

EFFECT OF RED YEAST RICE (*Monascus Purpureus*) EXTRACT TO THE TROMBOPOIETIN LEVEL IN DENGUE INFECTED PATIENTS

Muhammad Noor Diansyah, Suharto, Erwin Astha Triyono

Department of Internal Medicine, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Surabaya

ABSTRAK

*Trombositopenia merupakan komponen kunci dan parameter penting dalam infeksi dengue dari potensi perdarahan dan kondisi yang mengancam kehidupan. Angkak (red yeast rice) yang merupakan produk fermentasi beras dan ragi merah (*Monascus purpureus*), telah digunakan di Indonesia untuk meningkatkan jumlah trombosit pada infeksi dengue. Penelitian pra - klinis telah menunjukkan bahwa *Monascus purpureus* ekstrak dapat meningkatkan trombosit secara signifikan. Namun mekanismenya belum dipahami dengan jelas. Ia telah mengemukakan bahwa mekanisme tersebut karena efek anti - inflamasi atau stimulasi trombopoiesis. Desain penelitian yang digunakan adalah percobaan blind tunggal terkontrol acak. Subyek penelitian ini adalah pasien dengan infeksi dengue, yang dirawat di Ruang Rawat Inap Tropik infeksi RSUD Dr. Soetomo Surabaya. Total sampel 30 pasien yang dibagi ke dalam kelompok percobaan (n = 15) dan kelompok kontrol (n = 15). Kelompok percobaan menerima terapi standar untuk infeksi dengue dan ekstrak kapsul *Monascus purpureus* 3 kali 1 kapsul setiap hari selama 48 jam. Kelompok kontrol menerima terapi standar untuk infeksi dengue. Tingkat TPO diukur sebelum dan 48 jam setelah pengobatan. Analisis komparatif pada kedua kelompok menunjukkan bahwa antara 15 pasien dalam kelompok percobaan dan 15 pasien dalam kelompok kontrol, ada penurunan tingkat TPO (nilai median) dari 90 pg/mL menjadi 26,6 pg/mL, dan dari 130 pg/mL menjadi 66,8 pg/mL masing-masing. Kelompok percobaan mengalami penurunan yang lebih besar dari tingkat TPO (63,4 pg/ml) dibandingkan kelompok kontrol (63,2 pg/ml) tetapi secara statistik tidak signifikan (p = 0.916). (FMI 2013;49:220-225)*

Kata Kunci: *Monascus purpureus, Thrombopoietin, Infeksi Dengue*

ABSTRACT

*Thrombocytopenia is the key component and important parameter in dengue infection from the potency of bleeding and life threatening condition. Angkak (red yeast rice) a fermented product of rice and red yeast (*Monascus purpureus*), has been used in Indonesia to increase platelet count in dengue infection. Pre-clinical research has shown that *Monascus purpureus* extract can increase platelet count significantly. However the mechanism has not been understood clearly. It has been suggested that the mechanisms are due to anti-inflammatory effect or the stimulation of thrombopoiesis. Research design used single blind randomized controlled trial. The subjects of this research were patients with dengue infection, hospitalized in Tropical-Infectious Disease Ward, RSUD Dr. Soetomo Surabaya. The total samples were 30 patients who divided into trial group (n=15) and control group (n=15). Trial group received standard therapy for dengue infection and *Monascus purpureus* extract capsule 3 times 1 capsule daily for 48 hour. Control group received standard therapy for dengue infection. TPO levels was measured before and 48 hour after treatment. The comparative analysis on both groups shows that between 15 patients in trial group and 15 patients in control group, there are decrease of TPO level (median value) from 90 pg/mL to 26,6 pg/mL, and from 130 pg/mL to 66,8 pg/mL respectively. Trial group has greater decrease of TPO level (63,4 pg/ml) than control group (63,2 pg/ml) but statistically not significant (p = 0,916). (FMI 2013;49:220-225)*

Keyword: *Monascus purpureus, Thrombopoietin, Dengue Infection.*

Correspondence: Muhammad Noor Diansyah, Department of Internal Medicine, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Surabaya

INTRODUCTION

Dengue infection is an acute infection caused by dengue virus with antigen variants DEN - 1, DEN - 2, DEN - 3, DEN - 4. The infection is delivered by the bite of *Aedes Aegypti* mosquito and is endemic in tropical area such Indonesia. Around the world, 100 million cases were found each year, including 250,000-500,000 cases with the death of 24,000 cases (Gibbons & Vaughn 2002, World Health Organization and Special Programme for

Research and Training in Tropical Diseases 2009). Dengue infection in Indonesia is a serious health problem. The Ministry of Health of Indonesia stated the number of dengue fever patients in 2005 was 95,279 patients, with the number of deaths were 1,298 patients (Nasronudin 2007). Thrombocytopenia is a key component and essential parameters in dengue infection. Thrombocytopenia is also associated with bleeding manifestations, plasma leakage and severity of dengue infection. Severe thrombocytopenia was

observed in 74% cases of death due to dengue (Navarrete-Espinosa et al 2005). Several theories have been proposed by experts and researchers, the thrombosis damage in circulation, thrombosis aggregation, and trombopoiesis disorders of the bone marrow (Funahara et al 1987, Murgue et al 1997).

Thrombocytopenia in dengue infection can occur due to blockage in the process megakariopoiesis as a result of dengue virus infection on hematopoietic progenitor cells in the bone marrow, also thrombosis damage in peripheral as a result from inflammatory process, thrombosis destruction of the reticuloendothelial system, and also thrombosis aggregation in blood vessels (La Russa & Innis 1995, Murgue et al 1997). However, the cause of thrombocytopenia in dengue infection is still unclear.

The bone marrow plays an important role in the occurrence of thrombocytopenia in dengue infection. Trombopoietin (TPO) is a cytokine that can be isolated and identified as one of the growth factors being responsible for the trombopoiesis and megakariopoiesis process in the bone marrow. TPO stimulates megakariopoiesis, to precursors of megakaryocytes and thrombosis (Kaushansky 2005, von dem Borne et al 1998, Khorshed et al 2007). Angkak (red yeast rice) is a traditional medicinal plant of fermented rice and red mold *Monascus purpureus* (Danuri 2008).

In Indonesia *Monascus purpureus* extract is often used to increase thrombosis in dengue infection patients. Thrombosis increasing in dengue infection patients who were given *Monascus purpureus* extract therapy presumably through effects on megakariopoiesis increasing and trombopoiesis in bone marrow or anti-inflammatory effects of metabolites, monacolin K, ankaflavin and monascin (Tisnadaja 2006, Lin et al 2011).

The purpose of this research were analyzing the characteristics of research subjects who get dengue infection standard therapy with or without red yeast rice (*Monascus purpureus*) extract, the trombopoietin level in dengue infection patients before and after the therapy with or without red yeast rice (*Monascus purpureus*) extract, the trombopoietin level changes of dengue infection patients who get standard therapy dengue infection and red yeast rice (*Monascus purpureus*) extract with a control group. In addition, the increase of megakariopoiesis and trombopoiesis in the bone marrow or anti-inflammatory of metabolites become the important indicators of this research.

MATERIALS AND METHODS

This research was a single-blind randomized controlled trial. The population was patients in the Infectious-Tropical Diseases RSUD Dr. Soetomo Surabaya with the diagnosis of dengue infection based on WHO's diagnosis criteria in 1997. The samples were at least 30 patients included in this population. The inclusion criteria were patients of dengue infection based on WHO in 1997, positive results of anti-dengue IgG or IgM serology (the 5th day of fever), more than 13 years old or less than 65 years, fever admitted to the hospital about 5 days, willing to be involved in clinical trials with informed consent. Meanwhile, the exclusion criteria were dengue infection severe complications for instance sepsis, shock, moderate or severe bleeding (DHF grade III or IV), loss of consciousness; receive transfusions of whole blood, packed red cells, platelets, fresh frozen plasma during treatment and had platelet less than 20.000/mm³, if there were a bleeding and platelet transfusion.

Statistical analysis technique used SPSS 19.0 for collecting, recording, and calculating data. In order to see the data distribution (variables were normally distributed or not), Kolmogorov-Smirnov normality test was used. In order to analyze the basic data homogeneity, Chi-square test or Fisher's Exact test was used. In order to assess trombopoietin levels before and after with/ without red yeast rice extract (*Monascus purpureus*) in the treated group, two-tailed paired t statistical tests was used. In order to compare trombopoietin levels changes between the treated and controlled groups, two-tailed paired t test independent or the Mann-Whitney test with 5% or $p < 0.05$ significance level was used.

RESULTS

In this research, the proportion of dengue infection patients who came and were treated in RSUD Dr. Soetomo Tropical, the male sex was 76.67% and female was 23.33%. The mean age of the research subjects was 27.03 + 11.45 years, with the youngest aged 14 and the oldest 53 years. In the treatment group the mean age of patients with dengue infection were older than the control group, respectively 30.53 ± 11.63 years and 23.53 ± 10.49 years. These results showed no significant differences in the characteristics of sex ($p = 1.000$) and age ($p = 0.095$) in the research subjects. These results were consistent with researches conducted by Muharni et al. (2011) the proportion of male patients (70%) more than women (30%). Research conducted Cardier et al (2006) also indicated the proportion of male gender (64%) was more than women (36%) with a

mean age of 27.3 ± 6.21 years. Halstead research (2008) indicated there was no significant difference to the incidence of dengue for the gender. Halstead in his report wrote many studies conducted indicating there was no significant difference in the incidence of DHF/DSS by sex, with the male and female (MF ratio) of 1.18 in DHF without shock.

The mean temperature at the time the patient came was 37.73 ± 0.24 °C, the lowest temperature of 37.5 °C and 38.5 °C. The mean temperature in this research was similar to the average temperature on dengue infection research conducted by Thomas et al (2008) is 37.80 °C. In this research, the average summer day when it came to the hospital on day $4.63 + 0.61$, the fastest to the slowest - 3 and day 5. This result was slightly longer than the results of the research by Carlos et al (2005) which showed mean hot day while in the hospital 3.5 ± 1.4 days.

In this research of the results of initial laboratory tests of blood when the patient entered the hospital obtained a mean hemoglobin level of $14.36 + 1.54$ g/dL, with a mean number of leukocytes $4478.67 + 2197.23/\text{mm}^3$, the results of leukopenia $\leq 4000/\text{mm}^3$ occurred in 46.67% of patients. These results are similar to the research by Lee et al (2005) with leukopenia that occurred in 54.8% of patients with DHF, indicating leukopenia common in dengue infection. In this research, the mean hematocrit value on admission was $43.16 + 4.58\%$ with a maximum value of 50.5%. These results are higher than the mean hematocrit obtained in the research by Gomber et al (2001) in patients with DHF, ie $38.34 + 6.02\%$. Increased hematocrit $> 20\%$ of the initial value can describe plasma leakage in DHF.

In this research, all recipients had experienced thrombocytopenia while hospitalized with a mean platelet count on admission was $63\ 623 + 22948.53/\text{mm}^3$, with a minimum value and a maximum $97.000/\text{mm}^3$ $20.800/\text{mm}^3$, slightly above the average of platelets obtained in the research by Kittigul et al (2007), ie $60\ 722 + 48.350/\text{mm}^3$. The mean initial platelet count before treatment was $69\ 853 \pm 20203.07/\text{mm}^3$ in the control group and $57393.33 \pm 24485.75/\text{mm}^3$ in the treatment group. While the mean platelet count after treatment was $113,566.70 \pm 55680.15/\text{mm}^3$ in the control group and $108,173.30 \pm 33667.08/\text{mm}^3$ in the treatment group.

In this research, the increase in mean levels of SGOT $101.97 + 67.28$ U/L and SGPT $68.7 + 41.44$ U/L. Increased transaminase levels was also found in the research Villar-Centeno et al (2008) who obtained the results of the mean levels of AST 142.7 U/L and SGPT 90.8 U/L, an increase in serum transaminases occurred

in 60-90% of patients with DHF, and in most cases an increase in transaminases mild to moderate, but most small, 7-10% increased to 10 times the upper limit of normal values. In addition it was not found statistically significant differences between the levels of SGOT and SGPT in primary and secondary infections. It is concluded that elevated levels of transaminases not as a predictor of the severity of dengue infection. Average albumin level of $3.88 + 0.33$ g/dL. Lower below the average albumin levels in patients with DHF research conducted by Villar-Centeno et al (2008) is 3.95 g/dL. This may indicate the presence of disturbances began vascular permeability.

Serology IgM and IgG anti- dengue needed as a support to sharpen the diagnosis of dengue infection,. IgM antibody is an immunoglobulin isotype that appears first. Anti- dengue IgM was detected in 50% of patients are at day 3 to 5 fever, increased to 80% of patients on day 5 and 99% of patients on day 10. Peak levels of anti- dengue IgM occurred in the second week after the onset of the disease and usually decline to undetectable levels in 2-3 months later. While IgG anti- dengue generally detected at low titers at the end of the first week of fever, increased slowly, and the levels remained undetectable after a few months, and possibly for life. In secondary dengue virus infection, antibody titers increased rapidly. Immunoglobulin dominant isotope IgG anti- dengue is detected at high levels in the early phase of dengue infection and last up to 10 months or even a lifetime (Gubler 1996, World Health Organization 1997).

In this research, serology examination IgM and IgG anti- dengue fever day 5, to confirm the diagnosis of dengue infection. Of the 30 samples of the research showed that the serological test results of anti - dengue IgG positive was found in 19 patients (63.33%), the 8 patients in the group without *Monascus purpureus* and 11 patients in the group of *Monascus purpureus*. There were no patients with serological anti- dengue IgM negative. In this research, the percentage incidence of dengue infection due to secondary infections (63.33%) more than the primary infection (36.67%). Similar results were obtained in studies by Krishnamurti et al (2002) with 87% of cases of dengue fever due to secondary infections and 13% of cases are caused by primary infection. This shows that the percentage incidence of dengue is more common in secondary infections, according to the results shown in this research.

The results of this research indicate the percentage of patients with DHF grade 1 by 50% and DHF grade 2 by 50%. In the control group the proportion of subjects with DHF grade 1 and grade 2 respectively by 60% and

40%, while the treatment group, respectively 40% and 60%. There is no significant difference between the control group and the treatment group ($p = 0.281$), so it can be said that the severity of disease between the two groups was not significant.

DISCUSSION

TPO level before and after giving standard therapy of dengue infection with or without *Monascus purpureus* extract.

TPO levels obtained early in the control group was 130 (min - max: 25.2 to 801.1) pg/ml, while in the treated group by 90 (min - max: 15.4 to 979.4) pg/ml. From the results of the statistical analysis were not significantly different initial TPO levels in both research groups with $p = 0.351$. This suggested that the initial TPO levels in the two groups did not differ significantly. TPO levels in the control group before therapy without *Monascus purpureus* is 130 (min - max: 25.2 to 801.1) pg/ml, whereas after therapy without *Monascus purpureus* is 66.8 (min - max: 31.4 - 233.7) pg/ml. From the results of statistical analyzes found no significant differences in TPO levels before and after therapy without *Monascus purpureus* with $p = 0.089$.

Matondang et al (2004) in a research of patients with dengue infection were given standard therapy dengue infection showed a decrease in mean levels of TPO 395 pg/ml to 153.6 pg/ml, TPO levels started to decline on day 7 when the healing phase of dengue infection and reached normal levels (TPO levels below 50 pg/ml) on day 8 to day-to - 9 disease journey (Matondang et al 2004). Other findings in the research Matondang et al (2004) there is a negative correlation between platelet counts and TPO levels ($p = 0.000$) and TPO levels were correlated with the severity of thrombocytopenia in dengue infection. Similar results were also obtained in the research by Cardier et al (2006) which examines the role of TPO in dengue infection. Cardier et al, suggests that TPO is a cytokine that plays a role in the process trombopoiesis and thrombocytopenia associated with severe dengue infection. Decreased levels of TPO on dengue infection may be the natural history of the disease and associated with platelet count and the presence of an inflammatory process.

TPO levels in the treatment group before therapy *Monascus purpureus* is 90 (min - max: 15.4 to 979.4) pg/ml, whereas after administration of *Monascus purpureus* therapy was 26.6 (min - max: 6.6 to 321.7) pg/ml. From the results of statistical analyzes found significant differences in TPO levels before and after therapy *Monascus purpureus* with $p = 0.033$. So far not

obtained or the research literature that mentions the effect of *Monascus purpureus* extract against TPO levels in patients with dengue infection, this research is the first research ever conducted. Inflammatory process would increase the release of IL - 6 from macrophages or monocytes which in turn will affect the production of TPO in the liver. Thrombocytopenia conditions will also increase the levels of TPO in the blood because the bond between the TPO and its receptor (c - Mpl) on the wall of platelets is reduced. Increased levels of TPO will trigger the process trombopoiesis megakariopoiesis and bone marrow. After an increase in the number of platelets will be feedback mechanisms that will reduce levels of TPO (von dem Borne et al 1998, Kaushansky 2005).

The results in this research indicated a significant decrease in the amount of TPO in the treatment group ($p = 0.033$) which was consistent with an increase in the number of platelets. This was due to the effect of *Monascus purpureus* extract in patients with DHF which could influence an increase in the number of platelets through antiinflammatory effects or effects on processes and trombopoiesis megakariopoiesis the bone marrow that affects subsequent levels of TPO. Comparison between TPO group level changes with *Monascus purpureus* extract and TPO group level changes without *Monascus purpureus* extract

In the second test group, both treatment groups and the control group there were changes in TPO levels decrease respectively by 63.4 pg/ml (mean: 102.55 ± 168.42 pg/ml) and 63.2 pg/ml (mean: 202.13 ± 95.35 pg/ml). Statistical analysis of the results obtained greater reduction in TPO levels in the treatment group compared with the control group, but not statistically significant ($p = 0.916$).

Monascus purpureus contains monacolin K, which has a structural similarity with lovastatin and allegedly had the effect of statins resembles, in addition to other substances such as dimerumic acid (antioxidant), as well as metabolites ankavlafin and monascin which have anti-inflammatory effects (Lin et al 2011). *Monascus purpureus* extract on previous research shown to increase platelet counts presumably through effects on increasing megakariopoiesis and trombopoiesis in bone marrow or anti-inflammatory effects of metabolites (Tisnadaja 2006, Muharni et al 2011). The content of lovastatin on *Monascus purpureus*, can decrease the expression and function of proinflammatory cytokines IL - 6, IFN, and TNF in macrophages and decrease the formation of Reactive Oxygen Species (ROS) intracellular (Schönbeck & Libby 2004, Lin et al 2011, Arunachalam & Narmadhapriya 2011). But so far there

has been no data on the effect of statins on the production of TPO.

Based on existing literature, this was the first research examining the effects of *Monascus purpureus* extract on TPO levels and in this research obtained decreased levels of TPO changes in the treatment group was higher than the control group, but not statistically significant. The results of this research were expected to bring more information about the effect of *Monascus purpureus* extract in the treatment of dengue infection and might be an additional literature for further research.

The results of the evaluation of the *Monascus purpureus* extract effect

The results in this research demonstrate the effect of *Monascus purpureus* adversi extract obtained in the group treated dyspeptic complaints occurred in 5 patients (33.33%), not available dizziness complaints, complaints of headache was found in 3 patients (20%), and no symptoms are side effects other serious. Whereas in the control group dyspepsia found in 4 patients (26.67%) and headache in 2 patients (13.33%). There were no significant differences dyspeptic complaints ($p = 0.690$) and headache ($p = 0.624$) in both test groups.

Complaints and these symptoms can be caused not only by the effects of extract of *Monascus purpureus* but can also be a clinical symptom commonly found in patients with dengue infection (Erdogrul & Azirak 2004, Guglani & Kabra 2005). Analysis to compare with the control group. The analysis showed no significant differences found between the two test groups so that the symptoms that arise may still be a part manifestation of dengue infection.

Several limitations of the research that could affect the results of the research are firstly, this research was conducted in patients with dengue infection based on clinical and laboratory criteria of World Health Organization (1997). Diagnosis confirmation should be made early like dengue NS1 antigen test in order to get dengue infection diagnosis more quickly and accurately. Secondly, several confounding variables were not examined and measured in this research, such as viral factors (serotype, genotype), host factors (immunity, nutrition, and race). Thirdly, TPO level ideally was examined with shorter intervals and longer time until the healing phase. Material shortages and funding were the barriers of the examination.

CONCLUSION

Based on these results it can be concluded that firstly, in this research, the mean age of patients is twenty-seven years, the proportion of male patients is larger, the mean of days of fever in the hospital is on the fourth day, the proportion of secondary dengue infection is greater, the mean of initial platelet is about sixty three thousand. No statistically significant differences in the characteristics of the research subjects in both test groups. Secondly, TPO levels before and after giving *Monascus purpureus* extract decreases in *Monascus purpureus* extract group and the control group. Changes in TPO level decreasing in the group of patients with dengue infection given *Monascus purpureus* extract are greater compared with the control group but was not statistically significant.

ACKNOWLEDGMENTS

The author thanks to Laurentius Andre for his help during the making of this manuscript's lay-out.

REFERENCES

- Arunachalam C and Narmadhapriya D (2011). *Monascus* fermented rice and its beneficial aspects: a new review. *Asian Journal of Pharmaceutical and Clinical Research* 4, 29-31
- Cardier JE, Balogh V, Perez-Silva C, Romano E, Rivas B, Bosch N, Rothman AL (2006). Relationship of thrombopoietin and interleukin-11 levels to thrombocytopenia associated with dengue disease. *Cytokine* 34, 155-160
- Carlos CC, Oishi K, Cinco MT, Mapua CA, Inoue S, Cruz DJ, Pancho MA, Tanig CZ, Matias RR, Morita K, Natividad FF, Igarashi A, Nagatake T (2005). Comparison of clinical features and hematologic abnormalities between dengue fever and dengue hemorrhagic fever among children in the Philippines. *Am J Trop Med Hyg* 73, 435-440
- Danuri H (2008). Optimizing angkak pigments and lovastatin production by *Monascus purpureus*. *Hayati Journal of Biosciences* 15, 61-66
- Erdogrul O and Azirak S (2004). Review of the studies on the red yeast rice (*Monascus purpureus*). *Turkish Electronic J of Biotechnology* 2, 37-49
- Funahara Y, Ogawa K, Fujita N, Okuno Y (1987). Three possible triggers to induce thrombocytopenia in dengue virus infection. *Southeast Asian J Trop Med Public Health* 18, 351-355
- Gibbons RV and Vaughn DW (2002). Dengue: an escalating problem. *BMJ* 324, 1563-1566
- Gomber S, Ramachandran VG, Kumar S, Agarwal KN, Gupta P, Gupta P, Dewan DK (2001). Hematological

- observations as diagnostic markers in dengue hemorrhagic fever. A reappraisal. *Indian Pediatr* 38, 477-481
- Gubler DJ (1996). Serologic diagnosis of dengue and dengue haemorrhagic fever. *Dengue Bulletin* 20, 20-23
- Guglani L and Kabra SK (2005). T cell immunopathogenesis of dengue virus infection. *Dengue Bulletin* 29, 58-69
- Halstead SB (2008). Pathogenesis: risk factor prior to infection. In: Halstead SB (ed). *Dengue, Tropical Medicine Science and Practice*, London, Imperial College Press, p 219-256
- Kaushansky K (2005). The molecular mechanisms that control thrombopoiesis. *J Clin Invest* 115, 3339-3347
- Khorshed A, Abbas A, Kawy SA, Kholoussi N, El-Ghoroury EA, Salam HA (2007). Role of thrombopoietin in megakaryopoiesis and thrombopoiesis with relation to platelets ultrastructure. *J Med Sci* 7, 179-186
- Kittigul L, Pitakarnjanakul P, Sujirarat D, Siripanichgon K (2007). The differences of clinical manifestations and laboratory findings in children and adults with dengue virus infection. *J Clin Virol* 39, 76-81
- Krishnamurti C, Peat RA, Cutting MA, Rothwell SW (2002). Platelet adhesion to dengue-2 virus-infected endothelial cells. *Am J Trop Med Hyg* 66, 435-441
- La Russa VF and Innis BL (1995). Mechanisms of dengue virus-induced bone marrow suppression. *Baillieres Clin Haematol* 8, 249-270
- Lee IK, Liu JW, Yang KD (2005). Clinical characteristics and risk factors for concurrent bacteremia in adults with dengue hemorrhagic fever. *Am J Trop Med Hyg* 72, 221-226
- Lin CP, Lin YL, Huang PH, Tsai HS, Chen YH (2011). Inhibition of endothelial adhesion molecule expression by *Monascus purpureus*-fermented rice metabolites, monacolin K, ankaflavin, and monascin. *J Sci Food Agric* 91, 1751-1758
- Matondang AV, Widodo D, Zulkarnain I, Rengganis I, Trihandini I, Inada K, Endo S, Suhendro (2004). The correlation between thrombopoietin and platelet count in adult dengue viral infection patients. *Acta Med Indones* 36, 62-69
- Muharni S, Almahdy A, Martini RD (2011). Effect of the use of supplements *PSIDII folium* extract (*Psidium guajava* linn.) and red fermented rice (*Monascus purpureus*) in increase of thrombocytes at Dengue Hemorrhagic Fever (DHF) science in the installation of disease in hospital dr. M. Djamil Padang. Available from <http://pasca.unand.ac.id/id/wp-content/uploads/2011/09/ARTIKEL10.pdf>. Accessed March 20, 2012
- Murgue B, Cassar O, Guigon M, Chungue E (1997). Dengue virus inhibits human hematopoietic progenitor growth in vitro. *J Infect Dis* 175, 1497-1501
- Nasronudin (2007). Imunopatofisiologi infeksi virus dengue. In: Nasronudin, Hadi U, Vitanata M, Erwin A, Soewandjojo E, Soeharto (eds). *Penyakit Infeksi di Indonesia*, Surabaya, Universitas Airlangga Press, p 40-56
- Navarrete-Espinosa J, Gómez-Dantés H, Celis-Quintal JG, Vázquez-Martínez JL (2005). Clinical profile of dengue hemorrhagic fever cases in Mexico. *Salud Publica Mex* 47, 193-200
- Schönbeck U and Libby P (2004). Inflammation, immunity, and HMG-CoA reductase inhibitors: statins as antiinflammatory agents? *Circulation* 109, II18-II26
- Thomas L, Verlaeten O, Cabié A, Kaidomar S, Moravie V, Martial J, Najioullah F, Plumelle Y, Fonteau C, Dussart P, Césaire R (2008). Influence of the dengue serotype, previous dengue infection, and plasma viral load on clinical presentation and outcome during a dengue-2 and dengue-4 co-epidemic. *Am J Trop Med Hyg* 78, 990-998
- Tisnadjaja D (2006). *Bebas Kolesterol dan Demam Berdarah dengan Angkak*, Jakarta, Penerbit Penebar Swadaya, p 56-62
- Villar-Centeno LA, Díaz-Quijano FA, Martínez-Vega RA (2008). Biochemical alterations as markers of dengue hemorrhagic fever. *Am J Trop Med Hyg* 78, 370-374
- von dem Borne AE, Folman C, Linthorst GE, Porcelijn L, van den Oudenrijn S, van der Schoot E, de Haas M (1998). Thrombopoietin and its receptor: structure, function and role in the regulation of platelet production. *Baillieres Clin Haematol* 11, 409-426
- World Health Organization (1997). *Dengue Haemorrhagic Fever Diagnosis, Treatment, Prevention and Control*, 2nd ed., Geneva, World Health Organization, p 17-27
- World Health Organization and Special Programme for Research and Training in Tropical Diseases (2009). *Dengue Guidelines for Diagnosis, Treatment, Prevention and Control*, New Edition, France, World Health Organization, p 91-108