Zika Virus and Its Threads to Microcephaly

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ABSTRACT

The infection of ZIKV is through transplacental transmission. Polymerase chain reaction (PCR) detected the strains of ZIKV inside amniotic fluid only whereas in other fluids, serum and urine, revealed negative results. Microcephaly with numerous calcifications was discovered in the fetus of all cases. Ventriculomegaly and CNS structures anomalies were also discovered, though some structures such as subcortical nuclei of the brain and ascending dorsal columns of the spinal tract were well preserved.

Keywords: Microcephaly, ZIKV

ABSTRAK

Infeksi ZIKV melalui transmisi plasenta. Galur ZIKV dalam cairan ketuban dapat dideteksi menggunakan *polymerase chain reaction* (PCR), sedangkan pada cairan lainnya, yaitu serum dan urin, hasilnya negatif. Mikrosefali dengan kalsifikasi di semua kasus; lingkar kepala janin di bawah persentil kedua (34 cm). Ventrikulomegali dan struktur sistem saraf pusat abnormal, meskipun beberapa struktur seperti inti subkortikal otak dan jaras dorsalis medulla spinalis masih baik. **Dias Rima Sutiono, Cintya Pradnya Pratita. Virus Zika dan Hubungannya dengan Mikrosefali**

Kata kunci: Mikrosefali, ZIKV

INTRODUCTION

Zika virus (ZIKV) was originally found in 1947 within an experimental monkey in Ziika Forest, Uganda.[1] It becomes a hot topic after sudden outbreak in Brazil on May 2015.[2] ZIKV has similar characteristics to Dengue Virus (DENV). Both are from the *Flavivirus* family and spread by *Aedes sp.* such as *Ae. aegypti*, *Ae. Albopictus,* and *Ae. hensilii* mosquitoes.[2] The difference is only in the virion diameter and serotypes. DENV has four serotypes and 50 nm virion diameter, while ZIKV has only one serotypes and 10 nm smaller than DENV. ZIKV has a positive RNA with ~10.8 kb of length with 2 flanking non-coding regions (5' and 3' NCR) and a single long open reading frame encoding a polyprotein: 5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3' that is cleaved into capsid (C), precursor of membrane (prM), envelope (E), and seven non-structural proteins (NS). E is involved in various aspects of the viral cycle, mediating binding and membrane fusion.[3] The NS5 protein is the largest viral protein whose C-terminal portion has RNA-dependent RNA polymerase (RdRP) activity and the N-terminus is involved in RNA capping by virtue of its processing due to methyl transferase activity.[4]

The virus could spread among human through mosquito bite, blood or sperm.[5] After infection, the virus will be circulating inside bloodstream inducing the immune system to produce antibody. In five or six days after infection the virus will enter body's tissue. Blood is often examined to diagnose ZIKV infection by two methods, which are polymerase chain reaction (PCR) and serology.[5]

ZIKV infection shows mild, may be unrecognized,[2] symptoms after three until twelve days; such as rash, fever, joint pain, headache, and conjunctival hyperaemia.[2] Many are recovered after a week or longer and fatality is rare. Once infected, a person is likely to be protected from further infection.[7]

Blood transfusion from a diagnosed ZIKV-infection person is prohibited. Sexual intercourse is also prohibited in consideration of virus spread through sperm.[3] The virus is also suspected to be transmitted from a pregnant mother to the baby causing microcephaly.[2] ZIKV outbreak in Brazil presented seventeen cases of microcephaly confirmed to be related to ZIKV infection - two cases were miscarriage and the remaining fifteen cases were live births.[8] During 2014–2015, there were increased cases of central nervous system malformations in French Polynesia children after ZIKV outbreak.[2] Eighteen cases were reported including nine microcephaly cases compared to the national average of 0 to 2 cases of microcephaly per year.[9] World Health Organization (WHO) already advised a PHEIC (A Public Health Emergency of International Concern) regarding the ZIKV considering the finding of an increase in microcephaly cases of newborn and children.[10] Researches are conducted to prove and find the correlation between ZIKV and microcephaly.

Babies born with microcephaly will have head with occipital frontal circumference falls below the normal circumference for gestational age, sex and race. It is equal to or lower than standard deviations below mean (≤ -2SD) for age and sex; less than the second percentile and below -3SD is referred as severe microcephaly.[2] ECDC (The European Centre for Disease Prevention and Control) also stated that microcephaly is caused by the prematurely closed-skull since the fetal brain is not developed as the result of neurological impairment. Many causes of microcephaly

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are inherited genetic disorder, teratogenic drugs, malnutrition, hypoxic-ischemic lesions, and infections.[2] Infections are most commonly due to syphilis, toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus, and kinds of flavivirus. ZIKV is believed to be the flavivirus that will infect the baby of the infected mother through transplacental transmission.

CASE REPORTS

Mlakar J, *et al,*[11] and Calvet G, *et al,*[12] reported three identified cases of microcephaly associated with ZIKV infection of mothers.

Mlakar J, *et al,*[11] observed a dead fetus and the placenta of a 25-year-old woman who decided to terminate her pregnancy due to microcephaly detected by ultrasonography.[11] She is originally a European, worked as a volunteer and lived in Natal, Brazil. During her pregnancy she experienced a ZIKV disease-like symptom such as high fever followed by severe musculoskeletal and retroocular pain, itching, and generalized maculopapular rash. Ultrasonography performed at 14th and 20th week of gestation showed normal fetal growth and anatomy. However, signs of fetal anomalies started to show up at 29th week of gestation and at 32nd week of gestation; intrauterine growth retardation with normal amniotic fluid, fetal umbilical and uterine blood flows was confirmed by ultrasonography; a placenta measuring in 3.5 cm thickness with numerous calcifications, a head circumference below the second percentile for gestation or microcephaly, moderate ventriculomegaly and a transcerebellar diameter below second percentile. An autopsy of the organs of the fetus, especially brain and spinal cord (central nervous system), were conducted to confirm diagnosis by ultrasonography. Electron microscopic studies on brain's tissues, indirect immunofluorescence of the fetal brain and microbiologic investigation of the extracted RNAs from 10 mg placenta, lungs, heart, skin, spleen, thymus, liver, kidneys, and cerebral cortex using real-time PCR for the detection of ZIKV RNA (NS5) and one-step RT-PCR for the detection of the envelope (E)-protein coding region were also performed. ZIKV were found in the fetal brain sample with RT-PCR assay and ZIKV were detected 6.5×107 viral RNA copies per milligram of tissue.[11]

Other two cases observed by Calvet G, *et al,*[12] a 27-year-old and a 35-year-old women lived in the state of Paraiba, Northeast Region of Brazil. They also experienced ZIKV-disease like symptoms during their pregnancies similar to the first case. For the 27-year-old woman, no signs of fetal anomalies of her fetus detected by ultrasonography. At 27th week of gestation, the fetus presented microcephaly with calcification areas and was born with a head circumference of 30 cm. A relevant ventriculomegaly, asymmetry of hemispheres and hypo plastic cerebellum with complete absence of the cerebellar vermis were detected. Meanwhile, the ultrasonography of the 35-year-old woman's fetus at 25th week of gestation detected microcephaly, head circumference below third percentile, severe hypoplasia of cerebellar vermis and enlargement of the posterior fossa. The baby was born with severe ventriculomegaly, microphthalmia, cataract, and severe arthrogryposis in the legs and arms. Both women have no evidence of diabetes or blood-pressure-related disorders and did not report taking any medication (other than hydrocortisone for the younger), recreational drug use, alcohol consumption, or smoking during pregnancy. The younger one had not travelled outside her hometown during the previous few years and she had not had contact with any ill individuals. Also, she had no immunodeficiency or autoimmune disease. The older one had no relevant past medical history either of ZIKV or microcephaly. Probable ZIKV infection to the fetus was identified using real-time quantitative PCR (RT-qPCR) of the extracted RNA from amniotic fluid (amniocentesis) serum and urine.[12]

RESULTS

Externally observed, the fetus of the first case had microcephaly since its head circumference is 26 cm (1st percentile), smaller than normal 34 cm (Healthwise Staff, 2015). Observation of the brain and spinal cord (central nervous system) revealed microcephaly with a whole-brain weight of 84 g (4 SD below average), widely open sylvian fissures and a small cerebellum and brain stem. Almost complete agyria and internal hydrocephalus of the lateral ventricles were also observed. There were numerous variable-sized (filamentous, granular, and neuron-shaped) calcifications in the cortex and subcortical white matter in the frontal, parietal, and occipital lobes with focal involvement of the whole cortical ribbon, occasionally associated with cortical displacement. Diffuse astrogliosis was present with focal astrocytic outbursts into the subarachnoid space, mostly on the convexity of the cerebra; hemispheres. Activated microglial cells and some macrophages expressing HLA-DR were present throughout most of the cerebral gray and white matter. Scattered mild perivascular infiltrates composed of T-cells and some B-cells were present in the subcortical white matter. The brain stem and spinal cord showed Wallerian degeneration of the long descending tracts, especially the lateral corticospinal tract. The placenta confirmed focal calcifications in villi and decidua and no inflammation were found. Despite the growth retardation, the subcortical nuclei of the brain were quite well developed and the ascending dorsal columns of the spinal tract were well preserved. There were no relevant pathological changes in other fetal organs or in the umbilical cord or fetal membranes. Further observation on the brain tissues using electron microscope showed clusters of dense virus-like particles of approximately 50 nm found in damaged cytoplasmic vesicles. Indirect immunofluorescence showed granular intracytoplasmic reaction in destroyed neuronal structure. There were also groups of enveloped structures with bright interior, believed to be viruses from Flaviviridae family after negative staining were performed. The real-time PCR and one-step PCR revealed positive results for ZIKV from brain tissue and negative results for other flaviviruses.

Serum, urine, and amniotic fluid of the women from the second and third case were taken at the 28th week of gestation. Serology test was also conducted to detect the ZIKV antigen using anti-Zika-virus IgM. The infection of the fetus was confirmed as RT-qPCRs gave positive result for detection of ZIKV RNA strands and the alignments of the envelope and NS5 regions. The result of the serology test is positive in the amniotic fluid only; serum and urine were negative.

DISCUSSION

The identification by Mlakar J and Calvet G showed positive infection of ZIKV through transplacental transmission. In the second and third case, PCRs detected strains of ZIKV inside amniotic fluid only whereas in other fluid, which is in serum and urine, revealed negative

results. Amniotic fluid is swallowed by the fetus and will be released later,[13] consequently the fetus will positively become infected. Clusters of dense virus-like particles and groups of envelope with a bright interior were found and detected to be Flaviviridae family viruses after a negative staining. Additionally, auto-immune syndrome will succeed an ailment with Zika virus infection symptoms in prior days. Microcephaly with numerous calcifications was discovered in the all fetuses. Ventriculomegaly and the CNS structures anomalies were also discovered, though some structures such as subcortical nuclei of the brain and ascending dorsal columns of the spinal tract were well preserved.

CONCLUSION

The pathway of virus transmission from pregnant mothers to their babies is still not clear. There might be other mechanism than transplacental transmission.

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