

## CORRELATION BETWEEN TNF- $\alpha$ , IL-1 $\beta$ , PGE<sub>2</sub> AND sPLA<sub>2</sub> LEVELS WITH SEVERITY OF DENGUE HEMORRHAGIC FEVER

Purwati<sup>1</sup>, Endang Retnowati K<sup>2</sup>

<sup>1</sup> Department of Internal Medicine, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Surabaya

<sup>2</sup> Department of Clinical Pathology, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Surabaya

### ABSTRACT

*This study analyzed the correlation between the severity of dengue hemorrhagic fever patients with the immunological profile such as sPLA<sub>2</sub>, IL-1 $\beta$ , TNF- $\alpha$ , PGE<sub>2</sub>. This study is a cross sectional observational research performed between February 2009 – November 2009, comprising 45 patients hospitalized in the Tropical-Infection Ward Department of Internal Medicine Dr. Soetomo Hospital. The examinations of sPLA<sub>2</sub> (correlate enzyme assay), IL-1 $\beta$ , TNF- $\alpha$ , PGE<sub>2</sub> by EIA method were performed in the Department of Clinical Pathology, Medical Faculty and Institute of Tropical Disease Airlangga University. It was shown that there was no significant influence on the severity of dengue hemorrhagic fever, because there was no linear correlation. In this study, an increased level in grade 2 compared to grade 1 was found, but in grade 3 the level was decreased. Using Anova in this study a significant difference was found only in IL-1 $\beta$  ( $p=0.000$ ) on the severity of dengue hemorrhagic fever. A significant difference was found in grade 1 and 3 ( $p=0.040$ ), also in grade 2 and 3 ( $p=0.040$ ). This condition may be caused by a decrease in the inflammation process in grade 3, but the severity of dengue hemorrhagic fever could be influenced by virus strains, complement, thromboxane, leucotriene or other factors that could influence endothel permeability and capillary endothel dysfunction causing greater plasma leakage. No correlation was found between sPLA<sub>2</sub>, IL-1 $\beta$ , TNF- $\alpha$  and PGE<sub>2</sub> and the severity of dengue hemorrhagic fever, but in IL-1 $\beta$  a difference was found. this could be caused by a decrease of inflammatory process in grade 3. Further studies with different parameters which can cause the severity of the disease such as virus strains, complemen, thromboxane, leucotriene or other factors have to carried out.*

**Keywords:** IL-1 $\beta$ , TNF- $\alpha$ , PGE<sub>2</sub>, sPLA<sub>2</sub>, dengue hemorrhagic fever

**Correspondence:** Purwati, Department of Internal Medicine, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Surabaya

### INTRODUCTION

Dengue hemorrhagic fever (DHF) is one health problem in tropical areas. In Southeast Asia, with a total population of 1.5 billion, nearly 1.3 billion people are at risk for dengue virus infection. DHF is a major cause of hospitalization and death in children. DHF was first reported in Indonesia in 1968 in Jakarta and Surabaya. The incidence of dengue is increasing. Based on data from the Ministry of Health, in the early 1980s, the incidence of dengue around were 10000-30000 cases per year, and in the last ten years it increased by nearly approaching 30000-60000 cases per year. Currently dengue virus has spread to 29 provinces in Indonesia (Setiati 2006).

The proportion of DHF in age group above 15 years is increasing. The Ministry of Health in 1993 reported that DHF patients aged above 15 years reached 23.5%, while in 1997 increased to 35.5%. In East Java the number of adult DHF patients also tended to increase. In 1989 patients of 15-44 years age group reached 6.15%, and in

1999 the same age group rose to 34.54% (Soewandojo 2004).

Dengue virus infections are in fact a self limiting disease, but during the course of the disease, dangerous clinical complications often arise, such as plasma fluid leakage marked by hemoconcentration (elevated hematocrite), shock, and bleeding. Haemorrhage in dengue infection is mainly caused by disrupted platelet, either due to the decrease of its amount (thrombocytopenia) or quality (trombopathy).

Pathogenesis of dengue virus infection is still debated. Based on existing data, there is strong evidence that the imunopathological mechanism plays a role in the occurrence of dengue virus infection with various complications as mentioned above. From some known immune responses playing a role in the pathogenesis of dengue virus infection, the researchers tried to prove the role of several inflammatory mediators such as sPLA<sub>2</sub> (secretory Phospholipase-A<sub>2</sub>), IL-1 $\beta$  (Interleukin 1-beta), TNF- $\alpha$  (tumor necrosis factor-alpha), PGE<sub>2</sub>

(Prostaglandin E-2), in conjunction with the grade of severity of the dengue virus infection. It was also expected in this study to identify the pattern of sPLA2, IL-1 $\beta$ , TNF- $\alpha$ , PGE2 in various grades of severity in dengue hemorrhagic fever, which can be used to help finding the course of the disease and disease treatment.

This study aims to analyze the relationship between the grade of severity in patients with dengue virus infection with sPLA2, IL-1 $\beta$ , TNF- $\alpha$ , PGE2 and to increase the levels of insight about the role of these parameters, which can be used to assist clinicians in monitoring the development and progress of dengue fever, and to be a basis for further research in the field of infectious diseases, particularly dengue fever.

## MATERIALS AND METHODS

This research was an analytic observational study with cross sectional design. The study was conducted in February 2009 until November 2009. Research location was in Tropical Infectious Diseases Wards, Dr. Soetomo Hospital, Airlangga University Tropical Disease Institute and Department/Clinical Pathology Faculty of Medicine Airlangga University/Dr. Soetomo for the separation of plasma, platelet count examination, hematocrit, dengue IgG and IgM, NS1 (Non-Structural-1 Ag), plasma sPLA2 activity, Prostaglandins 2 plasma, IL-1 $\beta$ , and TNF- $\alpha$ .

Inclusion criteria were patients with a diagnosis of DHF by the WHO criteria (World Health Organization) in 1997: (a) sudden high fever, for no apparent reason, lasted continuously for two to seven days; and there are (b) bleeding manifestation, marked with one of the following: Rumpel Leede test/positive tourniquet, petechiae, echymoses, purpura, mucosal bleeding, epistaxis, bleeding gums, hematemesis and or melena; (c) thrombocytopenia (platelet count <100.000/ $\mu$ l); (d) hemoconcentration, which can be seen from the increased hematocrit of 20% or more, according to age and gender standards, or a decrease in hematocrit of 20% after fluid therapy.

The results of serological tests for IgG and or IgM anti-dengue were examined by using EIA (Enzyme Immunometric Assay) and/or NS-1 antigen was examined with a positive rapid test methods included in the study. Inclusion samples are classified according to grades of DHF, which is (a) DHF Grade I: Above symptoms plus positive tourniquet test, thrombocytopenia (<100,000), evidence of plasma leakage; (b) DHF Grade II: Above symptoms with spontaneous bleeding, thrombocytopenia (<100,000), evidence of plasma leakage; (c) DHF Grade III: Above

symptoms plus the failure of the circulation (cool and moist skin and nervous), thrombocytopenia (<100,000), evidence of plasma leakage; (d) DHF Grade IV: severe shock accompanied by blood pressure and pulse are not measurable, thrombocytopenia (<100,000), there is evidence of plasma leakage. Grades III and IV are also called SSD (Suhendro 2006).

Exclusion was performed if the sample patients had 2-7 days of fever caused by infection other than dengue, there was severe underlying diseases (hematological disorders, diabetes mellitus, chronic kidney failure, heart failure, liver failure, liver cirrhosis) and age less than 14 years.

Venous blood samples taken from patients treated at Tropic Wards, Dr. Soetomo Hospital, and taken by using a thermos cooler to the laboratory. Blood plasma was centrifuged and immediately separated into a number of aliquots. Samples were stored in -70°C at Tissue Bank Dr. Soetomo Hospital before treatment. Samples were taken into storage in a frozen state by using dry ice.

The examination of TNF- $\alpha$ , PGE2, and IL-1 $\beta$  was measured by using EIA (Enzyme Immunometric Assay) in units of pg/ml, with heparin as anticoagulant (Handojo 2003). In this study, sPLA2 parameters were measured using enzyme assay correlated with the unit/ml, and was used as anticoagulant.

Quality assurance of TNF- $\alpha$ , sPLA2, PGE2, IL-1 $\beta$  examination was performed with precision control by looking for the imprecision of duplicate samples checked within run. Accuracy control was not done, because there was no accuracy control materials and reagents was only for research.

Statistical analysis was performed as follows: the relationship of each parameter (TNF- $\alpha$ , sPLA2, PGE2, IL-1 $\beta$ ) with grades of DBD was analyzed with Spearman's correlation, the relationship of these four parameters with the grade of DBD was analyzed with multiple ordinal regression. Relations between four parameters with the grade of DHF (non linear): was analyzed by anova.

## RESULTS AND DISCUSSION

Based on this research that had been carried out in Dr. Soetomo Hospital. This study obtained as many as 45 patients with dengue hemorrhagic fever with details as follows: DHF grade 1 was found in 7 patients (15.6%), DHF grade 2 in 35 patients (77.8%) and DHF grade 3 in 3 patients (6.7%). In this research we did not find DHF grade 4 and the largest percentage was grade 2.

This was due to the community and medical workers alertness against dengue so their condition was mild when they visited the hospital. The sample of patients with dengue fever was 45 patients, consisted of 31 male patients (68.9%) and 14 female patients (31.1%) (Table 1).

Table 1. Characteristics of study subjects DHF patients

Characteristics	Range	Mean	SD
Age (years)	14 – 48	21.36	6.205
TNF- $\alpha$ (pg/ml)	73 – 809.7	199.7378	143.63442
IL-1 $\beta$ (pg/ml)	12.7 – 45.9	31.6333	6.45720
PGE <sub>2</sub> (pg/ml)	39.1 – 1350	238.0911	207.44473
sPLA <sub>2</sub> (U/ml)	37.8 – 195.9	97.4933	30.06433

The range of TNF- $\alpha$ , sPLA2, PGE2, and IL-1 $\beta$  levels was wide enough. This may be because the selection of the patients with fever between 2-7 days could cause such a state.

Quality assurance of TNF $\alpha$ , sPLA2, PGE2, and IL-1 $\beta$  levels was done by finding within run imprecision in the absorbance. Determination of imprecision was performed on different samples of patients with multiple or duplicate checks (Table 2).

Table 2. Imprecision within the run absorbance TNF $\alpha$ , sPLA2, PGE2, IL-1 $\beta$ .

	N	SD	CV
TNF $\alpha$	10	0.00732	2.00 %
sPLA2	10	0.01570	2.92 %
PGE2	5	1.34000	4.97%
IL-1 $\beta$	10	0.000803	2.12%

The results will have no meaning if there was no valid data, so in this study data validity was ascertained by quality assurance examination of TNF- $\alpha$ , sPLA2, PGE2, and IL-1 $\beta$  levels with precision control to measure imprecision.

Imprecision is an assessment of deviation from the mean value, the less the deviation (as determined by standard deviation/SD or coefficient of variation/SD), the closer the results of re-examination between one-another in the series. In general, deviations are acceptable for a particular examination parameters expressed by the coefficient of variation (CV). Examination variation coefficient should not exceed

5%, unless certain parameters are allowed up to 10% (PDS PATKLIN = Pehimpunan Spesialis Patologi Klinik 1995). The imprecision of TNF- $\alpha$ , sPLA2, PGE2, IL-1 $\beta$  is still below 5%.

#### Relationship level of IL-1 $\beta$ , TNF- $\alpha$ , PGE2 and sPLA2 with severity of dengue hemorrhagic fever.

In dengue virus infection, macrophages exposed to dengue virus increase the production and activation of proinflammatory cytokine secretion, various mediators and enzymes, phospholipase A2 (PLA2), and trigger the production of neutralizing antibodies or nonneutralization. Domination of non-neutralization antibody can even deliver and encourage the course of dengue infection by inducing various mediators. About an hour since the internalization of dengue virus, the activation of gene NF $\kappa$ B in macrophages occurs, resulting in increased production and secretion of IL-1 $\beta$ , followed by increased production and secretion of TNF- $\alpha$  and IL-6 in the next hour. Through the intercession of the Fc receptor, there will be more effective interaction and communication between macrophages and T lymphocytes. The T cells also produce and secrete pro-inflammatory cytokines so that more mediators and cytokines become involved. The effect of endothelial IL-1 $\beta$  was the occurrence of malfunctions, while TNF- $\alpha$  causes endothelial destruction. This situation may improve vascular permeability, encouraging plasma migration from the intravascular into extravascular.

Macrophage hyperactivity induced by dengue virus also causes increased production and secretion of PLA2 enzyme. PLA2 enzymes in the blood circulation will trigger metabolic arachidonic acid through cyclooxygenase path, acting as an inductor to increase synthesis of prostacyclin (PGI<sub>2</sub>), PGE2, thromboxane A<sub>2</sub>, and leukotriene. The impact of these four secondary mediators is very strong in influencing endothelial gap widening, increased vascular permeability. There is a great plasma displacement, encouraging dengue shock syndrome to occur (Catharina 2001, Chaturvedi et. al. 2000, Gill 2004, Hasteed 1989, Nasronudin 2007, Noisakran S et. al. 2008, Oppenheim & Ruscetti 2003). Based on this, it can be expected that there will be no correlation between elevated levels of TNF $\alpha$ , sPLA2, PGE2, IL-1 $\beta$  with a grade of DHF.

In this study, correlation levels of IL-1 $\beta$  with DHF grade of was found not linear with no significant difference ( $p = 0.929$ ). That condition can be identified from the data that DHF grade I had mean levels of IL-1 $\beta$  (Table 3). There was an increased concentration of DHF average in grade one and two, but the concentration decreased in grade three. This study showed significant difference only on IL-1 $\beta$  ( $p = 0.000$ )

on the grade DHF severity by using anova. Significant difference was found between DHF grades 1 and 3 in which the difference of mean 9.52381 with standard error 3.76532 ( $p = 0.040$ ), between DHF grade 2 and 3 where the difference of mean 3.28253 with standard error 13.78095 ( $p = 0.000$ ). In relations with these results, it was showed that the relationship was not linear. The situation can be attributed to a decrease inflammatory processes in DHF grade 3, and the severity of DHF grade may be influenced by complement, thromboxane, leukotrienes, or other factors that affect permeability endothelial and capillary endothelial damage, resulting in more severe plasma leakage.

In this study TNF- $\alpha$  levels with the grade of DHF was found not to have linear correlation with no significant difference ( $p = 0.922$ ). There was elevated average level from DHF grade 1 to grade 3, but it decreased in the third grade (Table 3). The situation can be attributed to the decrease of inflammatory processes in DHF grade 3, and the severity of DHF grade may be influenced by complement, thromboxane, leukotrienes, or other factors that affect endothelial permeability and capillary endothelial damage, resulting in more severe plasma leakage. PGE2 was affected by sPLA2 and this study found the same pattern of the increase and decrease of such parameters in the grade of DHF. It can be concluded that sPLA2 has effect on PGE2. In this study sPLA2 levels had no linear correlation with DHF grade with no significant difference ( $p = 0.709$ ). There was elevated levels of the average of one to two grades of DHF, but decreased in the third grade (Table 3).

The condition can be attributed to a decrease of inflammatory processes in DHF grade 3, and the severity of DHF may be influenced by complement, thromboxane, leukotrienes, or other factors that affect endothelial permeability and capillary endothelial damage, resulting in more severe plasma leakage. The condition can also be caused by specific immune response to viral infection, to produce TNF $\alpha$  due to the stimulation on B lymphocytes. B lymphocytes respond to DENV-3 antigen, but also against antigens DENV-1, DENV-2, and DENV-4 even in low concentrations. This allows the existence of cross responses between each of the different serotypes, so the response to the activation of T lymphocytes is also different (Ribardo DA et. al. 2002. Nasronudin 2007).

Effect of IL-1 $\beta$ , TNF- $\alpha$ , and PLA2 on the grade of severity of DHF was found not significant with respective values  $p = 0.402$ ,  $p = 0.589$ ,  $p = 0.959$ , so the effect of IL-1 $\beta$ , TNF- $\alpha$ , and PGE2 on the grade of DHF severity was not significant with each  $p = 0.332$ ,  $p = 0.594$ ,  $p = .696$ . In relations with the above data, there

was no linear correlation between these parameters with severity of DHF, as there are increased levels of grades 1 and 2, but there was a decline in third-grade levels. In this study the only significant difference was found in IL-1 $\beta$  ( $P = 0.000$ ) on the grade of severity of DHF by using anova. Significant difference was found between DHF grades 1 and 3 in which the difference of mean standard error of 9.52381 to 3.76532 ( $p = 0.040$ ), between DHF grade 2 and 3 where the difference of mean 3.28253 with standard error 13.78095 ( $p = 0.000$ ).

Table 3. Correlation between level of IL-1 $\beta$ , TNF- $\alpha$ , PGE2 and sPLA2 with the grade of severity of dengue fever.

DHF grade	TNF- $\alpha$ (pg/ml)	IL-1 $\beta$ (pg/ml)	PGE <sub>2</sub> (pg/ml)	sPLA <sub>2</sub> (U/ml)
I	139.9571	28.9571	175.8571	87.5429
II	216.4629	33.2143	258.5171	100.3486
III	144.1000	19.4333	145.0000	87.4000

In connection with these results, it can be that the relationship was not linear. The condition can be attributed to a decrease inflammatory processes, but the grade of severity of DHF can be affected by complement, thromboxane, leukotrienes, or other factors that affect endothelial permeability and capillary endothelial damage, resulting in more severe plasma leakage.

All four of these parameters have the same pattern on the grade of DHF. There is a higher level in DHF grade 2 compared with the first-grade and it decreases in the third grade. In this study, originally we expected the presence of significant correlation, because the more severe the grades of DHF severity, the higher the level of IL-1 $\beta$ , TNF- $\alpha$ , PLA2 and PGE2, but different circumstances occur in DHF grade 3.

## CONCLUSIONS

There is no correlation between levels of TNF- $\alpha$ , IL-1 $\beta$ , PGE2, and sPLA2 in DHF patients at various grades of severity of DHF, but at the level of IL-1 $\beta$  there are significant differences between DHF grades 1 and 3, and 2 and 3.

The levels of these four parameters increase in DHF grade 1 to 2, but in the third grade the level decreases. This can be attributed to lower inflammatory process, but the grade of severity in DHF can also be affected by complement, thromboxane, and leukotrienes.

Based on these results, further research should be conducted to determine the pattern of these parameters starting from the first day of fever up to the healing phase, and to identify the influence of other parameters that support the grade of severity, such as the examination of complement, thromboxane, leukotrienes and examination of the virus strains to support the grade of severity of DHF.

## REFERENCES

- Catharina 2001, 'Pathogenesis of dengue hemorrhagic fever and dengue shock syndrome', *Dengue Hemorrhagic Fever in Indonesia: the role of cytokines in plasma leakage, coagulation and fibrinolysis*, Nijmegen University Press, Dinsdag, pp. 15-23.
- Chaturvedi, UC, Agarwal, R, Elbishbishi, EA & Mustafa, AS 2000, 'Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis', *FEMS Immunology and Medical Microbiology*, vol. 28, pp. 183-188.
- Gill, GV & Beeching, NJ 2004, 'Dengue and yellow fever', in GV Gill & NJ Beeching (eds), *Tropical Medicine*, 5<sup>th</sup> edn, Blackwell Publishing, Massachusetts, pp. 262-266.
- Handojo, I 2003, 'Imunoasai untuk demam dengue atau demam berdarah dengue', *Imunoasai Terapan pada Beberapa Penyakit Infeksi*, Airlangga University Press, Surabaya, pp. 162-168.
- Hastead, SB 1989, 'Antibody, macrophages, dengue virus, infection, shock, and hemorrhage: a pathogenic cascade', *Reviews of infectious disease*, vol. 11, pp. S830-S839.
- Nasronudin 2007, 'Imunopatofisiologimolekuler infeksi virus dengue', in Nasronudin, U Hadi, Vitanata, EA Triyono, Bramantono, Suharto & E Soewandojo (eds), *Penyakit Infeksi Di Indonesia*, Surabaya, pp. 46-56.
- Noisakran, S, Perng, GC 2008, 'Alternate Hypothesis on the Pathogenesis of Dengue Hemorrhagic Fever (DHF)/Dengue Shock Syndrome (DSS) in Dengue Virus Infection Society for Experimental Biology and Medicine', pp. 401-408.
- Oppenheim, JJ & Ruscetti, FW 2003, 'Cytokines', in TG Parslow, DP Stites, AI Terr & JB Imboden (eds), *Medical Immunology*, 10<sup>th</sup> edn, San Fransisco, pp 48-166.
- Peters, CJ 2005, 'Dengue hemorrhagic fever/dengue shock syndrome', in Kasper, Braunwald, Fauci, Hauser, Longo & Jameson (eds), *Harrison's Principles of Internal Medicine*, 16<sup>th</sup> edn, New York, vol. I, pp. 1173-1174.
- Ribardo, DA, Kuhl, KR, Petreson, JW & Chopra, AK 2002, *Role of Metillin-like within Phospholipase A (2)-Activating Protein in Biological Function*. Retrieved April 2, 2002, from: file://A: DHF/endothel-Role of metillin-like region within phospholipase A (2).htm
- Setiati, TE, Wagenaar, JFP, Krui, MD, Mairuhu, ATA, Gorp, ECM & Soemantri, A 2006, 'Changing epidemiology of dengue haemorrhagic fever in indonesia', *Dengue Bulletin*, vol. 30, pp. 1-14.
- Soewandojo, E 2004, 'Tatalaksana demam berdarah dengue pada orang dewasa', in Suharto, U Hadi, & Nasronudin (eds), *Seri Penyakit Tropik Infeksi: Perkembangan Terkini dalam Pengelolaan Beberapa Penyakit Tropik Infeksi*, Airlangga University Press, Surabaya, pp. 113-129.
- Suhandro, Leonard, N, Khie, C & Herdiman, TP 2006, 'Demam berdarah dengue', in Aru WS, Bambang S, Idrus A, Marcellus SK & Siti S (eds), *Buku Ajar Ilmu Penyakit Dalam*, 4<sup>th</sup> edn, Jakarta, pp. 1731-1743.
- WHO 1997, 'Pengobatan DHF', *Demam Berdarah Dengue Diagnosis, Pengobatan, Pencegahan dan Pengendalian*, 2<sup>nd</sup> edn, EGC, Jakarta, pp. 34-46.