Association of angiopoietin-2 level and vascular endothelial growth factor level with dengue infection severity in children

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Research Article

Association of angiopoietin-2 level and vascular endothelial growth factor level with dengue infection severity in children

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ABSTRACT

Aim: This study aimed to determine the association of angiopoietin-2 (ANGPT-2) and vascular endothelial growth factor (VEGF) level with dengue infection severity in children. **Methods:** This study was observational method with cross-sectional study in 108 infected dengue children in M. Djamil Hospital, Padang. Level of ANGPT-2 and VEGF was examined by enzyme-linked immunosorbent assay (ELISA) using Human ANGPT-2 and VEGF ELISA Kit, Ray Biotech. Statistical analysis consisted of descriptive and comparative analyses. **Results:** The results showed an increase in ANGPT-2 levels according to the severity of the disease in pediatric patients with dengue infection, and the difference was statistically significant ($P < 0.05$). While VEGF levels did not differ significantly between groups. **Conclusion:** Angiopoietin-2 plays a significant role in plasma leakage in patients with dengue virus infection. Levels of angiopoietin-2 significantly increased along with the severity of the disease. The increasing of VEGF has no association with the severity of dengue virus infection.

KEY WORDS: Angiopoietin-2, Children, Dengue infection, Vascular endothelial growth factor

INTRODUCTION

Clinical manifestations of dengue virus infection vary widely, ranging from asymptomatic forms, undifferentiated fever, dengue fever (DF) as a mild form, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) which can cause death.[1] Many theories are trying to explain the variation of clinical appearance on dengue virus infection. However, there is no single theory that can explain the exact cause of increased vascular permeability in DF.

Angiopoietin (ANGPT) is a glycoprotein molecule that binds to a tyrosine kinase receptor (Tie-2). Angiopoietins consist of ANGPT-1 to ANGPT-4, but only ANGPT-1 and ANGPT-2 that clinically and widely published. Under normal circumstances, ANGPT-1 levels are higher than ANGPT-2. The bond between ANGPT-1 and Tie-2 receptors will trigger an electrical signal through the phosphatidylinositol-3-kinase (PI3K) pathway which results in recycling the pericardial cells to the endothelium so that endothelial integrity is maintained. ANGPT-1 also has the ability to stop the effects of vascular endothelial growth factor (VEGF) phosphorylation on VE-cadherin. However, in injury conditions, hypoxia and bacterial infections occur in an imbalance ratio of ANGPT-1 and ANGPT-2, as an increase in levels of ANGPT-2 expression. Increased levels of ANGPT-2 expression will lead to a shift of the Tie-2 receptor bond with ANGPT-1 resulting in endothelial integrity disorders and an increase in vascular permeability and plasma leakage.[2-4]

Several studies have shown an association of ANGPT with plasma leakage in certain diseases such as diabetic retinopathy,[5] impaired fetal growth,[6] preeclampsia,[7] pleural effusion,[8] tumors,[9] sepsis,[10] and acute respiratory distress syndrome.[2] The study of Van de Weg *et al.*[11] and Rampengan *et al.*[12] reported an increase in levels of ANGPT-2 in the DHF and DSS groups but not in DF and healthy children.

Another angiogenic protein that plays a role in plasma leakage is VEGF. The VEGF molecule has three receptors: Soluble vascular endothelial growth factor receptor (sVEGFR) 1, sVEGFR-2, and sVEGFR-3. VEGF levels reported significantly increased in

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DHF and DSS patients compared to controls.[13,14] The decrease in sVEGFR-2 levels in dengue virus infections due to the direct effect of dengue virus resulting in decreased sVEGFR-2 production and increased sVEGFR-2 expression on the surface of endothelial cells. This effect will result in elevated levels of free VEGF in the plasma that triggers the VE-cadherin phosphorylation and the occurrence of plasma leakage.[13] Free VEGF levels will also reinforce the effect of plasma leakage caused by ANGPT-2.[15] The levels of ANGPT-2 and sVEGFR-2 are reported to be the major markers of plasma leakage in DHF.[11] We, therefore, studied correlation Ang-2 and VEGF level with severity of dengue infection in children.

METHODS

Sample Preparation

This research used analytical observation method with cross-sectional approach on all dengue cases in pediatric ward and ambulatory ward, Dr. M. Djamil Hospital, Padang, from January 2016 to January 2017. All patients diagnosed with Dengue virus infection based on WHO 2011 clinical criteria and had positive results for dengue-serology (IgM alone or both IgM/IgG) were recruited in this study and further classified into 3 DF, DHF (I and II), and DSS (DHF III&IV). All subjects were treated according to the World Health Organization protocol. The study protocol was approved by the Committee for Medical Research Ethics of the Faculty of Medicine, University of Andalas. Written informed consent was obtained from the parents or legal guardians of the children.

Enzyme-linked Immunosorbent Assay (ELISA)

Blood was drawn 3 cc at the days of enrollment for evaluating complete blood counts, IgM and IgG anti dengue, and Ang-2 and VEGF level. Blood centrifuged for 5 min at 3000 rpm and plasma was stored at −80°C until they were used for assay. Plasma levels of Ang-2 and VEGF were measured using commercial ELISA kits (Human ANGPT-2 and VEGF ELISA Kit, Ray Biotech).

Statistical Analysis

Statistical analysis consisted of descriptive and comparative analyses. Descriptive analyses were used to analyze the characteristics and laboratory findings. We used ANOVA test to compare the Ang-2 and VEGF serum between three groups. All analyses were performed using the SPSS version 21.0.

RESULTS

There were 121 pediatric patients with dengue infection met the inclusion criteria; however, as much as 13 samples were excluded, consisted of 6 incompletes samples and 7 samples could not be examined since they were lysis. Finally, there were only 108 samples that were analyzed.

Demographic data and clinical characteristics can be shown in Table 1. The number of female samples is 58 people (53.71%) almost the same as male, 50 (46.29%). Most ages are 5–10 years. Most patients have good nutritional status (52.77%). In general, when come to the hospital, the child has a fever for 5 days. There were no significant differences between sex, age, and duration of fever in all three groups of disease severity. There was no statistically significant difference in hemoglobin levels across the three groups ($P = 0.1$). The hematocrit values of patients with DHF and DSS were higher than DF, and statistically, there was a significant difference between the three groups ($P = 0.016$). There was a significant decrease in platelet counts in patients with DHF and DSS compared with DF patients. Statistically, there was a significant difference between the three groups ($P = 0.01$).

Figure 1 shows the different levels of ANGPT-2 in each group based on the severity of the disease. In each group, there were some extreme values of ANGPT-2, indicating abnormal data distribution, and proven by Kolmogorov–Smirnov test ($P < 0.05$). Table 2 shows that the median grade of ANGPT-2 in the DSS group was higher than the DHF group and the median level in the DHF group was higher than the DD group. The differences in the levels of these three groups were statistically significant ($P < 0.05$). In the Mann–Whitney post hoc test there was also a significant difference between the ANGPT-2 levels between groups; in between DD group and DBD, DHF group with DSS as well as between group DD and DSS.

Figure 2 shows the differences in VEGF levels in each group based on the severity of the disease.

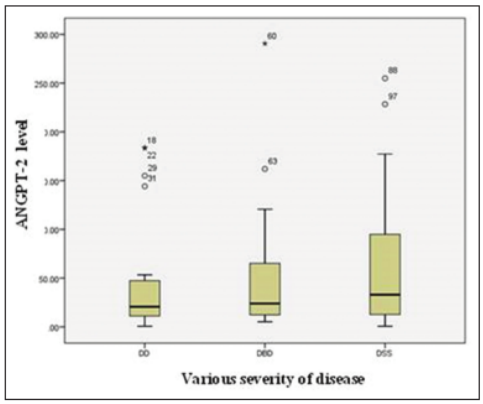

Figure 1: The difference of angiopoietin-2 levels in various severities of disease due to dengue virus infection

Table 1: Demographic and clinical characteristic

Characteristic	DF (*n*=34)	DHF (*n*=39)	DSS (*n*=35)	*P* value
Clinical characteristic				
Sex (%)				0.897*
Male	18	13	19	
Female	16	26	16	
Age, mean (SD), years old	7.87 (3.59)	7.07 (3.25)	6.19 (4.06)	0.165**
≤1	2	2	5	0.344***
1–5	8	9	12	
5–10	15	22	10	
>10	9	6	8	
Nutritional state				
Poor	10	15	13	
Good	21	18	18	
Excess/obesity	3	6	4	
Fever duration (days), median (minimum-maximum)	5 (2–6)	5 (2–8)	5 (3–6)	0.454***
Laboratory characteristic				
Hemoglobin g/dl, median (minimum-maximum)	12.2 (10.5–14.8)	13.3 (9.2–17.3)	13.5 (5.6–17.4)	0.010***
Hematocrit, % median (minimum-maximum)	37.5 (33–49)	41 (28–53)	41 (16–53)	0.016***
Thrombocytes, mm^3, median (minimum-maximum)	78,500 (13,000–247,000)	40,000 (13,000–133,000)	40,000 (4000–131,000)	0.001***

*Chi-square for trend test, **One-way ANOVA test, ***Kruskal–Wallis test

Table 2: Level of angiopoietin-2 in different levels of severity

Dengue severity level	*n*	Brinkman index pg/ml	*P* value
DF	34	397.45 (64.48–1895.07)	<0.001
DHF	39	558.52 (79.36–2797.71)	
DSS	35	1393.82 (58.52–6310.49)	

DF: Dengue fever, DHF: Dengue hemorrhage fever, DSS: Dengue shock syndrome

Table 3: Relation of VEGF level to severity of dengue viral infection in children

Dengue severity level	*n*	Indeks Brinkman pg/ml	*P* value
DF	34	20.59 (0.69–183.87)	0.471
DHF	39	23.97 (5.22–290.58)	
DSS	35	33.74 (0.69–254.93)	

DF: Dengue fever, DHF: Dengue hemorrhage fever, DSS: Dengue shock syndrome, VEGF: Vascular endothelial growth factor

In each group, there were some extreme values, indicating abnormal data distribution as evidenced by the Kolmogorov–Smirnov test ($P < 0.05$). Table 3 shows that median value of VEGF on DSS group is higher compare to DHF group and median value of DHF group is higher compare to DF group; however, statistically, those differences are not significance ($P > 0.05$).

DISCUSSION

In this study, there is a significant increase in ANGPT-2 levels along with the increasing severity of the disease due to dengue virus infection. The results from this study are similar to those obtained by Rampengan *et al.*[12] who reported that ANGPT-2 was instrumental in the pathogenesis of transient vascular leakage in DHF, an increase in ANGPT-2 caused the instability of the blood vessels, leading to increased vascular permeability that eventually led to the occurrence vascular leak. There is a significant increase in plasma

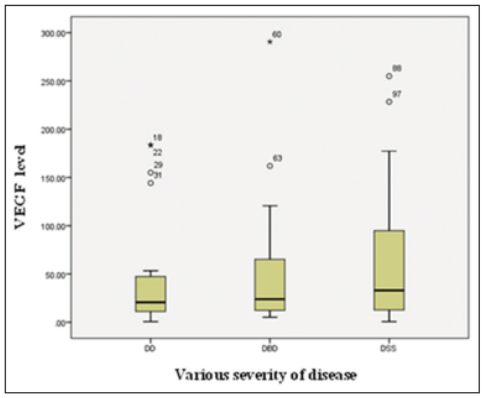

Figure 2: The differences in vascular endothelial growth factor levels at various severities of dengue viral infections

ANGPT-2 levels in samples with DHF and DSS but not on samples with DF. The mean ANGPT-2 levels in the DF group at the beginning of hospitalization were 2468.21 (SD 1534.55) pg/ml, in the DHF group,

3194.95 (SD 1572.02) pg/ml, and in the DSS 4005 group, 32 (1706.43) pg/ml. The level of ANGPT-2 decreased significantly in both DF, DBD, and DSS groups after 48 h of treatment.[12]

Roviezzo *et al.*[16] concluded that ANGPT-2 induced the formation of edema in rat claws and this effect was inhibited by the soluble form of the Ti1-2 receptors or ANGPT-1. The levels of NO and prostaglandin E2 did not increase, indicating that the action of ANGPT-2 did not affect this mediator. ANGPT-2 stimulates cell extravasation with minimal fluid, but in an on-going inflammatory state, it will reduce cellular tissue infiltration.[16]

The mechanism of increasing ANGPT-2 level and plasma leakage is a complex cascade. ANGPT-2 will cause instability of endothelial cell integrity and cause plasma leakage after released from WPB due to a stimulation of pro-inflammatory cytokines, thrombin, leukocyte and thrombocyte, circulatory alteration, or reduced tissue oxygenation. ANGPT-2 will bind to the Tie-2 receptor, causing Tie-2 receptor dephosphorization, thus inhibiting the release of the ANGPT-1/Tie-2 signal. ANGPT-2 does not cause receptor activation, so cascade of PI3K signals activation and Race pathway as caused by ANGPT-1 does not occur when Tie-2 receptors are occupied by ANGPT-2. This will result in unformatted of migratory signals, anti-permeability, and anti-inflammatory signals in endothelial cells then facilitate the plasma leakage.[15,17]

VEGF is a proangiogenic glycoprotein that promotes the proliferation, migration, and endothelial cell survival rate, but VEGF is also known as a strong inducer that increases vascular permeability, stimulates vasodilation, and induces fenestra in endothelial cells *in vivo* and *in vitro* and contributes to plasma leakage.[13] Endothelial dysfunction is believed to play an important role in the pathogenesis of plasma leakage, Van de Weg *et al.*[11] concluded in his study that there was an increase in ANGPT-2, endothelin-1, and MMP-2 and decreased levels of VEGFR-2 significantly associated with plasma leak.[11]

VEGF expression is triggered by various factors. The main factor that stimulates VEGF is tissue/cell hypoxia while other factors are estrogen, nitric oxide (NO), and growth factors (fibroblast growth factor-4), PDGF, tumor necrosis factor alpha, epidermal growth factor (EGF), transforming growth factor beta, keratinocyte growth factor, interleukin (IL)-6, IL-1β, and insulin-like growth factor-1.[18]

VEGF levels reported significantly increased in patients with DHF and DSS compared to controls,[13,14] but other studies reported that plasma VEGF levels are lower in DF and DHF groups than controls.[19,20] In this study, VEGF levels did not differ significantly in every level of severity between DF, DHF, and DSS groups. A study in Thailand, in 2005, on 31 patients of DF and 37 patients of DHF stated that plasma leakage in DHF patients could not be explained by increased VEGF.[14] Increased levels of VEGF are activated by the fibrinolysis process as evidenced by a significant association between plasma VEGF elevation and increased D-dimer.

CONCLUSION

ANGPT-2 plays a significant role in plasma leakage in patients with dengue virus infection. Levels of angiopoietin-2 significantly increased along with the severity of the disease. The increasing of VEGF has no association with the severity of dengue virus infection.

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