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Original Article

Association between interleukin-8 and severity of dengue shock syndrome in children

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Abstract

Background Dengue hemorrhagic fever (DHF) remains a major health problem in tropical countries. The case fatality rate (CFR) can be reduced from 45% to <1%, if dengue shock syndrome (DSS) is treated early and adequately. Early biomarkers for DSS outcomes in children are needed. Interleukin-8 (IL-8) might be one of the molecule, as it plays a role in the pathophysiology of DHF in children.

Objective To assess IL-8 levels in pediatric DHF patients at various stages of illness severity and to determine the correlation between serum IL-8 concentration on admission and DSS outcomes in children.

Methods A prospective cohort study was done in children with DSS who were admitted to the Pediatrics Department of Kandou Hospital, Manado. We measured subjects' serum IL-8 levels at the time of DSS diagnosis and followed-up subjects until there was improvement or deterioration. An association between IL-8 and DSS outcome was analyzed using univariable logistic regression test. An ROC curve and Chi-square test were used to analyze the prognostic value of serum IL-8 levels. Statistical significance was considered to be a P value of <0.05 (power 80, β=0.20)..

Results Fifty-eight children with DSS were included in this study. Twenty-seven subjects had clinical deterioration (to recurrent shock, prolonged shock or died). There was a significant association between elevated IL-8 levels and clinical deterioration in DSS (OR 116.7; 95%CI 18.0 to 756.0; P=0.0001). The ROC curve revealed an IL-8 cut-off level of 194.9 pg/mL, AUC 0.982, with sensitivity 89.3%, specificity 93.3%, positive predictive value (PPV) 92.6%, negative predictive value (NPV) 90.3%.

Conclusion There is an association between elevated early serum IL-8 level and a DSS deterioration. Further prognostic studies are needed to confirm the predictive ability of serum IL-8 level on DSS deterioration in children. **[Paediatr Indones. 2016;56:79-83.]**.

Keywords*: dengue shock syndrome, IL-8, marker, children*

Dengue hemorrhagic fever (DHF) remains a major health problem for 100 countries in tropical and subtropical regions.[1] The *World Health Organization* (WHO) reported that 2.5 billion people in the world live in endemic areas and are at risk of suffering from dengue fever (DF) or DHF.[1,2] The mortality rate from dengue shock syndrome (DSS) can increase to 40-45% if patients are not adequately treated early on. However, for patients with DSS who have adequate access to hospital care, the death rate can be reduced to <1%.[3] Effective antivirals and vaccines against the dengue virus that causes DHF have not been found, hence, therapy is limited to supportive treatment to manage plasma leakage and bleeding.[1] As such, it is necessary to find an early predictor of the occurrence of DSS or severe dengue virus infection, so that DHF can be treated early in the course of disease.

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The pathogenesis, with regards to the occurrence of dengue infection and its manifestations, has not been clearly explained. Endothelial cells, as well as pro-inflammatory and anti-inflammatory cytokines, in both innate and adaptive immunity are involved in the pathomechanism of DHF.[4] Cellular damage and cytokine activity may be the cause of plasma leakage, which tends to occur in for 24-48 hours in DHF patients.[5,6] However, some patients experience recurrent or prolonged shock. Based on empirical experience, endothelial cell damage may play a role in the pathomechanism of shock. Plasma leakage in DHF has been associated with a variety of cytokines.[7] In vitro, IL-8 concentration was increased in transendothelial electrical resistance (TEER) of human umbilical vein endothelial cells (HUVECs) and human endothelial cells-1 (HMEC-1).[8,9] In vivo, IL-8 has been associated with endothelial cell damage.[10,11] Interleukin-8 has been known to be associated with DHF and DSS.[10] Wong *et al*.[12] found an IL-8 cut-off point of >220pg/mL in patients that survived from septic shock, but an IL-8 cut-off point has not been studied in children with dengue. As such, we aimed to assess IL-8 levels in pediatric dengue patients, whose conditions improved or deteriorated. We also aimed to define a cut-off point of IL-8 to be used as a biomarker to predict clinical deterioration (to recurrent or prolonged shock or died) in children with DSS.

Methods

This prospective cohort study was conducted at the Department of Child Health, Prof. Dr R.D. Kandou Hospital, Manado from July 2012 to July 2014. Subjects were all pediatric patients aged 1-14 years and diagnosed with DSS, according to the WHO (2011) criteria.[2] We excluded children with bacterial infection and those who had received corticosteroid treatment or blood transfusion. The minimum required sample size was 38 (α= 0.05, β=0.2 r=0.4, P=0.8). At the time of diagnosis, blood specimens were examined for initial serum IL–8 levels (pg/mL) using the *Human CXCL8/IL-8 Immunoassay*. Subjects' age, sex, nutritional status, vital signs (temperature, pulse, blood pressure, breathing, and level of consciousness), and clinical symptoms were noted. Patients were followed up until there was improvement (cured) or deterioration (to recurrent or prolonged shock or died).

Data were first described as frequency, mean, median, standard deviation, and range. Univariable analysis with logistic regression test was used to assess for an association between serum IL-8 concentration and DSS outcome. Mann-Whitney test was used to compare initial serum IL-8 levels between the improvement and deterioration groups. Determination of prognostic value was done using a ROC curve and Chi-square test. Statistical significance was considered to be P<0.05. Data processing was done with *SPSS version 22.0 for Windows* software. The study was approved by the Ethics Committee for Health Studies at Sam Ratulangi University Medical School/Prof. DR. R.D Kandou Hospital, Manado.

Results

This study was conducted on 58 patients with DSS from July 2012 to July 2014. Twenty-seven subjects experienced deterioration of their condition, while 31 experienced improvement (**Figure 1**). No subjects dropped out of the study. In the DSS improvement group, there were 21/31 males and 10/31 females, while in the DSS deterioration group, there were 16/27 males and 11/27 females. The mean age of patients was 7.4 years in the DSS improvement group and 6.1 years in the DSS deterioration group. Normal nutritional status was observed in 19/31 children in the DSS improvement group and 20/27 children in the DSS deterioration group. Abdominal pain was

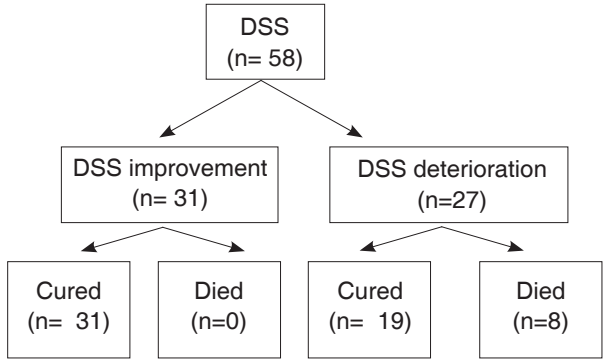

Figure 1. Outcomes of DSS

Table 1. Subjects' characteristics based on clinical course of DSS

Characteristics	Clinical course	
	DSS	
	DSS improvement (n=31)	DSS deterioration (n=27)
Gender, n		
Male	21	16
Female	10	11
Age, years		
Mean (SD)	7.4 (2.4)	6.1 (3.2)
Median (range)	7 (3-12)	5.5 (1-11)
Nutritional status, n		
Obese	5	2
Overweight	3	1
Normal	19	20
Undernutrition	4	3
Malnutrition	0	1
Abdominal pain, n		
Positive	19	25
Negative	12	2
Bleeding, n		
Mucosal	5	8
Non-mucosal	26	19
Fever day of shock, n		
Day 3	12	6
Day 4	16	10
Day 5	1	8
Day 6	2	3

Table 2. Clinical and laboratory characteristics of subjects based on clinical course

	Clinical outcomes		
	DSS improvement	DSS deterioration	
IL-8 level, pg/mL			
Mean (SD)	72.3 (61.2)	441.3 (200.72)	P=0.0001
Median (range)	60.8 (5.8-288.8)	447.0 (88.79-920.0)	

seen in 19/31 children in the DSS improvement group and in 25/37 children in the DSS deterioration group. Mucosal bleeding was found in 5/31 children in the DSS improvement group and 8/27 children in the DSS deterioration group. Subjects' fever duration was from 3 to 6 days, with resolution of fever most commonly occurring on the 4th and 5th days of fever in both groups (**Table 1**). Laboratory results showed higher levels of initial serum IL – 8 in the DSS deterioration group than in the improvement group (**Table 2**).

The results of the Mann-Whitney test analysis showed that the initial mean serum IL-8 levels were significantly higher in the DSS deterioration group (experienced recurrent shock, prolonged shock, or death) compared to those of the DSS improvement

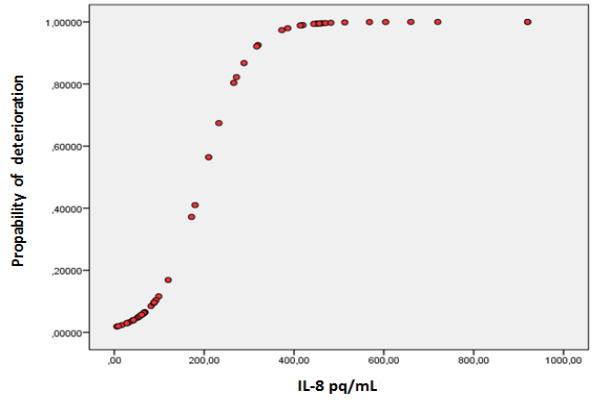

Figure 2. Scatterplot correlation of IL-8 and the deteriorations in DSS patient

(healthy) (mean 441.3 pg/mL *vs.* 72.3 pg/mL, respectively; P=0.0001). **Figure 2** shows statistically significant association between increased IL-8 and the DSS deterioration (P=0.0001). For predicting deterioration in DSS patients, ROC curve analysis revealed a cut-off IL-8 level of 194.9 pg/mL, with area under curve (AUC) of 0.982, 89.3% sensitivity, 93.3% specificity, 92.6% PPV, 90.3% NPV, and OR 116.7 (95%CI 18 to 756; P=0.0001) (**Figure 3**).

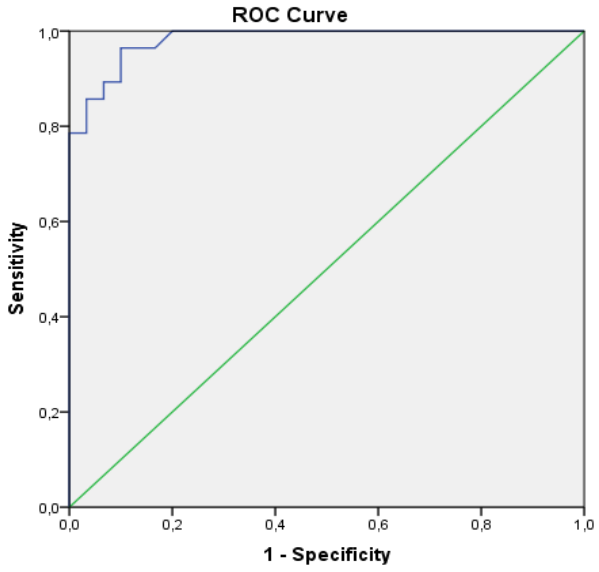

Figure 3. Area under curve IL-8 level between DSS improvement and deterioration groups

Discussion

In our study, we had more male than female DSS patients. A previous study found male more than female but there is no differences in the risk of males and females to experience DHF.[13] We found the majority of DSS cases to have normal nutritional status. A previous study reported that the majority of dengue and DSS cases have normal nutritional status.[14] Nutritional status is believed to affect the degree of disease severity, based on the theory that good nutrition increase the antibody response.[15] Excessive antigen and antibody reactions lead to more severe dengue infection.[1] However, the mechanism of the increased DSS severity in obese patients remains unclear. A study in El Salvador also found no significant relationship between nutritional status and DHF severity.[16] Our patients got shock for the first time on days 4 and 5 fever, similar to previous studies.[2,6] The pathomechanism of DHF involves leukocyte migration on day 5, and increasing numbers of immature neutrophils.[17]

Severe dengue infection is characterized by plasma leakage due to increased vascular permeability, coagulation of bleeding due to imbalance in hemostasis, or any other serious organ involvement.[13] Dengue viral infection induces endothelial cell production of cytokines, one of which is IL-8.[8] Increased IL-8 is associated with plasma leakage, endothelial cell damage, impaired coagulation, and fibrinolysis in dengue patients.[18] In our study, we found a significant relationship between IL-8 and the occurrence of deterioration in our DSS patients (**Figure 2**). The initial mean serum IL-8 level was higher in the DSS deterioration group (recurrent shock, prolonged shock, and/or death) than in the DSS improvement group (**Table 2**). Patients with higher IL-8 levels tended to have worse outcomes. Huang *et al*. found higher IL-6 and IL-8 levels in DSS and DHF patients compared to those with DF.[8] A study also reported higher IL-8 levels in DSS patients compared to DHF patients.[19] Another study observed that only patients with fatal shock had higher IL-8 levels.[20] These previous studies demonstrate that IL-8 plays an important role in predicting DHF or DSS outcomes.

We found that an initial serum IL-8 cut-off level of 194.9 pg/mL can predict DSS patient deterioration with OR 116.7, 89.3% sensitivity, and 93.3% specificity. This is a good result based on the Jouden index (more than 0.50).[21] These results suggest that predicting morbidity and mortality in DSS patients should not be based solely on clinical appearance at the time of diagnosis, but should be based on parameters indicating severity of ongoing endothelial cell damage. Patients with DSS may have better prognoses if there is little damage of endothelial cells, as mediated by IL-8. This statement is consistent with the empirical experience of clinicians. Armed with the patient's IL-8 levels, the clinician can change the management approach to improve outcomes of morbidity and mortality from DSS.

A strength of our study was its cohort prospective approach, which to our knowledge, has not been used

in previous studies. A limitation of the study was the lack of subject stratification based on duration of fever (length of dengue infection). Stratification is needed because if shock occurs in the early days of fever it sugests a high number of viral load and also high viremia.

In conclusion, there is an association between elevated early serum IL-8 level and DSS deterioration.

Conflict of interest

None declared.

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