Incidence Dengue Virus Infection in Children with Acute Fever in Endemic Area, Lesson from Primary Health Care in Western Java Indonesia

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Abstract Context: In endemic countries, most of dengue fever cases clinically resembles other diseases or just flu-like syndrome, it is important to detect dengue infection in patient with acute fever 1 to 4 day and which serotype of dengue virus that circulation to determine the risk of severe case of dengue to occur in primary health care. Aims: The aim of this study was to identify the incidence of dengue viral infection among children with acute fever in endemic area, and which serotype of dengue virus that circulating in community in Bandung, West Java, Indonesia. Settings and Design: Prospective cohort analysis study. Methods and Material: patients age 0 to 18 years old with acute fever from Sukajadi, Neglasari, and Padasuka Primary Health Centre form march to October 2018. These primary health centers represent three subdistricts with high dengue case every year in Bandung. Inclusion criteria: Fever at least $37.6^{o}C$, 1-4 days, with or not taking antipyretics. Name, age, and sex, nutritional status, and laboratorium examination that has been done by primary health centre was collect. Rapid NS1 antigen test is done as an additional check, multiplex real time RT-PCR assay for dengue virus (1-4) was perform from blood sample that NS1 antigen positive. Statistical analysis used: independent t-test analysis. Results: A total of 178 blood samples from patients with acute fever collected. 40 (22,5%) samples were positive with rapid NS1 antigen test, mean body temperature significantly higher in confirmed dengue virus cases (37,66 (±2,07) vs 38,33 (±0,83); p<0,005) while mean leucocyte count (8.964 (±5.369) vs 4803.91 (±2.197); p>0,005) and platelet count (239.218 (±99.402) vs 164.739,13 (±98.259); p>0,005) is lower in confirmed dengue virus cases. From 40 positive NS1 antigen test, 17 (42,5%) showed all serotype (Den 1-4) of dengue can be found, with 8 (47,05%) is Den 3. Conclusions: Incidence of dengue cases in patient with acute febrile in endemic urban area is quite high, and all serotype of dengue virus can be found, so the risk of secondary dengue virus infection is imminent.

Keywords: incidence, dengue virus, acute fever, serotype, endemic area

1. Introduction

Dengue is one of the most common arthropod-borne viral diseases in the tropical and subtropical regions of the world; annual incidence of 400 million infections and 100 million hospitalizations from dengue fever occur worldwide. Significant amount of mortality contributed by dengue in children (up to 21,000 deaths annually), particularly in untreated cases. [1,2] Dengue patients had variety of clinical signs and symptoms with varying age and severity. [3] The most frequently encountered is headache and body aches. In early phases of infection, the symptoms are very similar to flu. Classification of dengue fever severity according to World Health Organization (WHO) and divided into four categories according to the severity. [4]

Indonesia is reported as the second largest dengue fever among 30 endemic countries, with higher prevalence in several provinces, such as East Java, West Java, and Central Java. Incidence trend in Indonesia tend to increase. In 1968, the first 58 dengue cases were reported in Jakarta and Surabaya. Since then, the numbers had increased and many other geographical locations have also become endemic. A study in 2014 reported an annual dengue fever incidence increase, from 0.05 / 100,00 in 1968 to 35 - 40 / 100,000 in 2013. The highest incidence rate in Indonesia occurred in 2010 with the incidence of 85.7 / 100,000. Significant mortality was found in cases of dengue fever in Indonesia, with case fatality rate (CFR) reaching up to 41% in 1968. Despite the increasing

incidence, however, the CFR for dengue fever has been decreased into 0.73% in 2013. [5]

Laboratory tests in dengue patients, particularly complete blood count (CBC) may be altered during days 3 - 8. Due to specific pathophysiology of dengue virus, progressive leukopenia followed by thrombocytopenia and hemoconcentration due to plasma leakage may occur. Serological tests may be utilized in tandem with laboratory tests in order to confirm dengue infection. Several methods are available, such as NS1 antigen (sensitivity 76% and specificity 98%) or the dengue IgM antibody using ELISA method (sensitivity 90% and specificity 93%). A study by Chaloemwong et al. had found that patients with dengue had significantly lower leukocyte count and lower platelet count. [6] Ralapanawa et al. had concluded that hemoglobin values and acute phase platelet values may be utilized accurately in determining the severity of dengue fever. [7]

Serological type of dengue virus is categorized into four types: DENV-1, -2, -3, and -4. Dengue infection produces neutralizing antibodies (NAbs) against the four serotypes as immunological reaction. Serotype studies had revealed that DENV-1 was the most common form of dengue virus (41%) followed by DENV-2 (31%), DENV-3 (20%) and DENV-4 (7%). According to a seroprevalence study by Sasmono et al., the majority of children in urban population of Indonesia had multitype infection (previous infection with more than one dengue virus serotype); DENV-2 was the most prevalent serotype in dengue infection according to the study. [8] The aim of this study is to describe the epidemiology and virology (particularly the serotype) of dengue infection in children in urban area, specifically in Western Java.

2. Methods

2.1. Sampling Collection

We examine patients with acute fever from Sukajadi, Neglasari, and Padasuka Primary Health Centre. These primary health centers represent three subdistricts with high dengue case every year in Bandung. The data collection were performed in the period of March - October 2018.

2.2. Patient Criteria

Dengue fever patients in the aforementioned primary health centers were recruited for this study. The inclusion criteria for the patients in this study were:

- Fever >37.6°C for 1 - 4 days for all ages with or without previous usage of antipyretics
- The parents of the children are willing to consent
- Sociodemographic variables were collected (name, age, and sex)
- Nutritional status and laboratory examination have been performed by primary health center.

All of the data from the patients are confidential and only used for the purpose of this study.

2.3. Laboratory Examination

A blood sample is taken from the patients enrolled in the study by the laboratory worker. Routine blood work (Hemoglobin, leukocytes, hematocrit, and thrombocytes) and rapid NS1 antigen test were performed. Additional examination using multiplex real time RT-PCR assay (for dengue virus 1 - 4) was utilized to confirm the samples with positive results from rapid NS1 antigen test.

2.4. Statistical Analysis

The collected data will be analyzed using an independent t-test to compare the differences between patients with acute fever with positive NS1 results and different serotypes of dengue virus.

2.5. Ethical Statement

This study has been approved by the ethics committee of University of Padjadjaran.

3. Results

There were 178 blood samples collected from patients with acute fever from Sukajadi, Neglasari, and Padasuka primary health center. The majority of the patients had negative NS1 result (77.5%). There were 40 patients whom tested positive using NS1 test; the majority of these patients were in 5 - 14 years old age group (25%) and with normal nutritional status (72.5%). The CBC was performed in 23 patients (57.5%). Mean body temperature was significantly higher in confirmed dengue cases (37.66 ± 2.07 vs. 38.33 ± 0.83; p < 0.005). Mean leukocyte count was lower in confirmed dengue cases (8.964 ± 5.369vs. 4803.91 ± 2.197) and mean platelet count was lower in confirmed dengue cases (239,218 ± 99,402 vs. 164,739.13 ± 98,259). In both of the hematological profiles, however, was not significantly different (p > 0.005).

Table 1. Characteristics of patients with acute fever

No	Characteristics	NS 1 Negative n=138 (77,5%)		NS 1 Positive n=40 (22,5%)		P value
		n	%	n	%	
	Sex					
1	male	70	50,7%	18	45%	
	female	68	49,3%	22	55%	
	age					
	< 1 years old	1	0,7%	0	0%	
2	1-5 years old	30	21.7%	10	10%	
	5-14 years old	91	65.9%	25	25%	
	14-18 years old	16	11.6%	5	5%	

No	Characteristics	NS 1 Negative n=138 (77,5%)		NS 1 Positive n=40 (22,5%)		P value
		n	%	n	%	
3	Nutritional status					
	Severely wasted	17	12.3%	2	5%	
	Wasted	12	8,7%	4	10%	
	Normal	100	72,5%	29	72.5%	
	Overweight	7	5,1%	3	7,5%	
	Obesity	2	1,4%	2	5%	
4	Complete blood count :					
	done	43	31,2%	23	57,5%	
	not done	95	68,8%	17	42,5%	
5	Mean body temperature:					
	Mean ± SD	37,66 (±2,07)		38,33 (±0,83)		p=0.001
6	Mean leucoyte count:					
	Mean ± SD	8.964 (±5369)		4803.91 (±2.197)		P=1.00
7	Mean platelet count:					
	Mean ± SD	239.218 (±99.402)		164.739,13 (±98.259)		P=0.999

Dengue virus serotype was determined using nested PCR on patients with positive NS1 results. The majority of the patients had negative results on PCR test (57.5%). In seropositive results (using PCR test), the majority of the patients had DENV-3 (20%), followed with DENV-2 (10%), DENV-1 (5%), and DENV-4 (5%). Mixed DENV infection (particularly with DENV-1 and DENV-3) was found in one patient (2.5%).

Table 2. Dengue virus serotype based on nested PCR in patients with positive NS1 results

No	Serotype	Frequency	
		n	%
1	Negative	23	57,5%
2	DENV 1	2	5%
3	DENV 2	4	10%
4	DENV 3	8	20%
5	DENV 4	2	5%
6	Mixed DENV (DENV1; DENV 3)	1	2,5%
	Total	40	100

In positive serotypes using nested PCR test, mean leukocytes in DENV-3 infection was higher compared to the other serotypes and negative serotype. Mean platelet counts are lower in negative serotypes compared to the other dengue positive serotypes.

Table 3. Mean leukocyte and platelet count based on nested PCR for dengue virus serotype

No	Serotype	Mean	
		Leukocyte	platelet
1	Negative	4.672 (±2.079)	146.538 (± 65.252)
2	DENV 1	5.200	152.000
3	DENV 2	4550 (±3.042)	158.333 (±17.009)
4	DENV 3	6.075 (±6.075)	240.250 (±10.528)
5	DENV 4	3.400	239.000

Differences were reported in regards to mean leukocyte and platelet count between positive and negative dengue serotype using nested PCR. Mean leukocyte count was significantly lower in positive serotype compared to negative serotype (5.172 ± 2.509 vs. 7.884 ± 5.121; p = 0.017). Mean platelet count was higher in negative

serotype compared to positive serotype (203.000 ± 78.472 vs. 214.884 ± 101.524) although the difference was not significant (p = 0.339).

Table 4. Mean leukocyte and platelet count based on nested PCR for dengue virus results

No	NS 1 positive results (n=40)	Serotype		P value
		Positive (n=17)	Negative (n=23)	
1	Mean leucocyte count	5.172 (±2.509)	7.884 (±5.121)	p=0.017
2	Mean platelet count	203.000 (±78.472)	214.884 (±101.524)	p=0.339

4. Discussion

The test using CBC include several hematological profiles that may be affected in pathophysiological process of dengue infection. Considering the relatively limited resource setting in Indonesia, CBC is often ordered as a preliminary test to rule out dengue infection instead of rapid antigen tests. In settings with lack of diagnostic capability to detect accurately and rapidly the presence of dengue infection, the endemicity of dengue infection in Indonesia may be effectively maintained. [9] Several studies had recommended the usage of rapid detection tests (RDT) such as NS1 antigen tests in order to accurately and rapidly diagnose dengue infections. [10] According to a study by Wardhani et al. regarding hematological profiles of pediatric dengue patients in Surabaya, several values in CBC test may be more accurate in predicting the severity of dengue infection. In patients with dengue hemorrhagic fever, the hematocrit, thrombocytes, liver enzymes, and albumin were more prominent in pediatric patients with dengue hemorrhagic fever. Some signs and symptoms, such as gall bladder wall edema, ascites, and pleural effusion (as markers of plasma leakage) were more apparent in pediatric population compared to the adult population. The difference between of hematological indices between adults and children in the said study, however, was not significant. [11] Mean body temperature was significantly

higher in dengue confirmed cases. No significant differences, however, were found in hematological indices (particularly leukocyte and platelet counts); the cases with confirmed dengue, however, were had lower leukocyte and platelet counts compared to cases without confirmed dengue.

Rapid diagnosis may be achievable using NS1 test combined with PCR tests in order to confirm laboratory diagnosis of dengue. The PCR tests, however, suffer from discrepancy of confirmed positive tests when compared NS1 test. Klungthong et al. had found that percentage of dengue positive cases in ELISA tested negative with rates of 17 - 42% for all serotypes. [12] According to a study by the efficiency, sensitivity, specificity, positive and negative predictive values of NS1 Ag detection ELISA were 83.6, 73.5, 100, 100 and 70% respectively while for real time RT-PCR these were 87.3, 79.4, 100, 100 and 75% respectively. Maximum sensitivity of NS1 antigen detection ELISA was seen in two days of fever and that of real time RT-PCR in three days of fever. [13] The study result was concurrent with other previous studies, with 57.5% of prior positive cases using NS1 test were detected negative using PCR.

Dengue virus serotype has a significant role in diagnosis and prognosis of dengue infection cases. A study by Vicente et al. had found that DENV-2 had higher proportion of severe dengue compared to DENV-1 (OR 7.42; 95% CI 2.21 - 24.93). One of the possible explanation in such occurrence would be possible stimulatory effect brought by DENV-2 infection in alteration of nitric oxide production that may cause toxic and inflammatory effects. [14] In comparison, a study by Harapan et al. had stated that rapid change of serotype dominancy (serotype shift) in Indonesia in 2000s from DENV-3 to DENV-1 and DENV-2. [15] Additionally, antibody-dependent enhancement (ADE) of dengue virus infection may occur in endemic cases due to the possibility of multiple serotypes of present or previous history of dengue infection. [16] In this study, the majority of cases diagnosed with dengue fever were DENV-3 serotype. The shift in serotype was not apparent in the study location. Relatively specific study location may limit the generalizability of the seroprevalence result attained in this study in Indonesia.

5. Limitation

The distance between laboratory and source for blood samples might have increased the risk of false negative due to sample lysis. we did not perform further confirmation by the nucleic acid testing for dengue nor for other causes of fevers like Chikungunya, Zika or Rickettsia who might effect the results

6. Conclusion

Incidence of dengue cases in patient with acute febrile in endemic urban area is quite high, and all serotype of dengue virus can be found, so the risk of secondary dengue virus infection is imminent, Therefore it strongly suggest for the government to make a policy which supports the use of rapid NS1 antigen at primary health center especially in endemic area for early detection and prevention for the community.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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