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RESEARCH

IDENTIFICATION OF DENGUE VIRUS SEROTYPES AT THE DR. SOETOMO HOSPITAL SURABAYA IN 2016 AND ITS CORRELATION WITH NS1 ANTIGEN DETECTION

(Identifikasi Serotipe Virus Dengue di RSUD Dr. Soetomo Surabaya Tahun 2016 serta Kenasabannya dengan Deteksi Antigen NS1)

Jeine Stela Akualing¹, Aryati¹, Puspa Wardhani¹, Usman Hadi²

ABSTRAK

Serotipe virus dengue yang beredar terus mengalami perubahan dan berbeda di setiap daerah. Pergeseran serotipe maupun genotipe di dalamnya, mempengaruhi terjadinya wabah dengue di berbagai negara. Perbedaan serotipe diduga bernasab dengan deteksi antigen (Ag) non-structural 1 (NS1), namun belum banyak penelitian yang mendukung hal tersebut. Penelitian potong lintang dikerjakan sejak Februari-Agustus 2016 dan didapatkan 60 subjek infeksi virus dengue (IVD) dan 25 non-IVD. Ribonucleic acid (RNA) virus dengue diperiksa di semua subjek menggunakan Simplexa Dengue Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) termasuk identifikasi serotipe virus dengue dan pemeriksaan NS1 menggunakan uji cepat NS1 Panbio. Perbedaan perbandingan variabel kategorikal dianalisis dengan uji Fisher Exact. Kenasaban antara serotipe dengan deteksi Ag NS1 dianalisis dengan Chi-Kuadrat. RNA virus dengue terdeteksi di 43 dari 60 subjek IVD (71,7%). Serotipe terbanyak adalah DENV-3 (62,8%). Pergeseran dominasi serotipe telah terjadi di Surabaya, sebelumnya dari DENV-2 ke DENV-1 dan sekarang DENV-3, kemungkinan akibat mobilitas pejamu, transpor virus dan faktor geografis. Kepekaan uji cepat NS1 75% dan kekhasan 100%. Persentase deteksi NS1 antar serotipe berbeda bermakna ($p=0,002$). Deteksi NS1 lebih rendah pada DENV-1 dibandingkan DENV-2 ($p=0,007$) ataupun DENV-3 ($p=0,003$). Serotipe virus dengue bernasab dengan deteksi NS1 ($p=0,005$). Ciri serotipe maupun genotipe virus dengue kemungkinan mempengaruhi sekresi NS1. Telah terjadi pergeseran serotipe virus dengue di pasien IVD di Surabaya sehingga diperlukan surveillance berkesinambungan untuk memperkirakan terjadinya wabah. Serotipe bernasab dengan deteksi NS1. Salah satu penyebab hasil negatif palsu NS1 adalah perbedaan serotipe.

Kata kunci: Infeksi virus dengue, serotipe virus dengue, antigen NS1, serotipe DENV-3, serotipe DENV-1

ABSTRACT

Circulating dengue virus serotypes are still changing and differ between regions. Serotype displacement as well as genotype are affecting the occurrence of dengue outbreak. Serotype difference is suspected to be correlated with NS1 detection, but there is still lack of evidence to support this. A cross-sectional study was conducted in February-August 2016 on 60 dengue viral infection (DVI) and 25 non-DVI subjects. Dengue virus RNA was examined in all subjects using Simplexa Dengue Real-Time RT-PCR as well as serotype identification and NS1 using rapid test NS1 Panbio. Difference of proportion between categorical variables was analyzed using Fisher Exact Test. Correlation between serotype and NS1 detection was analyzed by Chi-Square. Dengue virus RNA was detected in 43 of 60 DVI subjects (71.7%). The majority serotype was DENV-3 (62.8%). Serotype displacement has been occurring in Surabaya, from DENV-2 to DENV-1 in the past, and now DENV-3, possibly due to host mobility, viral transport, and geographical factors. NS1 sensitivity was 75% and specificity 100%. NS1 detection between serotypes was significantly different ($p=0.002$). NS1 detection in DENV-1 was significantly lower than DENV-2 ($p=0.007$) or DENV-3 ($p=0.003$). Dengue virus serotype correlated with NS1 detection ($p=0.005$). Characteristics of serotype and genotype of dengue virus probably affected the secretion of NS1. Serotype displacement has occurred in DVI patients in Surabaya, thus continuous surveillance is needed in order to predict dengue outbreak. Serotypes correlate with NS1 detection. One of the causes concerning false negative results of NS1 is the serotype difference.

Key words: Dengue viral infection, dengue virus serotypes, NS1 antigen

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INTRODUCTION

Dengue virus infection (DVI) is an infectious transmitted disease caused by dengue virus consisting of 4 serotypes, namely DENV-1, DENV-2, DENV-3 and DENV-4 from Flavivirus genus. DVI has a wide clinical spectrum and the course of the disease is difficult to predict and also remains a global health problem.¹ The Incidence Rate (IR) of Dengue Hemorrhagic Fever (DHF) in Indonesia is continuously increasing along with the increase of severity. Case Fatality Rate (CFR) in East Java increased from 0.9% in 2013 to 1.15% in 2014.² Domination of circulating dengue virus serotypes is different between regions and is still changing.³⁻⁵ Dengue virus serotypes and genotypes displacement has been found in several countries when a DVI outbreak occurred.^{3,4} Diagnosis of DVI is established based on the detection of dengue virus ribonucleic acid (RNA) and non-structural 1 (NS1) antigen (Ag).¹ NS1 assay is commonly ordered by the clinicians to confirm the diagnosis of DVI, but a false negative result often occurs. The diagnostic sensitivity of NS1 varies according to several studies⁶⁻⁹ and serotypes difference probably has become one of the causes affecting the sensitivity of NS1 detection.^{7,9} Dengue virus serotype is suspected to be correlated with NS1 Ag detection.

A molecular epidemiology study of dengue virus in Surabaya (2005) found that DVI was dominated by DENV-2, followed by DENV-3 and DENV-4, while DENV-1 was not detected *et al.*¹⁰ Yamanaka⁵, reported that the displacement of serotype domination has occurred in Surabaya from DENV-2 in 2008 to DENV-1 in 2009. Serotypes displacement emerged with genotypes displacement and was associated with dengue outbreak. The proportion of DHF cases in Surabaya increased three fold after serotypes displacement in 2008.⁵ These indicate that a continuously monitoring for circulating dengue virus serotypes is crucial, particularly in predicting the risk of dengue outbreak.

Serotypes difference is suggested to be correlated with NS1 secretion, but there is still lack of evidence to support this. A certain serotype probably has a higher replication, thus the level of viremia and antigenemia are also high. Duyen⁷ revealed the association between serotypes and NS1 secretion⁷, but the study of Noor⁶ in Surabaya could not prove this.⁶

Dengue virus serotypes displacement is possibly still occurring, so that identification of current circulating dengue virus serotypes, particularly in Surabaya is still important. The correlation between dengue virus serotypes and NS1 Ag detection must be proven. This

study aimed to identify the circulating dengue virus serotypes in the Dr. Soetomo Hospital Surabaya in 2016 and its correlation with NS1 Ag detection.

METHODS

The study was conducted in February-August 2016 using a cross-sectional design, and samples were taken consecutively. Subjects consisted of acute phase DVI patients (2-7 day of fever) and non-DVI febrile patients in the Tropical and Infectious Diseases Wards, Department of Internal Medicine, Dr. Soetomo Hospital Surabaya. IgM and IgG anti dengue assays, Polymerase Chain Reaction (PCR) of dengue virus RNA and NS1 detection were performed in all subjects. DVI group was determined based on the 2011 World Health Organization (WHO) criteria and with at least one positive result of PCR or dengue serology assays. Non-DVI group was classified if patients had fever and had been proven to be caused by non dengue diseases, supported by the negative results of PCR and dengue serology assays. Immune status of DVI subjects was determined according to the ratio of IgM/IgG anti dengue using enzyme-linked immunosorbent assay (ELISA) method; primary DVI if >1.2 , secondary DVI if <1.2 .¹

Laboratory examinations were performed in the Clinical Pathology Laboratory, Dr. Soetomo Hospital Surabaya, and Airlangga University Hospital Laboratory, Surabaya, while the isolation of dengue virus RNA and PCR was performed in the Eijkman Biology Molecular Institute, Jakarta. RNA isolation was performed using MagNA Pure LC 2.0 instrument and MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche) reagent, dengue virus RNA detection and serotypes determination using Simplexa@ Dengue real time RT-PCR (Focus Diagnostics) and 3M Integrated Cycler instrument. IgM and IgG anti dengue examination were performed using Panbio Dengue Duo IgM and IgG capture ELISA (Panbio Diagnostics), Ag NS1 examination was done using immunochromatography rapid test NS1 Panbio (Panbio Diagnostics).

Statistical analysis was performed using SPSS ver 16.0. The difference of proportion between categorical variables was analyzed using Fisher Exact Test, and correlation between serotypes and Ag NS1 detection using Chi-Square. The strength of correlation was determined using coefficient of contingency (C). Sensitivity and specificity of NS1 were calculated using a 2x2 table. P-value <0.05 was considered as statistically significant, with a 95% confidence interval.

Table 1. The characteristics of subjects

Variables	DVI (n=60)	Non-DVI (n=25)
Age*	24.8 ± 10,8	34.7 ± 16.9
Sex:		
Males, n (%)	38 (63.3)	13 (52)
Females, n (%)	22 (36.7)	12 (48)
Day of febrile*	4.7 ± 1.1	5.51 ± 1.4
Positive PCR, n (%)	43 (71,7)	0 (0)
Positive NS1, n(%)	45 (75)	0 (0)
Positive IgM anti dengue, n(%)	44 (73.3)	0 (0)
Positive IgG anti dengue, n(%)	44 (73.3)	0 (0)
Primary DVI	20 (33.3%)	–
Secondary DVI	40 (66.6%)	–

*Mean ± SD, SD: standard deviation

RESULTS AND DISCUSSION

The results of this study revealed that 60 subjects were diagnosed as DVI. Dengue virus RNA was detected in 43 subjects (71.7%) and NS1 in 45 subjects (75%) (Table 1). Non-DVI group (n=25) consisted of typhoid fever (9), malaria (4), leptospirosis (4), urinary tract infection (6), hepatitis A (1) and morbili (1).

Identification of dengue virus serotypes revealed that DENV-3 was the predominant serotype found in 27 of 43 subjects with a positive PCR, followed by DENV-1 (8/43) and DENV-2 (7/43). Mixed serotypes infection of DENV-1 and DENV-3 was found in 1 subject. DENV-4 serotype was not detected in this study (Figure 1).

According to several studies, dengue virus serotypes displacement has been occurring in Surabaya, Indonesia in recent years (Table 2). In 2003 until 2007, it was dominated by DENV-2.¹⁰ In 2008, the prevalence of DENV-1 gradually increased and in 2010, only DENV-1 was found in Surabaya.^{5,11,12} The prevalence of

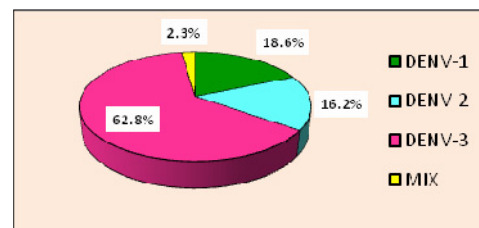


Figure 1. The percentage of identified dengue virus serotypes.

DENV-1 was high until 2012 along with the reduction of the prevalence of DENV-2. DENV-3 was not found in 2009–2012 and the prevalence of DENV-4 was low.^{5,11,13} Wardhani¹⁴ found that 4 serotypes circulated in Surabaya with DENV-1 as the predominant serotype, while the prevalence of DENV-3 which was not found in previous years started to increase.¹⁴ The increase of the prevalence of DENV-3 in Surabaya in 2012 was revealed by Fedik.¹⁵ This study revealed that the predominant serotype was DENV-3 (62.8%), while the prevalence of DENV-1 was decreased.

Table 2. The prevalence of dengue virus serotypes in Surabaya according to several studies

Years	Researchers	DENV-1	DENV-2	DENV-3	DENV-4	Mix	Total
2003–2005	Aryati ¹⁰	0	20 (80%)	4 (16%)	1 (4%)	0	25
2007	Yamanaka, <i>et al.</i> ⁵	0	46 (87%)	0	0	7 (13%)	53
2008–2009	Aryati, Wardhani ¹²	5 (20%)	13 (52%)	4 (16%)	3 (12%)	0	25
2009	Soegijanto, <i>et al.</i> ¹¹	79 (87%)	6 (6.5%)	0	6 (6.5%)	0	91
2010	Yamanaka, <i>et al.</i> ⁵	27 (100%)	0	0	0	0	27
2011	Soegijanto, <i>et al.</i> ¹³	182 (89.6%)	20 (9.8)	0	1 (0.5%)	0	203
2012	Soegijanto, <i>et al.</i> ¹³	79 (91.9%)	7 (8.1%)	0	0	0	86
2012	Wardhani ¹⁴	45 (67.2%)	8 (11.9%)	4 (6%)	6 (8.9%)	4 (6%)	67
2012	Fedik, <i>et al.</i> ¹⁵	2 (5.5%)	10 (27.8%)	11 (30.6%)	6 (16.7%)	7 (19.4%)	36
2016	This Research	8 (18.6%)	7 (16.2%)	27 (62.8%)	0	1 (2.3%)	43

The mechanism of serotypes displacement is unclear, probably due to host mobility, viral transport, or geographical factors. Every serotype has a different phylogenetic group which is known as genotype or subtype. Phylogenetic analysis showed that dengue virus can move from a far distance between countries or close distance between provinces. Several genotypes have a wide geographical distribution but others are only limited to a certain region.^{15,16}

Serotypes displacement is commonly accompanied by genotype displacement which is associated with the increase of DHF cases or dengue outbreak.^{5,13} The proposed mechanism is genotypes and serotypes displacement increasing the chance of occurrence of secondary heterotypic infection in endemic regions.⁵ Moreover, virus evolution is possibly happening along with the displacement that causes an increase in the transmission and virulence of dengue virus. Dewi¹⁷ investigated the genetic diversity of DENV-3 isolates obtained from a DHF outbreak in Palembang, Indonesia. The study revealed that the evolution of DENV-3 has occurred and contributed to the difference of DENV-3 transmission and virulence.¹⁷ DENV-3 epidemic happened in Jakarta, 1976 and was associated with the increase of DHF cases. The virus spread to Central Java in 1976 and Surabaya in 1977. This spreading caused an epidemic at that time.¹⁸

NS1 was only detected in 33 of 43 subjects with detectable dengue virus RNA. Positive PCR result can not guarantee that NS1 will always be positive and a negative PCR result can not guarantee that NS1 will always be negative (Table 3). This result was similar with previous studies.^{6,9} The sensitivity of dengue virus PCR was affected by PCR method, day of fever, and specimen handling. Sasmono¹⁹ showed that the sensitivity of Simplexa Dengue real-time PCR was 76.6%.¹⁹

Table 3. The results of NS1 compared to PCR in DVI subjects (n=60)

	Positive PCR	Negative PCR	Total
Positive NS1	33	12	45
Negative NS1	10	5	15
Total	43	17	60

Diagnostic sensitivity of NS1 in this study using the 2011 WHO criteria and positive results of PCR and/or IgM and/or IgG anti dengue ELISA as the gold standard was 75% (45/60) and 100% (25/25) specificity. Sensitivity of NS1 in primary DVI was 95% (19/20) and in secondary DVI 65% (26/40). The low sensitivity of NS1 was attributable to several possibilities, such as the presence of a specific antibody bound to NS1 leading to a low level of free NS1 that was difficult to be detected, particularly in secondary DVI, day of fever that was associated with viral burden and virulence of dengue virus in which a certain serotype has a high ability to replicate, causing a high level of viremia as well as antigenemia.^{7,9} This study aimed to prove whether serotypes difference correlated with the sensitivity of NS1 detection.

The percentage of positive NS1 between serotypes was significantly different (p=0.002). Percentage of positive NS1 in DENV-1 was lower than DENV-2 (p=0.007) as well as DENV-3 (p=0.003) (Figure 2). NS1 detection in primary DVI for each serotype was not significantly different, however in secondary DVI it was significantly different (Table 4 and 5). NS1 detection in DENV-2 and DENV-3 was still high even in secondary DVI. This indicated that the difference in NS1 detection was not only caused by humoral immune response, but also because of serotypes difference. There was a moderate and significant correlation between serotypes and the percentage of NS1 detection (p=0.005; C=0.447).

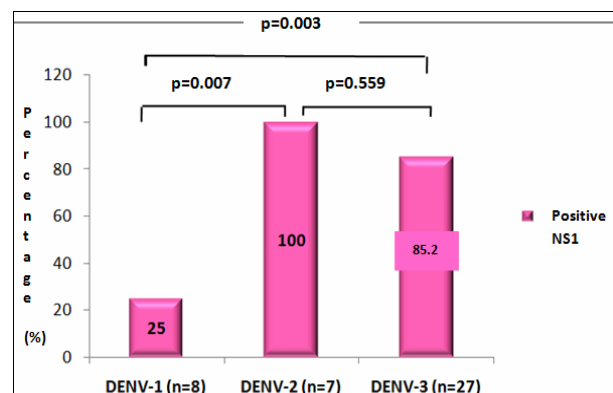


Figure 2. The percentage of positive NS1 in each dengue virus serotype.

Table 4. The difference of NS1 detection according to serotypes in primary DVI

	Primary DVI				p
	DENV-1 (n=1)	DENV-2 (n=4)	DENV-3 (n=7)	Mix (n=1)	
Positive NS1	0	4 (100%)	7 (100%)	1 (100%)	0.083

Table 5. The difference of NS1 detection according to serotypes in secondary DVI

	Secondary DVI				p
	DENV-1 (n=7)	DENV-2 (n=3)	DENV-3 (n=20)	Mix (n=0)	
Positive NS1	2 (28.5%)	3 (100%)	16 (80%)	0	0.027

Table 6. The distribution of dengue virus serotypes and positive NS1 in each serotype according to the day of fever

	Day of fever				
	D-3	D-4	D-5	D-6	D-7
DENV-1	0	2	2	1	3
NS1 (+) in DENV-1	0	0	1	0	1
DENV-2	1	4	2	0	0
NS1 (+) in DENV-2	1	4	2	0	0
DENV-3	8	8	6	4	1
NS1 (+) in DENV-3	8	7	5	2	1
Mixed	0	1	0	0	0
NS1 (+) in mixed serotypes	0	1	0	0	0

D: Day of fever

There was no difference in the proportion of dengue serotypes according to the day of fever ($p=0.233$). DENV-3 was detected in the subjects on the third until seventh day of fever, and NS1 detection in DENV-3 was higher than DENV-1 based on the day of fever (Table 6). This indicated that the difference in NS1 detection was not only caused by the day of fever when the samples were obtained, but also because of serotypes difference.

Previous studies revealed that there was a difference in the secretion of NS1 Ag between serotypes.^{20,21} The causes of the difference in NS1 secretion were not clearly understood, but the characteristics of the virus and genetics factor were considered to be the causes.^{22,23} The difference in NS1 detection between serotypes was probably associated with the affinity of anti-NS1 monoclonal antibody that was used in the commercial kit. The affinity of monoclonal antibody might be lower for certain serotypes, but further study is needed to prove this.⁹

The limitations of this study were that viral load and quantitative level of NS1 were not performed, so that the level of viremia and antigenemia could not be determined and correlated with the sensitivity of NS1 detection. NS1 examination was performed using an immunochromatography rapid test which has a lower sensitivity than ELISA (72.8% vs 83.2%)⁹, but NS1 rapid test has been widely used in many laboratories, particularly in limited facilities. The samples of DENV-1 and DENV-2 were few in primary and secondary DVI.

CONCLUSION AND SUGGESTION

Dengue virus serotypes displacement has occurred in DVI patients in Surabaya, so a continuously surveillance including serotypes and genotypes analysis is needed in order to predict dengue outbreak. Serotypes correlate with NS1 detection. One of the causes concerning false negative results of NS1 is serotype difference. Further studies are needed regarding the biological intrinsic factors of dengue virus affecting the secretion of NS1.

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