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Clinical Microbiology 2015: Genotype I and IV of dengue virus DEN-1 and its clinical manifestasions in Surabaya- Puspa Wardhani- Universitas Airlangga

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Abstract
Previous studies showed there has been a Dengue Virus (DENV) distribution shift from DENV-2 to DENV-1 in Surabaya. This study analysed genetic characteristics of DENV-1 and clinical manifestations. This was an analytical observational study with prospective cohort setting. The study was conducted in Dr. Soetomo General Hospital, from February to August 2012. DENV serotyping was done by real-time PCR using SimplexaTM 3M RT-PCR instrument. Viral load examination was done by two step real-time PCR in Applied Bio system 7500 instrument. Positive samples were cultured in C6/36 cells. Positive culture samples were genotyped using envelope gene sequence. Dengue serotype distributions in 2012 were DENV-1 (67.9%), DENV-2 (9.5%), DENV-3 (4.8%), DENV-4 (7.1%), mixed DENV-1 & 2 (2.4%), DENV-1 & 3 (5.9%), and DENV-1 & 4 (2.4%). DENV-1 consisted of genotype I (66.7%) and IV (33.3%), genotype II, III and V weren't detected. Comparation among DENV serotypes or DENV-1 genotype showed no significant differences in DF and DHF manifestations. In the children group, red blood cell count (RBC) was higher in DENV-1 genotype I than IV, but mean corpuscular haemoglobin (MCH), lymphocytes and albumin level were lower in genotype I. Viral load level was higher in genotype I than IV, unfortunately it had been not significant. By analysing E gene nucleotids sequences, each DENV-1 strain showed individual nucleotides and aminoalkanoic acid changes. From E gene sequences analysis, amount of aminoalkanoic acid subtitutions had no implication in DF and DHF manifestations.

Introduction

Dengue may be a self-limited, systemic virus infection caused by dengue virus (DENV), a member of the Flaviviridae family. Dengue poses an enormous public health challenges, with a worldwide burden of an estimated 390 million infections once a year occur across approximately 128 countries, with the potential for further spread. Four DENV serotypes (DENV-1, -2, -3, and -4) circulate in tropical and subtropical regions of the planet and are transmitted by Aedes mosquitoes because the vector.

The clinical manifestations of dengue range from asymptomatic or a light flu-like syndrome referred to as classic dengue (DF), to a more severe form referred to as dengue haemorrhagic fever (DHF) and therefore the potentially fatal dengue shock syndrome (DSS). DF generally characterized by acute febrile illness, often accompanied with severe headache, myalgia, arthralgia, rashes, leukopenia and thrombocytopenia. Unusual haemorrhage like gastrointestinal bleeding, hyper menorrhoea and large epistaxis sometimes occur. In DHF, the signs and symptoms during the first febrile phase are almost like those in DF. The distinct feature of DHF is that the increase in vascular permeability (plasma leakage) that differentiates DHF from DF. By the top of the febrile phase, DSS may occur, which is characterized by shock thanks to plasma leakage. Unusual manifestations (or expanded dengue syndrome) are increasingly reported with involvement of severe organ impairment like liver, kidneys, brain or heart. These may be associated with coinfections, comorbidities or complications of prolonged shock. The DENV genome consists of a ~10.7 kb single-stranded positive-sense RNA genome encoding 3 structural (C, prM/M, E) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) proteins. DENV has very diverse genetic characteristics. The four antigenic ally-related serotypes differ by ~25–40% at the aminoalkanoic acid levels. Within each serotype, there are several clusters of variants termed as genotypes which vary by ~6% and three at the nucleotide and aminoalkanoic acid levels, respectively. Dengue severity has been correlated with viral genetics. All four of the serotypes of DENV can cause severe and fatal disease, although DENV-2 and DENV-3 are more related to severe disease. In Indonesia, all four of the DENV serotypes are circulating, with the tendency of DENV-3 associated with severe diseases. However, thanks to the limited serotype data available in Indonesia, it's possible that other serotypes also contribute to the severity of the disease. Surabaya and Jakarta were the cities where dengue disease was first reported in Indonesia in 1968. Currently, all 34 provinces of Indonesia have reported dengue cases. Dengue disease is kind of common in urban areas in Indonesia, and it occurs annually, while periodic major outbreaks have occurred, like those reported in 1998 and 2004. In 2011, the East Java Provincial Health Office reported 1,008 dengue cases in Surabaya (incidence rate 36/ 100,000) with a case death rate of 0.70%. Although dengue in Surabaya has been reported, the clinical aspects of the disease and its correlation with virological factors have never been reported. Our study described the clinical features of dengue disease in Surabaya, combined with molecular analysis of DENV.

Materials and methods

Patient recruitment, sample collection and clinical and

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laboratory examinations

This cross-sectional study was performed from February to August 2012 in Surabaya, the capital city of East Java province, Indonesia. Surabaya is that the second largest city in Indonesia; it covers a neighbourhood of roughly 333,063 km2 and is inhabited by roughly 3 million people. Patients suspected of having dengue with fever >38˚C accompanied by at least one of the symptoms of dengue such as headache, rash, arthralgia, retro-orbital pain, malaise, signs of DHF or DSS, presenting at the Dr. Soetomo Central Hospital were invited to participate within the study and were enrolled upon obtaining written consent. Consent for minors was obtained from parents or legal guardians. Ethical clearance for this study was obtained from Airlangga University Medical Research ethics panel . Sera from dengue-suspected patients were collected during the 3–5 days of fever and subjected to serology tests and dengue antigen detection. Anti-dengue IgG and IgM detections were performed using Panbio Dengue Duo IgM and IgG Capture ELISA (Alere, Brisbane, Australia), which was also wont to determine the infection status (primary or secondary infection) according to manufacturer's protocol. Briefly, a positive IgM result (> 11 of Panbio units) was indicative of active primary or secondary infection. An IgG-positive result (> 22 Panbio units) was indicative of active secondary infection. Primary infection decided by positive IgM (> 11 Panbio units) and negative IgG (< 22 Panbio units), while secondary infection decided by positive IgG (> 22 Panbio units), which could be accompanied by elevated IgM levels. Detection of DENV NS1 antigen detection was performed employing a Panbio Dengue Early Rapid kit (Alere), consistent with the manufacturer's instructions. All of the patients underwent examination 2–4 times of complete blood count, aspartate aminotransferase (AST), alanine transaminase (ALT), and albumin. Occurrences of hepatomegaly, splenomegaly, ascites, and pleural effusion and per nephric fluid were examined using ultrasonography methods. Classification of the clinical manifestations of dengue was supported the WHO SEARO 2011 dengue guideline [6] and that we categorized patients.