

## Secondary infection and Den-3 serotype most common among dengue patients: a preliminary study

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### Abstrak

**Latar belakang:** Faktor risiko keparahan penyakit DBD meliputi virulensi virus, genetik, jenis infeksi, usia, status gizi, dan riwayat penyakit penyerta. Tujuan penelitian ini untuk mengetahui beberapa karakteristik yang dominan penderita infeksi virus dengue berdasarkan derajat keparahannya.

**Metode:** Penelitian ini merupakan penelitian observasional dengan rancangan cross sectional. Pengumpulan data dilakukan dengan pengambilan data derajat keparahan infeksi virus dengue, jenis infeksi dan penentuan serotipe virusnya. Pengumpulan data dilakukan terhadap 46 sampel yang dipilih dengan purposive sampling. Data derajat keparahan infeksi virus dengue diperoleh dari data rekam medik penderita, data jenis infeksi berdasarkan pemeriksaan rapid diagnostic test hasil penelitian Loka Litbang P2B2 Ciamis, dan pemeriksaan sampel serum darah menggunakan uji rt-pcr dilakukan untuk menentukan serotipe penderita virus dengue. Data disajikan secara deskriptif antara dua variabel.

**Hasil:** Karakteristik dominan penderita infeksi virus dengue berdasarkan derajat keparahannya adalah infeksi sekunder dari 46 penderita mendominasi sebanyak 37 orang (80,4%) dan 2 orang (5,5%) merupakan kategori berat. Penderita dengan serotipe Den-3 mendominasi sebanyak 26 orang (56,5%), dan 3 orang (11,52%) termasuk kategori berat.

**Kesimpulan:** Karakteristik dominan penderita infeksi virus dengue di lima rumah sakit Propinsi Jawa Barat berdasarkan derajat keparahannya didominasi oleh penderita dengan jenis infeksi sekunder dan serotipe Den-3. (*Health Science Indones 2010; 1: 14 - 19*)

**Kata kunci:** derajat keparahan infeksi virus dengue, jenis infeksi, serotipe.

### Abstract

**Background:** Severity of dengue infection comprises virus virulence, genetics, infection types, age, nutritional status, and history of co-existing diseases. The study aimed to identify the most common characteristics of dengue patient according to the severity of infection in West Java province.

**Methods:** This cross sectional design data was obtained from 46 samples taken with purposive sampling technique. Data of dengue virus infection severity were obtained from medical records of patients; type of infection was determined by rapid diagnostic test examination at Loka Litbang P2B2 Ciamis; and examination of blood serum sample used reverse transcriptase polymerase chain reaction (RT-PCR) to determine serotype of dengue virus patients. Data were analyzed by cross tabulation to describe distribution frequency between two variables.

**Results:** Most common characteristics of severe dengue patients were women secondary infection scores 37 (80.4%) from 46 dengue patients and 2 (5.5%) patients categorized as severe dengue. Based on serotype, Den-3 serotype dominated scores 26 (56.5%) and 3 people (11.52%) include severe dengue.

**Conclusion:** The most common characteristics of dengue patients at five hospitals in West Java province based on severity of dengue virus infection was dominated by secondary infection and Den-3 serotype. (*Health Science Indones 2010; 1: 14 - 19*)

**Key words:** severity dengue virus infection, type of infection, serotype.

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Dengue fever (DF) and dengue hemorrhagic fever (DHF) are grouped as re-emerging disease endemic mainly in tropical zone. They are caused by four serotypes of dengue virus (DEN1-4) that belong to *Flavivirus* genus and carried by *Aedes aegypti* mosquito.

Based on the *Ditjen Pengendalian Penyakit dan Penyehatan Lingkungan* reports (PP & PL), DHF cases fluctuates. In 1988 there were more than 40.000 cases, in 1998 there were 72133 cases, and in 2004 were 69017 cases. Incidence rate in 2008 reached 42,97/100.000 citizens, meanwhile Case Fatality Rate (CFR) in 1987 reached 4.6% then fluctuated between 2–3% in 1991–1999, less than 2% in 2000, the lowest in 2004 reached 1.12 % and 0.99% in 2008.<sup>1</sup>

There were 8923 dengue patients in West Java in 2003 and doubled to 17,448 in 2005 with 266 deaths. The first quarter of 2006, dengue patients were 11,175 people with 126 deaths (CFR 10.72%). Until 2007, all counties and municipalities in West Java Province had reported dengue outbreak.<sup>2</sup>

The results of serological survey held by Loka Litbang P2B2 Ciamis (2008) from 30 hospitals in 26 counties and municipalities in West Java Province showed 418 cases showed positive IgG and IgM. Based on screening, the majority came from Dr. Syamsudin Public Hospital in Sukabumi District, Gunung Djati Public Hospital in Cirebon Municipality, Karya Bhakti Public Hospital in Bogor municipality, Cibinong Public Hospital in Bogor District, and Bayu Asih Public Hospital in Purwakarta District.<sup>3</sup>

Risk factors for acute dengue virus are virulence, genetics, type of infection, age, nutritional status, and the history of co-existing diseases. The first dengue virus attack, the body will make specific immune response to dengue although not completely. This makes it possible for an individual to encounter a second infection. Because there are more than one virus dengue types, individual can be re-infected. With re-infection, the immune response will be more severe showing more violence symptom.<sup>4</sup>

This study aimed to describe the dominant characteristics of dengue patient according to the severity of disease.

## METHODS

A cross sectional study was carried out on dengue patients in five Public Hospital in West Java Province, namely Dr. Syamsudin Public Hospital in Sukabumi District, Gunung Djati Public Hospital in Cirebon Municipality, Karya Bhakti Public Hospital in Bogor municipality, Cibinong Public Hospital in Bogor District, and Bayu Asih Public Hospital in Purwakarta District, from December 2009 to May 2010. There were 116 cases with positive serology using rapid diagnostic test at the five hospitals. Data were obtained from 46 samples taken with purposive sampling technique that fulfill the inclusion criteria i.e. positive serology test, complete medical record, and positive RT-PCR test. Serology for IgG IgM was examined by using test on blood samples by rapid diagnostic test (RDT).

Laboratory confirmation through Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test was done to determine viral serotype. Viral RNA was extracted from 46 serum samples using Qiamp Viral RNA mini kit.® A two step nested reverse transcriptase (RT-PCR) was carried out on all first serum samples. In brief, cDNA copies of a portion of the viral genome were synthesized and amplified using two consensus primers designed to anneal to the four dengue virus serotypes. A second round of amplification was carried out using dengue serotype-specific primers, and the amplification products produced DNA with molecular sizes specific to each of the four dengue virus serotypes,<sup>5</sup> PCR products were resolved by 2 % (wt/vol) agarose gel electrophoresis and stained with SYBER SAFE. Negative and positive controls were included in all steps. Determination of disease severity were done according to medical records and classified according to WHO criteria based on medical data record diagnosis in each hospital.

Data was analyzed descriptively, cross tabulation presented between variable sex, age, type of infection, and serotype to the severity of dengue virus infection.

## RESULTS

There were 46 subjects categorized by age group, sex, type of infection and serotype virus based on severity of dengue virus infection.

Table 1. Several patient characteristics and severity of dengue infection

	Severity of dengue infection					
	DF		DHF I		DHF II	
	n	%	n	%	N	%
Age group						
0-9	1	4.0	1	5.9	1	25
10-19	13	52.0	2	11.8	2	50
20-29	4	16.0	6	35.3	1	25
30-39	6	24.0	5	29.4	0,0	0,0
40-49	1	4.0	1	5.9	0,0	0,0
50 or more	0	0.0	2	11.8	0,0	0,0
Gender						
Male	11	44.0	7	41.2	1	25
Female	14	56.0	10	58.8	3	75
Type of infection						
Primary	4	16.0	3	17.6	2	50.0
Secondary	21	84.0	14	82.4	2	50.0
Serotype						
Den-1	0	0.0	3	17.8	0	0.0
Den-2	6	24.0	7	41.2	1	25.0
Den-3	18	72.0	5	29.4	3	75.0
Den-4	1	4.0	1	5.8	0	0.0
Den-3,4	0	0.0	1	5.8	0	0.0

Based on age from all category severe dengue virus infection (DF, DHF I and DHF II) dominated by age group 10 – 19 years there were 17 patients (37.0%). DHF grade I most of patient on the age of 20 – 29 years scores 6 people (35.3%), differ from DF and DHF II the age categories are 10 – 19 years, 13 patients (52.0%) and 2 patients (50%). Depend on all

categorical severe dengue women were dominated than men that are from 46 samples are 27 (56.7%). Based on severity of dengue infection, women also dominated scores 10 patient (58.8%) DHF I and found 3 patients (75%) DHF II.

Table 1 also shown from 46 patient's dengue most common found as secondary infection scores 37 people (80.4%), nevertheless 9 people (19.6%) got primary infection. It is also found the same according to severe dengue, secondary infections are dominated on each category that is DF 21 patients (84%), 14 patients (82.2%) for DHF I, and 2 patients (50%) DHF II.

Den-3 serotype was dominated according to severity of dengue virus infection. Patient's DHF grade II scores 3 (75.0%), 5 (29.4%) DHF grade I and 18 (72.0%) for DF. There is one patient with double infection by serotype Den-3 and Den-4 whose include to category DHF grade I.

## DISCUSSION

The results show us based on age categories the highest percentage infected by dengue virus infection is 10 – 19 years. This result is similar to that found in Thailand that the highest patients from dengue virus infection happened to 10 – 14 years.<sup>6</sup> Patient's dengue virus infection dominated by differential age group 20–29 years, this was related with research in Nicaragua that internal bloody symptom generally happened to mature age group for 15.2 %.<sup>7</sup> Since 1968 to 1973, 95% DHF cases on children age group have been happen under the age of 15 in Indonesia. Although DHF cases had been happened at the age of 5–14 since 1993 - 1998, there was tendentiousness that the ages more than fifteen are increased.<sup>4</sup> The similar case also happened in Singapore that the average of age clustering dengue virus-infected been happened to the age of 14 in 1973 and increased in the age of 28 in 1994. This case improve that adults who infected by one strain are not immune the other strain dengue infection.<sup>8</sup>

According to sex categorical, between women and men are not different so far. Women found 58.7% meanwhile 41.3% for men. These findings were similarly with the research result at *Chon Buri Regional Hospital- Bangkok* in 1995 – 1998 i.e. 55% patients are women.<sup>9</sup> Besides that, research based on population in Mexico which compare dengue virus infection

rate according on sex, there was outcome that women are prone toward this virus.<sup>10</sup> Severity dengue virus infection from research result has shown that grade I and II DHF dominated by women. The same finding on research happened in Brazil where women more risky infected by dengue virus compared with men (RR = 1.53; OR = 3.2).<sup>11</sup> Men who infected by dengue virus lower intensive than women, it's related with research result that women mainly at the age 4 infected by DSS and fatal cases.<sup>12</sup>

The fact related secondary infection with infection degree still controversial because secondary infection cannot make clear automatically toward DHF etiology. Not all secondary infection caused severe dengue and on the contrary primary infection perhaps will cause it, for instant the case where primary infection could affect complicated is DSS,<sup>13</sup> the same result found in Sri Lanka there were bloody manifestation toward patient positive IgM.<sup>14</sup>

The severity of the disease is also affected by virus virulence, the virulence theoretical based on serotype differentiation which can be found at fatal cases. The virulence theoretical explained that the severe dengue cases happened because of the first dengue virus infection with violence virus. This theory appeared because not all secondary infection became severe dengue infection.<sup>15</sup>

The result showed serotype DEN-3 was dominated at each grade severity of dengue virus infection. This outcome on research related with epidemic dengue in Bangladesh that the result shown us DEN-3 was dominant. DEN-1 related with light dengue in Indonesia, meanwhile DEN-3 and DEN-2 were responsible with severe dengue cases. The difference of clinical manifestation is caused by different serotype occurred in Brazil, the result shown us that individual who infected by DEN-3 indicated with severe symptom than DEN-1 or DEN-2.<sup>16</sup>

In this research shown us that DEN-3 has dominate at each grade severity of dengue virus infection compared with another serotype, the result can be cleared based on phylogenetic and epidemiology study that pointed out toward

certain genotype virus has a capability to show epidemic DHF, <sup>17</sup> DEN-3 serotype has four different subtypes based on geographical differences. <sup>18</sup> Based on genetic analyzed, shown that strain DEN-3 potential to make the new genotype (genotype V). The genetic evolution from dengue virus in each serotype may obtain the generation of more virulence dengue virus or strains of dengue epidemic. <sup>19</sup>

The other factors that can be cleared so virus virulence also determined by sensitivity level from host itself that is the capability to improve dengue antibody in neutralizing virus with high virulence. There is a distinction in sequence of nucleic acid DNA, both interval and inner subtype that it makes a distinctive virulence virus and its potential epidemic. The transformation from amino acid system in E protein, virus can change sensitivity to hospes cell and implicate to virus replication and light or serious disease level. <sup>20</sup> Glycoprotein E contains in envelope as a part of the most antigenic virus. The biological capability from E protein makes effect to spectacular immune response and stimulates several antibodies. This protein is very significant because it can make an adherence of virus against cell target to grow the virus, make membrane fusion easier. <sup>21</sup> A macrophage that infected by dengue virus will release some kinds of mediator i.e. interferon IL-1, IL-6, IL-12, TNF and so on. Mediator and endotoxin are responsible against shock septic occurred, fever and improvement of capillary permeability. <sup>22</sup>

In conclusion, characteristics of dengue patients at five hospitals in West Java province based on severity of dengue virus infection were dominated by secondary infection and Den-3 serotype. The research results did not show the real condition because the researcher did not gain patient with DHF grade III and IV, so it's needed to do continuing research especially against the link of secondary infection with severity of dengue virus infection.

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