SERO-EPIDEMILOGICAL STUDY ON DENGUE VIRUS INFECTION IN FOUR INDONESIAN CITIES

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ABSTRAK


Kata Kunci: Virus Dengue, serotype, sero-epidemiologi

ABSTRACT

Dengue virus (DENV) infection is recognized as a major public health problem; >50 million persons are infected each year worldwide. Molecular epidemiology studies have investigated the possibility of a link between particular DENV genotype or cluster or particular clinical form of disease. Consequently, finding new viral genotypes in areas where they had been absent could be of epidemiologic and clinical interest. The aim of this study is to identify the serotype of dengue virus in Surabaya, Sidoarjo, Bangkalan, and Mataram. Human sera were obtained from 392 patients presenting clinical manifestations of dengue and tested for anti-dengue IgM antibodies (Becton-Dickinson). Dengue-infected samples were obtained during the first five days of the onset of fever and were processed for anti dengue IgM detection using IgM capture ELISA (MAC-ELISA). In Surabaya, 182 of 203 sample (2011) and 79 of 86 sample (2012) were D1 serotype. In Sidoarjo, 40 of 43 sample (2011) and all of 17 sample (2012) were D1 serotype. In Bangkalan, 23 of 24 sample (2013) and in Mataram all of 4 sample were D4 serotype. We can conclude that in Surabaya and Sidoarjo the most Dengue Viruses serotype is D1, while in Bangkalan and Mataram the most Dengue Virus serotype is D4.(FMI 2013;49:146-149)

Keywords: Dengue Virus, Serotype, Sero-epidemiological

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INTRODUCTION

Dengue virus (DENV) infection is recognized as a major public health problem; >50 million persons are infected each year worldwide (McBride & Bielefeldt-Ohmann 2002), and the incidence of severe, sometimes lethal, forms of the disease is increasing (Guzmán & Kouri 2002). Dengue viruses are mosquito borne flaviviruses with a single-stranded, non segmented, positive-sense RNA genome ≈11 kb in length. Four antigenically distinct serotype, DENV types 1 to 4, exist (Rice 1996). Infection with any serotype can lead to disease, ranging from mild infection, dengue fever (a generally mild disease with complete recovery), to severe forms (dengue hemorrhagic fever and dengue shock syndrome). Molecular epidemiology studies have investigated the possibility of a link between particular DENV genotype or cluster or particular clinical form of disease (Riccp-Hesse 2003, Messer et al 2003). Consequently, finding new viral genotypes in areas where they had been absent could be of epidemiologic and clinical interest. Emerging Infectious Diseases dengue epidemic are expanding rapidly. This emerging diseases continues to baffle and challenge epidemiologists and clinicians to study. Despite endemicity of 3 or more different dengue viruses, why
does severe dengue occur in some populations and not in others? Why are children principally affected in some areas and adults in others? How can severe dengue reliably be recognized early enough to permit appropriate therapy to be applied? (Halstead 2005).

During an infection with any of the 4 dengue viruses, the principal threat to human health resides in the ability of the infecting virus to produce an acute febrile syndrome characterized by clinically significant vascular permeability, dengue hemorrhagic fever (DHF). However, because at onset vascular permeability exhibits only subtle changes, how can a diagnostic be made early enough to begin life-saving intravenous treatment? In person with light skin color, the standard sphygmomanometer cuff tourniquet test has been widely used to screen children in outpatient settings; a positive result in an early warning of incipient DHF. Because of genetic diversity among humans, the tourniquet test as a screening tool requires widespread evaluation and validation (Halstead 2005).

Human are not uniformly susceptible to the DHF syndrome. HLA gene distribution correlates with increased susceptibility as well as with increased resistance (Stephens et al 2002). In addition, a powerful resistance gene is found in blacks (Guzmán et al 1990). Importantly, susceptibility to vascular permeability during a dengue infection is age-related. The susceptibility of young children to DHF precisely paralleled age-related changes in microvascular permeability measured in normal children and adult.

Dengue fever syndrome in susceptible adults may be contrasted to the innate susceptibility of children for vascular permeability syndrome during a secondary dengue virus infection. In Southeast Asia, the epicenter of DHF epidemics in children, dengue infection rates are falling, resulting in changing epidemiologic patterns of DHF. In Thailand, for example, the modal age at which children are hospitalized for DHF has steadily increased over the past decades. In addition, an increasing number of persons experience their first dengue infection at an older age, dengue fever cases are now appearing in adults (Guzmán & Kouri 2002).

MATERIALS AND METHODS

Seroepidemiology study had been done in Surabaya, Sidoarjo, Bangkalan and Mataram. Surabaya and Sidoarjo are part of East Java including in Java Island, and Bangkalan is part of Madura Island. Mataram city is part of West Nusa Tenggara province. There are 392 serum specimens from patients which had a clinical manifestation of dengue infection based on Criteria WHO-2009 from 2011 until January 2013. A number of 349 isolate were obtained from dengue patients sera in Surabaya (Dr. Soetomo Hospital) and Sidoarjo (Soerya Hospital) on 2011-2012; and 43 serum sample were collected in Surabaya, Bangkalan, and Mataram on January 2013.

Human sera were obtained from 392 patients presenting clinical manifestations of dengue and tested for anti-dengue IgM antibodies (Becton-Dickinson). The clinical samples corresponded with dengue cases reported during 2011-2013. Diagnosis of dengue virus infected was based on Criteria WHO 2009. Dengue-infected samples were obtained during the first five days of the onset of fever and were processed for anti dengue IgM detection using IgM capture ELISA (MAC-ELISA) as described by Vorndam et al

As a routine practice and with the idea of recording epidemic data, the suspected dengue sample already clinically diagnosed in community health centers were sent to Institute of Tropical Disease Airlangga University, Surabaya, Indonesia. In the laboratory, the presence of dengue virus was confirmed by MAC-ELISA and RT-PCR. Aedes albopictus C6/36 cells were grown in 48-well tissue culture plates as described by Igarashi (9). Briefly, 2x105 cells were plated in 1 ml of minimum essential medium (Gibco BRL, Grand Island, N.Y.) supplemented with 7% fetal bovine serum (Sigma Chemical Co., St Louis, Mo) and 1% glutamine, vitamins and nonessential amino acids. After 24 hours of cultures, 100 μl of every sera diluted 1:10 was added to the corresponding well. The mixture was then gently shaken and incubated for 60 minutes at room temperature. Cells were then washed with serum-free medium and cultured at 28 °C with complete medium for at least 10 days. Cells were harvested for RT-PCR diagnosis.

Total RNA was extracted either from 100 μl of serum or from cultured cells by using Trizol LS (GIBCO BRL, Gaithersburg, MD.) according to the manufacturers recommendations. Ethanol-precipitated RNA was recovered by centrifugation and air-dried. The RNA pellet was re-suspended in 50 μl of Diethylpyrocarbonate (Sigma)-treated water (DEPC water) and used as template for RT-PCR.

Synthetic oligonucleotide primer pairs were designed based on published sequence data for each of the four serotypes of dengue (10,11). Four fragments of an expected size of 482 bp (DEN-1), 392 bp (DEN-4), 290 bp (DEN-3) and 119 bp (DEN-2) were obtained by using the SuperScriptTM One Step RT-PCR kit in conjunction with PlatinumRTaq polymerase (Invitrogen, Life Technologies). A mixture of 5 μl of
RNA, 25 μM of sense and anti-sense PCR primers, and DEPC water to a total volume of 50 μl was incubated at 85 °C for 5 minutes and then chilled on ice. The tube-reaction mixture containing 2X PCR buffer containing 0.4 mM MgSO4 and Super Scrip TM RT/platinum R Taq Mix, as recommended by the manufacturer (Invitrogen TM Life Technologies), was added to the RNA and primers-containing tube. The reverse transcription reaction was performed at 50 °C for 30 minutes. Thermocycling began with a hot start at 9°C for 2 minutes followed by 40 cycles of annealing at 55 °C for 30 seconds, and extension at 72 °C for one minute and denaturing at 94 °C for 15 minutes. The PCR conditions for serotype assessment were as follows: 40 cycles of denaturing at 94 °C for 30 seconds, annealing at 55 °C for 1 minute, and extension at 72 °C for 7 minutes. The reaction mixtures were electrophoresed and visualized under UV light after ethidium bromide staining of the gels.

RESULTS

Table 1. Serotype of Dengue Virus Collected From Patient in Surabaya and Sidoarjo on 2011-2012

<table>
<thead>
<tr>
<th></th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surabaya</td>
<td>D1 182</td>
<td>40 79</td>
</tr>
<tr>
<td>Sidoarjo</td>
<td>20 3</td>
<td>7 0</td>
</tr>
<tr>
<td>Surabaya</td>
<td>D3 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Sidoarjo</td>
<td>1 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Surabaya</td>
<td>D4 1</td>
<td>0 0</td>
</tr>
<tr>
<td>Sidoarjo</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Table 2. Serotype of Dengue Virus Collected From Patient in Surabaya, Bangkalan Madura and Mataram Lombok on January 2013

<table>
<thead>
<tr>
<th>Sites</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surabaya</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Bangkalan</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Mataram</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
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</table>

DISCUSSION

Seroepidemiology of Dengue Virus Infection is a science of transferring Dengue Virus to other host of human being by biting of Aedes aegypti mosquito which has been supported by biotic and abiotic factor: such as an increasing population of Aedes aegypti in urban and sub urban environment due to the changing of raining season to sunrise. Dengue virus infection in human being can be found if the interaction among etiologic, host and environment has occurred (McBride & Bielefeldt-Olmann 2002). The etiologic is dengue virus which usually life in Aedes aegypti mosquito where it like life in clean water (Guzmán & Kouri 2002). Host is human being who has decreasing immunity due to very tired and getting more virulent virus from biting of Aedes aegypti mosquito which is supported by changing humidity of environment (Rice 1996). Since 1968 Dengue Virus Infection has been found in Indonesia, especially at Surabaya and Jakarta city. Firstly management of dengue virus infection very difficult to improve, therefore the higher mortality nearly 41,4 % had been found; but on the following years in five decades the mortality rates was becoming to decrease until 1,27 % on 2011. On January 2013, the outbreak of Dengue Virus Infection has been occurred and many cases of Children under five years has been found and one of them is a 21 days old baby suffered from DSS has been found, as a younger baby case. Beside it many cases babies below one years old has been found as severe dengue virus infection which shown clinical manifestation of bleeding and encephalopathy, therefore some of them cannot be help. It should be studied what kind Serotype of dengue virus was becoming predominant strain as a cause of severity?

As we know in the first decade the Serotype of Dengue 2 and 3 were predominant strain Den 2 tended to be neutralized stronger than other viruses. It was found in Surabaya before 2008 but on the year 2009, 2010, 2011 and 2012 there were changing of Serotype and foundDen 1 genotype IV showed a severity clinical manifestation with primary infection but on 2013 Serotype Den 1 and IV were found at Surabaya, Bangkalan and Mataram; especially Bangkalan and Mataram showed more Den IV. Phylogenetic analysis on Den V 1 in Surabaya showed that genotype I and IV consisted at the same time in 2012 although only genotype IV were isolate in 2011. There were differences between D 1 genotype I and IV. Analyzing the difference of genotype is important for vaccine development. Based on this result of study, monitoring the emergence of imported or mutated strain of Dengue Virus in human being and mosquito should always be continued. Therefore, continuous surveillance of circulating viruses is required to predict the risk of DHF and DF. This idea is very important for making procedure Update management of Dengue Virus Infection Cases to decrease Case Mortality in the outbreak situation and try to do new prevention method before Outbreak Occur.

CONCLUSION

In Surabaya and Sidoarjo the most Dengue Virus serotype is D1, while in Bangkalan and Mataram the most Dengue Virus serotype is D4.
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REFERENCES