Association of serologic and hematologic test results in dengue infant patients in RSUP. Dr. Hasan Sadikin Bandung

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Abstract. The incidence of Dengue virus infection is increasing every year and the progression of the disease is faster towards severe manifestations in infants than in children and adults. The clinical appearance is still challenging to make for the diagnosis of dengue fever, so routine blood examination becomes one of the further enforcement efforts. The gold standard is confirmatory tests for dengue, but this examination would be difficult in remote areas and also cost more. Research on serological testing and its association with routine blood testing in infant dengue-infected patients is still less publicized. The purpose of this study was to describe the connection between serological and routine blood test results of infant dengue infection patients in RSUP Dr. Hasan Sadikin. Observational design in dengue 56 infants with 2-12 months age range examined serologic test and routine blood examination. The results showed that serological testing tended to be on routine blood tests. It can be from differences in routine blood tests such as hemoglobin, hematocrit, and platelets. Also, there was also no difference in routine blood profile between reactive and non-reactive IgM groups. It suggests that routine blood examination results are still lacking for the diagnosis of dengue.

1. Introduction

Dengue infection is one of the significant public health problems in Indonesia. The number of sufferers and the extent of their spreading area increases with the increasing mobility and population density. The year 2007 was the year with the highest reported cases of dengue infection which reached 150,000 cases where 16.7% of cases were from Jakarta and West Java. The average reported cases of 95% reported in children <15 years and ≥5% of cases occurring in infants in the form of Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), the rate of dengue case mortality in infants is four times higher than in children and adults.

Clinical and laboratory manifestations can be seen based on three phases of dengue infection: the fever phase, the critical phase, and the healing phase. Or it can also be seen based on three clinical spectrum of Dengue infection based on WHO 2011 that is: dengue fever (Dengue Fever (DF)), dengue fever / DHF (Dengue Hemorrhagic Fever) and Dengue Shock Syndrome (DSS) shock syndrome. As well as the expansion syndrome of Dengue (Expanded Dengue Syndrome (EDS)). In the critical phase, clinicians should be alert to significant vascular leakage events as they progressively aggravate the condition of patients characterized by severe abdominal pain, ongoing vomiting, hepatomegaly, hematocrit increase, platelet decline (<20x10^9/L), serous effusions, membrane bleeding mucus and
decreased consciousness.4

In infants, the risk of DBD / SSD is higher than in children and adults. DHF / SSD usually occurs in a second infection by a virus with a different type of serotype than the first type of serotype that infects the child and adult. But in infants, DHF / SSD can occur during infection first time. This may be due to several unique conditions in infants that differentiate it from pediatric and adult patients such as the more fragile blood vessel integrity of infants, the presence of IgG from mothers who have previously suffered from Dengue (which would lead to an Antibody-Dependent Enhancement (ADE) mechanism) and/or a vertical virus infection of the mother.5,6 This study was conducted with the aim to provide an overview of the relationship between serological test results with routine blood examination of Dengue infant infants who underwent inpatient at Dr.Hasan Sadikin Hospital Bandung.

2. Methods

This research uses descriptive quantitative observational design with the cross sectional approach. The researcher collected data from the patient's medical record in the form of clinical manifestation record and laboratory test result the first day of undergoing the hospitalization of the infant patient who had been Dengue. Data are followed to obtain data of clinical manifestation and nadir of laboratory test result of Dengue Infection patient who underwent hospitalization in RSUP Dr.Hasan Sadikin Bandung in the period January 2011-December 2016. Data collection is after submitting and obtaining a letter of ethical release (No:347/UN6.C.10/PN/2017) and research permit from Dr.Hasan Sadikin General Hospital Bandung (No: LB.02.01/X.2.2.1/9448/2017). Inclusion criteria include infant diagnosed with dengue infection criteria. Patients with NS-1 and IgM and/or IgG positive were in the tested serologic group (31 patients) when infant came with fever, bleeding manifestation, hepatomegaly, low platelet, and leukocyte were hematologic group (25 patients). The laboratory data to be used is data at the time of admission or before admission, i.e., hematology test (Hb, Ht, leukocyte, platelet) and dengue diagnostic serology test (NS1 and IgM IgG anti-Dengue). Data were processed using SPSS 13.0 software.

3. Results

Of the total 136 Dengue infants treated at RSUP Dr. Hasan Sadikin in the period, 2011-2016 found 56 patients who meet the criteria of inclusion; the rest obtained the missing or incomplete medical records that make it into the exclusion criteria. Data on the distribution of age and sex as described in Table 1. The youngest patient was found to be two months old.

Table 1. Subjects profile.

| Variable                        | Dengue Infection and Fever (n=56) | Dengue Haemorrhagic Fever and Dengue Shock Syndrome (n=28) | Expanded Dengue Syndrome (n=4) |
|--------------------------------|-----------------------------------|-------------------------------------------------------------|-----------------------------|
| Age (months)                   |                                   |                                                             |                             |
| < 4                            | 7 12.5                            | 6 2                                                         |                             |
| 4-8                            | 39 69.6                           | 19 16                                                      | 3                           |
| 9-12                           | 10 17.9                           | 3 6                                                        | 1                           |
| Sex                            |                                   |                                                             |                             |
| Male                           | 24 42.9                           | 14 10                                                       |                             |
| Female                         | 32 57.1                           | 14 14                                                       | 4                           |
| Check-in – days since the first day of fever (days) |                             |                                                             |                             |
| 0-3                            | 13 23.2                           | 9 3                                                         |                             |
| 3-6                            | 43 76.8                           | 17 25                                                       | 1                           |
*Age grouping based on maternal IgG decayed presentation.

Results of hematologic examination of patients with Dengue infants are in Table 2. Laboratory results were in the highest level of hemoglobin and hematocrit, and the lowest level of leukocyte and platelets during hospitalization. Levels of hemoglobin, hematocrit, and platelet counts in both groups were significantly different (p-value<0.05), with Hb, hematocrit and platelet count in the serologically tested group being lower than those in the serologic group. The number of leukocytes in both groups did not show significant differences.

Table 2. Hematology test results.

| Variables            | Serologic tested group (n=31) | Hematologic group (n=25) | P value |
|----------------------|-------------------------------|--------------------------|---------|
| Hemoglobin (g/dL)    |                               |                          |         |
| Mean(SD)             | 10.70(1.26)                   | 11.81(1.74)              | 0.017   |
| Range                | 8.20-13.90                    | 8.90-16.00               |         |
| Leukocytes (cell/mL) |                               |                          |         |
| Mean(SD)             | 9,171(4,989)                  | 10,028(5,360)            | 0.499   |
| Range                | 2,000-23,600                  | 2,300-25,000             |         |
| Hematocrit (%)       |                               |                          |         |
| Mean(SD)             | 31.5(3.7)                     | 34.2(5.7)                | 0.052   |
| Range                | 24.0-39.0                     | 25.0-47.0                |         |
| Thrombocyte (cell/mL)|                               |                          |         |
| Median               | 32,000                        | 69,000                   | 0.023   |
| Range                | 5,800-151,000                 | 5,800-312,000            |         |

Results of hematologic serologic examination based on the serological reactivity of patients with Dengue infants are in Table 3. There were no major differences in levels of hemoglobin, leukocyte, hematocrit, and platelet counts in both groups (p-value>0.05). However, Hemoglobin, leukocyte, hematocrit and platelet count in the IgM reactive group being lower than those in the IgM non-reactive group.

Table 3. Hematologic test results based on IgM reactivity.

| Variables            | IgM Reactive (n=26) | IgM Non-Reactive (n=5) | P value |
|----------------------|--------------------|------------------------|---------|
| Hemoglobin (g/dL)    |                    |                        |         |
| Mean(SD)             | 10.68(1.35)        | 10.78(0.68)            | 0.830   |
| Range                | 8.20-13.90         | 9.90-11.80             |         |
| Leukocytes (cell/mL) |                    |                        |         |
| Mean(SD)             | 8,865(4,504)       | 10,760(7,492)          | 0.747   |
| Range                | 2,000-19,500       | 5,600-23,600           |         |
| Hematocrit (%)       |                    |                        |         |
| Mean(SD)             | 31.24(3.89)        | 32.84(1.85)            | 0.204   |
| Range                | 24.0-39.0          | 30.2-35.0              |         |
| Thrombocyte (cell/mL)|                    |                        |         |
| Mean(SD)             | 39,292(34,223)     | 74,400(52,647)         | 0.126   |
| Range                | 5,800-129,000      | 23,000-151,000         |         |

4. Discussions

Dengue is an acute disease caused by a virus of the genus Flavivirus called Dengue Virus (DENV) which has four different serotypes (DENV1, DENV2, DENV3 and DENV4). The disease is transmitted by mosquitoes of the genus Aedes that can become asymptomatic or develop into
symptomatic. Clinical spectrum of Dengue virus infection can be into asymptomatic, Dengue fever with or without accompanying bleeding, dengue hemorrhagic with or without shock and expansion syndrome Dengue manifestation.2,4,6 Various signs occur both the result of clinical and laboratory depend on the pathogenesis of the patient. In infants primarily, the symptoms of Dengue infection tend to experience progression towards severe symptoms more rapidly than children and adults. It is due to several reasons such as infant's hemodynamic ability not yet competent enough to compensate for the state of the plasma leak syndrome during Dengue infection and the Antibody-Dependent Enhancement (ADE) mechanism that occurs when the infant is infected. The second Dengue virus with a different serotype than the first infected times on her or her mother. In infants, there may also be a vertical transmission of the virus from mother to child through the placenta.3,5

Antibody-Dependent Enhancement is the mechanism by which viral replication becomes accessible and widespread, and the number of viruses generated becomes more due to immunoglobulin G in infants transmitted from the mother through the placenta that will directly bind to different dengue virus serotypes and will mediate viral endocytosis into dendritic cells. When the virus enters the cell dendritic, it interacts with Ig-like receptor B1 which has an inhibitory effect on FcR signaling to produce more competent specific antibodies to eradicate the virus, which in turn facilitates and facilitates the virus to be able to replicate freely and then infect other cells. With this ADE mechanism, the number of viruses produced will also infect more cells and consequently the reaction of these cells by generating more inflammatory mediators will result in the more significant manifestation of plasma leakage. It is what causes the severity of the disease higher in primary dengue infection in infants or secondary in children and adults with different types of serotype virus.5,7,8

Results of hematologic examination of patients with Dengue infants showed that the levels of hemoglobin, hematocrit, and platelet counts in both groups were significantly different (p-value<0.05). Hemoglobin, hematocrit and platelet counts in the serologic group being lower than those in the hematologic group. It shows that with proper hematological results, a relative examination was not performed, mainly based on the condition of hemoglobin and platelets.

In this study, data on the age of patients with Dengue infants were presented based on maternal IgG smelting presentation based on infant age. The incidence of DBD / SSD in infants is closely related to age at the time of maximum Dengue infection-enhancing activity. This critical period occurs about two months after the decline of Dengue-neutralizing maternal antibody to below its protective level. Dengue infections in infants aged 4-12 months tend to be severe because at this time lag may occur attachment of virions and non-neutralizing IgG that can facilitate the mechanism of ADE causing severe manifestations due to the emergence of 'cytokine storm.'

The data were at the worst value of hematology examination. Hematology examination based on IgM reactivity of patients with Dengue infants showed there were no significant differences in levels of hemoglobin, leukocyte, hematocrit, and platelet counts in both groups. It shows that the results of the hematologic examination alone cannot accurately diagnose dengue infection. But in the serologic group, patients with high platelet counts were found. However, these data suggest that good starting conditions can then progress to dengue fever, which may lead to the risk of death in infants.

However, hemoglobin, leukocyte, hematocrit and platelet count in the serologic group being lower than those in the dengue clinical group. It suggests that with a good hematological condition, IgM and/or IgG and/or NS-1 examination are relatively not performed. In Indonesia, dengue confirmatory testing is not yet a fixed procedure; it is still an option. It is because the facility to conduct those tests cannot be done in all regions in Indonesia. Tests can only be in sophisticated laboratories located in large cities. Also, the cost of performing is relatively expensive compared to routine hematologic examinations, so this examination becomes an option offered to the patient's family. In Table 3, the difference in platelet count is quite significant between the two groups although not statistically significant. The number of platelets can be one of the considerations whether serological tests need or not. However, if looking at the results of this study, then dengue confirmatory tests should be a mandatory procedure in Indonesia, especially in infant patients. Also, to reduce the risk of serologic
examination should be done despite the clinical conditions and the results of infant hematology in the normal range or good.

The limitation of this study is the number of medical record data of patients who lost up to 45.5% so it is unfortunate that the data of these patients cannot contribute to illustrate the manifestations in this study. This research is still lacking. It is recommended for further research to observe the clinical manifestation and laboratory manifestation until the results directly to the patient Dengue baby who is being treated each year to produce more comprehensive and reliable report and minimize bias. So that efforts to increase knowledge of medical personnel against clinical manifestations and laboratory of patients with Dengue infants can be achieved so that early detection, monitoring and effective management of patients with Dengue infants can be achieved to maintain or even decrease the mortality rate of infant dengue.

5. Conclusions

Results of hematologic examination of patients with Dengue infants showed that the levels of hemoglobin, hematocrit, and platelet counts in both groups were significantly different (p-value<0.05). It shows that with good hematological results, a relative examination was not performed, mainly based on the condition of hemoglobin and platelets. Hemoglobin, leukocyte, hematocrit and platelet count in the serologically reactive group being lower than those in the non-reactive group. It suggests that with an excellent hematological condition, the serological examination is relatively not performed.

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