RINGKASAN

Epidemiologi molekuler banyak dikembangkan untuk mencari berbagai faktor yang diperkirakan menjadi penyebab masih terus berkembangnya prevalensi infeksi virus Dengue di seluruh belahan dunia. Sekuensing dari berbagai regio di dalam genom virus Dengue tersebut penting dilakukan untuk menentukan variasi genetik dan untuk mengkarakterisasi subtipe (genotipe) di dalam masing-masing serotipenya.

Penelitian dilakukan pada 19 kota di Indonesia yang terdapat di pulau Sumatera, Batam, Kalimantan, Sulawesi, Papua, Jawa, Bali dan Lombok dari tahun 2003-2005. Pengambilan sample Demam Berdarah Dengue (DBD) dilakukan di Unit Pelaksana Fungsional (UPF) Ilmu Kesehatan Anak dan UPF Ilmu Penyakit Dalam di berbagai rumah sakit. Diagnosis DBD ditegakkan berdasarkan kriteria diagnosis menurut WHO 1997.

Pelaksanaan penelitian serologi dan PCR dikerjakan di laboratorium Dengue dan laboratorium Hepatitis Tropical Disease Center Universitas Airlangga. Pengerjaan isolasi kultur virus Dengue, sekuensing DNA dan analisis filogenetik dikerjakan di Laboratorium Virologi, Institute of Tropical Medicine, Nagasaki University, Jepang. Sebanyak 525 serum penderita DBD dengan kriteria WHO, 1997 dilakukan pemeriksaan IgM dan IgG antiDengue dan didapatkan dominasi infeksi sekunder sebanyak 57,14% (300/525), sedangkan infeksi primer sebanyak 12,57 % (66/525), kondisi *equivocal* sebanyak 4,20% (22/525) dan negatif sebanyak 26,09 % (137/525).

Sejumlah 192 sampel dilakukan *serotyping* dengan metode *Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)* yang dilakukan di *Tropical Disease Center (TDC)* Universitas Airlangga (Unair), terdapat dominasi DEN-2 yaitu sebanyak 65 sampel (65 %) dari 100 sampel positif PCR-nya kemudian diikuti oleh DEN-3 sebanyak 15 sampel (15 %), DEN-4 sebanyak 12 sampel (12 %) dan DEN-1 sebanyak 8 sampel (8 %). Analisis homologi dan analisis filogenetik dilakukan terhadap serotipe DEN-2 dan DEN-3 yang dominan dalam penelitian ini. Isolat dari Jawa Timur yaitu Surabaya, Malang, Pacitan, Jember terdapat homologi senada yang berkisar antara 77,4-97,5% namun terhadap Jakarta-1 dan sampel di luar Jawa (Aceh, Mataram, Batam, Gorontalo) terdapat homologi di bawah 50% . Analisis homologi untuk DEN-3 hanya terbatas pada

3 sampel yang memiliki kualitas elektroferogram yang bagus, yaitu Jayapura, Kendari dan Medan dengan nilai homologi yang cukup baik maupun terhadap referens (Martinique, Amerika Tengah) yaitu berkisar 83,3%- 92,4% .

Analisis filogenetik terhadap serotipe DEN-2 dari berbagai sampel menemukan adanya 2 *clade* yang berbeda . *Clade* yang pertama menunjukkan sampel dari Surabaya, Malang, Jember, Pacitan, Aceh, Mataram terdapat dalam satu gugusan .*Clade* yang berikutnya terdiri dari sampel Gorontalo, Batam dan Jakarta-1 bersama dengan referens yang dipakai pada penelitian ini yaitu Jamaica, Venezuela, Mexico dan Thailand.

Melalui proses isolasi virus dengan menggunakan *C6/36 Aedes albopictus cell monolayers* dalam *Eagle's minimal essential medium (E-MEM)* yang telah diperkaya dengan *2% fetal calf serum (FCS)* dan kemudian dilakukan sekuensing DNA, didapatkan genotipe Cosmopolitan untuk DEN-2 di Gorontalo-2005 dan genotipe I untuk DEN-3 Jakarta-2003 .

SUMMARY

Molecular epidemiology has been developed for finding many factors that may cause the high prevalence of dengue virus infection in the world. Sequencing from various regions inside the dengue virus genome is needed for confirmation of genetic variation and characterization of genotypes in each serotype.

The DHF samples were obtained from the Department of Pediatrics and Internal Medicine from various hospitals in 19 cities of Indonesia comprising Sumatera, Batam, Kalimantan, Sulawesi, Papua, Java, Bali and Lombok from 2003-2005. The DHF diagnosis was based on the 1997 WHO diagnostic criteria.

Serology and PCR testing were performed in the Dengue and Hepatitis Laboratory of the Tropical Disease Centre Airlangga University. Dengue virus isolation, DNA sequencing and phylogenetic analysis were done in the Virology laboratory, Institute of Tropical Medicine, Nagasaki, Japan.

IgM and IgG antiDengue testing were performed on 525 DHF sera fulfilling the 1997 WHO criteria showing a majority of secondary infection 57.14% (300/525), primary infection 12.57% (66/525), equivocal 4.20% (22/525) while negative 26.09% (137/525).

Serotyping using PCR was performed on 192 samples out of 525 sera, resulting in 100 PCR positive samples. From these, 65 samples (65%) showed a majority of DEN-2, followed by 15 samples (15%) DEN-3, 12 samples (12%) DEN-4 and 8 samples (8%) DEN-1.

Homology and phylogenetic analysis were performed to DEN-2 and DEN-3 serotypes which were dominant in this research. Samples from East Java showed similar homology ranging from 77.4% – 97.75% however, Jakarta-1 and samples from outside of Java (Aceh, Mataram, Batam, Gorontalo) showed homology less than 50%. Homology analysis to DEN-3 is limited to only three samples which showed good electropherogram quality, Jayapura, Kendari and Medan showing good homology inter-samples compared to reference (Martinique, French West Indies) ranging from 83.3% - 92.4%.

Phylogenetic analysis to DEN-2 serotype from various samples showed two different clades. The first clade was from samples of Surabaya, Malang, Jember, Pacitan,

Aceh, Mataram. The other clade was from Gorontalo, Batam, Jakarta-1 together with the reference (Jamaica, Venezuela, Mexico, Thailand) used in this study.

Dengue virus isolation using C6/36 *Aedes albopictus* cell monolayers in Eagles's minimal essential medium (E-MEM) supplemented with 2% fetal calf serum (FCS) and followed by DNA sequencing, resulted in Cosmopolitan genotype for DEN-2 from Gorontalo 2005 and genotype-I for DEN-3 from Jakarta 2003.

ABSTRACT

Molecular epidemiology is needed to solve the problem for endemic Dengue Hemorrhagic Fever in Indonesia. This research has been carried out consisting of 525 Dengue Hemorrhagic Fever sera, according to the WHO criteria. These sera were collected of 19 cities in Indonesia comprising the islands of Sumatera, Batam, Kalimantan, Sulawesi, Papua, Java, Bali and Lombok from 2003 until 2005.

The immune response profile was as follows 57.14% (300/525) secondary infection , 12.57% (66/525) primary infection, 4.20% (22/525) equivocal and 26.09% (137/525) negative. From 192 PCR samples, 100 (52%) sera were positive, consisting of 65% DEN-2 , 15% DEN-3 , 12% DEN-4 and 8% DEN-1.

Homology analysis showed nucleotide differences in capsid region DEN-2 serotypes, while DEN-3 serotypes were relatively consistent. Phylogenetic analysis using envelope (E) gene revealed that the Cosmopolitan genotype from Gorontalo in 2005, is currently circulating locally, with the potential to cause a severe hemorrhagic disease. Members of this genotype were closely related to viruses from Malaysia, Singapore, Thailand, Philippines and Australia. The isolate from Jakarta, 2003 showed DEN-3 with genotype-I. This genotype was similar to the isolates from Indonesia 1978, 1985, and also from Thailand 1992, Philippines 1997, Fiji 1992.

These results showed that four serotypes are circulating in Indonesia but have a different distribution in each island, dominated by DEN-2, followed by DEN-3, DEN-4 and DEN-1. Cosmopolitan genotype from DEN-2 was similar to Southeast Asia countries. It was also revealed that genotype-I from DEN-3 showed no change over the years since 1978.

Keywords : DHF, DEN-2 virus, DEN-3 virus, DNA sequencing, homology analysis, phylogenetic analysis