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Use of rapid immunochromatographic test to detect dengue infection in community-based patients in Indonesia

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Abstract

Severe dengue virus (DENV) manifestations commonly occurred in secondary infections. Serology assay using rapid immunochromatographic test is one of diagnostic modalities used in community setting. The aim of this research was to evaluate the use of a serial rapid immunochromatographic test in establishing DENV infection in community patients. This cross-sectional study was conducted in Clinical Microbiology Laboratory Department of Microbiology Faculty of Medicine Universitas Indonesia Jakarta using paired stored sera from community-based DENV patient collected in 2010. Samples with positive nonstructural protein 1 (NS1) result were subjected to hemagglutination inhibition (HI) assay. Serial NS1, IgM, IgG, clinical features, and virus serotype result from previous study were taken as secondary data and compared with HI assay result as gold standard. For rapid immunochromatographic test vs HI analysis, both results were classified as 'Primary Infection' and 'Secondary Infection'. A total of 25 samples fulfilled the inclusion criteria. The proportion of primary and secondary infection according to Bioline SD Dengue Duo was 44% and 56%, respectively. In the other side, 23 samples (92%) were classified as secondary infection by mean of HI assay; the rest was primary infection. The highest agreement rate between serial rapid immunochromatographic test and HI was 68%. The rapid test can detect IgM and IgG as early as on 3rd day of fever. The results of rapid immunochromatographic test were in accordance with HI if it was examined within 3-7 day of fever and therefore can replace HI for determining DENV infection whether primary or secondary.

Keywords: DENV infection, HI assay, Rapid immunochromatographic test, Community-based, Indonesia.

Introduction

Indonesia is a highly endemic country for dengue virus (DENV) infection.[1] In 2010, there were 80,065 cases of confirmed DENV, with the incidence rate of 34.29 per 100.000 population and case fatality rate (CFR) of 0.93%.[2] In 2013, the dengue haemorrhagic fever (DHF) incidence in children was 35-40/100.000 while the CFR was 0.73%.[3] Cohort study[4] revealed that among acute febrile illness in children age 2-15 years old in Asia, the prevalence of probable and laboratory-confirmed DENV case was 29.5% and 12,5%, respectively. Study in adults[5] showed that the proportion of secondary DENV infection was 65%. Similar study[6] in Bandung, West Java, Indonesia, stated that asymptomatic DENV infection was three times more than symptomatic cases. Another cohort study[7] in Western Java, Indonesia, revealed that the overall proportion of DENV infection among fever adult patient was 12.4%.

DENV has four antigenically distinct serotypes, and all of these were reported circulating in Indonesia.[8] Clinical spectrum of DENV infection ranges from asymptomatic infection, dengue fever, DHF, to expanded dengue syndrome.[9] Severe manifestation, including DHF and dengue shock syndrome, mostly occurred in patients with secondary DENV infection.[10]

Accurate and efficient diagnostic kits are important for patient care, surveillance, and research of pathogenesis and vaccine efficacy.[11] An example of DENV diagnostic modalities is serology examinations.[12] Serology methods are considered to be more feasible to be conducted in community settings and daily practices, compared to molecular methods. However, the disadvantage of serology technique, such as hemagglutination inhibition test, was not practical as it needs acute and convalescent serum with skilled laboratory technician.[13] Ideally, the acute phase serum

Practice Points

- Dengue infection is still prevalent in Indonesia.
- An accurate and rapid diagnostic tool is needed to help physicians establishing dengue diagnosis especially in community setting.
- Hemagglutination inhibition (HI) is capable in establishing primary or secondary dengue infection but it consumes times and resources.
- Secondary dengue infection were predominant in this study.
- The Bioline SD Dengue Duo rapid immunochromatographic test has the same performance compared with hemagglutination inhibition in determining type of dengue infection in community patients if it is done within 3-7 day of fever.

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should be collected on 0-5 days after fever onset and the convalescent serum should be collected on 14-21 days after fever onset.[9]

Primary and secondary DENV infection should be identified because severe DENV manifestations tend to occur in patient with secondary infection.[14] Secondary infection can cause severe manifestations, especially when someone gets infected for the second time with different DENV serotype other than previous infection.[10] This phenomena is called antibody-dependent enhancement (ADE), where antibody elicited in previous infection fails to neutralize recent infection by different DENV serotype.[12]

A number of commercial products offer serology examination using single serum, to differentiate primary and secondary infection, by detecting the appearance of IgM antibody.[15] IgM antibody can be detected four days after the onset of fever. IgM and IgG seroconversion from paired samples could confirm DENV infection, while detection of IgM antibody in single serum sample from a patient with appropriate symptoms could support probable diagnosis. Unfortunately, in secondary infection, there is anamnestic antibody response, which causes higher elevation of IgG anti-DENV antibody than IgM.[12] So, a timely and properly collected single serum sample is needed in order to differentiate primary and secondary infection.

This study was conducted to assess the performance of serial rapid immunochromatographic assay in determining type of DENV infection in community. The kit used for testing was Bioline SD Dengue Duo™ (Standard Diagnostic, Korea) which can detect NS1 antigen and IgM-IgG antibody simultaneously. Previous study[16] revealed that this assay had 88.65% and 98.75% of sensitivity and specificity, respectively. Evaluation by Centre for Disease Control and Prevention (CDC), however, showed that this assay had 60.9% (95%; CI 58.2 – 63.6%) of sensitivity and 90% (95%; CI 88.3 – 91.7%) of specificity.[15]

This study was done as a complement for other research entitled 'International Study on Biomarkers and Gene Expression Patterns in Patients with Dengue Virus Infection' conducted by The Indonesian Study Centre, Jakarta. The ethical clearance was obtained from Health Research Ethics Comittee Faculty of Medicine Universitas Indonesia/Dr. Cipto Mangunkusumo General Hospital, number 63/PT.02.FK/ETIK/2010.[17] In this study, the results of rapid immunochromatographic test was compared with hemagglutination inhibition (HI) test.[18] The HI was used as comparison because this method had been applied for determination of type of DENV infection in seroepidemiology study, though it has less specificity for DENV.

Materials and methods

This was a cross sectional study conducted in Clinical Microbiology Laboratory Faculty of Medicine Universitas Indonesia Jakarta, from July 2013 until June 2014. The study population was the sera from patients with suspected DENV infection, living in the DENV endemic communities in Jakarta. The sera was already collected from the previous study by Dewi *et al.*[19] and stored at –80°C freezer. The inclusion criteria were: i) Sera from patients who presented with fever (≥38°C) lasting for 2 days with one of the following symptoms: headache, retro-orbital pain, myalgia, rash, leukopenia, and/or bleeding manifestation, collected for 7 days consecutively and stored in -80°C freezer in Clinical Microbiology Laboratory FMUI; and ii) Sera with positive NS1 antigen result, tested by rapid immunochromatographic assay Bioline SD Dengue Duo™.

Sample fulfilled the inclusion criteria were subjected to HI assay,[20] for determination of the type of infection. The HI results was interpreted in accordance to WHO 1997 Dengue Guidelines (Table 1).[18]

The NS1, IgM, IgG results, virus serotype, and clinical features were adopted from previous studies.[17,19] The NS1 antigen, IgM, and IgG antibody were determined using rapid immunochromatographic test as explained elsewhere.[19] The result was interpreted after 15 minutes according to Table 2 as follow: when there was only IgM band that visible, the sample was classified as Primary Infection, while there was visible IgG band with or without IgM band, the sample was classified as Secondary Infection.[21,22] Each sample underwent assessment on daily interpretation of type of infection related to the result of serial immunochromatography assay (Table 2) based on the fever day 1 until fever day 7. In the analysis comparing Bioline SD Dengue Duo and HI, the result of both examinations were classified as "Primary" and "Secondary". Moreover, we also evaluated the daily positive proportion of NS1, IgM, and IgG antibody.

Results

A total of 98 stored sera were marked as eligible for HI test but only 25 sera fulfilled the inclusion criteria. All of 25 sera were sample with positive NS1 result, which had been confirmed by RT-PCR. One sample had negative RT-PCR but still included in analysis because the NS1 was positive. The clinical DENV criteria was determined according to WHO 1997 classification. Proportion of DF and DHF were 16 cases (64%) and 9 cases (36%), respectively. Of 25 samples, 7 were DENV-1 and the other 7 were DENV-2; the rest were DENV-3 (5 samples) and mix infection (5 samples, including 3 samples of DENV-1 and DENV-3 mix

Table 1: Interpretation of HI Assay[18]

Antibody response	Interval between 1st & 2nd sample	Convalescent Titre	Interpretation
≥4-fold rise	≥7 days	≤1:1280	Acute flavivirus infection, primary
≥4-fold rise	Any specimen	≥1:2560	Acute flavivirus infection, secondary
≥4-fold rise	<7 days	≤1:1280	Acute flavivirus infection, either primary or secondary
No change	Any specimen	>1:2560	Recent flavivirus infection, secondary
No change	≥7 days	≤1:1280	Not dengue
No change	<7 days	≤1:1280	Uninterpretable
Unknown	Single specimen	≤1:1280	Uninterpretable

Table 2: Interpretation of Rapid Immunochromatograpy Test[22]

Result		Interpretation
IgM	IgG	
(+)	(-)	Primary infection
(+)	(+)	Secondary infection
(-)	(+)	Secondary infection suspected
(-)	(-)	Not dengue

infection, 1 sample of DENV-1 and DENV-4 mix infection, and 1 sample of DENV-1, DENV-3, and DENV-4 mix infection). There were 14 patients who came on the 1st day of fever and 11 patients came on the 2nd day of fever.

All of 25 paired sera were subjected to HI assay for determination of type of infection, according to WHO 1997 classification. There were 2 (8%) samples of primary DENV infection and 23 (92%) samples of secondary DENV infection. On the other side, the rapid test detected 11 (44%) samples categorized as primary infection and 14 (56%) samples classified as secondary infection. Statistical test showed no difference between HI and rapid test in determining type of infection. The interval day between acute and convalescence serum ranged from 5 to 30 days.

For daily serial examination, the rapid test can detect the NS1 and IgM antibody as early as on 1st day of fever. On the other side, the rapid test can detect IgG antibody as early as on 3rd day of fever. The rapid test can detect IgM and IgG antibody from > 50% of all samples on the 6th day of fever (Figure 1).

When comparing the proportion of NS1, IgM, and IgG antibody between primary and secondary infection based on the rapid test result, we found that both in the primary and secondary infection, the positivity of NS1 was higher in secondary infection when the sample was checked on the 2nd day of fever. But on the 7th day of fever, the positive proportion of NS1 was higher in samples with primary infection while in secondary infection, the NS1 declined more rapid. This result showed that in primary infection, NS1 could be detected longer from blood than in second-

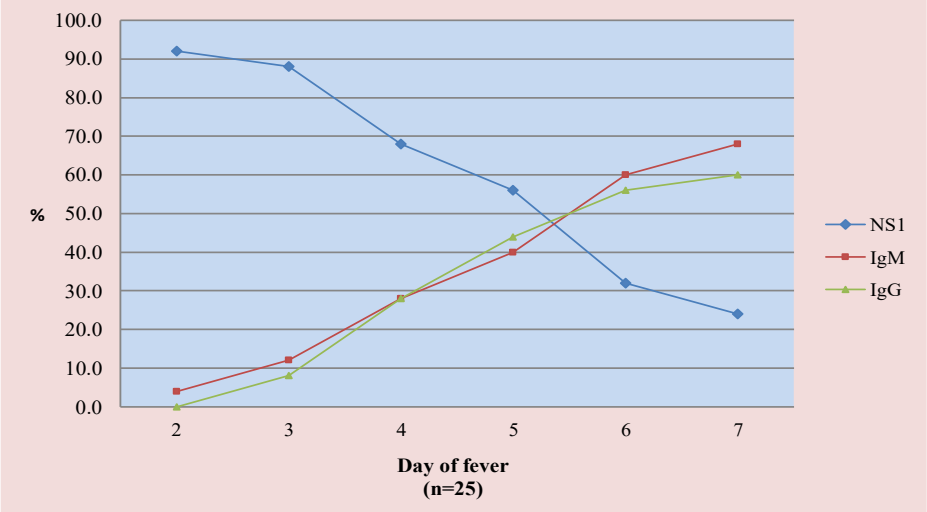

Figure 1: Positive proportion of daily NS1, IgM, IgG result according to rapid immunochromatography assay

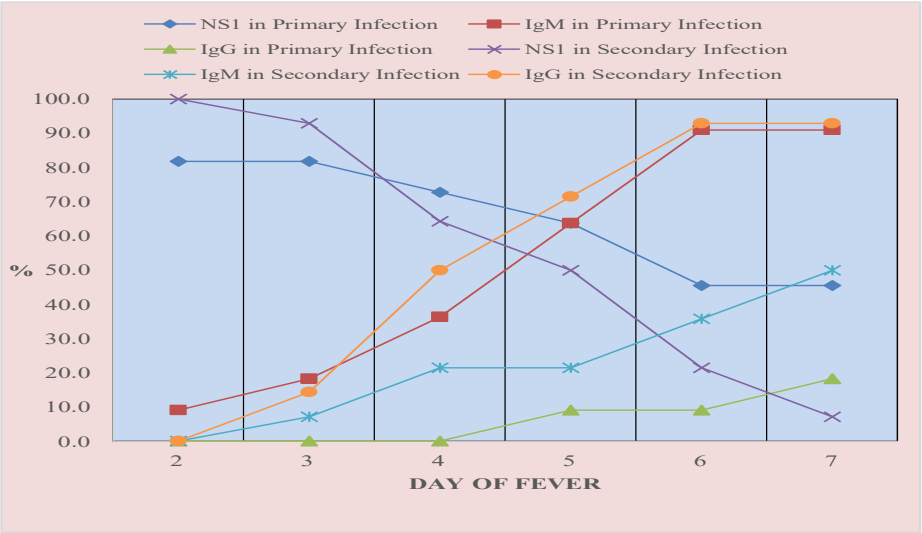

Figure 2: Comparison of positive proportion of serial NS1, IgM, and IgG result, between primary and secondary infection, according to rapid immunochromatography assay

ary infection. The similar phenomenon was also observed when comparing positive proportion of IgG between primary and secondary infection on the 7th day of fever (Figure 2).

Discussion

DENV is still a burden for tropical countries, such as Indonesia. Severe dengue manifestations were commonly occured in secondary DENV infection. Thus, a rapid and accurate diagnostic tool is needed to guide the physicians in the community setting. In this study, we used stored samples with positive NS1 result. So all of the samples were considered as DENV samples and thus we didn't assess the sensitivity and specificity of the rapid test. We found that there was no significant difference between HI and the rapid immunochromatographic test when our samples were examined within 7 days of fever. Both assays were able to determine that secondary DENV infection was dominant in tested samples. Since the type of infection were differentiated by the presence of IgM and IgG, the capability of this rapid kit to detect those antibodies become important. The rapid test showed fair accuracy (>70%) when conducted on 5th day of fever (data not shown). This result was also supported by the finding that this rapid kit can detect IgM and IgG antibody from >50% samples on the 6th day. This was also in accordance with study from Shih et al which revealed that in patient who came on the 5th day of fever, the positivity rate of the IgM was 88.89%.[23] Another study also showed that the highest IgM antibody positivity (83%) was detected on the 5th day of fever.[24] Therefore for patients with 5-day fever, accompanied by signs and symptoms relevant with DENV infection, detection of IgM and IgG anti-DENV antibody should be considered.

Our study revealed that the NS1 and IgM antibody could be detected as early as on the 1st day of fever, while the IgG antibody could be detected as early as on the 3rd of fever. Another research[23] showed that the NS1, IgM, and IgG could be detected as early as on the 1st day of fever with different proportion for each parameter respectively. The highest NS1 proportion was detected less than 3rd day of fever. Based on the result, we suggested testing NS1 antigen in suspected DENV cases with fever less than 3 days with signs and symptoms related to DENV infection.

The other finding in study was the dominance of secondary DENV infection. Both HI assay and the rapid test showed the prevalent of secondary DENV infection, even though in different proportion. It was possible that for the samples which were defined as primary DENV infection by the rapid test but detected as secondary infection by HI assay, the IgG couldn't be detected within 7 day of fever by the rapid test. As mentioned in another study,[22] the rapid IgG score correlated significantly with the HI titre ≥1:2560. It could be that the titre of the IgG antibody did not reach the threshold; thus the samples were classified as primary infection.

The advantage of rapid test was mainly in its practical aspect, so it could be done in all health care facilities, where the results could be obtained in 10-15 minutes. Therefore, the diagnosis could be established quickly, the patient could be managed appropriately, and the type of infection could also be identified. Apart from those, the rapid diagnostic test results should be interpreted carefully considering patient's clinical condition.

This rapid diagnostic test was generally able to define the type of DENV infection since the 2nd day of fever but the accuracy rate was very low. This accuracy rate reached the highest point on the 7th day of fever. This was in accordance with previous study[25] which stated that the positivity rate of the rapid test in detecting antibody increased along with the day of fever. The benefit in clinical practice was that the diagnosis of DENV based on IgM and IgG examination using rapid test could be done as early as on the 3rd day of fever, before the patient entered the critical phase.

Conclusion

This rapid immunochromatographic test was useful in determining type of DENV infection in DENV endemic community if conducted within 7 days of fever. The rapid test could also determined the type of DENV infection as early as on the 2nd day of fever. In DENV endemic countries, NS1 antigen detection test could be useful to detect DENV infection within 3 days of fever and IgM/IgG antibody detection on 5-7 days of fever.

Competing interest

The authors declared that there is no conflict of interest.

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