



The Role of Angiotensin-2 on Plasma Leakage in the Critical Phase and Convalescence of Dengue Virus Infection in Children

Rinang Mariko^{1*}, Nice Rachmawati¹, Fitrissia Amelin¹, Sri Rezeki Hadinegoro²

¹Department of Pediatrics, Faculty of Medicine, Andalas University, General Hospital of Dr. M. Djamil, Padang, Indonesia;

²Department of Pediatrics, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

Abstract

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***Correspondence:** Rinang Mariko, Department of Pediatrics, Faculty of Medicine, Andalas University/General Hospital of Dr. M. Djamil, Padang, Indonesia.
E-mail: rinang.mariko@yahoo.com

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BACKGROUND: The worst complication of dengue hemorrhagic fever (DHF) that leads to death is hypovolemic shock due to plasma leakage from intravascular to extravascular space caused by endothelial dysfunction.

AIM: This study is aimed to analyze the difference of Angiotensin-2 (Ang-2) level in DHF patients related to plasma leakage between critical phase and convalescence.

METHODS: One hundred and ten children with DHF confirmed serologically with positive IgM anti-dengue or IgM and IgG anti-dengue were recruited in this study. We examine the clinical manifestations, laboratory parameters (platelet count, AST and ALT levels), and molecular test (Ang-2). The molecular indicator was tested by Enzyme-Linked Immunosorbent Assay. Data were collected between July-November 2019. This is an observational study with cross-sectional comparative design.

RESULTS: In our study, we found correlation of clinical symptoms (abdominal pain, persistent vomit, and hepatomegaly) with the occurrence of plasma leakage in DHF patients. We also found that Ang-2 level is significantly higher in children with DHF during critical phase compared to convalescence ($p < 0.01$).

CONCLUSION: There is a significant difference of Ang-2 level in DHF patients during the critical phase and convalescence.

Introduction

Dengue infection remains one of the health sector's problems in Indonesia due to the high incidence and mortality rate. In 2005, Indonesia was the biggest contributor of dengue infection in South East Asia (53%) with total of 95.270 cases and 1.298 death cases. Based on data from the Ministry of Health of the Republic of Indonesia, there were 126.675 dengue hemorrhagic fever (DHF) cases with 09% case fatality rate [1]. The worst complication of DHF that leads to death is hypovolemic shock due to plasma leakage from intravascular to extravascular space caused by endothelial dysfunction. The severity of the symptoms caused by dengue virus infection related to the severity of plasma leakage [2], [3].

Angiotensin-1 (Ang-1) and Angiotensin-2 (Ang-2) have functions in maintaining vascular function and integrity. Angiotensin-1 is reported not only to play a role in stabilizing blood vessels during angiogenesis but also in inhibiting the increase of vascular permeability, as well as having anti-inflammatory effects. Ang-1 m-RNA molecules expressed on periendothelial cells play a role in maintaining blood vessel stability,

inhibiting plasma leakage, suppressing inflammatory gene expression, and preventing the recruitment and migration of leukocytes [4].

In contrast to Ang-1, Ang-2 which is selectively expressed on endothelial cells, once it binds to the Tie 2 receptor (Tyrosine Kinase 2) will result in a signaling disorder of Ang-1/Tie 2 so that vascular permeability dysfunction occurs [5], [6]. Elevated Ang-2 levels have been reported to be associated with vascular leakage and increased mortality in critically ill children and adults with septic shock [7].

Methods

This study is an observational study with a comparative cross-sectional design. The study was conducted during July-November 2019 at the Pediatric Ward of RSUP Dr. M. Djamil Padang, four nearby hospitals, Biomedic laboratory of the Medical Faculty of Andalas University, and the central laboratory of RSUP Dr. M. Djamil Padang.

Population

The study population was patients with dengue virus infection (DHF and DSS) age 1–15 years old hospitalized at RSUP Dr. M. Djamil Padang and four hospitals nearby that fit 2011 WHO criteria and confirmed serologically with positive IgM anti-dengue or positive IgM and IgG anti-dengue in July–November 2019. Subjects were part of the population that met inclusion and exclusion criteria. The inclusion criteria were willing to be included in the study (with consent gained from the parents) and signed the informed consent form. The exclusion criteria were having other bacterial or viral infection based on clinical and laboratory examination, receiving corticosteroid therapy, and nutritional status overweight or obese.

Sampling method

The sampling method was consecutive sampling, the research subjects were obtained based on the order of admission to the hospital. From the results of calculations using the sampling formula, the sample size for each group was determined at 32 people, making the total sample size used for both groups was 64 people. The results of the study recorded by filling in a case record form. The recording included subject data (identity, history taking, physical and laboratory examinations), notes on inclusion-exclusion criteria, and a consent form obtained from the family. The appointed officers who worked on patients screening, taking and storing subject's samples in hospitals, and providing care and treatment in the hospitals were also recorded.

Data analysis

The data obtained were analyzed using a computer system in the form of tables and charts. Univariate analysis was performed on each variable from the research results. In the categorical variables, the data are displayed in the form of a frequency distribution, the data included in this study were gender, mucosal bleeding, and hepatomegaly. In numeric variables, the data are displayed in the form of a central tendency, namely the mean value, middle value, and standard deviation. The data to be analyzed in this study were day of fever, hematocrit, platelet count, AST, ALT, and Ang-2 level. Bivariate analysis was performed to see the mean difference between day of fever, hematocrit, platelet count, AST, ALT, and Ang-2 level between DHF patients with shock and without shock. First, the data were analyzed using the normality test to determine the normality of the data using the Shapiro Wilk test ($n < 50$), then continued with bivariate analysis, if the data is normally distributed then the analysis is carried out using the dependent t test, but if it is known to be not normally distributed then it is carried out using Mann-Whitney test with a confident interval (CI) of 95%

and $\alpha = 0.05$. The conclusion from the test results if the p value ≤ 0.05 then H_0 is rejected, meaning there is a difference in the mean between the independent variable and the dependent variable.

Results

Subjects characteristics

This study was conducted to determine the model of the predictor score system for the occurrence of shock in dengue hemorrhagic fever based on clinical symptoms, laboratory and molecular parameters. The research was conducted at the Children's Ward of RSUP Dr. M. Djamil Padang, the Biomedical Laboratory of Medical Faculty of Andalas University and the central laboratory of RSUP Dr. M. Djamil Padang. The data collected were 110 samples.

The characteristics of the subject are presented in Table 1. As the table shown, from all children suffering from DHF with evidence of plasma leakage, 44.3% were male and 55.7% were female, while from the total children suffering from DHF, 46.4% were male and 53.6% were female. The average age of DHF patients with plasma leakage was 3.67–10.17 years and DHF patients without plasma leakage was 2.38–10.08 years. Statistically, there was no difference in sex and age between DHF with plasma leakage compared to without plasma leakage ($p > 0.05$). Based on nutritional status, 29 children were undernourished, 63 children with normal nutritional status, and 18 children were overweight. Children in both groups were dominated by children with normal nutritional status, namely 53.2% and 67.7%. Based on statistics, there was no significant difference of nutritional status between DHF with plasma leakage compared to those without plasma leakage ($p > 0.05$).

Table 1: Subject characteristics

Characteristics	Plasma leakage		p-value
	Yes (n = 79)	No (n = 31)	
Sex, n (%)			0.632
Male	35 (44.3)	16 (51.6)	
Female	44 (55.7)	15 (48.4)	
Age (years), mean \pm SD	6.92 \pm 3.25	6.23 \pm 3.85	0.338
Nutritional status, n(%)			0.181
Undernourished	21 (26.6)	8 (25.8)	
Normal	42 (53.2)	21 (67.7)	
Overweight	16 (20.3)	2 (6.5)	

*p < 0.05 significant.

Association between clinical symptoms and laboratory results with plasma leakage

Table 2 shows that there was no association between the duration of fever, mucosal bleeding, platelets, AST, and ALT level with the occurrence of plasma leakage ($p > 0.05$). However, there was an association of abdominal pain, persistent vomiting, and hepatomegaly with plasma leakage ($p < 0.05$).

Table 2: Association between clinical symptoms and laboratory results with plasma leakage

Variable	Plasma leakage		p-value
	Yes (f%) (n = 79)	No (f%) (n = 31)	
A. Clinical symptom			
Duration of fever			
≥4 days	68 (86.1)	27 (87.1)	1.000
<4 days	11 (13.9)	4 (12.9)	
Mucosal bleeding			
Yes	16 (20.3)	2 (6.5)	0.141
No	63 (79.7)	29 (93.5)	
Abdominal pain			
Yes	45 (57.0)	6 (19.4)	0.001*
No	34 (43.0)	25 (80.6)	
Persistent vomit			
Yes	40 (50.6)	5 (16.1)	0.002*
No	39 (49.4)	26 (83.9)	
Hepatomegaly			
Hepatomegaly	50 (63.3)	8 (25.8)	0.001*
Normal	29 (36.7)	23 (74.2)	
B. Laboratory result			
Platelet			
≤100.000/mm ³	78 (98.7)	30 (96.8)	1.000
>100.000/mm ³	1 (1.3)	1 (3.2)	
AST			
Abnormal	68 (86.1)	21 (67.7)	0.053
Normal	11 (13.9)	10 (32.3)	
ALT			
Abnormal	52 (65.8)	18 (58.1)	0.589
Normal	27 (34.2)	13 (41.9)	

*p < 0.05 significant.

In Table 2 clinical symptoms such as mucosal bleeding (27.4%), abdominal pain (75.8%), persistent vomiting (64.5%), and hepatomegaly (80.6%) were more common in children with DSS than in children with DHF (p < 0.05%). Statistically, there was no difference in the duration of fever with the occurrence of DHF with shock or without shock (p < 0.05). However, there were significant differences in mucosal bleeding, abdominal pain, persistent vomiting, and hepatomegaly in DHF with shock and without shock (p < 0.05).

Role of Ang-2 toward plasma leakage in critical phase and convalescence

The role of Ang-2 toward plasma leakage in critical phase and convalescence shown in Table 3.

Table 3: Role of Ang-2 toward plasma leakage in critical phase and convalescence

Variable	Plasma leakage		p-value
	Critical phase mean ± SD	Convalescence mean ± SD	
Ang-2 level (pg/ml)	630.56 ± 57.29	450.38 ± 67.85	<0.001

*p < 0.05 significant.

Table 3 shows that the mean Ang-2 level in the critical phase was 630.56 ± 57.29 pg/ml, while in the convalescence there was a decrease to 450.38 ± 67.85 pg/ml. The results of statistical tests show that there is a role of Ang-2 in the occurrence of plasma leakage in the critical phase with p < 0.001 (p < 0.05) (Figure 1).

Discussion

Characteristics of research subject

Although number of female subjects is slightly higher in Table 1, we can conclude that there were no

significant differences in the terms of gender and age between DHF with and without plasma leakage groups. This is similar to the research by Sumarmo in 2002 which stated that there is no gender difference in DHF patients. Research in the Directorate General of PP and PL, Ministry of Health of the Republic of Indonesia in 2008 also showed the same results where there was no sex difference in children suffering from dengue virus infection [8], [9].

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 2–11 years and study of Mayetti that the age of 5–10 years was the group most suffering from dengue compared to other age groups. Similar to this study, there were no age differences that were statistically significant between both groups [8], [10].

From the nutritional status category, subjects with good nutritional status were affected the most by dengue infection rather than the ones with undernourished and overweight state. Overall, the number of well-nourished patients were seen more in the plasma leakage group rather than subjects with undernourished and overweight state, thus there was a statistically significant difference between nutritional status and the incidence of dengue virus infection. Until now, the relationship of nutritional status and dengue virus infection is still debated and requires further research [11]. Research by Setiati *et al.* found that DHF rarely occurs in malnourished children. A strong inflammatory response is one of the factors causing DHF [12]. Research by Maron *et al.* found that lack of nutrition can suppress immune responses mediated by cell, thereby reducing the risk of dengue virus infection [13].

Difference of clinical symptoms and laboratory results in DHF with plasma leakage and without plasma leakage

There were no significant differences in the duration of fever, mucosal bleeding, platelet count, and AST and ALT levels, while we found significant differences in symptoms of abdominal pain, persistent vomit, and presence of hepatomegaly in both groups. Fever is found in all DHF patients either with evidence of plasma leakage or not [14]. Research in India found that 100% of DHF patients had fever but 45% of them were found at the first hospital admission [15].

Vasculopathy, platelet deficiency and dysfunction, and defects in the coagulation pathway are some of the factors that could cause bleeding. Decreased platelet production and increased platelet damage lead to thrombocytopenia in DHF patients. Disorders of platelet function make the vessel become more brittle, making the bleeding to occur more easily [16]. Choudury *et al.* found that

55% of DHF patients presented with bleeding, while Nagaram found that mucosal bleeding occurred in 24% of children with DSS and 1.7% in children with DHF [14], [15].

In addition to fever, abdominal pain and vomiting are also symptoms associated with DHF and part of warning signs in dengue infection. Nagaram found 73 cases with complaints of abdominal pain and 115 cases with vomiting, 38.2% had abdominal pain and 60.4% had persistent vomiting in DHF patients while in DSS patients 96% had abdominal pain and 100% had persistent vomit [14]. 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 high sensitivity and specificity in predicting the occurrence of severe dengue infection which partly similar to this study that found that abdominal pain and persistent vomiting significantly associated with the presence of plasma leakage in DHF patients [17].

The Nagaram study also showed that 100% DSS patients were found to develop hepatomegaly while in all DHF patients were found as much as 77%, thus hepatomegaly is associated with severe form of DHF [14]. Similar to this study, hepatomegaly is significantly different in group with plasma leakage compared to group without plasma leakage. Hematocrit, platelet count, AST, and ALT level will change according to the severity of dengue infection. The more severe dengue infection occurs, the more increase of hematocrit, AST, and ALT level and decrease of platelet count. Thrombocytopenia in DHF patients is due to bone marrow suppression, destruction of platelets, and increase of platelet consumption from interactions between platelets and endothelial cells damaged by dengue virus infection [18]. Ralapanawa *et al.* obtained platelet cut off of 105,000 to predict the progress of patients of DHF with sensitivity of 80% and specificity of 50%. Besides, platelet levels were found to be decreased starting on the 5 day in DHF patients with shock [19]. Valentino *et al.* concluded that there was a significant relationship between platelet level and clinical degree of dengue infection [20]. In the contrary, we did not find significant difference of platelet level between DHF patients with plasma leakage and without plasma leakage.

Transaminase levels usually increase up to 9 days after the onset of fever and gradually return to normal level within 2 weeks. A study by Jagadishkumar *et al.* in 2012 showed that elevation of liver enzymes helps in recognition of severe forms of dengue infection, whereas much as 17.27% of children had >10 fold increase in liver enzymes and commonly observed in DHF and DSS groups [21]. Conversely, we found that there was no significant difference in AST and ALT levels in DHF with and without plasma leakage.

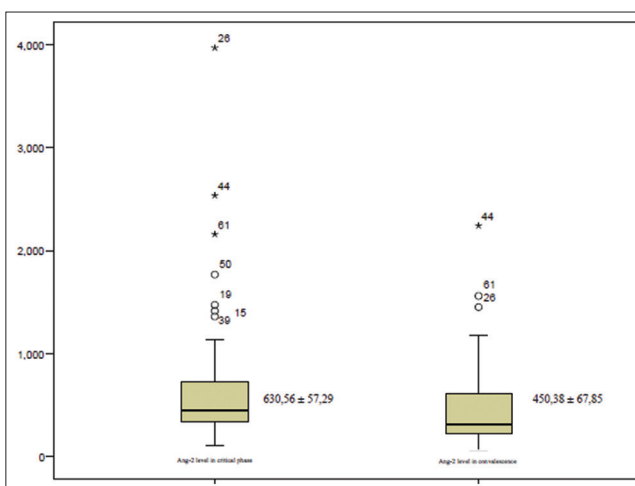


Figure 1: Boxplot of Ang-2 level in critical phase and convalescence

Role of Ang-2 toward plasma leakage in critical phase and convalescence

In 2008, Giuliano *et al.* found that increase level of Ang-2 associated with plasma leakage and increase mortality in critically ill children [7]. Study by Rampengan in 2015 showed Ang-2 level at admission was 2,486.21 pg/dl, 3,194.95 pg/dl and 4,005.32 pg/dl in DF, DHF and DSS group, respectively. This result show increasing plasma leakage with increasing severity of dengue viral infection. After 48 h, the level of Ang-2 decrease significantly in all groups. These findings might reflect the role of Ang-2 in vascular leakage in dengue viral infection. Increase of Ang-2 level could trigger vascular instability causing an increase in vascular permeability and plasma leakage [22]. Michels *et al.* in 2012 also found that DSS and DHF patients had higher serum Ang-2 level at the time of initial treatment compared to when they discharged [23]. Our finding is consistent with previous studies, the mean Ang-2 level in the critical phase was 630.56 ± 57.29 pg/ml, while in the convalescence there was a decrease to 450.38 ± 67.85 pg/ml. The results of statistical tests show that there is a role of Ang-2 in the occurrence of plasma leakage in the critical phase.

The mechanism for increasing Ang-2 level and plasma leakage is a complex cascade. Binding of Ang-1 with the Tie-2 receptor via the phosphatidylinositol-3-kinase AKT pathway will cause stability of blood vessels by recruiting pericyte into new blood vessel 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 inhibit Ang-1 signaling to Tie-2 and increase blood vessel instability [24].

Conclusion

There is no relationship between gender, age, nutritional status, duration of fever, mucosal bleeding,

platelet count, transaminase level, and the occurrence of plasma leakage, meanwhile there is a relationship between symptom of abdominal pain, persistent vomit, hepatomegaly and the occurrence of plasma leakage. Angiopoietin-2 level related to plasma leakage in critical phase and convalescence, thus Ang-2 level can be used in predicting plasma leakage or shock in dengue virus infection.

References

- Information Centre of Ministry of Health of Republic of Indonesia. DHF Situation. Indonesia: Information Centre of Ministry of Health of Republic of Indonesia; 2016.
- Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev.* 1998;11(3):480-96. <https://doi.org/10.1128/CMR.11.3.480> PMID:9665979
- Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol.* 2002;10(2):100-3. [https://doi.org/10.1016/S0966-842X\(01\)02288-0](https://doi.org/10.1016/S0966-842X(01)02288-0) PMID:11827812
- Siner JM, Bhandari V, Engle KM, Elias JA, Siegel MD. Elevated serum angiopoietin 2 levels are associated with increased mortality in sepsis. *Shock.* 2009;31(4):348-53. <https://doi.org/10.1097/SHK.0b013e318188bd06> PMID:18791490
- Kranidioti H, Orfanos SE, Vaki I, Kotanidou, Raftogiannis M, Dimopoulou I, et al. (Angiopoietin 2 is increased in septic shock: Evidence for the existence of a circulatory factor stimulating its release from human monocyte. *Immunol Lett.* 2009;125:65-71.
- Kumpers P, Lukasz A, David S, Horn R, Hafer C, Faulhaber-Walter R, et al. Excess circulating angiopoietin-2 a strong predictor of mortality in critically ill medical patient. *Clin Care.* 2008;12(6):147. <https://doi.org/10.1186/cc7130> PMID:19025590
- Giuliano JS Jr., Lahni PM, Bigham MT, Manning PB, Nelson DP, Wong HR, et al. Plasma angiopoietin-2 levels increase in children following cardiopulmonary bypass. *Intensive Care Med.* 2008;34(10):1851-7. <https://doi.org/10.1007/s00134-008-1174-9> PMID:18516587
- Sumarmo PS. Infeksi virus dengue. In: Sumarmo PS, Garna H, Hadinegoro SR, editors. *Penyunting Buku Ajar Ilmu Kesehatan Anak dan Penyakit Tropis.* Jakarta: Balai Penerbit Fakultas Kedokteran Universitas Indonesia; 2002. p. 176-208.
- Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan Kementerian Kesehatan. Laporan akuntabilitas kinerja DITJEN PP dan PL tahun 2012; 2012.
- Mayetti M. Hubungan gambaran klinis dan laboratorium sebagai faktor risiko syok pada demam berdarah dengue. *Sari Pediatr.* 2010;11(5):367-73.
- Trang NT, Long NP, Hue TT, Hung LP, Trung TD, Dinh DN, et al. Association between nutritional status and dengue infection: A systematic review and meta-analysis. *BMC Infect Dis.* 2016;16:172. <https://doi.org/10.1186/s12879-016-1498-y> PMID:27097934
- Setiati TE, Soemantri AG. Blood Lactic Acids as a Predictor of Mortality in Severe Dengue Haemorrhagic Fever in dr. Kariadi Hospital-Semarang, Central Java, Semarang: Universitas Diponegoro; 1997. p. 10-9.
- Marón GM, Clará AW, Diddle JW, Pleités EB, Miller L, Macdonald 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):324-9. <https://doi.org/10.4269/ajtmh.2010.09-0365> PMID:20134012
- Nagaram P, Piduru P, Munagala V, Matli V. Clinical and laboratory profile and outcome of dengue cases among children attending a tertiary care hospital of South India. *Int J Contemp Pediatr.* 2017;4(3):1074-80.
- Choudhury J, Mohanty D, Routray S. Clinical profile and outcome of dengue fever and dengue haemorrhagic fever in pediatric age group. *Int J Contemp Pediatr.* 2016;3(2):442-44. <https://doi.org/10.18203/2349-3291.ijcp20161025>
- Hasan S, Jamdar SF, Alalowi M, Al Ageel Al Beajji SM. Dengue virus: A global human threat: Review of literature. *J Int Soc Prevent Communit Dent* 2016;6(1):1-6. <https://doi.org/10.4103/2231-0762.175416> PMID:27011925
- Adam AS, Pasaribu S, Wijaya H, Pasaribu AP. Warning sign as a predictor of dengue infection severity in children. *Med J Indones.* 2018;27(2):101-7.
- Rasyada A, Nasrul E, Edward Z. Relationship between hematocrit value and platelet count in patients with dengue hemorrhagic fever. *J Kesehatan Andalas.* 2014;3(3):115. <https://doi.org/10.25077/jka.v3i3.115>
- Ralapanawa U, Alawattagama A, Gunrathne M, Tennakoon S, Kularatne S, Jayalath T. Value of peripheral blood count for dengue severity prediction. *BMC Res Notes.* 2018;11(1):400. <https://doi.org/10.1186/s13104-018-3505-4> PMID:29925425
- Valentino B, Riyanto BB, Dewi RM. Hubungan Antara Hasil Pemeriksaan Darah Lengkap Dengan Derajat Klinik Infeksi Dengue Pada Pasien Dewasa Di Rsup Dr. Kariadi Semarang; 2012.
- Jagadishkumar K, Jain P, Manjunath VG, Umesh L. Hepatic involvement in dengue Fever in children. *Iran J Pediatr.* 2012;22(2):231-6. PMID:23056891
- Rampengan NH, Daud D, Warouw S, Ganda LJ. Serum angiopoietin-2 as marker of plasma leakage in dengue viral infection. *Am J Clin Exp Med.* 2015;3(1):39-43.
- Michels M, van der Ven AJ, Djamiatun K, Fijnheer R, de Groot PG, 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 Med Hyg.* 2012;87(5):943-6. <https://doi.org/10.4269/ajtmh.2012.12-0020>
- Yuan HT, Khankin EV, Karumanchi SA, Parikh SM. Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium. *Mol Cell Biol.* 2009;29(8):2011-22. <https://doi.org/10.1128/MCB.01472-08> PMID:19223473