Vol 25 No. 1 November 2018

p-ISSN 0854-4263
e-ISSN 4277-4685

INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

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INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
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2018 Nov; 25(1): 1-128
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

IgA ANTI-DENGUE PROFILE IN SAMPLES WITH POSITIVE DENGUE PCR OR NS1

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ABSTRACT

Dengue Virus Infection (DVI) causes several clinical manifestations and requires varied diagnostic instruments. IgA anti-dengue as one of the diagnostic markers of DVI is suspected to have a shorter lifespan and greater sensitivity in detecting secondary infections compared to IgM anti-dengue. This study was conducted using 34 sera with positive RT-PCR or NS1 dengue virus. Samples were examined by a reverse flow immunochromatographic method using AIM Dengue IgA Assure Rapid Test and will be analyzed its profile toward the day of fever, serotype, severity, platelet count, and type of infection. The overall sensitivity of IgA anti-dengue was 61.76% (n=34); in which IgA anti-dengue detected 14.29% primary and 66.67% secondary cases. IgA anti-dengue detected DEN1, DEN2, DEN3, and Mixed DEN1 – DEN3 virus serotype respectively 55.56%, 22.22%, 16.67%, and 5.56% (n=20). The day of fever was dominated by day-4 and day-5 respectively 28.57% (n=21). IgA anti-dengue was detected in DD, DHF grade I, II, and III 42,86%, 28.57%, 19.05%, and 9.52% (n=21) respectively. IgA anti-dengue detected in all levels of platelet count, it detected 60% in < 50,000 cell/mm^3, 30% in 50,000 - 100.000 cell/mm^3 and 10% in > 100,000 cell/mm^3 platelet count sample (n=20). In conclusion, IgA anti-dengue showed a good performance, applicable as a diagnostic marker of DVI.

Key words: Dengue virus infection, IgA anti-dengue, dengue virus serotype, type of infection dengue virus, dengue virus severity, AIM Dengue IgA Assure Rapid Test

INTRODUCTION

Dengue Virus Infection (DVI) is an infectious disease that has a high incidence and prevalence especially in tropical regions such as Indonesia. This disease is caused by the dengue virus and transmitted by Aedes aegepty and Aedes albopictus. This disease still becomes the major cause of morbidity and mortality in tropical and sub-tropical countries around the world.[1]

The prevalence of DVI around the world is 50 to 100 million cases with 22,000 deaths.[2] The number DVI cases in Indonesia is increasing along the rainy season. In mid-December of 2014, 71,668 people were recorded suffering from DVI and 641 of them died.[3] This number is lower than the previous year (2013) in which 112,511 people suffered from DVI with 871 death.[3]

Dengue virus infection causes symptomatic and asymptomatic DVI. Symptomatic of DVI consist of non-specific fever, Dengue Fever (DD) and Dengue Hemorrhagic Fever (DHF). Dengue hemorrhagic fever is divided into stage I and stage II based on the presence or absence of bleeding. While DHF is divided into stage III and stage IV based on the presence or absence of shock.[1]

Dengue virus infection patients are often found in a bleeding and shock stage that can lead to multiple organ failure and even death. Early diagnosis and appropriate treatment are the keys to DVI treatment. Currently, routine serological diagnosis of DVI is performed using IgM, IgG and NS1 dengue virus derived from blood or serum specimens. IgM anti-dengue used as a sign of acute dengue infection can be detected on the 6th day and can remain positive until the 10th day after fever which may lead to late diagnostic.[4] In several cases, IgM anti-dengue can persist for several months after onset offever that tends to cause a false-positive result.[5]

Currently, the use of Immunoglobulin A(IgA) anti-dengue as the diagnostic marker of DVI has been developed. A study conducted by Ahmed *et al*. showed that IgA anti-dengue had total 99.4% sensitivity rate.[6] Another study conducted by Tan *et al*. showed IgA anti-dengue had been detected 92.8% in secondary infection, 77.4% in primary infection and had an 86.7% total sensitivity.[7]

IgA anti-dengue as a diagnostic marker as DVI is still not widely used in Indonesia although its diagnostic kit is available.[8] This study aimed to find

out IgA anti-dengue profile towards days of fever, dengue virus serotype, platelet count, dengue severity and type of infection.

METHODS

This study was conducted during March-September 2017 using 34 RT-PCR or NS1 positive sera. IgA anti-dengue assay was then performed in these samples. Samples with positive IgA anti-dengue were analyzed about its profile towards days of fever, virus serotype, platelet count, severity and type of infection. Days of fever were determined when body temperature was increased above 37.5°C and counted from 1st till 7th day. Serotype dengue virus was classified into DEN-1, DEN-2, DEN-3, and DEN-4. The severity of DVI was determined based on 2011 WHO classification. Type of infection consisted of either primary or secondary infection.[1]

Laboratory examinations were performed as the Infection Division of Clinical Pathology Department, Dr. Soetomo Hospital Surabaya. The RT-PCR assay was performed using Simplexa Dengue Real-Time PCR (Focus Diagnostic) while NS1 assay was performed using SD Bioline Dengue Duo (Standard Diagnostics). IgA anti-dengue assay was performed using AIM Dengue IgA Assure Rapid Test.

RESULTS AND DISCUSSION

This study revealed that IgA anti-dengue assay was positive in 21 (61.7%) samples (n=34), thus analyze its profile was only performed toward these 21 samples (Table 1).

Immune response begins when the dengue virus enters the bloodstream. IgM anti-dengue starts to rise around the 3rd to 5th day after a fever, and then increases in 1-3 weeks, and can be detected for up to 3 months, even for more than 8 months.[9] IgG anti-dengue arises about 2 weeks after the onset of fever in primary infection while in secondary infection IgG begins to increase sharply on 3rd to 5th day after fever.[1] IgA anti-dengue starts on day 5 after fever but is shorter than IgM anti-dengue.[9] A previous study by Decker *et al*. stated that IgA anti-dengue could be detected one day after the appearance of IgM anti-dengue.[10]

This study showed that the day of fever was dominated by the 4th and 5th day with 28.57% positive rate followed by the 3rd, 7th, and 6th day (Figure 1). IgA anti-dengue positivity decreased after the 5th day.A study conducted by Decker showed that IgA anti-dengue had a 45-day lifespan compared with anti-dengue IgM that has a 90-day lifespan, thus IgA anti-dengue had a potential lower false positive value.[10]

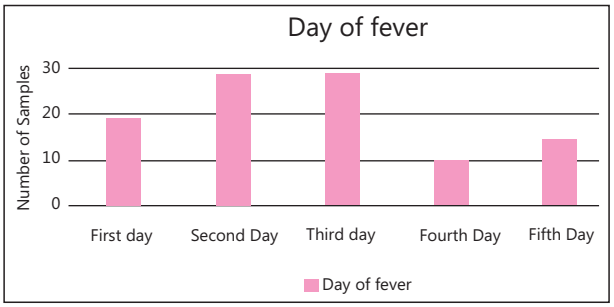

Figure 1. IgA anti-dengue profile toward day of fever on the 2nd day, 3rd day, 4th day, 5th day

Dengue Virus (DEN) has 4 strains (DEN 1, DEN 2, DEN 3, DEN 4). The structure of this serotype is very similar, but antibodies can not provide cross-protection.[1] All dengue serotypes are found in Indonesia. The dominance of the dengue virus serotype is somewhere dynamic and the shift of dominance takes place over time.[9] Research by Ipain West Java showed that DVI was mostly caused by DEN-3 and DEN-2 serotypes.[11] Research by Andriyoko *et al*. using 27 samples found that dengue was dominated by DEN-3 followed by DEN-2.[12] Research conducted by Aryati *et al*. showed domination of DEN-1 followed by DEN-3 virus.[13]

This study showed dominance of 10 (55.56%) samples with DEN-3 serotype followed by 4 (22.22%) samples with serotype DEN-2 and 3 (16.67%) samples with serotype DEN-1 and 1 (5.56%) sample with mixed serotype DEN-1 and DEN-3 (Figure 2). The variation of serotype dominance proved that dengue virus has moved to its vector host.[13] Research conducted by Aryati *et al*. showed that there was a change of serotype dominance occurring in Surabaya between 2005-2009 and 2012.[13] Dengue virus infection was dominated by DENV-2 in 2009, while in 2012, it was dominated by DENV-1 serotype and finally in 2016-2017 it was dominated by DEN-3.[14]

Dengue severity is associated with secondary infection.[1] Halstead proposed a secondary heterologous infection hypothesis that said dengue occurred when a person was re-infected with dengue virus with different serotypes. This re-infection caused anamnestic antibody reactions resulting in high concentrations of immune complexes.[15] This study showed IgA anti-dengue was found 9 (42.86%) in samples with DHF grade I followed by 6 (28.57%) DHF II and 4 (19.05%) DHF III and 2 (9.52%) DD (Figure 3).

Table 1. Characteristics of samples

IgA anti-dengue	Total (n=34)	Percentage (%)
Positive	21	61.76
Negative	13	38.24
Total	34	100.00
Samples characteristics	**Total (n=21)**	**Percentage (%)**
Day of fever		
3	4	19.05
4	6	28.57
5	6	28.57
6	2	9.52
7	3	14.29
Total	**21**	**100.00**
PCR and/or NS1		
PCR (+)	3	14.29
NS1 (+)	3	14.29
PCR dan NS1 (+)	15	71.43
Total	**21**	**100.00**
Virus serotype		
DEN-1	4	22.22
DEN-2	3	16.67
DEN-3	10	55.56
DEN-4	0	0.00
Mixed	1	5.56
Total	**18**	**100.00**
Severity		
DD	2	9.52
DHF Grade I	9	42.86
DHF Grade II	6	28.57
DHF Grade III	4	19.05
DHF Grade IV	0	0.00
Total	**21**	**100.00**
Platelets		
> 100.000	2	10
50.000 - 100.000	6	30
< 50.000	12	60
Total	**20**	**100**
Type of infection		
Primary	3	14.29
Secondary	14	66.67
Unknown	4	19.05
Total	**21**	**100.00**

This study showed decreasing platelet counts in a total of 20 samples with positive IgA anti-dengue. The platelet profile was dominated by 12 (60%) samples with <50,000 cells/mm^3, platelet counts followed by 6 (30%) samples with 50,000 - 100,000 cells/mm^3 platelet counts, and 2 (10%) samples with

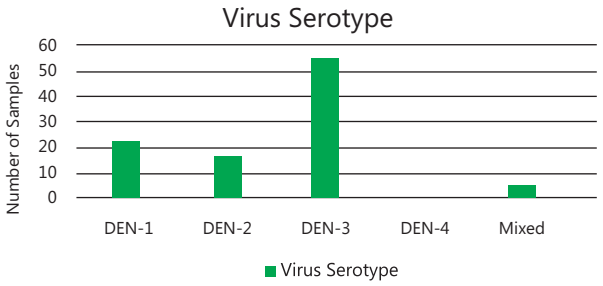

Figure 2. IgA anti-dengue profile toward dengue virus serotype

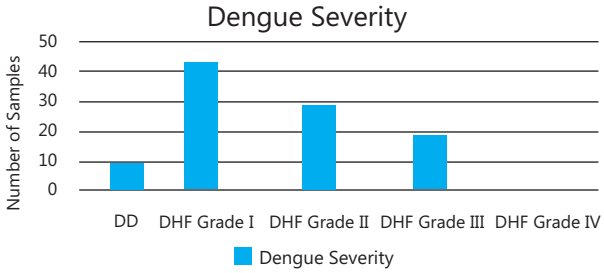

Figure 3. IgA anti-dengue profile toward dengue severity

> 100,000 cells/mm^3 platelet counts (Figure 4). This study showed that IgA anti-dengue could be found in samples with varying levels of platelet counts.

This study showed that IgA anti-dengue had a higher sensitivity (66.67%) in secondary infection compared to (14.29%) in primary infection. There were 4 (19.05%) samples with unknown dengue type of infection which means IgG and IgM anti-dengue both showed negative results (Figure 5). Whether the negative results of IgM and IgG anti-dengue could be caused by IgM/IgG was not established yet which mostly appeared at the beginning of primary infection.[15] Decker *et al.* stated that IgA anti-dengue appeared at the same time or one day after the arise of IgM anti-dengue. This study showed that IgA anti-dengue could be detected even when IgG or IgM anti-dengue had not been formed.[10]

CONCLUSION AND SUGGESTION

Dengue virus infection has a broad spectrum of clinical manifestation. IgA anti-dengue has been developed to become one of its diagnostic markers. AIM Dengue IgA Assure Rapid Test showed a good performance and can be used as an early diagnostic instrument for DVI.

Based on the results of this study, it is recommended to classify the positivity level of IgA anti-dengue results to obtain more detailed data about IgA anti-dengue profile towards days of fever, serotype, severity, platelet count, and type of infection

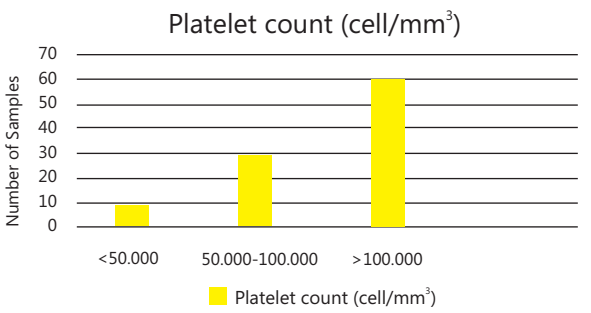

Figure 4. IgA anti-dengue profile toward platelet counts

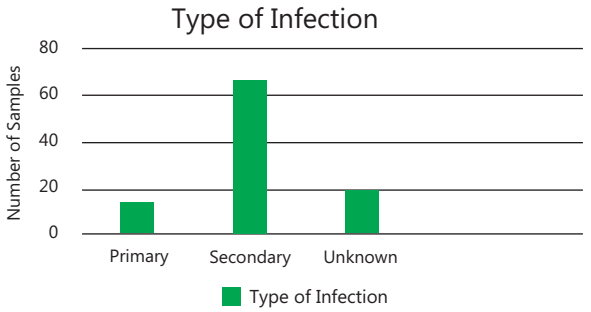

Figure 5. IgA anti-dengue profile toward types of infection

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