Primer Design for Sequencing DENV-4 to be used in Molecular Epidemiology of Dengue Viruses in Jakarta

Nadia Tita Indriasti,[1] Beti Ernawati Dewi[2]

[1]Medical Science Program, Faculty of Medicine Universitas Indonesia
[2]Department of Microbiology, Faculty of Medicine Universitas Indonesia

Abstract

This study is aimed to recognize the characteristic of dengue patients in Jakarta, to identify the genotype of dengue virus serotype 4 (DENV-4) in Jakarta and to design primer for DENV-4. This cross-sectional study involved 100 patients with positive result of NS-1 examination. This research was conducted at the Department of Microbiology, Faculty of Medicine, Universitas Indonesia. The specimen were collected from March until December 2010, while the virus sequencing was perfomed between January and October 2011. The patients were categorized based on RT-PCR confirmation results, gender, age and domicile. The identification of serotipe of dengue viruses is determined by RT-PCR examination. The genotipe of DENV-4 is determined by sequencing of whole genome of DENV-4 by using Genetyx software. The primer design for DENV-4 was carried out by using Primer Designer program. The highest prevalence of DF was found among patients of 14-20 years old (41,4%) and those who lived in East Jakarta. The ratio of DF between male and female is not significantly different. The predominant serotype circulating in Jakarta was DENV 2. The genotype of serotype 4 circulating in Jakarta in 2010-2011 was type II. Primer design for dengue serotype 4 had a size of 406-692 base pairs, no hairpin developed, dimer formation was within two and Tm difference ranged from 0 to 8^{0}C.

Keywords: *DHF, serotype 4, genotype, primer*

Desain Primer DENV-4 untuk Sekuensing yang akan Digunakan pada Epidemiologi Molekuler Virus Dengue di Jakarta

Abstrak

Penelitian ini bertujuan untuk mengidentifikasi karakteristik pasien demam berdarah dengue di Jakarta dan mengidentifikasi genotipe virus dengue serotype 4 (DENV 4) di Jakarta serta membuat desain primer DENV-4. Penelitian dilakukan secara potong lintang pada 100 pasien dengan hasil pemeriksaan NS-1 positif. Penelitian dilakukan di Departemen Mikrobiologi, Faculty of Medicine, Universitas Indonesia. Pengambilan data dilakukan pada bulan Maret-December 2010, virus sequencing dilakukan pada bulan Januari-Oktober 2011. Sampel dibagi berdasarkan hasil konfirmasi RT-PCR, jenis kelamin, usia dan tempat tinggal. Identifikasi virus dengue dan penentuan serotipe menggunakan RT-PCR. Penentuan genotipe DENV-4 dilakukan dengan melakukan sekuensing whole genome DENV-4 menggunakan software Genetyx. Pembuatan primer DENV-4 dilakukan dengan program Primer Designer. Prevalensi demam berdarah dengue tertinggi terdapat pada usia 14-20 tahun (41,4%) dan pada pasien yang bertempat tinggal di Jakarta Timur. Ratio penderita perempuan dan laki-laki tidak menunjukkan perbedaan bermakna. Genotipe DENV-4 yang bersirkulasi di Jakarta adalah tipe II. Desain primer DENV-4 memiliki ukuran 406-692 pasang basa, tidak ada hairpin, formasi dimer dengan dua dan Tm yang berbeda yang berkisar antara 0-8^{0}C.

Kata Kunci: *DBD, serotipe 4, genotipe, primer*

Introduction

Over the past few decades, dengue infection has become a major public health concern throughout the world. This infection severely affects more than 100 countries in tropical and sub-tropical regions, such as South East Asia countries including Indonesia. Dengue fever (DF) is the initial manifestation of this infection. Even though the disease is self-limiting and benign, it may also progress to the lethal forms known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). There are four types of dengue virus that have been identified; they are DENV-1, DENV-2, DENV-3 and DENV-4. DENV-3 is the main cause of dengue cases in Indonesia, accounted for 37% of total cases in 2004. Other serotypes like DENV-1, DENV-2 and DENV-4 are also present. DENV-4 cause 19% of the case.

Early recognition of dengue infection using recent methods such as dengue NS-1 detection and RT-PCR is essential in order to enhance recovery and downsize the number of mortalities. By recognizing the molecular biologic properties of this virus, the sensitivity and specificity of these detection procedures will increase and more accurate. Besides that, identification of molecular biologic properties of dengue virus can be applied as a base of vaccine development in the future. There are a range of methods to analyze molecular biology of dengue virus such as gene cloning, nucleotide sequencing and gene expression. In the year 1973, there was a study that revealed the genotype of DENV-4 in Indonesia. It shows that DENV-4 of Indonesian strain belonged to genotype 2.[2]

In this study, we would like to recognize the characteristic of dengue patients and to identify the genotype of DENV-4 in Jakarta, in the year 2010. Futhermore, we also design primer for DENV-4.

Method

This cross sectional study involved 100 patients with fever less than two days or 48 hours administered in hospitals in Jakarta with positive result of NS-1. We started collecting the specimen in March-December 2010, while the virus sequencing was perfomed in January-October 2011. We did the research at Department of Microbiology, Faculty of Medicine, Universitas Indonesia.

The viral isolation was from serum of the patient. We extract the viral RNA and continue with RT-PCR examination to detect the dengue viruses and determine the serotipe. The sequence results of DNAs DENV-4 was analyzed with Primer Designer and Genetyx software. We also designed the primer for DENV-4.

Results and Discussion

This study reported the characteristic of dengue patients and the epidemiology in Jakarta 2010, phylogenetic tree of DENV-4 and primer design for this serotype. The highest prevalence of DF was found among patients of 14-20 years old (41,4%) but according to chi-square test, this result was not statistically significant ($\chi^2(4) = 2.642$, $p = 0.619$). The number of DHF patients decreased with age.

Cuban DHF/DSS outbreak in 1981 reported that hospitalized dengue patients were highly dominated by group of age between 8 and 11 years old and the number decreased greatly until nearly the baseline among mid-teens.[3] DHF is the most prevalent among pediatrics, particularly 10 years old and below. The possible explanation is the children generate higher number of cytokines rather than adults. However, children under 14 years old did not participate in this research, as blood sample collection was not feasible. The highest prevalence of DF was found among patients of those who lived in East Jakarta (Table 1). Nevertheless, the precise information about dengue distribution in Jakarta is not available yet, but according to the chi-square test this result was not statistically significant ($\chi^2(2) = 1.532$, $p = 0.509$).

The infection rate of DHF between male and female has been shown to equal. However, immune responses of female are stronger than male;[4] thus, cytokines are produced in greater number. In addition, the capillary bed of female are tend to increase its permeability more than male. In previous study, it shows that the ratio of milder form of DHF was predominated slightly by male. Meanwhile, dengue of grade III, IV and fatal DSS was more prominent in female.[5] However, in accordance with chi-square test our result is not statistically significant (p>0,05). Therefore, there was no significant relationship between male and female patients ($\chi^2(1) = 0.265$, $p = 0.607$).

Tabel 1. Characteristic of Suspected Dengue Patients

Tabel1. Characteristic of Suspected Dengue Patients

Characteristic	Suspected N (%)	Confirmed positive n (%)	Confirmed Negativen (%)
Sex (100)			
Female	49 (49)	35 (71,5)	14 (28,5)
Male	51 (51)	34 (66,7)	17 (33,3)
Age (87)			
14-20	36 (41.4)	28 (77,8)	8 (22,2)
21-30	32 (36.8)	21 (65,6)	11 (34,4)
31-40	10 (11.5)	6 (60)	4 (40)
41-50	7 (8,1)	4 (57,1)	3 (42,9)
51-60	2 (2.3)	1 (50)	1 (50)
Area (78)			
West Jakarta	0 (0)	0 (0)	0 (0)
Central Jakarta	8 (10.2)	7 (87,5)	1 (12,5)
East Jakarta	65 (83.3)	45 (69,2)	20 (30,8)
North Jakarta	5 (6.4)	4 (80)	1 (20)
South Jakarta	0	0 (0)	0 (0)

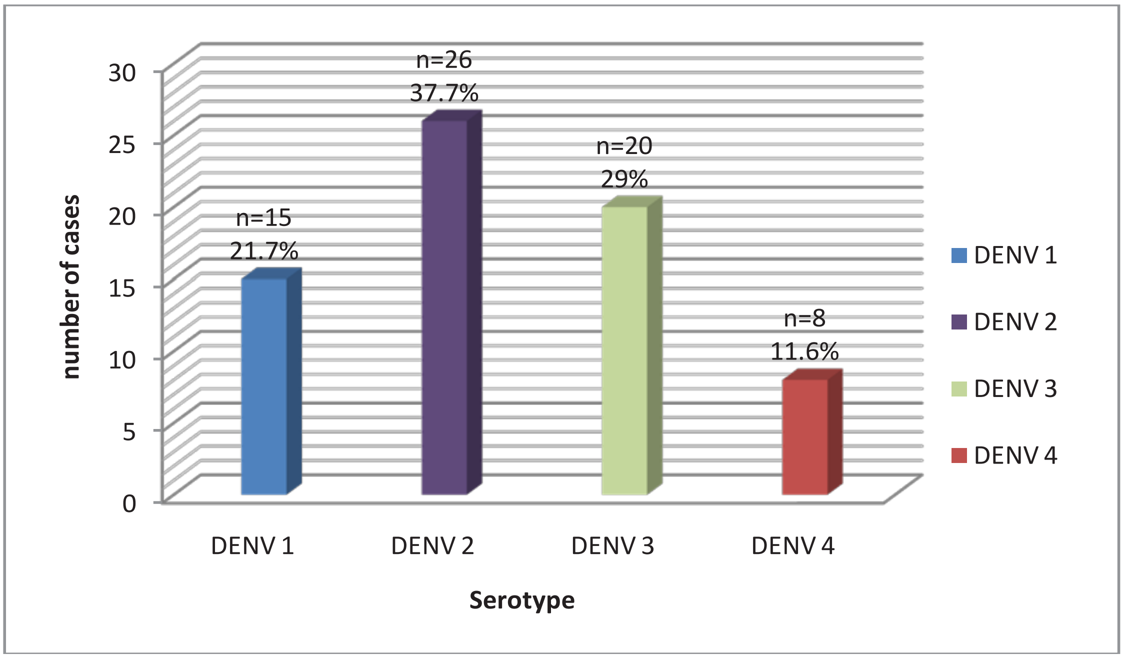

Figure 1. Serotype of Positive Dengue Patients

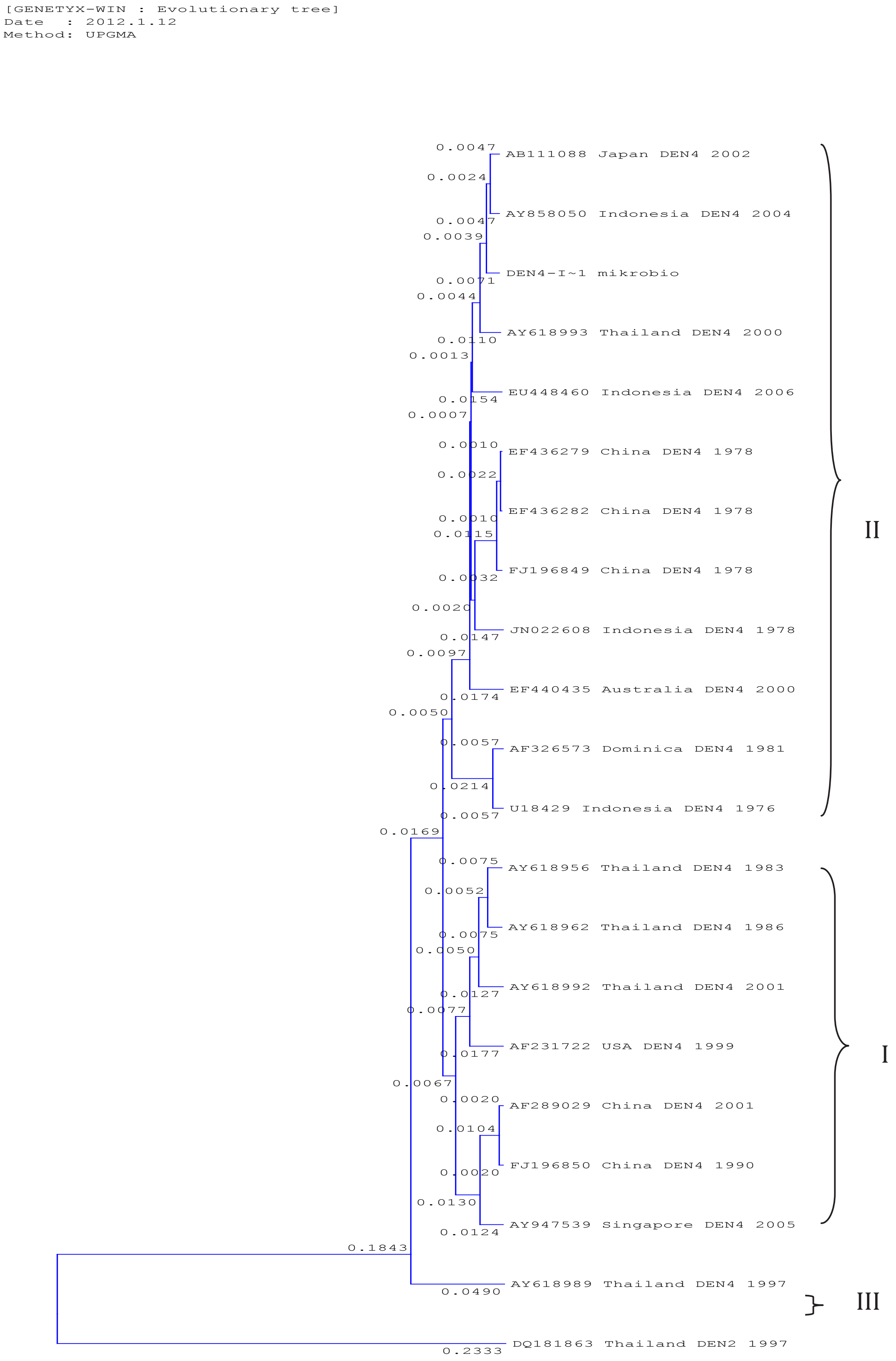

Figure 2. Phylogenetic Tree of DENV 4

The most dominant serotype was DENV 2 (37%). The least serotype was DEN-4 with 8 patients (Figure 1). It shows the same result with the data of 2004 which stated that the predominant circulating serotypes in Indonesia were DENV 3 and DENV 2.[6]

Phylogenetic tree of DENV 4 was carried out by comparing the gene sequence of DENV 4 envelope in this study with the envelopes from gene bank. By using Genetyx software, we found that DENV 4-I, Indonesian strain, was grouped to genotype II. It had the same genotype with Indonesian DENV-4 discovered in 1976, 1978, 2004 and 2006. Primer design is required to determine genotype. In this study we amplified and sequenced Singapore strain DEN4 GQ398256 whole genome provided by Gene bank to design overlapping primer. Then, we analyzed hairpin and dimer formation using PRIMER software. We found that there was no hairpin developed and the dimer formations are between 1 and 2. Tm difference ranged from 0 to 8^0C.

Tabel 2. Primer Used for Whole Genome DENV-4 Sequence Amplification

Nucleotide Position	Sequence 5'-3'	Size (bp)	Tm (0C) Difference	Hair Pin	Dimer	Information
10-29	TCTGTGTGGACCGACAAGGA	460	10C	-	2	Forward
451-470	GGTTCGCCATCTCTTGTTGA					Reverse
351-370	CAGGAAGGAGATAGGTCGCA	576	-	-	2	Forward
908-927	GGCGACTAGCATCATCAGGA					Reverse
766-785	ACATGGATGTCATCGGAAGG	527	40C	-	2	Forward
1274-1293	CTTCGCACATGTCACAACTC					Reverse
1150-1169	GCAACAAGATGTCCAACGCA	561	70C	-	2	Forward
1692-1711	CCTCCTGAGATCCTAGCACT					Reverse
1600-1619	GTAGTAGTCCTTGGATCGCA	499	60C	-	2	Forward
2080-2099	AATGCACTCTCTCCAACACC					Reverse
1985-2004	GGCGTATCATCTCATCTACC	533	-	-	2	Forward
2499-2518	ACTGTTCTGTCCAAGTGTGC					Reverse
2370-2389	GACATGCATAGCTGTTGGAG	618	60C	-	2	Forward
2969-2988	GATTGCCGCCGACATTAATC					Reverse
2720-2739	AAGGCAAGAGAGCACTCACA	642	80C	-	2	Forward
3343-3362	CAGCACCATTGCGTGACCAG					Reverse
3180-3199	ACAGCACAATTACCGTCAGG	527	20C	-	2	Forward
3688-3707	ATGGCTAGGTGAATCTGTCC					Reverse
3513-3532	GCTGCTATGCCTGACCTTGT	626	20C	-	1	Forward
4120-4139	CAAGATCTCCTTGAGGCTCC					Reverse
4092-4111	GCCAGTGTACCTAATGACTC	496	60C	-	2	Forward
4569-4588	CTCCTTCAGACAGTGTGGCT					Reverse

4360-4379	GTGAAGCAGGATGAAGATGG	472	40C		1	Forward
4813-4832	CCTGGTTCTATGGCTAAGAC					Reverse
4768-4787	GGATGGAGGCTCGGAGACAA	647	-	-	2	Forward
5396-5415	TCTAGCCGCGACACTGCAAG					Reverse
5229-5248	ACGTGGATTGCCAATCCGTT	671	30C	-	1	Forward
5881-5900	CCTCTTCTCTGAGCGGCACT					Reverse
5663-5682	AGAGAGTGATCCAGTTGAGC	532	10C	-	2	Forward
6176-6195	GTAAGAGATACCGGCAGAAG					Reverse
5971-5990	GAAGATCATGCTCACTGGAC	477	70C	-	1	Forward
6429-6448	CCAGCTTGGCCTTAGAGGAA					Reverse
6546-6565	GCTTGTAGCTCTACTAGGTG	441	70C	-	2	Forward
6968-6987	TATGGTGTGTCTCAGCATGG					Reverse
6795-7814	GACCATTCTCACCATCATCG	406	80C		1	Forward
7182-7201	TTGCCTGCAATCCTGGACCT					Reverse
7179-7198	AATAGGTCCAGGATTGCAGG	557	70C	-	2	Forward
7717-7736	CCTCTGGACACCGCATGCTT					Reverse
7503-7522	CTACTTGGCAGGAGCTGGAC	569	-	-	1	Forward
8053-8072	GGCTCCACCATCTTCAAGAC					Reverse
7908-7928	CATGGCTACTTATGGCTGGA	692	-	-	2	Forward
8581-8600	GTGTCTGTCATGGCCAACTG					Reverse
8396-8415	GGAGAAGGCTTCAGACATTG	604	-	-	2	Forward
8981-9000	CAGCCACATGTACCAGATTG					Reverse
8827-8846	GCCAGTGAAGCTGTGAATGA	559	10C	-	2	Forward
9367-9386	CCATATGTTCCAACCTGTCC					Reverse
9323-9342	GAGCGGTGATGGACATCATA	470	30C	-	1	Forward
9774-9793	CCTGCGAGATTCTGGCTCTT					Reverse
9600-9619	CCTCTTCTTGAACGACATGG	464	10C	-	2	Forward
10045-10064	CACGAATGGACTGGAGTCTT					Reverse
9950-9969	GGTCAATCCACGCTCATCAC	679	80C	-	1	Forward
10610-10629	AATCCATCTCGCGGCGCTCT					Reverse

Conclusion

The highest prevalence of DF was found among patients of 14-20 years old and those who lived in East Jakarta. The ratio of DF between female and male was nearly the same. The predominant serotypes in Jakarta were DENV-3 and DENV-2. Genotype of DENV-4 circulating in Jakarta was identified as type II. Primer design for dengue serotype 4 was using genome of Singaporean strain. It had size of 406-692 base pairs, no hairpin developed, dimer formation was within two and Tm difference ranged from 0 to 8^0C.

References

1 World Health Organization. Special program for research and training programme in tropical diseases, 2004.

2 Lanciotti RS, Gubler DJ, Trent DW. Molecular evolution and phylogeny of dengue-4 viruses. Journal of General Virology. 1997;78:2279-84.

3 Kouri GP, Guzmán MG, Bravo JR, Triana C. Dengue haemorrhagic fever/dengue shock syndrome: lessons from the Cuban epidemic, 1981. Bull World Health Organ. 1989;67(4):375-80.

4 Halstead SB. Immunological parameters of togavirus disease syndromes. In: Schlesinger RW (ed). Togaviruses: biology, structure, replication. New York: Academic Press; 1980. p.107-73.

5 Halstead SB, Nimmannitya S, Cohen SN. Observations related to pathogenesis of dengue hemorrhagic fever. J Yale Biology and Medicine. 1970;311-28.

6 Departemen Kesehatan RI. Kasus demam berdarah dengue di Indonesia 2011. [cited on July 2011] Available from URL:http://www.depkes.go.id/index.php/berita/info-umum-kesehatan/772-kasus-demam-berdarah-dengue-di-indonesia.html.