Determination of Genotype of Dengue Virus Serotype 1 by Using Primer Design

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Abstract

Dengue fever has become a worldwide health problem. This disease occurs more and more frequently and often cause death, especially in some Asian countries including Indonesia. The purpose of this study was to determine the genotype of dengue virus serotype 1 in Indonesia by using primer design as a base to take part in the development of diagnostics and vaccines of the dengue virus. This research consisted of 100 respondents; male and female aged 14-60 years. All samples were selected by consecutive sampling and dengue viruses used in this study were randomly selected in March-December 2010. The next step was sequencing process in January-October 2011 in the Department of Microbiology FKUI by using cross sectional design. The result of this study was dengue virus serotype 1 strains from Indonesia belonged to genotype 4.
Keywords: *genotype of dengue virus serotype 1, Indonesia, diagnostic, vaccine*

Penentuan Genotipe Virus Dengue Serotipe 1 Menggunakan Desain Primer

Abstrak

Saat ini, demam berdarah sudah menjadi masalah kesehatan di seluruh dunia. Penyakit tersebut makin sering terjadi bahkan seringkali menyebabkan kematian khususnya di beberapa negara Asia termasuk Indonesia. Tujuan penelitian adalah untuk mengetahui genotipe virus dengue serotype 1 di Indonesia menggunakan desain primer sebagai dasar untuk turut ambil bagian dalam pengembangan diagnostik dan vaksin dari virus dengue. Riset ini terdiri atas 100 responden; laki-laki dan perempuan berusia 14-60 tahun. Sampel dipilih secara konsekutif dan virus dengue yang digunakan dalam riset ini dipilih secara acak pada bulan Maret-Desember 2010 dilanjutkan dengan proses sequencing pada bulan Januari-Oktober 2011 di Departemen Mikrobiologi FKUI dengan desain cross sectional. Hasil penelitian ini adalah virus dengue serotype 1 strain Indonesia termasuk genotype 4.
Kata kunci: *genotipe virus dengue serotipe 1, Indonesia, diagnostik, vaksin.*

Background

Dengue is an arboviral infection, with 100 million cases every year in the tropical areas of the world. Dengue is a primer disease in tropic areas; the cycle of the virus involves human and *Aedes aegypti*, a domestic, day-biting mosquito that prefers to feed on humans. Dengue virus may cause an illness that ranges from nonspecific viral syndrome to severe and fatal hemorrhagic disease that can cause death called dengue hemorrhagic fever (DHF).

DF/DHF is caused by the dengue viruses, which belong to the genus Flavivirus, family Flaviviridae.[1] There are four antigenically related but distinct dengue virus serotypes (DENV-1, DENV-2, DENV-3 and DENV-4), all of which causes DF/DHF.[2] These serotypes can be distinguished by serological method.

The incidence of DF has elevated considerably worldwide in the last few decades; 2.5 billion people are currently having risk to be infected by dengue virus. This disease is now endemic in more than 100 countries in many areas including Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. The most seriously affected areas are South-east Asia and the Western Pacific.[1] Based on data collected from the Indonesian Ministry of Health from January to April 2004, there were a total of 58,301 cases of DF and DHF, with 658 deaths occured. These patients were mainly from Jakarta, Bali and East Nusa Tenggara. DENV-3 is categorized as the primary circulating virus serotype (37%), with DENV-4 (19%), DENV-2 and DENV-1 are also present.[3]

Gene cloning, nucleotide sequencing, gene expression and other advanced technology have played a major role in finding more information about molecular biology of dengue virus. In 2008, a study found that several serotypes of dengue virus isolated from Indonesia had different genotypes including DENV-1. Klungthong *et al*[4] stated that DENV-1 from which was isolated in 1988, belonged to genotype II.

In vaccine strategy, there are genotype variations within each serotype. Distinct genotypes have been identified that may affect to strategy of diagnostic and vaccine development. In diagnostic and vaccine development, information about molecular of dengue virus is highly needed. Therefore, in this research, the molecular epidemiological of DENV-1 from dengue infected patients in Jakarta 2010 will be identified in order to compare the current data with previous data collected in the previous years as a base of diagnostic and vaccine development in the future.

Methods

The study design for this research is cross sectional. Dengue patients who participated in this study were collected consecutively. All dengue virus collected from participants were selected randomly. The data used in this study was adopted from secondary data collected from previous research.

Specimen collection starts from March 2010 to December 2011, followed by sequencing process from January 2011 to October 2011 at Department of Microbiology Faculty of Medicine Universitas Indonesia.

Populations for this research were patients with fever less than 48 hours in West and East Jakarta. Inclusion criteria includes adult (>14 years old) with fever measured by rectal temperature of > 38^{o} that occurs less than 48 hours. In addition, patients must fulfill the WHO criteria for dengue fever and agree to the informed consent. Exclusion criteria includes patients with mixed infection, pregnant women, and patients with other comorbidities

All patients who had sign informed consent for participating in the research study had their blood serum checked to find the presence of NS1 antigen of dengue virus. Patients who got negative result were not hospitalized and patients with positive result were admitted to hospital and examined by RT-PCR. When RT-PCR results were positive, RNA virus was isolated from the plasma, and cDNA was produced and the DNA was amplified and sequenced. Finally, the sequence results were analyzed using software called Genetyx and Primer Designer.™ The determination of dengue virus genotype 3 is using RT-PCR. Data were processed using Genetyx version 5.1. We also use software called Primers to create primers for sequencing.

Table 1. Characteristic of Dengue Patients

Characteristic	Suspected Patients		Confirmed Positive		Confirmed Negative	
	Total	%	n	%	n	%
Sex (100)						
Female	49	49	35	71.43	14	28.57
Male	51	51	34	66.67	17	33.33
Total	100		69		31	
Age (87)						
14-20	36	41.38	28	77.78	8	22.22
21-30	32	36.78	21	65.63	11	34.38
31-40	10	11.49	6	60	4	40
41-50	7	8.05	4	57.14	3	42.86
51-60	2	2.3	1	50	1	50
Total	87		60		27	
Total Area (78)						
West Jakarta	0	0	0	0	0	0
Central Jakarta	8	10.26	7	87.5	1	12.5
East Jakarta	65	83.33	45	69.23	20	30.77
North Jakarta	5	6.41	4	80	1	20
South Jakarta	0	0	0	0	0	0
Total	78		56		22	

Results and Discussion

In last few decades, the incidence of DF ascended considerably. DHF or dengue shock syndrome (DSS) is a growing global health problem. The genetic of dengue virus varies in every area. Thus, we need to find genetic variation of dengue virus from all serotypes in Jakarta in 2010 in order to support the development of diagnostic modalities and vaccines.

This research provides 100 suspected dengue patients. It is clearly seen that both male and female have similar percentages of suspected dengue cases, with slightly higher number in females. RT PCR further confirms these and the results were similar. There is a careful study showed that males predominate slightly among those who experience milder disease, however, in severe cases, females are more predominant.[5] This study suggested that there is evidence that females have stronger immune response than males[6] leading to a greater cytokines formation. Furthermore, the permeability of capillary bed of females is more improved than that of male. Chi-square test were performed, the data did not reach significant result. Therefore, there is no relationship between gender and dengue infection ($p = 0.607$).

Suspected dengue patients are also divided into five age groups. The highest numbers of suspected and tested positive dengue patients were recorded in the younger age group (14-20 years old). In the Cuban DHF/DSS outbreak of 1981, hospitalized patients peaked at age 8 to 11 years and decreases dramatically to the near baseline among mid-teens.[7] Based on this fact, DHF is restricted in most instances to the pediatric age group, mostly children of 10 years and younger. The possible explanation is that the capillaries of children are more prone to cytokine-mediated increased permeability than are those of adults. However, this study did not include children of 10 years and younger because it requires a great

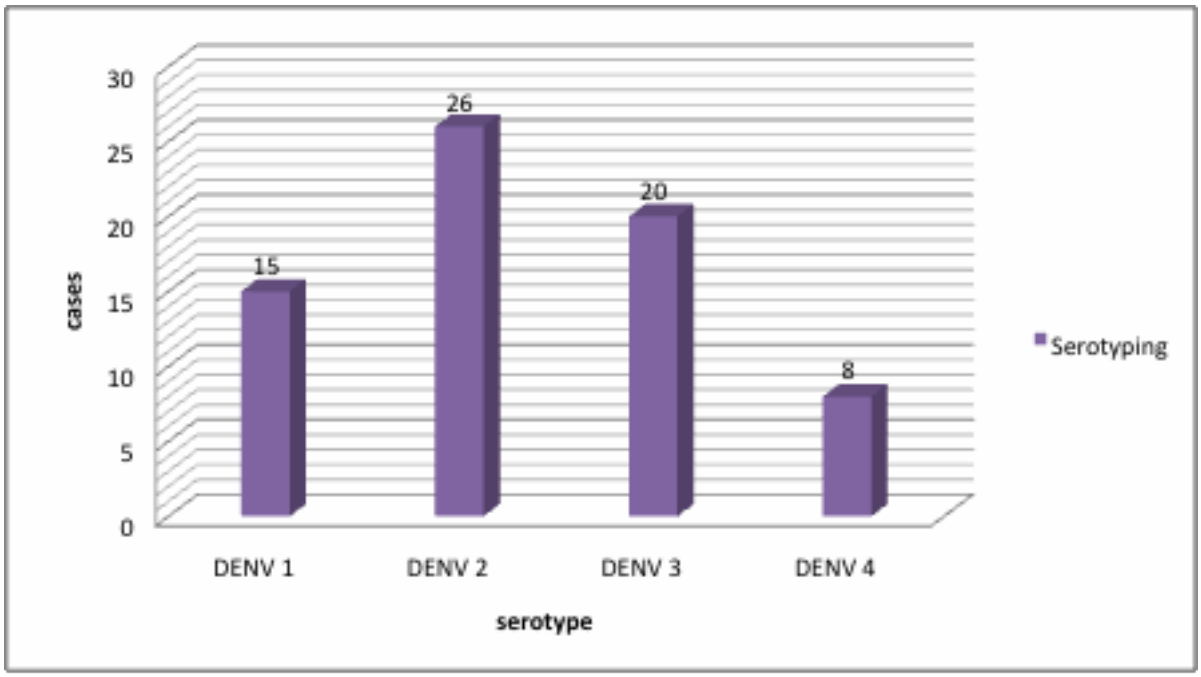

Figure 1. Serotype of Dengue Patients

amount of blood to be tested. However, according to chi-square test in data analysis, this result is not significant ($p>0.05$).

In Jakarta, there are a total of 65 suspected dengue cases, with a significantly high number of 45 cases in East Jakarta. Meanwhile, Central and North Jakarta showed the occurrence of 7 and 4 dengue cases, West Jakarta only record 1 case and South Jakarta was the only area which showed an absence of dengue case. However, there is no specific data about the spread of dengue cases in the 5 areas for the present.

Based on figure 1, the most common serotype of dengue cases in Jakarta in 2010 was DEN-2 with 37% of the population, followed by DEN-3. This result is identical with the previous data collected by the Ministry of Health. According to that data, DEN 3 and DEN 2 were the main circulating virus serotype in Indonesia in 2004.[4]

Figure 2 shows the position of gene sequences from several countries. In this study, several DENV 1 envelopes have been compared with other envelopes acquired from gene bank. After being analyzed using Genetyx software, it was found

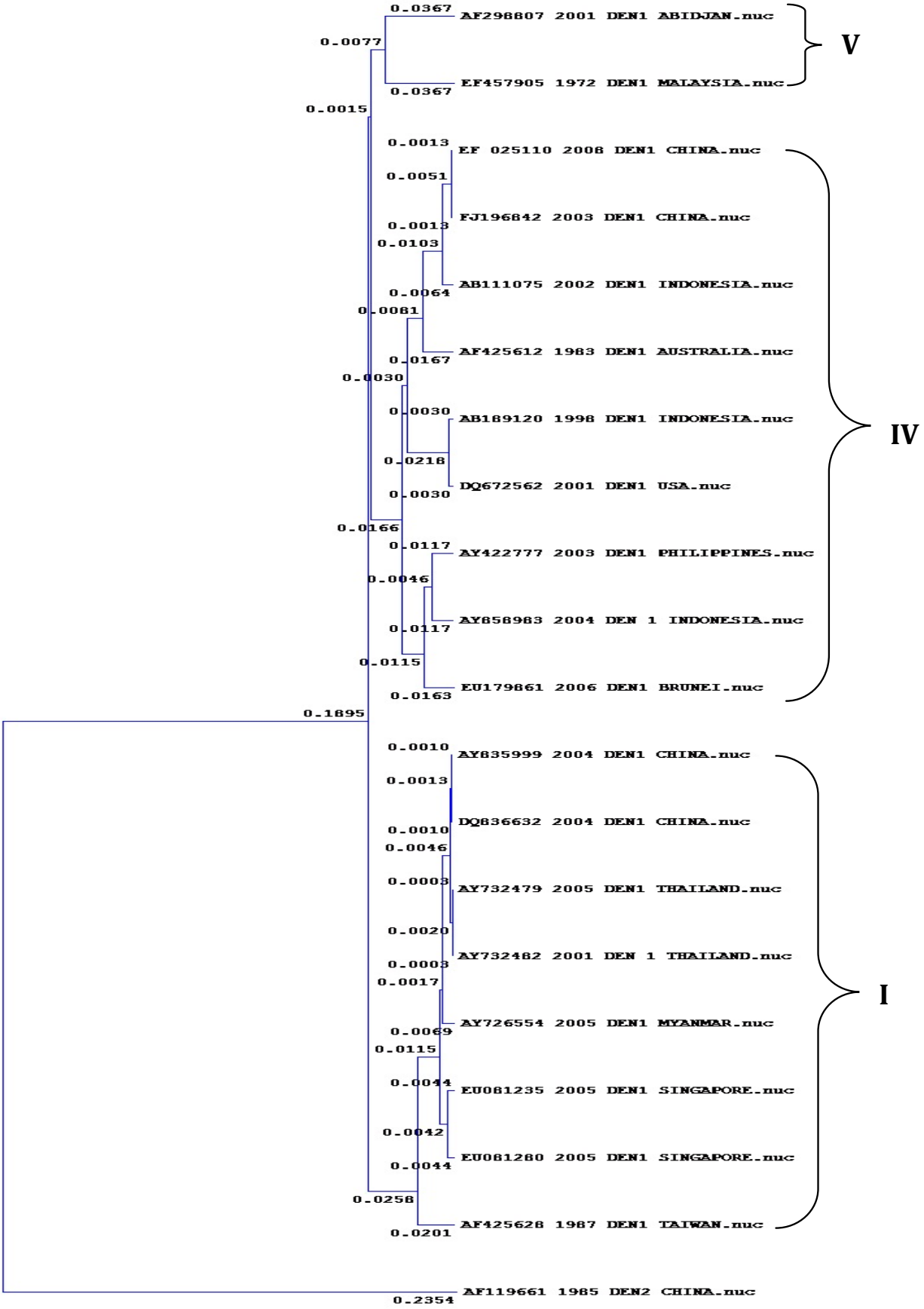

Figure 2. Phylogenetic Tree

that all Indonesian strains were categorized as genotype IV. This result was not identical with the previous data isolated in 1988, which showed that DENV 1 belonged to genotype IV.

In order to determine genotypes, primers should be designed. The design used whole genome data of DEN 1 which is provided in gene bank. To amplify and sequence whole genome of DEN 1, overlapping primers have been designed with PCR products from 457bp-678bp. Hair pin and dimer formation were also analyzed using primer software. It was found that all of the primer pair did not develop hair pin and Tm difference ranged from 0°-6°C the formation of the dimer was 1 to 2.

Table 2. Primer Design of DENV-1

Nucleotide Position	Sequence 5' – 3'	Size (bp)	Tm (°C) Diff	HairPin	Dimer	Information
10 – 29	TCTACGTGGACCGACAAGAA	526	3	-	2	Forward
517 – 536	ACATGTTGACACCTGCAGAG					Reverse
423 – 442	CCACAGTCCTGACGTTCCAT	499	1	-	2	Forward
905 – 924	GGAGTCACCAGCATCAGCAA					Reverse
827 – 846	CCAGGATTCACGGTGATAGC	538	1	-	2	Forward
1346 – 1365	TGGACGGTAACGATCACAGA					Reverse
1214 – 1233	CGAACATTCGTGGACAGAGG	623	2	-	2	Forward
1818 – 1837	GTGCACAGGCTCATTCAAGT					Reverse
1670 – 1689	AAGAAGCAGGAAGCAGTCGT	544	6	-	2	Forward
2195 – 2214	CCTCCTATAGAACCGAAGTC					Reverse
2029 – 2048	CAACATTGAAGCGGAGCCAC	568	6	-	2	Forward
2578 – 2597	CTGATCGAATTCCACACACC					Reverse
2365 – 2384	GAAGTTCACACCTGGACAGA	601	4	-	2	Forward
2947 – 2966	GTCGATGGTCACACACTTGG					Reverse
2809 – 2828	CACTACCTTCATCATCGACG	512	2	-	1	Forward
3302 – 3321	GTGGTTCTAAGAGATGGTCC					Reverse
3218 – 3237	CCGTGGCACTTAGGCAAGTT	593	1	-	2	Forward
3792 – 3811	TGCCACCAGACTCAATCCAA					Reverse
3634 – 3653	GATCAGGCTATCGATCATGG	678	5	-	2	Forward
4293 – 4312	CCAGGAGACCTTAGCTGCTT					Reverse
4013 – 4032	TTATGCCTGTCCACGACTTC	472	1	-	2	Forward
4466 – 4485	AAGAGAGTCGCCGGTATTGA					Reverse
4404 – 4423	ATGACACGATCACCATCCTC	489	1	-	2	Forward
4874 – 4999	GGGTTCCACTTGTTGTCACCA					Reverse

4841 – 4860	AATGTACAGACAGCGCCAGG	526	2	-	2	Forward
5296 – 5315	AAGTGGCGTGACACATAAGG					Reverse
5244 – 5263	GGTATCAGACAACAGCAGTG	667	5	-	2	Forward
5892 – 5911	CCTTCCAATTCTTCCTCTCC					Reverse
5826 – 5845	AAGATGGTCCAGAGCGTGTC	500	2	-	2	Forward
6307 – 6326	AGTATGTTCTGGCATCCAGC					Reverse
6140 – 6159	GGAGATCTACCTGTCTGGCT	510	3	-	2	Forward
6631 – 6650	TTGAGGACATCACGCAGAGT					Reverse
6458 – 6477	CTGGTTATGCTGCACAACTC	484	2	-	2	Forward
6923 – 6942	CAGGCAGAACGTGGATGTAG					Reverse
6811 – 6830	ATTGACAGTTGCAGCCAATG	616	0	-	2	Forward
7408 – 7427	GTCCTGTGGCCAGTGTGATG					Reverse
7234 – 7253	AAGGACGGCAGCCGGAATAA	494	5	-	1	Forward
7709 – 7728	AACACGCAGTGTCGAGAGGA					Reverse
7681 – 7700	GGATAGATCTGAAGCCAAGG	462	1	-	2	Forward
8124 – 8143	TACCACACTCGGCATATAGG					Reverse
8075 – 8094	GATATTGGTGAGTCCTCTCC	459	4	-	2	Forward
8515 – 8534	TGACCATGGATGAGGCTGAT					Reverse
8400 – 8419	TTGGCCAGAGGATAGAGAAC	526	2	-	2	Forward
8807 – 8926	GTAGACGCATGTGGCACATT					Reverse
8866 – 8885	GGACCTTGTGCGCAGAGAGA	480	4	-	2	Forward
9327 – 9346	GACATCCATCACGGTTCCAT					Reverse
9229 - 9248	CACTGACATCATGGAACCTG	487	5	-	2	Forward
9697 – 9716	GCTGGTGGAAGTGGTGTGAG					Reverse
9592 – 9611	TGCAACAGCCTTAACAGCCT	601	3	-	2	Forward
10174 – 10193	CAATGAGCCTTCTCACTTGG					Reverse
9941 – 9960	AGTCGCACCACCTGGTCGAT	511	0	-	2	Forward
10433 – 10452	CCACGATGGAGCTACAGGCA					Reverse

Conclusion

The most prevalent dengue viruses in Jakarta during 2010 are DENV-2 and DENV-3. After being analyzed using Genetyx software, it was found that all Indonesian strain belongs to genotype IV.

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