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Research Report

Serotype and Clinical Performance of Dengue Virus Infection on the Year 2009

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ABSTRACT

Dengue hemorrhagic fever is one of the important health problem in Indonesia, mortality rate is becoming decrease but many dengue shock syndrome cases is very difficult to be help. Previous study showed that some of DEN 2 and DEN 3 virus cases could show a clinical performance of severe dengue virus infection such as dengue shock syndrome. There are four serotype of dengue virus infection can cause primary and secondary infection. The aim of this research is to know the relationship between clinical performance of dengue virus infection and serotype dengue virus and also to know the role of primary and secondary infection and age of dengue virus cases. A prospective analytic observational study, which was conducted in Dr. Soetomo hospital since January 2009. RT-PCR was used to attempt to identify the infecting serotype from dengue virus isolated using vero cell. Antibody responses were measured by ELISA and clinical manifestation were measured with the WHO criteria 1997. Dengue serotype identification by RT-PCR was 70 patients. Virus types were DEN-2 65(92.8%), DEN-1 3(4.2%), and DEN-3 2(2.8%). Patients with DEN-1 genotype IV were more trend severe disease DSS and unusual infection. Commanly usually secondary exposure cause more severe clinical manifestation than primary exposure (p = 0.035) but in this study found that all of DEN-1 genotype IV, primary or secondary infection to show severe clinical manifestation of dengue virus infection. We can conclude that DEN-2 was the most dominant serotype in Dr. Soetomo Hospital. On Primary and secondary infection, DEN-1 genotype IV showing more severe than DEN-2 and DEN-3.

Key words: *Serotype, Clinical Performance, Dengue Virus Infection*

INTRODUCTION

Dengue hemorrhagic fever is one of the important health problem in Indonesia, although the mortality rate has been decreased but many dengue shock syndrome cases is very difficult to be solving handled. Natural course of dengue virus infection is very difficult to predict of the earlier time of severity occur; It is may be due to the new variant of dengue virus that infect a child could be severe and can not be identified earlier.

Previous study show that some of DEN 2 and DEN 3 virus cases could show a clinical performance of severe dengue virus infection such as dengue shock syndrome.

Based on Halstead hypothesis, the severe dengue virus infection could be correlated with secondary infection. The infant cases show a severe clinical manifestation.

Thailand and Cuba, many cases of dengue virus infection were identified as secondary infection and some of them showed dengue shock syndrome, but this case did not finded in other countries. Moren (1980) found that the differences of growing dengue virus in monocyte could be a predictor of severity or mild cases for dengue virus infection.

There are four serotype of dengue virus infection which can cause primary and secondary infection. Clinical performance of secondary infection to show more severe clinical manifestation than primary infection of dengue virus. The role of serotype and age of cases will influence the severe clinical manifestation of dengue virus infection.

Based on of the clinical and bio medical problem that mention above the researcher want to identify serotype dengue virus infection that circulating in Surabaya; to know the relationship between clinical performance of dengue virus infection and serotype dengue virus and also to know the role of primary and secondary infection and

age patient/age virus of dengue virus cases. The study had been done in Dr. Soetomo Hospital on 2009.

MATERIAL & METHOD

Prospective and analytic observational study had been done in Dr. Soetomo Hospital and the ethical clearance was conduct on January 01, 2009. The population of this research is all cases of dengue virus infection that become in patient at Tropical ward of children, diagnosis were done based on WHO 1997. Cases of dengue virus infection were collected & involving in research based on inform concern. All of these cases were examined for IgM & IgG anti dengue virus and then followed by PCR examination to identify dengue virus serotype.

Blood examination should be done everyday. X-Ray examination were also done base on clinical performance of Pleural Effusion & Ascites. Data of all cases dengue virus infection should be analyze using method of Kruskal Walles & Mann Whitney and Regression Logistic multivariet.

RESULT & ANALYSIS

150 cases of primary and secondary of dengue virus infection were studied. Dengue virus was isolated from vero cell and 120 samples have positive CPE. 70 samples were found as serotype by doing RT-PCR examination.

Table 1. Age Distribution of Dengue Virus Infection

	Clinical Performance & Diagnostic				
Age (Year)	DF	DHF	DSS	Unusual	Total
1–4	9	10	5	0	24
5–14	22	16	5	3	46
Total	31	26	10	3	70

Table 2. Sex Distribution of Dengue Virus Infection

	Clinical Performance & Diagnostic				
Sex	DF	DHF	DSS	Unusual	Total
Boy	15	8	5	2	30
Girl	16	18	5	1	40
Total	31	26	10	3	70

Age and sex distribution of 70 cases dengue virus infection showed that: school age children especially girl were found more prevalence suffering from DHF than pre school age (see table 1 & 2)

Table 3. Distribution of Serotype Dengue Virus Based on Age

	Serotype of Dengue Virus				
Age (Year)	DEN 1	DEN 2	DEN 3	DEN 4	Total
1–4	2*	20	2	0	24
5–14	1	45	0	0	46
Total	3	65	2	0	70

Serotype DEN2 was dominant than DEN1 & DEN3 (see table 3) had exposured 40 cases of school age children which clinical performance of DF 14 cases, DHF 19 cases, DSS 7 cases. All of them showed secondary immune response and 5 cases of school age children and 20 cases pre school age showed primary immune respon (see table 7).

Table 4. Distribution of Immune Response of Dengue Virus Infection Based on Age

	Immune response of Dengue Virus Infection		
Age (Year)	Primary	Secondary	Total
1–4	11 (45,8%)	13 (54,2%)	24 (100%)
5–14	15 (32,6%)	31 (67,4%)	46 (100%)
Total	26 (37,1%)	44 (62,9%)	70 (100%)

This table 4, give information that the school age children showed more higher cases suffering from secondary of dengue virus infection.

Table 5. Distribution of Clinical Performance of Dengue Virus Infection

	Clinical Performance & Diagnostic				
Type of Infection	DF	DHF	DSS	Unusual	Total
Primary	16	7	1*	2	26
Secondary	15	19	9	1	44
Total	31	26	10	3	70

Mann-Whitney; $p = 0{,}035$*
* = *significant* ($p<0{,}05$)

Table 6. Distribution of Serotype on Clinical Performance of Dengue Virus Infection

	Clinical Performance & Diagnostic				
Serotype	DF	DHF	DSS	Unusual	Total
DEN 1	0	0	**2**	**1**	**3**
DEN 2	30	26	7	2	65
DEN 3	1	0	1	0	2
DEN 4	0	0	0	0	0
Total	31	26	10	3	70

Kruskal-Wallis: *p = 0,03**

* = *significant (p<0,05)*

Serotype DEN 1: there ware only 3 cases (see table 3) consisted of 2 cases had age 1–4 years and 1 had age 5–14 years. They showed a severe clinical performance as DSS 2 cases and 1 case as unusual case (see table 6).

Serotype DEN 1 was usually mild case but this study 1 case showed a severe clinical performance as DSS and identified as primary infection (see table5). The second case was identified as secondary dengue virus infection and the third case was an unusual case which showed secondary of dengue virus infection (see table 7). Based on Yamanaka this serotype DEN 1 might be have genotype IV or mention as DEN 1 genotype IV.

Table 7. Distribution of Primary and Secondary infection and Serotype that were correlated with clinical Performance of Dengue Virus Infection

	Clinical Performance & Diagnostic				
Type of Infection	DF	DHF	DSS	Unusual	Total
Primary					
DEN 1	0	0	1*	0	1
DEN 2	16	7	0	2	25
DEN 3	0	0	0	0	0
DEN 4	0	0	0	0	0
Total	16	7	1	2	26
Secondary					
DEN 1	0	0	1	1	2
DEN 2	14	19	7	0	40
DEN 3	1	0	1	0	2
DEN 4	0	0	0	0	0
Total	15	13	9	1	44

DISCUSSION

This study found that 65.7% cases of Dengue virus infection were on school age group children and more higher than pre-school age group (34.3%). It correlated with previous study based on explanation that many mosquito of Aedes Aegypti and Albocpitus were found surrounding the school where the pupil got education every day and playing football, baseball where the mosquito can bite one or more pupil especially if the sanitation and hygiene of school were not routine controlled for the population of larva.

In pre-school age group children showed primary infection of dengue virus 45.8% were more found and showed a clinical performance of DHF. However the secondary infection more found in school age group children (67.4%). The study in Asia & Latin America found there were a trend to be increase dengue virus infected cases in older than younger.

The outbreak of DHF cases in Bangladesh on 2000 found that all of DHF cases death had age more than 5 years old. In school age group children, there were secondary infection of dengue virus with clinical performance of dengue fever (67.4%).

E Ong (2008) study in Singapore has showed there were increasing secondary infection in children with age 1–5 years (0.77%) and age 6–10 years (6.7%). And had a high risk of dengue virus infection in school age group children. DEN 2 serotype were dominant and then followed by DEN 1 & DEN 3.

In this study DEN 1 genotype IV has showed a clinical performance of DSS (DHF grade III & IV) with primary infection and unusual case of DHF with showed primary & secondary infection.

Study in Thailand, DEN 2 serotype showed more severe of clinical performance and it was influenced by serotype and had a special strain. DEN 2 in South East Asia had showed more severe than DEN 2 in Latin America. DEN 2 in Africa were very rare causing health problem in human being.

Study in Singapore 2008 found serotype DEN 2 was dominant and followed by serotype DEN 1 & DEN 3. Our study in Surabaya 2009 had found serotype DEN 1 genotype IV, this showed a severe clinical performance as Dengue Shock Syndrome and unusual DHF cases with primary and secondary dengue virus infection. Some studies in Thailand showed serotype DEN 2 more severe clinical performance; it was influenced by dominant serotype and special strain of Dengue Virus.

In South East Asia serotype DEN 2 were dominant than Latin America and in Africa. In Africa serotype DEN 2 was rarely found and did not make health problem in human being.

Leit Meyer 1999 found that the differences dengue virus virulence based on structure protein E, PRM, NS1 & NS3. Protein E was a first antigen that influencing to entry and attachment endosom by fusion method and making virion. The differences of amino acid structure at the specific area can cause changing of antigen for attachment can cause replication of virus.

Rico Herse 1990 showed the distribution of DEN 1 based on Protein E & NS1 was found in: genotype I America & Africa; genotype II Srilanka; genotype III Japan; genotype IV South East Asia, Australia and Mexico; genotype V Taiwan and Thailand.

Manoa 2004 found the differences of clinical performance serotype DEN 1, such as DEN 1 genotype V virus can show

a clinical performance of dengue fever and DEN 1 genotype IV showed a clinical performance of DHF & DSS in some cases cannot be help and death. Some of them showed as primary infection dengue virus. This DEN 1 genotype IV were also found in Indonesia. This phenomena also has founded in this study.

Halstead hypothesis give information that secondary infection dengue virus can cause a severe clinical performance of dengue virus infection, it maybe due to ADE for promoting dengue virus to entry in many target cell of organ making children become a case of dengue virus infection with a severe clinical performance.

In this study, serotype DEN 1 genotype IV can promote a severe clinical performance of DSS & unusual DHF infection whatever with primary or secondary infection of Dengue virus.

Laili 2004, DEN 1 genotype IV one of genotype dengue virus that had been found in Indonesia and had shown a severe clinical performance. The other DEN 1 with difference genotype had shown a mild clinical performance. However secondary infection of Dengue virus showed more severe clinical performance than primary infection dengue virus.

CONCLUSION

1. Dengue virus infection in Dr. Soetomo Hospital Surabaya has more cases with DEN 2 following by DEN 1 and DEN 3
2. Serotype dengue virus influence to clinical performance of dengue virus infection especially DEN 1 genotype IV can cause severe clinical performance significant.
3. Secondary infection of dengue virus showed more severe clinical performance than primary infection dengue virus based on statistical analysis. However in this study one case of DEN 1 genotype IV with primary infection tend to have more severe clinical performance of dengue virus infection and this case could not be handled.

RECOMMENDATION

1. The future study focusing on genotype and the differences of structure dengue virus which to learn the pathogenesis of dengue virus infection.
2. Study should be done in many cities in Indonesia.

REFERENCE

Alison I, Janet M, Alexandra G, et al, 2007. Differential functional avidity of dengue virus specific T-cell clones for variant peptides representing heterologous and previously encountered serotypes. *J Virol* 81:10081–10091.

Angel B, Maria G, Samantha H, et al, 2002. Diagnosis of dengue virus infection by detection of specific immunoglobulin M and IgA antibodies in serum and saliva. *Clinl Diagn Lab Immunol* 10: 317–322.

Angel B, Samantha N, Perez L, et al, 2006. Serotype specific differences in clinical manifestations of dengue. *Am J Trop Med Hyg* 74:449-456

Aryati 2003.Aspek Laboratorium demam berdarah dengue. Dalam: Soegijanto S. Demam berdarah dengue. Surabaya: Airlangga University Press, hlm 117–132.

Balmaseda A, Samantha N, Perez L, et al, 2006. Serotype specific differences in clinical manifestations of dengue. *Am J Trop Med Hyg* 74:449–456

Bhamarapravati N. Pathology of dengue haemorrhagic fever. In: Thangcharoen P, editors. Monograph on dengue/dengue haemorrhagic fever WHO. New Delhi: 1993.p72–79.

Branch SL, Paul NL, 1999. Evaluation of four methods for detection of immunoglobulin M antibodies to dengue virus. *Clinl Diagn Lab Immunol* 6:555–557.

Chaturvedi UC, Nagar R, Shrivastava R, 2006. Macrophage & dengue virus: friend or for? *Indian J Med Res* 124:23–40.

Chen HC, Hofman FM, Kung JT, Lin YD, 2007. Both virus and tumor necrosis factor alpha are critical for endothelium damage in a mouse model of dengue virus induced hemorrhage. *J Virol* 81:5518–5526.

Chareonsirisuthigul T, Kalayanarooj S, Ubol S, 2007. Dengue virus (DENV) antibody-dependent enhancement of infection upregulates the production of anti-inflamatory cytokines, but suppresses anti-DENV free radical and pro-inflamatory cytokine production, in THP-1 cells. *J Virol* 88:365–375.

Dinas Kesehatan Propinsi Jawa Timur, 2001. Situasi kasus DBD dan kebijaksanaan program P2 DBD Propinsi Jawa Timur tahun 2002. Pertemuan kewaspadaan dini DBD bagi dokter spesialis dan dokter Puskesmas Propinsi Jawa Timur.

Eva H, Elsa V, Leonel P, Erick S, 2000. Clinical, epidemiologic, and virologic features of dengue in the 1998 epidemic in Nicaragua. *Am J Trop Med Hyg* 63:5–11.

Gubler DJ, 1998. Dengue and dengue hemorrhagic fever. *Clinical Microbiology Review,* 11:480–496.

Guha D, Schimmer B, 2005. Dengue fever: new paradigms for a changing epidemiology. *Emerging Themes in Epidemiology,* 2: 1–10.

Halstead SB, Heinz FX, Barrett AD, Roehrig JT, 2005. Dengue virus: molecular basis of cell entry and pathogenesis. *Vaccine* 23:849-856

Halstead SB. Pathophysiology and pathogenesis of dengue hemorrhagic fever. In:Thangcharoen P, editors. Monograph on dengue/dengue haemorrhagic fever WHO. New Delhi: 1993.p80–99.

Harris E, Videa E, Perez L, et al, 2000. Clinical, epidemiologic, and virologic features of dengua in the 1998 epidemic in Nicaragua. *Am J Trop Med Hyg,* 63:5–11.

Huang KJ, Chen SC, Liu HS, Lin YS. Manifestation of thrombocytopenia in dengue-2 virus infected mice. Journal of General Virology 81:2177–182.

Hung NT, Lan NT, Lei HY, et al, 2005. Association between sex, nutritional status, severity of dengue hemorrhagic fever, and immune status in infant with dengue hemorrhagic fever. *Am J Trop Med Hyg* 73:370–374

John RS, 2005. Understanding dengue pathogenesis: implications for vaccine design. *Bull Who* 83:308–314.

Kalayanarooj S, Nimmanntiya S, 2005. Is dengue severity related to nutritional status. *South Asian J Trop Med Public Healh* 36:378–385.

Kao LC, King C, Chao D, Chang J, 2004. Laboratory diagnosis of dengue virus infection: current and future perspectives in clinical diagnosis and public health. *J. Microbiol Imunol* 38:5–16 .

Karen C, Jennifer L, Eva H, 2006. Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. *J Virol* 80:11418–11431.

Leitmeyer K, David W, Douglas M, et al, 1999, Dengue virus structural differences that corelate with pathogenesis. *J Virol* 73:4738–4747.

Malloy M, Alan LR, 2005. Altered cytokine responses of dengue specific CD4 T cells to heterologous serotype. *J Immunol* 175:2676–2683.

Matheus S, Deparis X, Labeau B, Lelarge J, 2005. Discrimination between primary and secondary dengue virus infection by immunoglobulin G avidity test using a single acute-phase serum sample. *J Clin Microbiol* 43:2793–2797

Nasrum M, Sabran A, 2006. Tehnik identifikasi serotipe virus dengue dengan uji reverse transcription polymerase chain reaction (RT-PCR). *J Med Nus* 27:68–72.

Pervin M, Tabassum S, Ali M, 2004. Clinical and laboratory observations associated with the 2000 dengue outbreak in Dhaka, Bangladesh. *Dengue Bulletin* 28:96–106.

Seneviratne SL, Malavige GN, Silva HJ, 2005. Pathogenesis of liver involvement during dengue viral infections. *J Trop Med Hyg* 100:608–614.

Seema, Jain SK, 2005. Molecular mechanism of pathogenesis of dengue virus: entry and fusion with target cell. J Clin Bio 20: 92–103.

Shu PY, Huang JH, 2004. Current advances in dengue diagnosis. *Clinl Diagn Lab Immunol* 11:642–650.

Soegijanto S, 1999. Masalah penyakit DBD di Indonesia. Dalam: Soegijanto S. Demam berdarah dengue. Surabaya: Airlangga University Press, hlm 5-9.

Sriprom M, Pongsumpun P, Yoksan S, et al, 2003. Dengue haemorrhagic fever in Thailand, 1998–2003: primary or secondary infection. *Dengue Bulletin* 27:36–46.

Takol C, Siripen K, Sukathida U, 2007. Dengue virus (DENV) antibody-dependent enhancement of infection upregulates the production of anti-inflamatory cytokines. *J Virol* 88:365–375.

Vaughn DW, Green S, Kalayanarooj S, et al,2000. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 181:2–9.

Veeradej VW, Endy TP, Samakoses R, et al, 2003. Transplacentally transferred maternal-infant antibodies to dengue virus. *Am J Trop Med Hyg* 69:123–128.