Is There a Link between Zika Virus and Microcephaly In Neonates?

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Abstract
With Zika virus spreading worldwide, a lot of attention is drawn to researching its pathogenesis and etiology. It has also been noticed by various research groups and such health agencies such as CDC and WHO that there might be a connection between ZIKV and microcephaly, due to the spiking number of cases of microcephaly reported in areas with affected patients. Temporal and geographical data from the affected ZIKV areas, including Brazil and French Polynesia, suggests a connection between microcephaly and the virus. Tests of amniotic fluids of pregnant women with reported Zika virus infection and microcephalic fetuses revealed the presence of viral RNA. Zika was also found in placenta and fetal brain tissue collected from two miscarriages and two newborns. Evidence presented in this review is sufficient to prove an existent causal relationship between Zika virus and prenatal and neonatal microcephaly.

Introduction

Zika virus (ZIKV) is an emerging disease that has gone from a mild endemic, circulating only locally in Africa and parts of Asia, to a worldwide pandemic, spreading rapidly throughout the Americas. (Lupton, 2016; Paixão, et. al. 2016).

ZIKV is an arbovirus which belongs to the genus Flavivirus, family Flavivirdae. It is known to be transmitted by mosquitoes of the Aedes species – such as Ae. luteocephalus, Ae. aegypti, Ae. africanus, Ae. alboictus, and Ae. hensilli, which are usually found in areas with warm climate; however, Ae. albopictus inhabits as far north as the Great Lakes in the U.S. (Fellner, 2016) Ae. aegypti is considered to be the most efficient vector for ZIKV, which creates a bigger threat of spread of the virus, because this specie is characterized by an easy adaptation to human environments, indoor and daytime feeding, preference of feeding on humans, and an ability to breed in extremely small amounts of water. (Bell, et. al. 2016) As all other flaviviruses, including Dengue virus, Yellow fever virus, West Nile virus, Japanese encephalitis virus, Tick-Borne encephalitis virus, ZIKV carries a positive single-stranded RNA, which encodes a polyprotein that is then processed into three structural proteins: the envelope, the capsid, and the precursor of membrane. It also encodes seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. (Al-Quahtani, et. al. 2016).

ZIKV was discovered in 1947, in Uganda's Zika forest, near Entebbe, during regular surveillance for sylvatic yellow fever in Macacamulatta rhesus monkeys. (Paixão, et. al. 2016) Shortly after, in 1952, the first case of ZIKV infection in humans was reported in Uganda and the United Republic of Tanzania. For the next 4 decades, ZIKV had spread to equatorial Asia (India, Pakistan, Malaysia, Indonesia, etc.), but infections were mild, sporadic, and without large epidemics. (Al-Quahtani, et. al. 2016) However, in 2007 the first major outbreak of ZIKV infection occurred on Yap Island in the Federated States of Micronesia, where well-nigh 75% of population was infected with the virus. (Fellner, 2016; Al-Quahtani, et. al. 2016) Next major epidemic of ZIKV was reported in 2013 in French Polynesia. The most recent outbreak of ZIKV happened in Brazil in 2015, with first confirmed case reported in May 2015. (Al-Quahtani, et. al. 2016).

Due to an increasing number of microcephaly cases being reported in the ZIKV affected areas, WHO and Brazilian Ministry of Health have confirmed possibility of a link between ZIKV outbreaks and such severe fetal neurological defects as microcephaly and recommended that all pregnant women residing in or traveling to the affected areas should take precautions to avoid contact with possible vectors. (Calvet, et. al. 2016) Having a virus as a cause of birth defects is not surprising (for example, cytomegalovirus or rubella virus can be a source of microcephaly and other anomalies), but there has never been a time when a virus caused an epidemic of congenital birth anomalies. (Rasmussen, et. al. 2016) The CDC has advised that all infants, whose mothers traveled or resided in areas affected by ZIKV during pregnancy, should be suspected for congenital ZIKV infection if either intracranial calcifications or microcephaly were detected at birth or prenatally or if mother's ZIKV testing results were positive. (Fleming-Dutra, et. al. 2016) Can there really be a connection between Zika virus and neonatal microcephaly?

Methods

This systematic review was composed after reviewing and evaluating clinical data reports of Zika virus outbreaks in Haiti, French Polynesia, Philippines, and Cambodia, case-studies of infants and fetuses with presumed or confirmed ZIKV infection, and review articles; this data was obtained via searching engines as MEDLINE and ProQuest, or directly from published sources.

Discussion

Zika Virus: Diagnosis and Treatment

ZIKV infection starts in dendritic cells near the site of inoculation (if mosquito-mediated), which is followed by spreading to lymph-nodes and the bloodstream. (Al-Quahtani, et. al. 2016) Replication of flavaviral RNA occurs in both cellular cytoplasm and nucleus. Infectious ZIKV appears in human blood as early as day 1 of illness onset, and can stay in blood as late as day 11 after onset. (Hayes, 2009) The incubation period for ZIKV has been determined to be 3-12 days. The outcome of infection depends

on competition between viral replication and the host immune response. (Al-Quahtani, et. al. 2016) Usually, human infections with ZIKV are mild, and lethal outcomes are rare; common symptoms include fever, arthralgia and muscle pain, conjunctivitis, rash, and headaches, lasting up to one week. (Pastula, et. al. 2016) However, 80% of ZIKV are asymptomatic, which makes it extremely hard to diagnose and treat.

In addition to diagnosis based on epidemiological circumstances and clinical symptoms, there are not so many laboratory diagnostic tests available, because ZIKV as a pandemic is very recent. Existent assays for ZIKV diagnosis include use of reverse transcriptase-polymerase chain reaction (RT-PCR) to detect viral nucleic acid in the blood and other body fluids, such as saliva or urine. This test is useful only if performed on serum collected within day one to day three of symptom onset or on saliva or urine collected up to day five of symptom onset. Serological tests using enzyme-linked immunosorbent assays (ELISA) are able to detect the presence of anti-Zika virus immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, which develop in human organism by the end of the first week of infection. (Al-Qahtani, et. al. 2016; Fellner, 2016) However, as specific as these two test are (compared to NAT or reduction neutralization assay, for example), cross-reaction with related flaviviruses, such as West Nile and yellow fever, and dengue, is very common; therefore, additional tests should be performed to exclude those options.

Microcephaly

Microcephaly (micro- small; cephal- head) is a congenital condition which is diagnosed when newborn children have a smaller brain and skull compared to those of others of the same sex and age (See Fig. 1). (NINDS, 2016) Microcephaly can be a result of both environmental and genetic causes. Most common environmental factors that lead to microcephaly in infants are radiation, lead and mercury intoxication, in utero exposure to alcohol and drugs, and infections, such as TORCHES. (Calvet, et. al. 2016; Teixeira, et. al. 2016) Common genetic causes are autosomal recessive microcephaly, Aicardi-Goutières syndrome, Rett syndrome, and chromosomal trisomy. (Calvet, et. al. 2016) Frequently, microcephaly is associated with mental retardation, Down's syndrome, and neurometabolic syndromes.

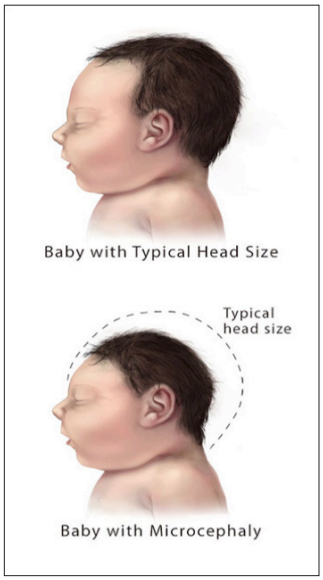

Figure 1 Microcephaly in an Infant (Bottom) Compared With an Infant With a Normal Head Size (Top) Source: CDC

There is no treatment available in order to return a baby's head to a normal size and shape; and all treatment techniques focus on decreasing the impact of accompanying disabilities. (NINDS, 2016) Therefore, understanding whether there's indeed a relationship between maternal infection with ZIKV and congenital microcephaly and the mechanism, in which the virus affects prenatal development, is extremely important for decreasing the number of microcephaly cases.

Evidence for a Link between ZIKV and Microcephaly

After the emergence of ZIKV in Brazil in 2015, a 20-fold average annual increase of microcephaly cases was reported. In 2015, there has been 1248 new suspected cases – prevalence of 99.7 per 100 000 livebirths. (Ventura, et. al. 2016) On November 28, 2015 Brazil declared congenital ZIKV to be responsible for the microcephaly epidemic. The very first alert was raised by the State Secretariat of Health of Pernambuco, Brazil in October 2015 after analyzing the obvious spike in microcephaly cases: from January to July 2015 there had only been 6 cases reported, 6 cases in August, 11 in September, and 39 in October. Mothers of 38 newborns were interviewed, 24 of whom reported having a rash during pregnancy. They also reported not to have had any common exposure to pesticides, drugs, alcohol abuse, radiation, or any other possible teratogenic factors. (Teixeira, et. al. 2016) As ZIKV has spread to other states in Brazil, 49th epidemiologic week had the highest rate of cases of microcephaly reported – 900 cases in a week; women delivering then would have been in the first trimester of pregnancy during the peak of ZIKV outbreak. (Kleber de Olivera, et. al. 2015; Teixeira, et. al. 2016) This observation suggests a very strong association, both geographic and temporal, between ZIKV and microcephaly epidemics.

In addition, a retrospective study, using serologic and statistical data, of 2013-2014 outbreak of ZIKV disease in French Polynesia identified that 1% of mothers infected with ZIKV in the first trimester, had given birth to neonates with microcephaly, which suggests a prevalence to be 50 times as high as the estimated baseline. (Rasmussen, et. al. 2016) Yet, this study was based on small numbers of patients, fetuses were not tested for ZIKV, and other brain anomalies were not included in the study.

Eight-eight pregnant women in Brazil, who reported having an onset rash for the previous 5 days, were tested and evaluated for ZIKV RNA. There were 72 with positive test results and 16 negative. Ultrasonography of 42 of those testing positively and the 16 negative ones revealed fetal brain anomalies were present in 29% of the former and 0% of the latter. (Rasmussen, et. al. 2016) Yet, this study is very limited by lack of tests for other viruses in mothers' serum and amniotic fluid for ZIKV or other viruses. Also, there were no postnatal observations of the patients.

Link between congenital ZIKV and microcephaly is strongly supported by histopathologic evaluation of 4 formalin-fixed, paraffin-embedded tissue samples from two newborns, born at 36 and 38 weeks of gestation, who died shortly after birth and had microcephaly, parenchymal calcification, microglial nodules, gliosis, brain cell necrosis, and two miscarriages (lost at 11 and 13 weeks gestation) with chorionic villi with calcification, fibrosis, and focal villitis. All four mothers reported having clinical signs of Zika during the first trimester of pregnancy – fever and rash. Laboratory testing was performed by the CDC; the samples included brain tissues from both newborns, placenta of one newborn, and products of conception from the two miscarriages. These samples were tested by ZIKV RT-PCR and all four cases turned out positive for NS5 and envelope genes. Further sequence analysis revealed the highest matches with ZIKV strains isolated from Brazil in 2015. The same samples were also tested for viral antigens by immunohistochemistry using a mouse polyclonal anti-Zika virus antibodies, and viral antigens were found in glial cells and neurons of one newborn and in chorionic villi of one of the miscarriages. RT-PCR for dengue was performed and tests results were negative, which eliminates the possibility of cross-reaction of the virus. (Martines, et. al. 2016) Also, the mothers' tests for rubella, herpes simplex, HIV, toxoplasmosis, and cytomegalovirus were negative, which points at ZIKV infection as the cause of microcephaly and fetal demise.

Another piece of evidence that supports the suggestion that ZIKV can negatively affect fetal CNS and cause microcephaly was obtained by a research team at Hospital Geral Roberto Santos in Salvador, Brazil. There, tissues and fragments of a female fetus, delivered after fetal demise in the 32nd week of gestation, were examined and tested for congenital ZIKV. Fetus had signs of microcephaly and arthrogryposis. Samples of cerebral cortex, medulla oblongata, cerebrospinal and amniotic fluids tested positive by ZIKV-specific RT-PCR, which targets viral NS5. Samples from other organs, such as extracts from the heart, lungs, and liver, didn't test positive and appeared to be undamaged. Also, the mother's tests for HIV, HTLV, hepatitis C, toxoplasmosis, rubella virus, and cytomegalovirus during the 4th gestational week were negative, which leaves responsibility for fetal brain anomalies to the virus. (Sarno, et. al. 2016) However, this study is limited by not performing histopathological analysis of the sample tissues.

In addition, a different group of researchers tested the amniotic fluid of two pregnant women with microcephalic fetuses. Both women had normal fetal ultrasounds in the first trimester, but ultrasounds done later on in weeks 25 and 27 revealed severe microcephaly (head circumference below the third percentile), hypoplasia of the cerebellar vermis, and posterior fossa enlargement in one fetus and asymmetry of hemispheres, and hypoplastic cerebellum with the cerebellar vermis completely missing. Both mothers did not report being on any medications during pregnancy, alcohol and drug use, or smoking. Tests for autoimmune and immunodeficiency diseases, and TORCHES were negative as well. However, they both reported having ZIKV infection symptoms at 10 and 18 weeks of gestation, respectively; also, they haven't reported travelling outside of Brazil during the previous years, or being in contact with any infected individuals. The amniotic fluid from each patient was then tested for any non-human genomic sequences, and ZIKV genome was identified in both samples. (Calvet, et. al 2016; Schuler-Faccini, et. al. 2016) The samples were also tested with RT-PCR for dengue and chikungunya, and the results were negative. ELISA test identified the presence of anti-ZIKV IgM in both samples as well. (Calvet, et. al. 2016) This study proves that ZIKV infection can cross the placental barrier. And since the ultrasounds at earlier gestation stages (during the first trimester) appeared to be normal but during the second trimester the fetuses started to develop signs of microcephaly and other brain anomalies (after the mothers being infected in the first trimester), the link between ZIKV and microcephaly is evident.

Also, according to Shepard, a pioneer in the field of teratology, in order to consider something a teratogen, there has to be an association of a rare environmental exposure with a rare defect. (Rasmussen, et. al. 2016) Considering the stats for microcephaly obtained in Brazil would not qualify because exposure to ZIKV wasn't rare. Yet, ZIKV is a rare exposure for the people that spent only a short time in the ZIKV affected areas and gave birth to infants with such brain anomalies. Considering microcephaly statistics in the United States of America, it can be assumed to be a rare defect, with only 6 cases in 10000 live-born infants. One of the recent reports in the U.S. illustrates this connection: a pregnant woman traveled to Mexico, Belize, and Guatemala during her 11th gestation week. She was tested positive for ZIKV IgM after her return. Ultrasonography and MRI of the fetus was performed during gestation weeks 19 and 20 and severe brain anomalies were diagnosed; the head circumference had dropped from the 47th percentile during the 16th week of gestation to the 24th percentile at gestation week

20. The pregnancy was terminated in the 21st gestation week, but considering the trend of the head circumference decrease, microcephaly would have developed if the pregnancy continued. (Rasmussen, et. al. 2016).

Mechanisms of infection of the fetal brain by Zika virus

The most recent research suggests that ZIKV is able to infect human neural progenitor cells, dysregulate transcription and stunt their growth. Human induced pluripotent stem cells (hiPSCs) were used as an in vitro model to investigate the mechanism of ZIKV infection and impact on neural cells. This study was able to determine that cells of different cell lines have variation in their susceptibility to the virus; hiPSCs showed one of the highest permissiveness levels. Further investigation of hiPSCs established that human neural progenitor cells (which can further be differentiated into cortical neurons, astrocytes, and oligodendrocytes) are a direct target of a ZIKV. Within 72 hours after being infected with ZIKV, total number of viable cells was observed to be reduce by 29.9 +/- 6.6%. Levels of caspase-3, involved in apoptotic pathway regulation, in infected hNPCs were found to be significantly higher than those in control group. (Tang, et. al. 2016) However, it is hard to conclude if all strains of ZIKV affect human embryotic brain cells in the same manner, for only one strain of virus was used in this research. In addition, assessing the level of neurotropism expressed by ZIKV is intricate because Zika infects other types of cells as well.

Conclusion

Evidence linking frequent occurrence of microcephaly in regions with ongoing ZIKV is deficient and scarce, for the pandemic is very recent and emerging. There are still many questions that remain unanswered, such as whether there's a range of associated birth defects, how this range is affected by the timing of fetal exposure to the virus and severity of maternal infection, if such range exists, and whether severe anomalies are only limited to the CNS.

However, based on the data available for analysis, it may be concluded that ZIKV can cross the placental barrier and exhibit higher tropism to a specific range of tissues. The accumulation of geographical and temporal and rare exposure-rate defect associations between maternal ZIKV infection and microcephaly in fetuses and neonates, and multiple case-study findings support a causal relationship present between the two events. Yet, more research must be conducted to have a better understanding of the effects and mechanisms of infection and to be able to propose possible treatment solutions.

List of Acronyms

Arbovirus	Arthropod-borne virus
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-Linked Immunosorbent Assays
HIV	Human Immunodeficiency Virus
hiPSC	Human Induced Pluripotent Stem Cell
hNPC	Human Neural Progenitor cell
HTLV	Human T-Cell Lymphotropic Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NAT	Nucleic Acid Amplification Test
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
TORCHES	Toxoplasmosis, rubella, cytomegalovirus, herpesvirus, and syphilis
ZIKV	Zika Virus

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