profile of severity of thrombocytopenia in dengue infection is shown in figure 7.

Tabel 2. Profile of TPO Levels in DF and DHF

Day	DHF			DF			Hypotetics Test*
	Mean (SD)	Median	Min. – Max.	Mean (SD)	Median	Min. – Max.	
3	616,05 (378,80)	627,60	257,60 - 951,40	320,00 (0)	320,00	320,00 - 320,00	p = 1
4	700,65 (582,20)	612,60	131,10 - 2.740,00	383,45 (299,71)	446,30	64,10 - 922,80	p = 0,07
5	436,40 (246,20)	347,35	158,50 - 983,80	456,37 (308,50)	397,70	57,60 - 1089,00	p = 0,9
6	368,40 (254,30)	354,50	46,30 - 882,80	332,55 (271,22)	219,85	0 - 1083,00	p = 0,7
7	408,80 (411,60)	395,10	0 - 1.566,00	207,98 (241,75)	176,50	0 - 841,40	p = 0,6
8	304,75 (265,40)	194,40	0 - 967,60	211,89 (349,88)	131,15	0 - 1.305,00	p = 0,1
9	218,10 (187,30)	225,20	0 - 509,00	225,92 (462,94)	27,10	0 - 1.265,00	p = 0,2

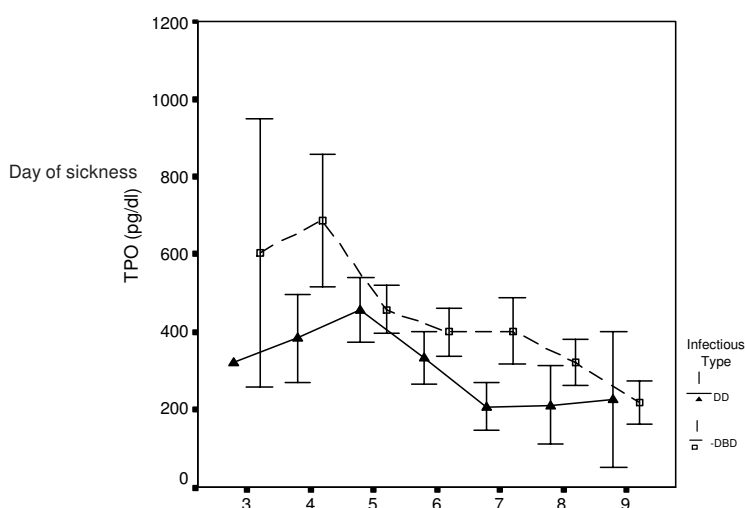SD = Standard deviation, * Mann Whitney test for means of two independent groups

Figure 4. Profile of Plasma Trombopoietin Levels in DF dan DHF

Figure 5. Diagram of Platelet Counts and Plasma TPO Levels in Dengue Infection

TPO Plasma Level Profile at Nadir Point of Platelet Count

TPO plasma levels at nadir point of platelet counts in DHF group were ranged between 173.1 and 967.6 pg/ml with mean value of 523.3 (median value of 304 pg/ml). In DF group it was ranged between 57.6 and 755.3 pg/ml with mean value of 339 pg/ml (median value of 304 pg/ml). Statistical analysis found significant difference between these two groups (p= 0.03). Profile of severity of thrombocytopenia in DHF and DF infection is shown in figure 8. Correlation analysis using Spearmann test found significant inverse correlation between TPO plasma levels and nadir point platelet counts (p=0.01).
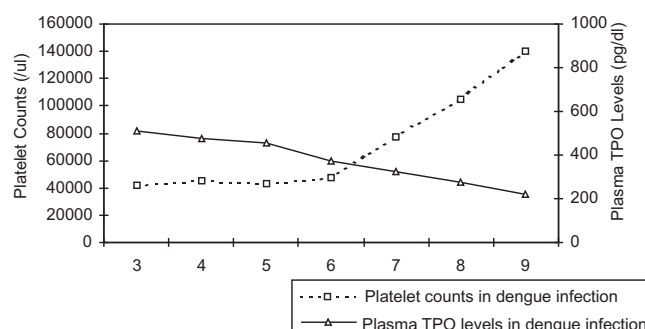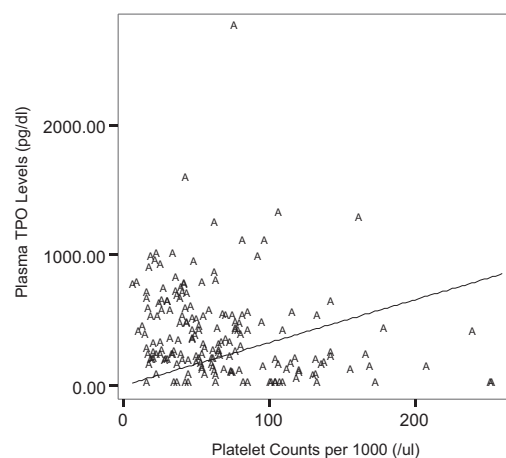

Figure 6. Correlation Between Plasma TPO Levels and Platelet Counts in Dengue Infection (DF and DHF)
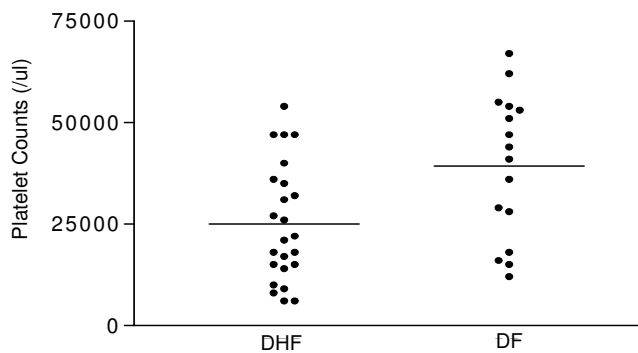
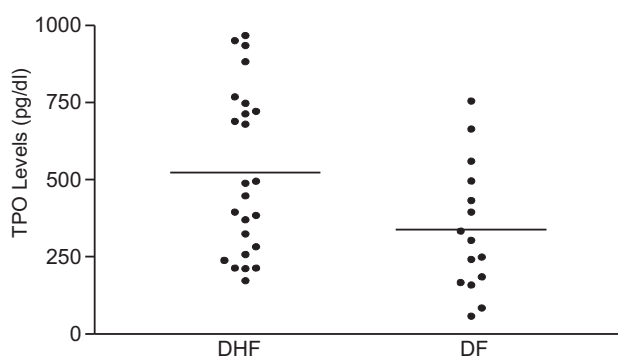Figure 7. Profil of Severity of Thrombocytopenia According Type of Infection

Figure 8. Profile of Plasma TPO Levels and Thrombocytopenia in DF and DHF

DISCUSSION

In this study, subjects in age group of 20 to 25 years old had the largest proportion compared to the other age groups. Table 1 showed primary infection had shifted from younger patient age group of 14 – 19 years old to age group of 20 – 25 years old. In the beginning of an epidemic disease in a country, age distribution showed the largest proportion in age group under 15 years old (86-95 %). The following epidemics documented increased young adult patients. It had been reported since 1984 the proportion of patients whose age more than 15 years old had increased. Based on the theory of secondary infection, it means that second infection caused by different type of virus affected the older patients than before.

Thrombocytopenia has critical role in pathogenesis of DHF. Previous studies reported DHF in adults without shock, the platelet counts would have mild to moderate decrease on 3rd day until 7th day of course of illness and reached normal level on 8th or 9th day.

The possible mechanism of thrombocytopenia in DHF were thrombopoiesis disturbance, platelet dysfunction and increased platelet destruction due to peripheral sequestration. [7,8,9] Platelet aggregation disturbance was supported by studies done by Mitrakul and Srichaikul et al. They found increased

thromboglobulin and PF4 which were also signs of increased platelet degranulation during acute phase of DHF infection. [9,10] Thrombocytopenia might also due to the presence of antibody anti VD on platelet-binding VD. [11], and platelet consumption during ongoing coagulopathy process. [12,15] Some studies had reported significant correlation between platelet counts and pleural effusion as one of plasma leakage manifestation in DHF. [13,14,15] Plasma leakage occurred after few days of fever and reached maximum when the fever had disappeared. At the same time, complement was activated and platelet counts markedly reduce in circulation. [13]

This study, the author tried to observe humoral thrombopoiesis regulation by clinical and laboratory observation during clinical course of dengue infection. TPO plasma levels were classified into two subgroups which were thrombocytopenic phase on 3rd to 7th day and normal platelet counts phase on 8th to 9th day of illness. Thrombocytopenia phase was considered as early phase while normal platelet counts phase as convalescent phase. It is shown in figure 3.

Bone marrow has important role in mechanism of thrombocytopenia. Suppression on hematopoietic system occurred during dengue infection. Published research reported that during early phase of disease, bone marrow described hypocellularity and attenuation of megacaryocytes maturation. In fact, on 5th day, bone marrow celullarity and megacaryocytes had increased.[7,16,17,18] Megacaryoblast were found and accelerated megacaryocytopoiesis was just like as described in the recovery phase after acute suppression. Meanwhile, the platelet counts began to increase during convalescent phase around 6th or 7th day and reached normal on 8th to 10th day course of disease. [9,10,20] From these results, it indicated that basic mechanism of thrombocytopenia during early phase of dengue infection was bone marrow suppression. This also indicated a lesion on pluripotential progenitor cells or direct influence of virus on progenitor cells. Mechanism of bone marrow suppression in dengue infection might involved 3 main factors: 1) Direct lesion of progenitor cells by dengue virus; 2) infected stromal cells;3) changes in bone marrow regulation.[12]

From this study, TPO plasma levels were observed increasing during early phase indicated the presence of thrombopoiesis stimulation activity in plasma as normal response to thrombocytopenia. It seemed that TPO was main thrombopoiesis regulator in dengue viral infection. In relation to thrombocytopenia, bone marrow suppression was presumed as main factor that caused

thrombocytopenia during early phase of disease. This was supported by fact that free TPO plasma levels in circulation was high in bone marrow failure. On 8th and 9th day where the subjects were at convalescent phase, increased platelet counts were observed and thrombopoiesis stimulation activity decreased. The limitation of study was the absence of bone marrow aspiration to document any evidence of bone marrow suppression during early phase of disease.

So far, there was only one published research known by the author which had reported observation of humoral factors that responsible in thrombopoiesis regulation in DHF. The study observed 11 subjects during acute phase which was from 3rd day to 8th day course of disease. The results were different from this study and showed no increased plasma TPO levels even when severe thrombocytopenia occurred. On 5th and 6th day of illness, rapid increase of plasma TPO levels was seen and followed by increased platelet counts. It was presumed in that study that there were temporary changes in humoral thrombopoiesis regulation which might be due to damage of lymphoid tissue. Reticuloendothelial system had been known as main regulator of thrombopoiesis. Dengue viral infection affected the lymphoid tissue. The different results may be caused by different methods of plasma TPO levels examination and number of study samples.[25]

The presence of thrombopoiesis stimulation activity in dengue infection was supported by this study as seen in figure 4 described profile of plasma TPO levels according to platelet counts. It showed significant decrease of mean TPO levels at platelet counts above 100,000/mm^3 which was from 395 pg/ml to 153.6 pg/ml (p=0.00). These results suggested decreased platelet counts stimulated TPO secretion as humoral substance needed to induce platelet production. On the other hand, increased platelet counts could enhance binding of TPO to platelet receptor and made plasma TPO levels decrease. Clinical data on humoral thrombopoiesis regulation had indicated the presence of stimulating factor and inhibiting factor involved in this process. Clinical observation suggested that either in human or animals responded thrombocytopenia by producing more TPO to stimulate platelet production proportionally.[22-24] The feedback mechanism will maintain adequate platelet counts in normal condition. Shreiner et al found the majority of patients with normal platelet counts did not showed any thrombopoiesis stimulation activity compared to control group.[26]

During clinical course of disease, mean plasma TPO levels was seen increased on 3rd day, reached maximum on 4th day and decreased on 7th day (convalescent phase). This results were conformed to mean platelet counts which decreased on 3rd day, reached minimum on 4th day and 5th day, then back to normal on 8th day. It indicated that TPO levels was in accordance with pathogenesis of dengue infection. It was assumed that suppression of hematopoiesis caused by direct interaction with virus which lasted 3 - 4 days. The bone marrow became hyperplastic and cytopenia recovered. Severity of bone marrow suppression in DF was similar to those occurred in DHF infection.[7] In this study, it seemed that TPO activity could increase platelet production and when platelet counts reached normal at convalescent phase, plasma TPO levels decreased. Statistical analysis found no significant correlation of mean TPO levels between DHF and DF group. It might be caused by large standard error that made standard deviation was overlapping.

Statistical analysis revealed inverse correlation between plasma TPO levels and platelet counts in dengue infection. It means TPO levels tended to increase while platelet counts were decreased.

From the severity of thrombocytopenia point of view, there was significant difference between DHF and DF group (p=0.00). Sutaryo found the platelet count of DF infection in children did not decrease as much as in DHF. [26] Khrisnamurti et al found severity of thrombocytopenia during acute phase in DHF was significantly different with those in DF infection.[13] The same result was also reported by Kalayanaroj et al.[28] There was significant correlation between severity of thrombocytopenia and TPO levels (p=0.00).

Mitrakul et al observed that in 11 patients with DHF, severity of thrombocytopenia also condated with disease severity in early phase.[9] The same results was also reported by Isarangkura et al.[29] Krishnamurti et al reported severity of thrombocytopenia had significant correlation with plasma leakage manifested by pleural effusion index.[15] Nimmnatya reported correlation between platelet count and hemoconcentration. [10] In severe case of DHF with shock, increased hematocrit was ranged 30 –70 % and platelet counts below 50,000/ul. It seemed that plasma leakage also indicated disease severity. Further research with large sample size is required to investigate correlation between thrombocytopenia and disease severity.

Limitation of this study was the variation in sample sizes being examined each day. The subjects were

observed until the platelet counts reached above 100,000/mm^3, therefore duration of observation was different for each subject. Thus, the result of this study is specific only for population with similar subject characteristics, and cannot be generalized in wide population. Further research with larger sample size and more specific study design is required to investigate the correlation between of TPO levels and platelet counts during clinical course of disease.

CONCLUSION

Increased plasma TPO levels during early phase of disease indicated thrombopoiesis stimulation activity in response to thrombocytopenia which occurred in dengue viral infection in adults patients. TPO plays critical role as thrombopoiesis regulator in dengue viral infection. There was significant inverse correlation between platelet counts and plasma TPO levels in dengue viral infection. Plasma TPO levels correlated with severity of thrombocytopenia in DF and DHF infection.

Further research is required to investigate the relation between various parameters of other thrombopoiesis regulators such as IL-6, IL-11 during clinical course of dengue viral infection

Further longitudinal study is required to investigate the influence of platelet antibody, DIC and bone marrow suppression on clinical course of dengue viral infection specially in severe DHF

Further research is required to observe plasma TPO levels in severe DHF

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