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DIFFERENCE OF ANGIOPOIETIN-2 LEVEL IN DENGUE HEMORRHAGIC FEVER WITH AND WITHOUT SHOCK

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ABSTRACT

Purpose: *Dengue infection is one of health sector problem in Indonesia, as the incidence and mortality rate due to dengue infection in Indonesia has escalated over the years. The purpose of this study is to analyse the difference of angiopoietin-2 level in dengue hemorrhagic fever with and without shock*

Methods: *One hundred and ten children with dengue hemorrhagic fever were recruited into this study. We examine the clinical manifestation, laboratory parameter (platelet, hematocrit, AST and ALT) and molecular test (Ang-2 and sVE-Cadherin). The molecular indicator was tested by Enzyme-Linked Immunosorbent Assay (ELISA).Data were collected between July 2016 until June 2018. This is an observational study with cross-sectional comparative design in children with dengue virus infection in RSUP DR M Djamil Padang. The data obtained were analyzed using a computer system in the form of tables and graphs.*

Results: *In our study we have found correlation of clinical symptoms (mucosal bleeding, abdominal pain, persistent vomiting, and hepatomegaly), hematocrit levels, and decreased platelet levels between DHF with shock compared to DHF without shock. Interestingly, there is a significant difference in the average rate of Ang-2 between DHF patients with shock compared to DHF without shock($p < 0.05$). However, there was no difference in the average rate of sVE-Cadherin. The average levels of Ang-2 in DHF patients with shock is 739,66±55,21 pg/ml while in DHF alone is 497,90±220,68 pg/ml.*

Conclusions: *There was a difference in the average level of Ang-2 between DHF patients with shock and without shock*

KEYWORDS: *Dengue Haemorrhagic Fever, Children, Ang-2, Sve-Chaderin*

Article History

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INTRODUCTION

Dengue infection is one of health sector problem in Indonesia, as the incidence and mortality rate due to dengue infection in Indonesia has escalated over the years and is still very high. In 2005, Indonesia became the highest contributor to dengue infection in Southeast Asia (53%) with a total of 95,270 people and 1,298 deaths. According to the Ministry of Health of the Republic of Indonesia in 2015 there were 126.675 cases of DHF with deaths 0,9%.[1] The incidence rate of

dengue virus infection in West Sumatra was 69,16% in 20105 with case fatality rate 0,61%. The incidence of dengue hemorrhagic fever in RSUP M. Djamil Padang in 2007 reached 259 cases during 1 year with shock incidents of 46%.[2]

Dengue virus infection is an infectious disease caused by dengue virus and is transmitted by the Aedes aegypti mosquito. Dengue virus (DEN-V) consists of 4 serotypes namely DEN-1, DEN-2, DEN-3, and DEN-4 (Ministry of Health, 2009). The study of Megariani et al. in Padang city, by using RT-PCR, it has been found that infection by DEN-2 was the highest case of 46.15%, followed by DEN-1 and DEN-4, each 15.38% respectively. Infection by DEN-3 was found to be 11.54%.[3]

The most feared complication of DHF is death. Death in children is caused by hypovolemic shock due to plasma leakage from intravascular to extravascular space due to endothelial dysfunction. The severity of symptoms caused by dengue virus infection is related to the severity of plasma leakage.[4-5]

There is no theory to date that can explain DHF completely since there is no animal model that can fully show reactions and symptoms as in humans after infected by the dengue virus.[6] Various theories such as virulence theory, viral load, antibody dependent enhancement (ADE), innate immunity, T-cell mediated apoptosis, cytokine storms, autoimmune and genetic have been suggested by experts. There are three systems in the body that are most involved in the pathogenesis of DHF, namely the immune system, liver, and vascular capillary endothelium.[7-11]

In dengue infection the liver is also involved. Liver cell damage is due to inflammatory process and the formation of antigen-antibody complexes, also by the pathogenesis of dengue infection. Infected hepatocytes increase the activity of AST and ALT, release VEGF and reduce the synthesis of clotting factors.[12-14] Manifestations of bleeding, enlargement of the liver and increase in AST and ALT were significantly more common in the DSS group than in the DHF group.[15-16]

Hepatomegaly in dengue infection results from the direct influence of the dengue virus on the liver. Data from previous study also shows that hepatomegaly plays a role as a risk factor for severe dengue virus infection.[17] Angiopoeitin-1 and Angiopoeitin-2 maintain vascular function and integrity. Angiopoietin-1 is reported to not only play a role in vascular stabilization during angiogenesis but also plays a role in inhibiting the increaseof vascular permeability and has anti-inflammatory effects.[18] The Ang-1 m-RNA molecule expressed on periendothelial cells plays a role in maintaining vascular stability, inhibits plasma leakage, suppresses expression of inflammatory genes and prevents recruitment and migration of leukocytes. Unlike Ang-1, Ang-2 which is selectively expressed in endothelial cells when binding to the Tie 2 receptor will result in signaling disorders of Ang-1/Tie 2 making disruption on vascular permeability.[19-20] Based on the description of the background, the researchers wanted to find out whether there was a difference of Angiopoietin-2 level in dengue hemorrhagic fever with shock and without shock.

METHODS

This study is an observational study with a comparative cross-sectional design. Blood samples were collected in the Pediatric Division of RS Dr M Djamil Padang. Routine blood tests, AST, ALT, IgG and IgM anti-dengue were tested at the Clinical Pathology laboratory of RS Dr. M Djamil Padang. Angiopoietin-2 examination was carried out in the Biomedical laboratory of the Medical Faculty of Andalas University, Padang. The study was conducted for 1 year.

The study population was patients with dengue virus infection (DHF and DSS) who were hospitalized at RSUP Dr. M. Djamil Padang according to WHO 2011 criteria. Subjects were part of the population that met the inclusion and exclusion criteria. The inclusion criteria were patients with dengue hemorrhagic fever who had received informed consent

from parents to participate in the study. Exclusion criteria are patients suffering from other viral or bacterial infections based on clinical and laboratory examinations, receiving corticosteroid therapy, malnourished, and obese.

The data obtained were analyzed using a computer system in the form of tables and graphs. Bivariate analysis was carried out to see the difference between the day of fever, hematocrit, platelet, AST, ALT and Ang-2 of the DHF patients with and without shock. First the data is analyzed using normality test to determine the normality of the data using the Shapiro Wilk test (n <50), then followed by bivariate analysis, if the data is normally distributed then the analysis is carried out using the dependent t test, but if it is known not normally distributed then Mann-Whitney test was done with confident interval (CI) 95% and $\alpha = 0.05$. The conclusion of the test results if the value of $p \leq 0.05$ then H_0 is rejected, meaning that there is a difference in the mean between the independent variables and the dependent variable.

Results

Subjects Characteristic

The study was conducted in the Children's Ward of Dr. RSUP M. Djamil Padang, Biomedical Laboratory in Medical Faculty of Unand and the central laboratory of RSUP Dr. M. Djamil Padang. The collected data is as many as 110 samples.

The characteristics of the research subjects are presented in table 1 below.

Table 1: Characteristics of the Study Subject

Characteristics	Dengue Infection		P Value
	DSS (n=62)	DHF (n=48)	
Sex,n(%) Male Female	30 (48,4) 32 (51,6)	21 (43,8) 27 (56,3)	0,771
Age (years), mean±SD	6,56±3,29	6,94±3,61	0,573
Nutritional status,n(%) Undernourished Normal Overweight	14 (22,6) 33 (53,2) 15 (24,2)	15 (31,3) 30 (62,5) 3 (6,3)	0,039*

***p<0,05 is Significant**

Table 1 is known to have no difference of sex and age between DHF and shock compared to DHF without shock (p>0.05). However, there was a difference of nutritional status between DHF with shock compared to DHF without shock (p <0.05).

The Difference of Clinical Symptoms (Duration of Fever, Mucosal Bleeding, Abdominal Pain, Persistent Vomiting And Hepatomegaly) between DHF with Shock Compared to Without Shock

The difference of clinical symptoms (duration of fever, mucosal bleeding, abdominal pain, persistent vomiting and hepatomegaly) in DHF with shock and without shock can be seen as follows.

Table 2: Difference of Clinical Symptoms (Duration of Fever, Mucosal Bleeding, Abdominal Pain, Persistent Vomiting and Hepatomegaly) in DHF with Shock and without Shock

Variables	Dengue Infection		P Value
	DSS (n=62)	DHF (n=48)	
Duration of fever, n(%) ≥ 4 days < 4 days	56 (90,3) 6 (9,7)	39 (81,3) 9 (18,8)	0,274
Mucosal bleeding, n(%) Yes No	17 (27,4) 45 (72,6)	1 (2,1) 47 (97,9)	0,001*
Abdominal pain, n(%) Yes No	47 (75,8) 15 (24,2)	4 (8,3) 44 (91,7)	0,000
Persistent vomit, n(%) Yes No	40 (64,5) 22 (35,5)	5 (10,4) 43 (89,6)	0,000*
Hepatomegaly, n(%) Hepatomegaly Normal	50 (80,6) 12 (19,4)	8 (16,7) 40 (83,3)	0,000*

***p<0,05 Significant**

Table 2 shows that there is no difference of duration of fever with the occurrence DHF with shock (p> 0.05). However, there is a difference of mucosal bleeding, abdominal pain, persistent vomiting and hepatomegaly in DHF with shock (p <0.05).

A Difference of Laboratory Results (Hematocrit, Thrombocyte, AST, ALT) Levels in DHF with Shock and Without Shock

A difference of laboratory results (hematocrit, thrombocyte, AST, ALT) levels in DHF with shock and without shock can be seen as follows

Table 3: Difference of Laboratory Results (Hematocrit, Thrombocyte, AST, ALT) Levels in DHF With Shock and without Shock

Variables	Dengue Infection		P Value
	DSS (n=62)	DHF (n=48)	
Hematocrit, n(%) > 40% ≤ 40%	54 (87,1) 8 (12,9)	30 (62,5) 18 (37,5)	0,005*
Platelets, n(%) < 50.000/mm^3 ≥ 50.000/mm^3	52 (83,9) 10 (16,1)	5 (10,4) 43 (89,6)	0,000*
AST,n(%) ≥ 48 U/L < 48 U/L	54 (87,1) 8 (12,9)	35 (72,9) 13 (27,1)	0,103
ALT,n(%) ≥ 30 U/L < 30 U/L	44 (71,0) 18 (29,0)	26 (54,2) 22 (45,8)	0,106

***p<0,05 Significant**

Table 3 showed that there was no difference of AST and ALTlevels in DHF with shock and without shock (p> 0.05). However, there was a platelet and hematocrit difference in DHF with shock and without shock (p <0.05).

A Difference of Angiopoietin-2 in Dengue Hemorrhagic Fever with and Without Shock

The difference between the results of Ang-2 examination between DHF patients with shock compared to those without shock can be seen as follows.

Table 4: The difference of the Results of Ang-2 Examination between DHF Patients with Shock Compared to those without Shock

Variable	Dengue Infection		P Value
	DSS (n=62) mean±SD	DHF (n=48) mean±SD	
Ang-2 (pg/ml)	739,66±55,21	497,90±220,68	0,036*

Table 4 shows the mean level of Ang-2 in DHF patients with shock is 739.66 ± 55.21 pg/ml while DHF alone is 497.90 ± 220.68 pg/ml. The results of statistical tests revealed that there were differences in the average levels of Ang-2 among DHF patients with shock compared to those without shock ($p < 0.05$). Mean sVE-Cadherin levels in DHF patients with shock is 5.93 ± 4.87 ng/ml while DHF alone is 5.86 ± 4.811 ng/ml. The statistical test results revealed that there was no difference in mean sVE-Cadherin levels between DHF patients and shock compared to those without shock ($p < 0.05$).

DISCUSSIONS

Characteristics of Research Subjects

The characteristics of the research subjects in the form of gender and age group showed there were no significant differences between the groups with shock and without shock while the nutritional status showed significant differences. This study found that girls had more DHF than men, and there was no relationship between dengue virus infection and gender, as well as the severity. This is similar to the research by Sumarmoin 2002 which stated that there is no gender difference in DHF patients. Research in the Directorate General of PP & PL of the Ministry of Health of the Republic of Indonesia in 2008 also showed the same results where there were no sex differences in children suffering from dengue virus infection.[21-22]

Age of 6 years old is the average age obtained in this study. This is in accordance with the study of Sumarmo which found that the most DHF patients in Indonesia were aged 5-11 years and Mayetti's study also found that the age of 5-10 years was the group most suffering from dengue compared to other age groups.[2,21] Similar to this study, there were no age differences that were statistically significant between the three groups of the disease severity.

The proportion of patients with dengue virus infection in this study was more in the case of good nutritional status rather than the under nutrition status and overweight. Overall, the number of well-nourished patients were seen more in severe disease (DSS) than the undernourished and overweight patients, thus there was a statistically significant difference between nutritional status and the severity of the disease ($p < 0.05$). The meta-analysis study conducted by Trang et al. stated that the incidence of DHF is more common in immune competent and well-nourished children, but there is no significant difference between nutritional status and the incidence of dengue virus infection. Until now, the relationship of nutritional status with dengue virus infection is still in debate and requires further research.[23] Research by Setiati et al. found that DHF and DSS rarely occur in malnourished children. A strong inflammatory response is one of the factors causing DHF and DSS.[24] Research by Maron et al. found that lack of nutrition can suppress immune responses mediated by cells, thereby reducing the risk of dengue virus infection.[25]

A Difference of Clinical Symptoms (Duration of Fever, Mucosal Bleeding, Abdominal Pain, Persistent Vomiting and Hepatomegaly) in Dengue with Shock and Without Shock

There are no significant differences in the duration of fever and the occurrence of DHF with shock, while there is a significant difference between mucosal bleeding, abdominal pain, persistent vomiting and hepatomegaly with the occurrence of DHF with shock. Fever is found in all patients with DHF either with shock or without shock.[26] Research in India found 100% of the DHF patients had a fever but 45% of them were found at the first hospital admission.[27]

Vasculopathy, platelet deficiency and dysfunction, and defects in the coagulation pathway are some of the factors causing bleeding. Decreased platelet production and increased platelet damage can cause thrombocytopenia in DHF. Disorders of platelet function cause blood vessels to become brittle and cause bleeding.[28] Choudhury et al found 55% of bleeding cases.[27]Nagaramfoundmucosal bleeding in 24% of cases in children with DSS and 1.7% in children with DHF.[26] In addition to fever, abdominal pain and vomiting is also a symptom that is often found in DHF and is a warning sign in dengue cases. Abdullah et al found that there was a significant relationship between persistent vomiting, fluid accumulation and mucosal bleeding with the severity of dengue infection and had a high sensitivity and specificity in predicting the occurrence of severe dengue infection.[29]

Nagaram found 73 cases with complaints of abdominal pain and 115 cases with vomiting. In DHF patients there were 32.8% cases of abdominal pain and 60.4% of cases of vomiting while in DSS patients there were 96% cases of abdominal pain and 100% of cases of vomiting. Research conducted by researchers also found that there was a relationship between abdominal pain and vomiting with the occurrence of DHF with shock. Although dengue virus is a non-hepatotropic infection, liver injury often occurs, ranging from mild dysfunction to increasing liver enzymes manifested with jaundice and even fulminant liver failure.[26]Bilquiss et al's research in 2012 found that AST levels were higher than ALT levels. Hepatomegaly was found in all DHF and DSS patients, with the most severe damage to the liver found on DSS. The study found 100% DSS patients had increased AST levels >5 times while DHF patients in 61.5% case.[30]

The Nagaram study obtained 100% hepatomegaly condition in the DSS case group and 77% in the DHF group.[26] Zhang et al.'s (2014) study found that hepatomegaly in children with dengue infection had a 5 times greater risk of death compared to children infected with dengue without the discovery of hepatomegaly.[31]Yulianto et al research in 2016 included 188 patients who met the study criteria, 56 (30%) had DD diagnoses, 58 (31%) DHF and 74 (39%) DSS.[32] Multivariate analysis showed abdominal pain (OR 5.06, 95% CI; 1.72; 14.87). From the review above compared to this study, there was a difference of mucosal bleeding, abdominal pain, persistent vomiting and hepatomegaly in DHF with shock ($p < 0.05$).

A Difference of Laboratory Results (Hematocrit, Thrombocyte, AST, ALT) Levels in DHF with Shock and without Shock

Hematocrit, platelet, AST and ALT levels will change according to the severity of dengue virus infection. The more severe dengue virus infection that occurs, the more increase of hematocrit, AST and ALT levels and the decrease of platelet levels that are statistically significant will occur. This study found a significant difference between hematocrit and platelet levels between the two groups of DHF with or without shock. Differences in hematocrit levels that indicate significant plasma leakage occurs in the more severe disease group which causes the risk of hypovolemic shock. Hematological disorders in patients infected with the dengue virus will change starting on the third day of fever and will return to normal on day 11.[21] The hematocrit level is the concentration (showed in percentage) of erythrocytes in 100 mL of

blood. The hematocrit value will increase (hemoconcentration) due to an increase in blood cell or a decrease in blood plasma volume. Conversely, the hematocrit value will decrease (hemodilution) due to the decline of blood cells or an increase in blood plasma levels.[33] The hematocrit value> 40% is found more in DSS patients, about 44% of cases, while the <40% hematocrit value is more common in DHF patients, about 76% of cases. Research conducted by researchers obtained the same results as Nagaram's research in India where obtained <40% more hematocrit values were found in DHF patients, while hematocrit values were> 40% in DSS patients. In this study, platelets were found to be lower in the DSS group than in the DHF group.[26]

The pathophysiology of thrombocytopenia in DHF cases is caused by depression of the bone marrow, destruction of platelets and increased consumption of platelets from interactions between platelets and endothelial cells damaged by dengue virus infection.[34] In patients with DSS platelets <50,000 were obtained as much as 53.5% of cases with p-value <0.005.[26] Ralapanawa et al. obtained platelet cut-off of 105,000 to predict the progress of patients to DHF with a sensitivity of 80% and specificity of 50%. Ralapanawa et al. found platelet levels decreased starting on the fifth day in DHF patients who experience shock.[35] Valentino et al. concluded that there was a significant relationship between the number of platelets and leukocytes with the clinical degree of dengue infection, so that the number of platelets and leukocytes could be considered to help determine the clinical degree of dengue infection.[36] Similar to research conducted by researchers wherein DHF patients with shock platelet level <50,000 in as many as 83.9% of cases, there was a significant difference of platelets and hematocrit in DHF with shock compared to DHF without shock (p <0.05).

Increased of AST and ALT were found more in DHF patients with shock than without shock, but there were no significant differences. Hepatomegaly and liver dysfunction are more frequent and more severe in DSS than in DHF. Fadilah's study confirmed that T lymphocytes were activated in vivo as a response to dengue virus infection and the activation rate was higher in DSS than DHF but there was no significant relationship between T lymphocytes and AST or ALT serum values in DHF cases. This is due to several factors, changes in lymphocyte T liver enzyme levels do not occur simultaneously, reduced T lymphocytes begin 3 days after the onset of fever, where transaminase levels usually increase up to 9 days after the onset of fever and gradually return to normal values in 2 week.[37] In line with the recovery of the disease, liver function will show improvement, in DHF will return to normal values, while in DSS will also show improvement even if it does not reach normal numbers.[38]

The Difference in the Results of Ang-2 Examination in Dengue Patients with Shock and without Shock

Hematological parameters are considered as potential predictors, one of which is Angiopoietin. This study obtained Ang-2 levels of DHF patients with higher shock than those without shock (p<0.05). This indicates that the higher the level of Ang-2, the more severe the disease due to dengue virus infection. The main complications of dengue infection mainly affect the vascular system, including plasma leakage which will cause hypovolemic shock and potentially turn to become a DSS. Angiopoietin can be considered a potential predictor for DSS in patients suffering from dengue infection. This is in accordance with the research conducted by Rampengan et al. who found that an increase in Ang-2 levels could trigger vascular instability, causing an increase in vascular permeability and plasma leakage. The study found an increase in Ang-2 levels in DSS patients compared to DHF patients and dengue fever.[39]

The mechanism for increasing Ang-2 levels and plasma leakage is a complex cascade. Binding of Ang-1 with the Tie-2 receptor via the phophatidylinositol-3-kinase (PI3K) AKT pathway will cause stability of blood vessels by recruiting pericyte into new blood vessels and maintaining intercellular contact. Ang-1 also has anti-inflammatory properties by

regulating surface molecular adhesion. The binding of Ang-2 to the Tie-2 receptor can inhibit Ang-1 signaling to Tie-2 and increase blood vessel instability.[40] Mitchel et al. in 2012 also found that DSS and DHF patients also had higher serum Ang-2 levels at the time of initial treatment than when they discharged and even when compared to the control group. A release of Ang-2 from WPB is caused by activation of endothelial cells by proinflammatory cytokines, direct interaction of the virus with endothelial cells, the release of mast cell products and procoagulant factors such as thrombin.[41]

Literature that has written normal levels of Ang-2 in children as far as the knowledge of researchers has not yet existed. Research by Rampengan et al found the highest Ang-2 levels in DSS compared to DHF and dengue fever with results of 2,486.21 pg/dl in dengue fever, 3,194.95 pg/dl in DHF and 4,005.32 pg/dl on DSS. In the research conducted by researchers concluded that if the level of Ang-2 $\geq$ 427.56 pg/ml, then the subject will experience DSS.[39]

Increased levels of Ang-2 are also associated with death in sepsis. David et al found that Ang-2 concentrations have been reported as predictors of mortality in critically ill patients. Ang-2 cut-off value of 2.86 ng/mL predicts the development of severe sepsis with a sensitivity of 75% and specificity of 56%, the cut-off value of Ang-2 3.56 ng/mL predicts the development of septic shock with a sensitivity of 73% and specificity 68%, and the Ang-2 cut-off value of 5.1 ng/mL predicted mortality with 80% sensitivity and 76% specificity. This study is important since it can estimate the level of Ang-2 as a biomarker in clinical practice so that it can be used in clinical decision making regarding prognosis.[42]

CONCLUSIONS

There is a significant difference in mucosal bleeding, abdominal pain, persistent vomiting, and hepatomegaly in DHF with shock compared to DHF without shock. There was a significant difference between hematocrit and decreased of platelet in DHF with shock compared to without shock but there was no difference in AST and ALTlevel in DHF patients with shock and DHF without shock. There is a difference in the average rate of Ang-2 among DHF patients with shock compared to without shock.

REFERENCES

1. *Pusat Data danInformasiKementerianKesehatan Indonesia: Situasi DBD. 2016.*
2. *Mayetti. Hubungangambaranklinisdanlaboratoriumsebagaifaktorrisikosyokpadademamberdarah dengue.Sari Pediatri.2010;11:367-373.*
3. *Megariani, Mariko R, Alkamar A, Eka Putra A. Ujidiagnostikpemeriksaan antigen nonstrukturaluntukdeteksidiniinfeksi virus dengue padaanak. Sari Pediatri. 2014;12:121-127.*
4. *Gubler DJ. Dengue and dengue haemorrhagic fever. Clin Microbial Rev. 1998;11(3):480-496.*
5. *Gubler DJ. Epidemic dengue/dengue haemorrhagic feveras a public health, social and economic problem in the 21 st century. Trends Microbiol. 2002;10(2):100-103.*
6. *Kyle JL, Beatty PR, Harris E. Dengue virus infect machrophages and dendritic cells in a mouse model of infection. J.Iinfect.Dis. 2007;195(12):1808-1817.*
7. *Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemorraghic fever. CurrOpinInfect.Dis. 2006;19(5),429-436.*

8. *Clyde K, Kyle JL, Harris E. Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. J.Virol. 2006;80(23):11418-11431.*
9. *Lei HY, Yeh TM, Liu HS, Lin YS, Chen SH, Liu CC. (2001).Immunophatogenesis of dengue virus infection. J. Biomed Sci. 2001;8(5):377-388.*
10. *KuraneI. Dengue hemorrhagic fever with special emphasis on immunopathogenesis. Comp Immunol Microbial Infect Dis. 2007;30(5-6):329-340.*
11. *Noisakran S, Perng GC. Alternate hypothesis on the pathogenesis of dengue hemorraghic fever (DHF)/dengue syok syndrome (DSS) in dengue viral infection. Exp Bio Med. 2008;233(4):401-408.*
12. *Pancharoen C, Rungasarannont A, Thisyakorn U. Hepatic dysfunction in dengue patients with various severity. J Med Assoc Thai. 2002;85(1):S298-301.*
13. *Lin CF, Wan SW, Chen MC, Lin SC, Cheng CC, Chiu SC, et al. Liver injury caused by antibodies against dengue virus nonstructural protein 1 in a murine model. Lab Invest. 2008;88(10):1079-1089.*
14. *Wahid SF, Sanusi S, Zawawi MM, Ali RA. A comparison of the pattern of liver involvement in dengue hemorraghic fever with classic dengue fever. Southeast Asian J Trop Med Public Health. 2000;31(2):259-263.*
15. *Parkash O, Almas A, Jafri SM, Hamid S, Akhtar J, Alishah H. Severity of acute hepatitis and its outcome in patients with dengue fever in a tertiary care hospital Karachi, Pakistan (South Asia). BMC Gastroenterol. 2010;10:43.*
16. *Supriatan M. GangguanFungsiHatipadaDemamBerdarah Dengue. Tesis. Semarang. Program PendidikanDokterSpesialis I FakultasKedokteranUniversitasDiponegoro. 2004.*
17. *LedikaMA, Setiabudi D, Dhamayanti M. Association between clinical profiles and severe dengue infection in children in developing country.American Journal of Epidemiology and Infectious Disease. 2015;3(3):45-49.*
18. *Siner JM, Bhandari V, Engle KM, Elias JA, Siegel MD.Elevated serum angiopoietin 2levels are associated with increased mortality in sepsis. Shock. 2009;31:348-353.*
19. *Kranidioti H, Orfanos SE, Vaki I, Kotanidou, Raftogiannis M, Dimopoulou I, et al.Angiopoetin 2 is increased in septic shock: evidence for the exixtence of a circulatory factor stimulating its release from human monocyte. Immunol Lett. 2009;125:65-71.*
20. *Kumpers P, Lukasz A, David S, Horn R, Hafer C, Faulhaber-Walter R, et al.Excess circulating angiopoetin-2 a strong predictor of mortality incritically ill medical patient. Clin Care, 2008;12:147.*
21. *Sumarmo PS. Infeksi virus dengue. Dalam: Sumarmo PS, Garna H, Hadinegoro SRH, penyunting Buku ajar ilmu kesehatan anak dan penyakit tropis Edisi pertama Jakarta: Balai Penerbit Fakultas kedokteran Universitas Indonesia. 2002:176-208.*
22. *DirektoratJenderalPengendalianPenyakitdanPenyehatanLingkunganKementerianKesehatan. Laporanakuntabilitaskinerja DITJEN PP dan PL tahun 2012.2012.*

23. *Trang NTH, Long NP, Hue TTM, Hung LP, Trung TD, Dinh DN, et al. Association between nutritional status and dengue infection: a systematic review and meta-analysis. BMC Infectious Diseases. 2016;16:2-11.*
24. *Setiati TE, Soemantri AG. Blood lactic acids as a predictor of mortality in severe dengue haemorrhagic fever in drKariadi Hospital–Semarang, Central Java. Semarang. Universitas Diponegoro. 1997;10-19.*
25. *Maron GM, Clara AW, Diddle JW, Pleites EB, Miller L, Mac Donald G, et al. Association between nutritional status and severity of dengue infection in children in El Salvador. Am J Trop Med Hyg. 2010;82(2):324-329.*
26. *Nagaram PP, Pidugu P, Munagala VK, Matli VV. Clinical abd laboratory profile and outcome of dengue cases among children attending a tertiary care hospital of South India. Int J ContempPediatr.2017;4(3):1074-1080.*
27. *Choudhury J, Mohanty D, Routray SS. Clinical profile and outcome, of dengue fever and dengue haemorhagic fever in pediatric age group. J IntContempPediatr.2016;3(2):442-444.*
28. *Hasan S, Jamdar SF, Alolawai M, Beajii SMAAA. Dengue virus: A global human threat: Review of literature. J IntSocPrev Community Dent. 2016;6(1):1–6.*
29. *Abdullah SA, Pasaribu S, Wijaya H, Pasaribu AP. Warning sign as a predictor of dengue infection severity in children. Med J of Indonesia. 2018;27:101-107.*
30. *BilquissShabbir et al. Acute Abdominal pain in dengue fever. PJHMS. 2012;6(1).*
31. *Zhang H, Zhou YP, Peng HJ, Zhang XH, Zhou FY, Liu ZH,Chen XG. Predictive symptoms and signs of severe dengue disease forpatients with dengue fever: A meta-analysis. BioMed Research International. 2014;1-10.*
32. *Yulianto A, Laksono IS, Juffrie M.Faktor prognosis derajatkeparahaninfeksi dengue. Sari Pediatri. 2016;18(3):198-203.*
33. *Thomas M.Moleculer Mechanism of angiopoeitin 2 mediated destabilization of the vascular endothelium. Disertasi. Program DoktoralUniversitas Freiburg.2008.*
34. *Rasyada A, Nasrul E, Edward Z. Hubungannilaihematokritterhadapjumlahtrombositpadapenderitademamberdarah dengue. JurnalKesehatanAndalas. 2014;3(3):343-347.*
35. *Ralapanawa U, Alawattegama ATM, Gunratne M, Tennakoon S, Kularatne SAM, Jayalath T. Value of peripheral blood count for dengue severity prediction. BMC Res Notes. 2018;11:400.*
36. *Valentino B, Riyanto B, Dewi RM. Hubunganantarahasilpemeriksaandarahlengkapdenganderajatklinikinfeksi dengue padapasiendewasa di RSUP Dr.Kariadi Semarang. JurnalMediMedikaMuda.2012.*
37. *Fadilah S, Abdul wahid, Sanusi S, Zawawi M, Ali R. A comparison of the pattern of liver involvement in dengue hemorrhagic fever with classic dengue fever.Southeastt Asian J Trop Med Public Health.2000:259-63.*
38. *Tambolkar SA. Liver functions as a marker of severity of disease in children with dengue fever. Webmed Central Paediatrics. 2015;6(8):1-7.*
39. *RampenganNH, Daud D, Warouw S, Ganda LJ. Serum angiopoietin-2 as marker of plasma leakage in dengue viral infection. American Journal of Clinical and Experimental Medicine. 2015;3(1):39-43.*

40. *Yuan HT, Khankin EV, Karumanchi A, Parikh SM. Angiopoetin 2 is a partial agonist/antagonist of Tie-2 signaling in the endothelium. Mol Cell Biol. 2009;29:2011-2022.*

41. *Mitchel M, van der Ven AJAM, Djamiatun K, Fijnheer R, de Groot P, Griffioen AW, et al. Imbalance of Angiopoietin 1 and Angiopoietin-2 in severe dengue and relationship with thrombocytopenia, endothelial activation and vascular stability. Am J Trop Hyg. 2012;87(5):943-946.*

42. *David S, Mukherjee A, Ghosh CC, Yano M, Khankin EV, Wenger JB, et al. Angiopoietin-2 may contribute to multiple organ dysfunction and death in sepsis. Crit Care Med.2012;40:3034 –3041.*