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Case Report

Clinical features and virological confirmation of perinatal dengue infection in Jambi, Indonesia: A case report

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ABSTRACT

The hyper-endemicity of dengue in Indonesia poses a significant threat of dengue virus (DENV) vertical transmission during pregnancy. A 29-year-old female at 38 weeks of pregnancy presented to hospital with acute fever and later confirmed with DENV infection. Due to signs of fetal distress, the neonate was delivered by emergency caesarean section. The mother developed a dengue critical phase post-caesarean with excessive bleeding and required blood transfusion. During the 6th day of life, the neonate was diagnosed and later confirmed with dengue. Next-generation sequencing of DENV RNA isolated directly from sera of both mother and neonate revealed identical DENV-2 whole-genome sequences. Plaque reduction neutralization test (PRNT) detected anti-dengue antibodies in both mother and neonate. Altogether, our data confirmed the occurrence of vertical transmission. Dengue vertical transmission during pregnancy may lead to severe manifestation, hence early diagnosis, close monitoring, and prompt intervention are critical.

Background

Dengue is a growing global health problem with an estimated 390 million infections per year worldwide (Bhatt et al., 2013). Dengue has been found in increasing numbers in all 34 provinces in Indonesia, posing significant risk to ~250 million population and placing a high economic and disease burden on the country (Wahyono et al., 2017).

Dengue virus (DENV) infection can occur in any age group, including pregnant women and their neonates. The DENV-infected pregnant women are considered to be a vulnerable group, having a higher risk of developing severe dengue, hemorrhagic complications, and death, though the mechanisms are still unclear (Charlier et al., 2017). Here, we report a case of symptomatic maternal dengue infection and subsequent symptomatic disease in the neonate confirmed through whole-genome sequencing with evidence of anti-DENV antibody transfer, suggesting vertical transmission.

Case presentation

A 29-year-old female was admitted to Siloam Hospital, Jambi, Indonesia with symptoms of fever that began 3 days prior, joint and muscle pains, headache, and loss of appetite. She was 38 weeks pregnant with her second child. She had a spontaneous vaginal delivery for her first pregnancy. Her vitals were normal upon admission and she tested positive for DENV NS1 antigen with a platelet count of 149,000/μL. Serological testing using Panbio Dengue Duo IgG/IgM rapid tests resulted positive for anti-DENV IgG and IgM and further confirmed using Panbio Dengue Duo IgG/IgM ELISA. Other laboratory results were normal. The fetus showed a fetal heart rate of 170 bpm, demonstrating fetal distress. Due to continued signs of fetal distress observed as increased fetal heart rate and reduced movement, the patient underwent emergency caesarean section the next day.

A female neonate weighing 2.9 kg was delivered. The amniotic fluid was stained with meconium and the neonate displayed signs

Abbreviations: BWA, Burrows-Wheeler Alignment; BPM, beats per minute; DENV, dengue virus; ICU, intensive care unit; NGS, next-generation sequencing; NS1, non-structural protein 1.

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of respiratory distress at birth with 1st- and 5th-minute Apgar scores of 6 and 8, respectively. The neonate's vital signs returned to normal and had normal lab results during the next 4 days of life. During the caesarean section, the mother lost 200 mL of blood. Post-caesarean section, her platelets began decreasing, reaching their lowest on the 2nd day post-caesarian section at 59,000/μL. On the 6th day post-caesarian, her symptoms and laboratory results returned to normal and she was discharged from the hospital two days later.

On the neonate's 6th day of life, her temperature rose to 38.6 °C and she began showing signs of respiratory distress with increased respiratory rate and cyanotic extremities. She was then admitted to neonatal ICU. There were no signs of mosquito bite in this specialized containment. Laboratory results demonstrated a plummeting platelet count to 23,000/μL and on the 2nd day of fever, she tested positive for DENV NS1 antigen. The platelet count continued to drop as far as 11,000/μL and she received a total of 150 mL of thrombocyte concentrate transfusion spread over the next 3 days. Serological test using Panbio anti-DENV IgG/IgM rapid assay results were negative. However, when we tested using ELISA, the results were positive only for anti-DENV IgM. There were no signs of hemorrhage, pleural effusion, or ascites. On the 10th day of life and 5th day post-fever onset, the platelet count returned to normal at 256,000/μL. She was discharged from the hospital the next day.

During the observation, blood samples were collected from both mother and neonate during their viremic phase at the third day of fever for the mother and second day of fever for the neonate. Serum samples were separated from venous blood and used for RNA extraction using QIAamp Viral RNA Mini kit (Qiagen). An

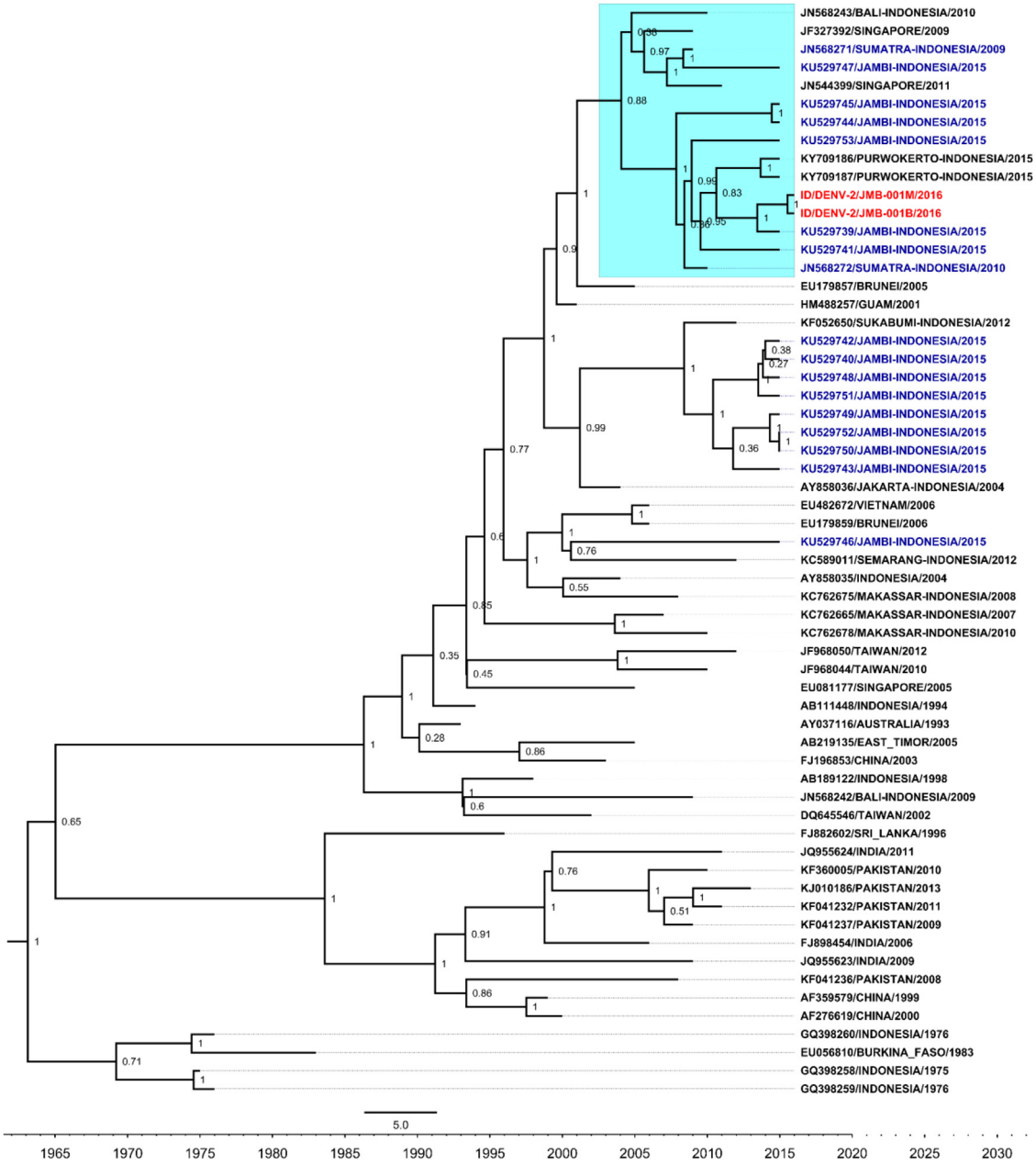

Figure 1. Maximum Clade Credibility (MCC) tree of DENV-2 Cosmopolitan genotype grouping generated by Bayesian inference method as implemented in BEAST using TN93 evolution model, gamma parameter rates, and molecular clock analysis from the Envelope gene sequences. The Jambi perinatal isolates (red font) were grouped into a Jambi-Sumatran clade (highlighted) together with other isolates from Sumatra 2009-2010 and Jambi 2015 (blue font). The posterior probabilities of the clades were indicated as numbers in the node labels. Whole-genome sequences of the DENV-2 isolates have been deposited in GenBank with accession number MK411558 and MK411559.

initial DENV detection and serotyping was performed using Simplexa Dengue real-time RT-PCR (Sasmono et al., 2014) and revealed the presence of dengue virus serotype 2 (DENV-2) in both samples. An in-depth analysis was performed using next-generation sequencing (NGS) where RNAs were extracted from 6 × 140 μL of serum sample, pooled, and used as template in NGS library preparation using TruSeq RNA library preparation kit (Illumina) using a metagenomic approach. Library was run in Illumina MiSeq NGS system and the resulting reads processed using BWA alignment and SPAdes de novo assembly bioinformatic softwares. The DENV-2 was detected in 0.14% and 0.02% of total sequence reads with 75× and 20× depth for mother and neonate samples, respectively. A total of 95% DENV genome coverage was achieved for both samples. Sequence identity analysis of the genome revealed a 100% identity (GenBank accession numbers MK411558 and MK411559). The DENV-2 sequences were genotyped as Cosmopolitan, according to Twiddy, et al. (Twiddy et al., 2002) (Figure 1).

The titer of anti-DENV antibody in serum samples was measured using a standard dengue Plaque Reduction Neutralization Test (PRNT) (Timiryasova et al., 2013) where 2-fold serially diluted serum samples was performed and challenged with the parental strains of the recombinant CYD vaccine viruses from all four DENV serotypes (Sasmono et al., 2018). Serum samples from the mother showed a detectable level of antibodies to all four serotypes in high levels. A similar profile, at a much lesser degree, was observed in the neonate's serum (Supplementary Table).

Discussion

In this case report, the mother was admitted to hospital with mild dengue symptoms which later developed into severe manifestation. The confirmation of DENV infection using NS1 antigen detection increased the awareness of the medical staff to monitor the fetal distress status which led to the decision to conduct caesarean section delivery. The elective decision to safeguard the fetus from potential harm of dengue-related risks has been described previously (Chye et al., 1997), although hazards of surgical intervention in patients with acute DENV infection are possible (Thaithumyanon et al., 1994).

Severe manifestations of dengue in the mother were observed within 3 days post-caesarean section, with evident thrombocytopenia and blood loss requiring blood transfusion, as has been observed elsewhere (Chye et al., 1997). The neonate showed evidence of dengue infection in her 6th day, with thrombocytopenia and positive DENV-NS1 antigen detection. However, with careful monitoring and intervention, the mother and neonate's conditions were improved and did not last to long-term sequalae. The clinical manifestations of the DENV-infected neonates are variable, from asymptomatic disease, typical dengue symptoms such as fever and rash, signs of plasma leakage such as ascites and pleural effusion, to dengue shock syndrome (Sinhabahu et al., 2014; Yang et al., 2015; Arragain et al., 2017). Dengue symptoms in the mother, obstetric complications associated with dengue, and clinical outcomes of the neonate have been described in a systematic review on maternal dengue and pregnancy outcomes (Pouliot et al., 2010).

The occurrence of dengue mother-to-child transmission has been confirmed through the detection of IgM/IgG antibodies, NS1 antigen, viral isolation, and nucleic acid (Sinhabahu et al., 2014; Arragain et al., 2017; Ribeiro et al., 2013). We performed a metagenomic NGS approach to sequence the whole genome of the DENV. The genomes sequences derived from both mother and neonate showed identical sequences, inferring the vertical transmission of DENV. Phylogenetic analysis revealed that the DENV-2 was classified into Cosmopolitan genotype and closely-related to strains circulated in Jambi (Haryanto et al., 2016). To our knowledge, our study is the first to confirm dengue perinatal transmission using NGS technology.

The antibody profiles of both mother and neonate were highly similar showing evidence of antibody transfer from mother to neonate. Low titer of antibodies (DENV neutralizing antibodies) in the neonate may be the cause of negative IgG testing using rapid test and ELISA, which may be explained by the lower sensitivity of the assays compared to PRNT. It was also of note that the mother was likely to acquire secondary infection, shown by the presence of antibodies to all four DENV serotypes. The presence of all four anti-DENV antibodies in the neonate confirmed the occurrence of antibody transfer, since natural DENV infection in the neonate would be likely to generate primary infection and antibody to the particular serotype. Studies reported greater levels of anti-DENV antibodies in infants due to vertical transmission (Chau et al., 2009; Castanha et al., 2016; Kliks et al., 1988). However, the lower level of IgG transferred to the neonate may be explained by the findings that the transfer of total IgG is decreased as the level of maternal IgG increased (Castanha et al., 2016) as observed by the high levels of maternal IgG in our study (Supplementary Table).

Although cord blood analysis was not performed, the evidence of positive DENV NS1 antigen in both mother and neonate and the identical DENV-2 genome sequences derived from both specimens indicated the occurrence of vertical transmission. The evidence of mosquito bite was ruled out since the mother and neonate were cared for in the ICU with containment from pathogens and germs, including mosquito. The onset of fever within 7 days of onset also supported the diagnosis of vertical transmission, as also observed in other case reports (Phupong, 2001; Bopeththa et al., 2018).

In conclusion, we report the dengue vertical transmission and confirmed it using DENV NS1 antigen, and DENV RNA detection (and whole-genome sequencing), and anti-DENV IgG/IgM antibodies. Dengue poses significant risks to women in pregnancy and the apparent vertical transmission to neonates should be taken into account while incorporating a vigilant disease management.

Authors' contributions

SH, RTS set the conceptual design of the study; SH, GIVWU, MSS, H performed clinical care, diagnosis, data collection, and analysis on patients' status. BY, RFH, DD, RIK performed the experiments and data analysis; HT analyzed the NGS data and performed bioinformatic analyses; RTS, SH, BY, MSS made the first draft of the manuscript; RTS contributed to the generation of final manuscript.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.07.019.

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