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Research Report

MOLECULAR SURVEILLANCE OF DENGUE VIRUS SEROTYPE USING POLYMERASE CHAIN REACTION IN SURABAYA 2013

Teguh Hari Sucipto[1], Amaliah Labiqah[1], Siti Churrotin[1], Nur Laila Fitriati Ahwanah[1], Kris Cahyo Mulyatno[1], Soegeng Soegijanto[1], Tomohiro Kotaki[2], Masanori Kameoka[2], Eiji Konishi[2]
[1] Institute of Tropical Desease, Universitas Airlangga
[2] Center for Infectious Disease, Kobe University Graduate School of Medicine, Japan

ABSTRACT

Dengue is one of the infectious diseases which is endemic in the tropical and sub-tropical country. The disease found in Indonesia Surabaya, 1968. The symptoms of Dengue virus infections are two kinds, first DF (Dengue Fever), second DHF (Dengue Hemorrhagic Fever). This infectious disease transmitted by Aedes aegypti mosquito. Mosquitoes breed in clean water areas. More than 100,000 cases of DF/DHF occurred in Indonesia every year. The purpose of this study were to provide information and the spread of dengue virus types in Surabaya from January 2013 to September 2013. The analysis technique used to determine the type of dengue virus infection was used PCR (Polymerase Chain Reaction). The results obtained 69% DENV-1, 27% DENV-2 isolates, 4% isolates DENV-3, and 0% DENV-4 isolates.

Key words: *Dengue, DENV, Surabaya 2013, Polymerase Chain Reaction*

ABSTRAK

Dengue merupakan salah satu penyakit infeksi yang endemik di daerah tropis dan sub tropis. Penyakit ini ditemukan di Indonesia pada tahun 1968 tepatnya di kota Surabaya. Gejala infeksi virus Dengue ada 2 macam, yaitu DF (Dengue Fever) dan DHF (Dengue Hemorrhagic Fever). Penyakit infeksi ini ditularkan melalui nyamuk Aedes aegypti, nyamuk ini berkembang biak pada daerah air yang bersih. Lebih dari 100.000 kasus DF/DHF terjadi di Indonesia setiap tahunnya. Tujuan dari penelitian ini adalah untuk memberikan informasi persebaran dan tipe virus dengue yang ada di Surabaya pada periode Januari 2013 sampai dengan September 2013. Teknik analisis yang digunakan untuk menentukan tipe infeksi virus dengue adalah menggunakan PCR (Polymerase Chain Reaction). Hasil isolat yang diperoleh 69% DENV-1, isolat DENV-2 27%, isolat DENV-3 4%, dan isolat DENV-4 0%.

Kata kunci: *Dengue, DENV, Surabaya 2013, Polymerase Chain Reaction*

INTRODUCTION

Dengue fever (DF) is a kind of infectious diseases that is distributed in the tropical and sub-tropical country.[1] This infectious disease is transmitted by *Aedes aegypti* mosquitoes, mosquitoes breed in clean water areas. This infectious diseasehas been found in 18 and 19 centuries ago.[2] Later, in 1953–1954 has been reported that the presence of DHF (Dengue Hemorrhagic Fever) in Manila Philippines. In, 1958 was found in Bangkok Thailand. In the 1960's has been found in Malaysia, Singapore, and Vietnam. This was due to the increasing influence of geographical and the density of *Aedes aegypti*.[3]

Dengue virus infection in human may be subclinical and clinical, with mild symptoms such as fever / flu - like syndrome or Dengue Fever (DF).[4] Dengue Fever are limited and rarely fatal. However, it is becoming high risk if the infection develops into Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS) can turn into death. Hemorrhagic Fever (DHF) is caused by vascular permeability that is characterized by capillary leakage, thrombocytopenia and hypovolamic shock.[5]

In Indonesia, Dengue Hemorrhagic Fever (DHF) occurred for the first time as an outbreak in Surabaya, in 1968. Dengue fever has spread to all regions of the province with the number of cities infected were increasing. More

than 250,000-500,000 cases of DF / DHF occurred in the world each year.[6] Genetically, there are 4 types of dengue viruses, they are DENV-1, DENV-2, DENV-3 and DENV-4. Until now, has not been found effective antivirus for dengue.

The purpose of this study was to provide information and the spread of dengue virus types in Surabaya from January 2013 to September 2013. The analysis technique used to determine the types of dengue virus infection was used PCR (Polymerase Chain Reaction).

MATERIALS AND METHODS

Population Sample

Epidemiological studies has conducted in Surabaya, East Java. Samplings were conducted at Soerya Children's Hospital and Maternity Sepanjang Sidoarjo accompanied by a certificate of Ethics from LPPM (Institute for Research and Community Service) Airlangga University. 800 serum samples were obtained. The study populations were all patients with Dengue Hemorrhagic Fever (DHF) which met the sample criteria according to WHO (World Health Organization) 2009.

Collection of Samples and the Diagnosis of Dengue

Blood samples were taken from DENV - infected patients diagnosed with IgG and IgM examination. Diagnosis based on WHO 2009 criteria[7] consists of clinical and laboratory criteria were as follows, sudden high fever, with no apparent reason, lasted continuously for 2–7 days, there were manifestations of bleeding, including testing positive tourniqued, petechical, ecchymosis, epistaxis, bleeding gums, and haemasthasis or malena, liver enlargement, shock, marked rapid and weak pulse, and pulse pressure, hypotension, feet and hands cold, moist skin and the patient was restless. Based on laboratory criteria, thrombocytopenia (100.000/mm^3 or less), haemoconcentration, can be seen from the increase in hematocrit of 20% or more, according to age and type of gender, or a decrease in hematocrit of 20% after fluid therapy.

Sampling operational procedures were as follows, the doctor asked the willingness to research subjects to participate. If the doctor was willing to coordinate with the hospital laboratory personnel to take the patient's blood, hospital laboratory personnel take as much as 8–10 cc blood of patients put in the tube on ice, prepared the shipment of samples to researcher at Airlangga University in LPT, then the sample were being examined at LPT Airlangga University.

Processing of Samples

Serum used for viral culture, formed after the CPE (Cytopathic effect) by viral infected examination of molecular biology ranging from the extraction of RNA, RT-PCR examination, and PCR using primers specific to determine the serotype of the virus.

Isolation of Virus

Blood serum taken from patients, inoculated into cell cultures (vero cell). Cells were grown for 2–3 passages, the first time the passage of time was 5–7 days. After positive cells showed CPE or infected, the culture fluid were collected and molecular biological examination can be done.

Extraction of RNA

RNA extraction using Trizol solution, 200 mL liquid culture, then added a solution of 800 mL Trizol, then mixed with pipette for several times and incubated in the mixture and incubated at room temperature. 200 mL chloroform was added to the mixture and allowed to stand at room temperature for 5 minutes, then centrifuged 12,000 rpm for 15 min at 4ºC. 500 mL of supernatant was taken and then added 500 mL 2 - propanol as much into a new Eppendorf tube, vortex and left at room temperature for 10 minutes. Centrifuge was repeated 12,000 rpm for 10 min at 4ºC. Supernatant layer then removed slowly by pipette carefully so that the RNA formed is not fetched. Ethanol 70% in increments of 1 mL was added to the sediment above the vortex and in this phase deposition can be saved or resumed by a centrifuge 12,000 rpm for 10 min at 4ºC. Disposal repeated supernatant layer was then dried with a vacuum pump for 10 minutes. Pellet suspended with DW as much as 10 mL, ready for further examination cDNA synthesis using (Reverse Transcriptase Polymerase Chain Reaction) RT-PCR method and PCR method for DNA synthesis.

Synthesis of DNA by PCR

Reactions for cDNA synthesis using TS primer (type-specific) is TS1 (5´-CGTCTCAGTGATCCGGGGG-3´), TS2 (5´-CGCCACAAGGGCCATGAACAG-3´), TS3 (5´-TAACATCATCATCATGAG ACAGAGC-3´), and TS4 (5´-CTCTGT TGTCTTAAACAAGAGA-3´), and then incubation for 3 phase, phase 1 were incubated at 65ºC for 5 minutes. Phase 2, incubated at 50ºC for 45 minutes and incubated at 85ºC for 5 minutes. Then, phase 3 incubated 37ºC for 20 minutes.

Doing PCR using primers D1 (5´-TCAATATGCTGAAACGCGAGAA-3´) and TS (type-specific) is TS1, TS2, TS3, and TS4 together in a 1.5 mL Eppendorf tube. Principles of PCR consisted of three stages: denaturation of double-stranded DNA, subsequent annealing (annealing) primer on the target DNA, primer extension last (primer elongation) at the presence of DNA polymerase. The results of DNA that occurs was ex-potential accumulation of the specific target DNA, approximately 2^n where n is the number of cycles set in the PCR process. At this stage PCR was performed in 35 cycles, the temperature and time used for denaturation 94°C for 30 s, annealing 55ºC for 60 sec and extension 72ºC for 2 min.

Electrophoresis

Validation was the process of DNA replication can be done with electrophoresis gel using ethidium bromide

which has been given to tracer tape from serotype to be searched.

RESULTS AND DISCUSSION

Virus isolation resulted from the sample collection from Soerya Children's Hospital and Maternity Sepanjang Sidoarjo from January 2013 to September 2013 were 68 virus isolates obtained from total of 800 serum samples.

In January obtained 34 isolates of dengue viruses which consists 31 isolates DENV-1, 1 isolate of DENV-2, and 2 isolates of DENV-3, in January 2013 rainfall was very high so dengue virus isolates obtained higher than other months (February to September) 2013. Due to higher rainfall was a good time for *Aedes Aegypti* mosquitoes to breed. From February to June, 16 isolates obtained (February DENV-3 by 1 isolates, March DENV-1 as 6 isolates, 4 isolates as DENV-1 April, May DENV-1 3 isolates, and June DENV-1 2 isolates) due to rainfall was very low in so the decline of dengue vector breeding. On July, 12 isolates obtained DENV-2, while on Aug. 5 isolates obtained DENV-2, and in September 1 isolates obtained DENV-2.

In figure 2 it can be seen that every month in 2013 (from January to September) there were cases of dengue and there are many different types of dengue virus every month. Dengue Virus Type 1 (DENV-1), from January to September 2013 still dominate as before. Based on research conducted by Yamanaka (2011) DENV-1 type of dengue virus was dominated since 2009 to 2010.[8] As well as in 2011 and 2012, has done research that DENV-1 obtained 90.3% and 93.2% but this result was still in the process of publication. This caused by a secondary infection that occurs when a carrier mechanism and progression of the virus increases immunity. Figure 4 was the result of electrophoresis showed positive samples of fragments coding genes NS1 protein of infected DENV-1 (band at 482 bp).

Dengue virus type 2 (DENV-2), figure 3 was the result of electrophoresis showed positive samples of fragments coding genes NS1 protein of infected DENV-2 (band at 119 bp) using agarose gel 1.5% and DNA ladder for marker, in July, August, and September dominates like in 2007, specifically in April, 53 isolates were obtained. From June 2008 to April 2009 there were 68% infections due to DENV-2.[8] Dengue virus type 2 (DENV-2) appeared back in July, August, and September 2013 did not find the results

Table 1. Result of Band Electrophoresis (bp)

No.	Type of Dengue Virus	bp
1	DENV-1	482
2	DENV-2	119
3	DENV-3	290
4	DENV-4	392

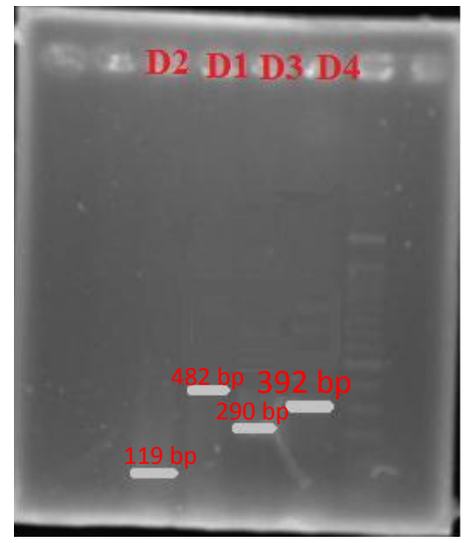

Figure 1. Serotype DENV

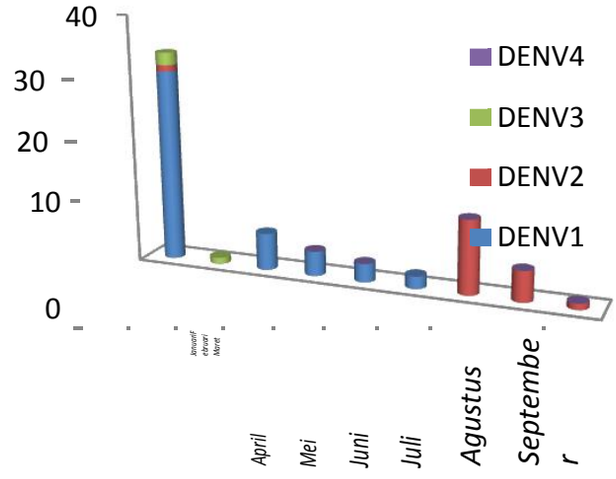

Figure 2. Diagram of Dengue Virus Isolates in Surabaya 2013

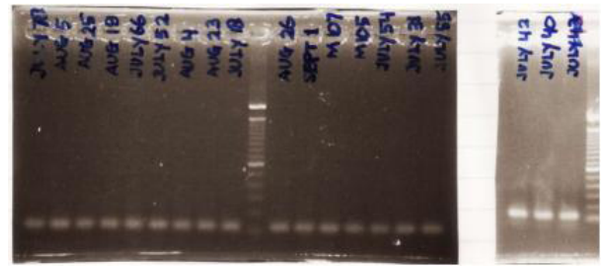

Figure 3. The results of electrophoresis of DNA fragments coding genes NS1 protein of dengue virus serotype 2 (DENV-2) at position 119 bp.

Figure 4. The results of electrophoresis of DNA fragments coding genes NS1 protein of dengue virus serotype 1 (DENV-1) at position 482 bp

of dominance (the previous month). Was this indicate a change of dominance back of dengue virus type 1 to type 2 as it happened in 2008 to 2010?

Dengue Virus Type 3 (DENV-3), for so long has been conducted research at the Institute of Tropical Disease, Airlangga University from 2007 to 2012 has never gained DENV 3 isolates. However, in January 2013 gained 2 isolates DENV-3 and February 2013 1 isolates obtained DENV-3, it has been published by Kotaki (2013).[9] In 2008, Ong et al., has published the same thing, namely the Jakarta DENV-3 of samples collection.10 At the time of the 69 samples obtained 15 isolates, these isolates comprised 10 isolates DENV-3, two isolates DENV-2, two isolates DENV-4, and 1 isolates DENV-1. Based on these publications it can be seen that actually has been dominated DENV-3 infection. Thus, it was possible that this DENV-3 cycle will return again and especially in Surabaya.

Dengue Virus Type 4 (DENV-4), no DENV-4 isolates obtained from January to September 2013. The possibility of this was due to declining circulation and spreading viral evolution.

CONCLUSION

DENV 1 dominance in Surabaya (from January 2013 to September 2013) was still ranked first, followed by DENV 2, DENV 3 gained 2 isolates in January 2013 and 1 isolate of DENV 3 in February. Not obtained isolat DENV 4.

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