American Journal of Clinical Medicine Research, 2017, Vol. 5, No. 4, 55-58
Available online at http://pubs.sciepub.com/ajcmr/5/4/4

DOI:10.12691/ajcmr-5-4-4

Impact of Reactive Dengue NS1 Antigen with Time for Hospital Admission in Dengue Viral Infection Patient

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Abstract Dengue is the most rapidly spreading mosquito-borne viral disease worldwide with estimated 30-fold increase in incidence over the last five decades. The recent study had found an increase in the number of admissions of cases of dengue fever with reactive NS1 antigen. The aim of this study was to evaluate the utility of NS1 antigen as a diagnostic marker and to determine whether there is a higher risk of severe dengue infection for patient with positive NS1 antigen. This was a retrospective analysis study conducted in 7 referral Hospitals in Bandung, West Java, Indonesia. The medical records of all the individuals admitted to the Hospital from 1 January to 31 December 2015 were evaluated. We used a questionnaire as a tool for data collection consisted of the epidemiological, clinical manifestation, laboratory results and final diagnosis data that were collected during the period of hospitalizing. In 2015 there were 4.096 cases of dengue viral infection hospitalized. Most of the patients with positive NS1 antigen were admitted at early phase (0-2 days) and regular phase (3-5 days). Platelet count >100.000/mm3 was significantly associated with positive NS1Ag assay, while lower thrombocytopenia (platelet count <50.000/mm^3) was significantly associated with positive IgM dengue antibody. Severe cases of dengue viral infection of DHF significantly more often happen on patient with IgM antibody dengue positive (P<0.005). NS1 Ag assay is useful, sensitive and specific for the diagnosis of dengue detection, with good sensitivity and specificity during acute phase when detectable antibodies still negative.

Keywords: *dengue, NS1, hospital admission, surveillance*

Cite This Article: Riyadi Adrizain, and Djatnika Setiabudi, "Impact of Reactive Dengue NS1 Antigen with Time for Hospital Admission in Dengue Viral Infection Patient." *American Journal of Clinical Medicine Research*, vol. 5, no. 4 (2017): 55-58. doi: 10.12691/ajcmr-5-4-4.

1. Introduction

Dengue is the most rapidly spreading mosquito borne viral disease worldwide with estimated 30-fold increase in incidence over the last five decades. [1] Infection with any 1 of 4 dengue viruses produces a spectrum of clinical illness ranging from a mild undifferentiated febrile illness to dengue fever (DF), dengue hemorrhagic fever (DHF), a potentially life-threatening disease. [2] The morbidity and mortality of DHF can be reduced by early hospitalization. Since early hospitalization and careful supportive care can reduce the case-fatality rate of DHF, the rapid identification of patients at risk for developing DHF is desirable in regions where dengue viral infection (DVI) is endemic. [3]

Dengue virus is a member of flaviviridae family, with substantial genetic diversity shown by the presence of four serotypes (DENV 1 to 4). The genome consists of single-stranded positive-sense RNA which encodes three structural (C, prM/M, E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). [1]

Laboratory confirmation is important due to difficulties in making accurate diagnosis due to broad spectrum of clinical presentation. Dengue IgM and IgG antibodies dengue kits are widely used for diagnosing of dengue infection in routine laboratories, however, there are variations in detection limit during acute phase of the disease from 4-5 days for IgM and 1-14 days for IgG antibodies to become detectable [2,4]. The commercial NS antigen detection assay has been increasingly used and are becoming the tool of choice in Indonesia among clinicians to confirm DENV infection because of earlier detectable results [5,6].

The recent study had found an increase in number of admissions of cases of dengue fever with reactive NS1 antigen. In one side it has a benefit for early intervention at early stage of illness but in the other hand it resulted in increased burden of hospital with DVI with mild symptoms like DF cases especially during epidemic period. [1]

The aim of this study was to evaluate the utility of NS1 antigen as diagnostic marker and to determine whether there is higher risk of severe dengue infection for patient with positive NS1 antigen.

2. Materials and Methods

2.1. Study Design and Subjects

This was a retrospective analysis study that was conducted in 7 referral Hospitals in Bandung, West Java, Indonesia. The medical records of all the individuals

admitted to the Hospital from 1 January to 31 December 2015 were evaluated. We used a questionnaire as a tool for data collection consisted of the epidemiological, clinical manifestation, laboratory results and final diagnosis data that were collected during the period of hospitalizing. Individual with dengue diagnosis based on clinical and serological criteria were included in the study. This study is part of hospital-based surveillance: an accuracy, adequacy and timely dengue case report in Bandung, West Java, Indonesia 2015 research.

2.2. Definitions

The case definition of dengue based on the 1997/2011 World Health Organization (WHO) classification [7,8]. For this study, Dengue fever (DF) was operationally defined as a mild cases. The dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) were grouped together, and defined as a severe case. [11] Laboratory diagnosis divided into confirmed cases using NS 1 antigen dengue rapid test and probable cases using IgM antibody dengue rapid test. The variable of number of days between the onset of illness and the time of admission was categorized into early (0-2 days), regular (3-5 days), and late (6 or more days) admission. [11] These categories were arbitrarily derived from clinical phase and period of dengue: febrile, critical and recovery.

2.3. Statistical Analysis

Dengue viral infection (DVI) incidence by age sub group was calculated. A statistical analysis was performed to evaluate the association between Serological test used with time of admission, clinical manifestation and final diagnosis. For univariate analysis, a chi-square or exact fisher test were performed for the categorical variables. p-values lower than 0,05 were considered to be significant, The Health Research Ethic Committee Medical Faculty of Padjadjaran approved the study. A data analysis was performed with the software program SPSS 13.0 for windows.

3. Results

From 1 January to 31 December 2015 there were 4.096 cases of dengue viral infection that hospitalized. 2.115 cases were ≤15 years of age and 1.981 cases were >15 years old. Cases that admitted in early phase of illness is 22.8% (Table 1) and statistically more often in pediatric (≤15 year) patient, while delaying admission (≥6 days fever at home) more often in adult (>15 year) patient (p<0,005).

Most of patient with positive NS1 antigen was admitted at early phase (0-2 days) and regular phase (3-5 days) while on late phase there is no statistically significant impact on positive NS1 antigen with time of admission.

The comparison of NS1ag assay and IgM with platelet count can be seen on Table 2. Platelet count in the range of <10.000/mm^3, 10-20.000 /mm^3, 20-50.000/mm^3, and >50.000/mm^3 were seen in 42 (1.54%), 235 (11.32%), 544 (26.2%), and 1254 (60.94%) cases respectively. Platelet count >100.000/mm^3 was significantly associated with positive NS1Ag assay, while lower thrombocytopenia (platelet count <50.000/mm3) was significantly associated with positive IgM dengue antibody, while there is no significantly different between NS1 antigen and IgM antibody for severe trombocytopenia.

This study also showed that there is no significant difference of prevalence severe bleeding between NS1Ag assay positive and IgM antibody dengue positive and no significant difference on unusual manifestation in dengue viral infection with neurological manifestation between patient with NS1 antigen and IgM antibody dengue positive (P>0.005).

Table 1. Duration of Fever Prior to Admission of Dengue Viral Infection Cases

Days of Fever	<15 year	≥15 year	p-value
0-2 days	573	363	p<0.001*
3-5 days	1406	1214	p=0,589*
≥6 days	232	308	p<0.001*

*Chi-Square Test, significant if p<0,005.

Table 2. Duration of Fever Prior to Admission of Dengue Viral Infection Cases and NS1 antigen (+)

Days of Fever	≤15 year		>15 year		p-value
	Number of Patient	NS 1 (+)	Number of Patient	NS 1 (+)	
0-2 days	573	369	363	147	p<0.001*
3-5 days	1406	305	1214	165	p<0.001*
≥6 days	232	16	308	10	p=0.050*

*Chi-Square Test, significant if p<0,005.

Table 3. Comparison of NS1 Antigen Assay and Platelet Counts

Platelet range	NS (+)	IgM (+)	p-value
<10.000/mm^3	12	30	p=0.009*
>10.000-20.000/mm3	95	140	p=0.008*
>20.000-50.000/mm^3	225	319	p<0.001*
>50.000-100.000/mm^3	312	361	p=0.063*
>100.000-150.000/mm^3	221	150	p<0.001*
>150.000/mm^3	147	63	p<0.001*

*Chi-Square Test, significant if p<0,005.

Table 4. Severe Bleeding Manifestation in Dengue Viral Infection

Severe Bleeding Manifestation	NS 1 (+)		IgM (+)		p-value
	Present	Not Present	Present	Not Present	
Hematemesis	7	114	10	156	p=0.932*
Melena	10	153	9	188	p=0.508*
Hematemesis melena	0	118	1	166	p=1.0**

*Chi-Square Test, significant if p<0,005;
**Exact fisher test, significant if p<0,005.

Table 5. Unusual Manifestation in Dengue Viral Infection

Unusual Manifestation	NS 1(+)	IgM (+)	p-value
Seizure	7	19	p=0.080**
Loss of consciousness	0	11	

**Exact fisher test, significant if p<0,005.

Table 6. Diagnosis of Dengue Viral Infection in Hospitalized Cases

Diagnosis	NS 1(+)	IgM (+)	p-value
Viral syndrome	1	1	p<0.001**
DF	363	411	p<0.001*
DHF	296	601	p<0.001*
DSS	21	56	p=0.032*

*Chi-Square Test, significant if p<0,005;
**Exact fisher test, significant if p<0,005.

Table 7. Outcome of Dengue Viral Infection in Hospitalized Cases

Outcome	NS 1(+)	IgM (+)	p-value
Improvement	954	1039	p=1.00**
Died	2	3	

**Exact fisher test, significant if p<0,005.

This study showed in Table 6 that severe cases of dengue viral infection of DHF significantly more often happen on patient with IgM antibody dengue positive (P<0.005) while DSS case was not significantly different. while mild cases DF significantly more often happen in IgM antibody dengue positive, while there is no differences in viral syndrome. There were five death cases (0.24%) of serological positive dengue viral infection, and among them 2 cases positive for NS1Ag assay and 3 cases positive for IgM antibody dengue, there no significantly different between both groups.

4. Discussion

The revised WHO 2011 dengue guidelines have emphasized the need for early diagnosis and treatment to reduce the mortality due to severe diagnosis and treatment to reduce the mortality due to severe dengue infection. [8] The classical methods for confirmation diagnosis of dengue are viral isolation, serotype identification, antibody detection test (IgM and IgG dengue), haemagglutination inhibition (HI) test or neutralization test but all there these are time consuming and do not help in the confirmation of diagnosis at an early phase of illness. With good sensitivity of NS1 antigen detection in day 2 until 4 of illness and maximum sensitivity on day 2 (94, 6%) and specificity 100% [2], could be a benefit for early diagnosis, in the other hand can make an early unnecessary admission for dengue viral infection patient [1,9,10].

This study showed the majority of cases admitted during period between the third and fifth day from onset of disease, children patient more often admitted in early phase, data also showed there is a significantly impact of positive NS1 antigen assay in early phase with early admission, this may occur because as parent and physicians are so worried about DHF, unnecessary hospitalization of children will take place. This phenomenon especially occurs in regions with a high incidence of DHF cases. [11] Other study in Southeast Asia found that cases of dengue adults showed mild signs and symptoms. [12] In contrast, a majority of the severe cases occur in children aged 2-15 years. Adults apparently acquire immunity from primary infection and avoid DHF. [13,14] Furthermore, the case fatality rate (CFR) among children during late admission exceeded 1%. This can emphasizes practice of early admission among children patient. [11]

There are earlier study that showed Free NS1 levels in plasma correlated with viremia levels and were higher in patients with DHF than in those with DF. An elevated free NS1 level (≥600 ng/mL) within 72 hours of illness onset identified patients at risk for developing DHF, Immune complex formation with secreted (sNS1) and sNS1 binding to endothelial cells have been proposed as potential factors in DHF pathogenesis. Whether high circulating levels of antibody-bound or free sNS1 protein following peak viremia play a direct role in the pathogenesis of DHF is unknown and will require further investigation, however in our study the predominant mode of presentation in patient who were NS1 Ag positive was non-severe dengue infection manifestation. This study showed NS1 Ag test was not an early predictor of severe dengue infection even though it helped in confirmation of diagnosis at an early stage of illness, because overall efficiency of the tests was found to be 83.64% and specificity was 100% and maximum sensitivity of NS1 antigen detection was on D2 of illness compare with Dengue IgM and IgG ELISA kits that were widely used for diagnosis of dengue infection in routine laboratories but there are variations in detection limit during acute phase of the disease. [15] After the onset of symptoms, it usually takes 4–5 and 1–14 days respectively for anti DENV IgM and IgG antibodies to become detectable, depending on whether the patient has primary or secondary infection.

This study also showed the real need for the treating physicians to recognize the early warning sign and it should remain the only indicator for admission rather than results of laboratory test because we found there was significance relation between NS1 ag positive with severe manifestation and platelet count, this is maybe because the factor that associated is immunological responded that knowns as antibody dependent enhancement (ADE). It is also important from treating physicians to educate patients or parents to educate them regarding home care treatment and identification of symptoms and warning sign of dengue viral infection, especially during epidemics.

This study is retrospective analysis and diagnosis was confirmed by rapid test of NS1 antigen or dengue IgM antibody, we cannot determine plasma level of the secreted NS1 or plasma level of IgM antibody that can be correlated with viremia levels and severity of disease. Virus isolation and serotype identification was also not done in the present study. Different serotype maybe has a different severity manifestation.

5. Conclusions

NS1 Ag assay is useful, sensitive and specific for the diagnosis of dengue detection, with good sensitivity and specificity during acute phase when detectable antibodies still negative. However from this study cannot be used as an early predictor of severe dengue infection, and criteria to hospitalized the dengue viral infection should be based on clinical warning sign.

Acknowledgements

This study was supported by an Academic Leadership Grant (ALG) from Universitas Padjadjaran.

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