Paediatrica Indonesiana

VOLUME 55 March • 2015 NUMBER 2

Original Article

Quantitative NS1 antigen and the severity of dengue virus infections

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Abstract

Background Dengue infection is one of the main cause of morbidity and mortality in children in Indonesia. Since it is known that earlier treatment and supportive therapies can decreased case fatality rate from dengue hemorrhagic fever (DHF), identification of children who have risks to develop to DHF must be quickly identified, mainly in areas of endemic.

Objective To find a correlation between increased quantitative secreted nonstructural protein-1 (sNS1) with clinical course of severe dengue infections.

Methods This was a cross-sectional study conducted on children with dengue infections in Tropical Infections Division of Child Health Department, Sanglah Hospital, Denpasar. Detection of the dengue antigen was made by examining sNS1 quantitative immuno-assay. Analysis correlation of Spearman test was used to look the relationship between increased quantitative sNS1 with clinical course of severe dengue infections.

Results There was a positive relationship between quantitative sNS1 and clinical course of severe dengue infections with a value of r = 0.903, P=0.001. Increased sNS1 level had a positive correlation with more severe dengue infections.

Conclusions Quantitative sNS1 titer has a strong positive correlation with clinical course of severe dengue infections. **[Paediatr Indones. 2015;55:87-90.]**

Keywords: *dengue infection, severity, sNS1*

Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are severe and potentially fatal complications of infection by dengue virus. Prevalence of dengue shock syndrome still reported high in Sardjito Hospital Yogyakarta. Between January 2002 until August 2003 it was reported that 41% patients DHF had dengue shock syndrome. *World Health Organization* reported that DSS occurs in 35.2% of DHF cases.[1,2] Crude fatality rate of DHF in Indonesia was 4.6%.[3] Early intervention and effective management of plasma leakage, with plasma fluid substitution (plasma expander) or with electrolyte solution, in a fast, proper, and adequate way, can reduce the fatality of DHF due to prolonged shock.[3,4]

Early identification of patients that have potential risks to develop into severe DHF or DSS is very important to prompt supportive treatment to reduce the number of deaths.[5,6] Until recently, there is still no examination of dengue infections that can lead us to predict whether this infection will growth to be more severe degrees. Dengue virus nonstructural

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protein-1 (NS1) was found at the time of dengue viral replication.[7] The NS1 protein examination display in the form of membrane associated protein (mNS1) and secreted form (sNS1). The sNS1 is related to the pathogenesis of severe dengue virus infections. A preliminary study indicates the involvement of NS1 in viral RNA replication.[8] The level of secreted form of NS1 (sNS1) in plasma has a strong correlation with viral titer, where higher level of sNS1 is found more frequent in patients with DHF compare to dengue fever.[9,10] Therefore the primary objective of this study was to determined a correlation betwen quantitative sNS1 measurement with dengue infection clinical course, from mild to severe form.

Methods

This study was a cross sectional study, performed at the Pediatric Tropical Infections Division of Sanglah Hospital in Denpasar, on children with suspected dengue infection. Inclusion criteria was children who had fever on the 4th day, with two or more of the following symptoms: headache, retroorbital pain, myalgia or athralgia, maculopapular rash, petechiae, and positive torniquet test. An informed consent was provided by parent or caregivers. Children whose caregivers refused to provide any informed consent were excluded. Sample size was calculated for a correlation test with α= 0.05, power = 80 %, with correlation coefficients from the previous studies was 0.6, revealed the minimum subjects for the study was 37.

Quantitative NS1 examination was a measurement of NS1 antigen titer with immuno-assay method using one-step sandwich ELISA microplate formats, the *Platelia™ Dengue NS1 Ag* test (*Biorad Laboratories Marnes-La-Coquette, France*). Enzyme immuno-assay examination was performed using 50γL serum samples that were incubated directly and simultaneously with 50γL diluents (phosphate buffer) and 100γL diluted conjugated (anti- NS1 Mab coupled with horseradish peroxydase) for 90 minutes using a microplate in temperature of 37°C. After 30 minutes, microplates were washed and complex immune response was detected by a change of color. Then discoloration was seen through its optical density, read with spectrophotometer that has been programmed with settings on 450/620 nm to detect sNS1 free antigen in the samples.

Severity of dengue infections can be seen through its clinical course that manifests as clinical diagnosis that met with WHO 1997 criteria,[1] which were classified as dengue fever (DF), DHF grades 1,2,3, and 4. Dengue infection that accompanied with shock was defined as DHF grades 3 and 4.

Data was analysed using Spearman correlation test because data was not normally distributed. This analysis was performed to find the relationship between the quantitative NS1 levels with DF, DHF grades 1, 2, 3, and 4. Ethical clearance was proven by the Research dan Development Board of Udayana University Medical School and a research permit was provided from Sanglah General Hospital.

Results

During the study period a total of 37 subjects with dengue infections fulfilled the eligible criteria, consisted of nine subjects-with DF, 13 subjects with

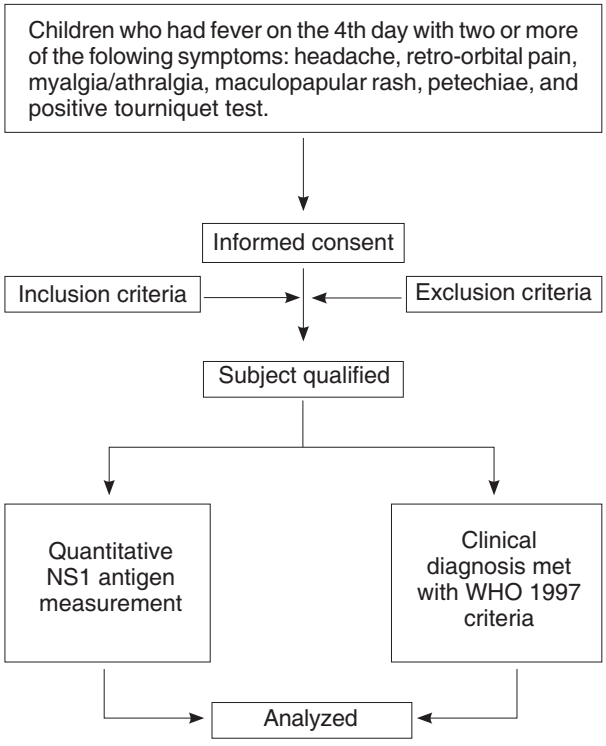

Figure 1. Study scheme

DHF grade 1, two subjects with DHF grade 2, nine subjects with DHF grade 3, and 4 subjects with DHF grade 4. The study scheme is shown in **Figure 1**.

Characteristics of the study subjects are shown in **Table 1**. The mean age of the study subjects was 86.7 (SD 3.7) months, with 20/37 subjects were girls. The mean leukocyte count, hematocrit level, and platelet counts, were 4.62 (3.06 SD)/μL , 43.77 (SD 5.12)%, 69.12 (SD 22.91)/μL, respectively.

Table 1. Characteristics of study subjects

Characteristics	N=37
Mean age (SD), months	86.97 (3.7)
Gender	
Girls, n	20
Mean WBC (SD),/μL	4.6 (3.1)
Mean hematocrit (SD),%	43.8 (5.1)
Mean platelets (SD),/μL	69.1 (22.9)
Diagnosis, n	
DF	9
DHF grade 1	13
DHF gradel 2	2
DHF grade 3	9
DHF gradel 4	4

DF=dengue fever, DHF=dengue hemorrhagic fever

The correlation between sNS1 levels with clinical spectrum of dengue infections is performed on **Figure 2**. Spearman correlation analysis showed a significant correlation between the quantitative sNS1 levels with severity of dengue infections with r=0.903 (P value=0.001).

Discussion

Dengue virus (DENV) nonstructural protein-1 (NS-1) is a secreted glycoprotein that is absent from viral particles but accumulates in the supernatant and on the plasma membrane of cells during infection. A previous study indicates the involvement of NS1 in viral RNA replication.[7] Secreted NS1 (sNS1) level in plasma correlates with viral titer, where it was found higher in patients with DHF than dengue fever.[8,9] Non structural protein 1 (NS1) level increases within 72 hours after onset of the disease (viremia phase), which indicates the risk of evolving disease towards DHF. In a study, it was found that the cut -off value of free sNS-1 titer to develop into DHF was 600ng/mL using capture ELISA method with r=0.6.[8]

In addition, a previous study revealed that the NS1 protein can be directly involve in the course of the disease that become more severe through its ability to stimulate dendritic cells to produce cytokines, such as TNF- α, IL- 1, and IL- 6.[11] Also heterologous infection phenomena associated with antibodies is predicted as a major component in the pathogenesis of severe complications in dengue virus infection. In secondary dengue infection, antibodies are formed in a complex with dengue virus. Those two pathogenesis mechanism show the ability of sNS1 to induce the occurrence of more severe dengue infection.[11,12]

Similar results with previous studies was obtained in this study, that showed strong positive correlation

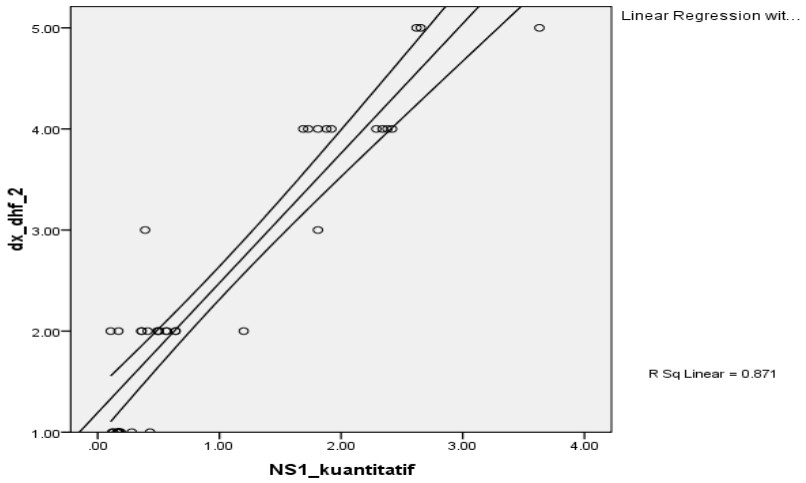

Figure 2. A correlation curve between sNS1 levels with severity of dengue infections
dx_dhf 2: DHF diagnoses: 1= DF, 2= DHF grade 1, 3= DHF grade 2, 4 = DHF grade 3, 5 : DHF grade 4

(r = 0.903, P = 0.001) between sNS1 quantitative titer with severe clinical course of dengue infections. Positive correlation indicates that the higher sNS1 level, the severer the clinical course. Our finding shows higher correlation between sNS1 titer and the severity of dengue infections compared to a previous study[8] due to different laboratory tool used. The previous study used *Pan-E Dengue ELISA* (*Pan-Bio Diagnostic*, Brisbane, Australia) while our study was using *Platelia™ Dengue NS1 Ag-ELISA* (*Biorad Labororatories Marnes-La-Coquette*, France). *Pan-E dengue ELISA* (*Panbio®*) showed higher sensitivity in confirming DENV-1 and DENV-3 infections. The *Platelia™* NS1 kit (*Biorad®*) was more sensitive in the detection of DENV-1 and DENV-2 infections.[9] Indonesia has highest prevalence of DENV-2 infections, this might explain the ~~was~~ different results with the previous study. Same explanation can be also applied for some outliers seen in the scattered plot. The outlier plots might represent other viral serotype than DENV-2 infection.

The limitation of our study was that blood samples for that sNS1 quantitative measurement were taken at day fourth of fever, while previous studies have shown that the peak of serum sNS1 level was reached on fever day third.[8,9] Other limitation was we did not perform RT-PCR to distinguished serotypes of the dengue viral.

We conclude that quantitative sNS1 measurement has a strong positive correlation with the severity of dengue infections

Acknowledgment

Our sincere gratitude to the subjects of the study, their parents, physician, and nurses in charge at Jempiring Ward Sanglah Hospital, nonethless I Gde Raka Widiana, MD for his help in constructing methodology and statistical analysis in this study.

Conflict of interest

None declared.

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