Review: Evidence of Neurological Sequelae in Children With Acquired Zika Virus Infection

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ABSTRACT

Limited information is available on health outcomes related to Zika virus infection acquired during childhood. Zika virus can cause severe central nervous system malformations in congenitally exposed fetuses and neonates. *In vitro* studies show the capacity of Zika virus to infect neural progenitor cells, induce central and peripheral neuronal cell deaths, and target different brain cells over the course of brain development. Studies of postnatally infected mice and nonhuman primates have detected degradation of neural cells and morphologic brain cell changes consistent with a broad neuroinflammatory response. In addition, case reports of central nervous system disease in adults and in adolescents secondary to Zika virus infection suggest that Zika virus may have a broader impact on neurological health beyond that observed in congenitally exposed newborns. Long-term neurological complications have been observed with other acquired flaviviral infections, with clinical symptoms manifesting for years after primary infection. The extent to which postnatal Zika virus infection in humans negatively affects the central and peripheral nervous systems and causes long-term neurological damage or cognitive effects is currently unknown. To better understand the potential for neurological sequelae associated with acquired Zika virus infection in children, we reviewed the biological, clinical, and epidemiologic literature and summarized the evidence for this link. First, we review biological mechanisms for neurological manifestations of Zika virus infection in experimental studies. Second, we review observational studies of congenital Zika virus infection and case studies and surveillance reports of neurological sequelae of Zika virus infection in adults and in children. Lastly, we discuss the challenges of conducting Zika virus-neurological sequela studies and future directions for pediatric Zika virus research.

Background

In 2016, the World Health Organization designated the Zika virus (ZIKV) epidemic as a Public Health Emergency of International Concern because of the emerging association between microcephaly and ZIKV infection during pregnancy.[1] Although many studies have evaluated the biochemical pathways for congenital ZIKV infection and related severe neurodevelopmental fetal and neonatal outcomes, research on acquired ZIKV infection in children (i.e., ages one month to 18 years) is lacking. The few existing epidemiologic reports describe incidence rates of acquired ZIKV infection among symptomatic children only and predominantly include probable cases based on clinical signs and symptoms, rather than laboratory-confirmed cases. Limited information is available on health outcomes related to acquired ZIKV infection in children.

ZIKV, like other neurotropic viruses such as cytomegalovirus and rubella, causes severe central nervous system (CNS) malformations in congenitally exposed fetuses and neonates. *In vitro* studies of brain cells and organoids show that although ZIKV targets forebrain-specific neural progenitor cells (NPCs), it has been shown to induce both central and peripheral neuronal cell death and may affect different brain cells over the course of brain development.[2-5] Human brain development includes key processes of proliferation and migration of glial progenitor cells that continue to differentiate and mature throughout childhood and adolescence. These cells are important for developing neural pathways and myelination.[6] The

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neurotropic properties of ZIKV and its impact on developing neural cells raise concerns about the potential neurological sequelae of postnatal ZIKV infection.

Studies of postnatally infected mice and nonhuman primates have detected degradation of neural cells and morphologic brain cell changes consistent with a broad neuroinflammatory response.[7-10] In addition, case reports of CNS disease in adults and adolescents secondary to ZIKV infection suggest that ZIKV may have a broader impact on neurological health beyond that observed in congenitally exposed newborns.[11] Long-term neurological complications have been observed with other acquired flaviviral infections, such as West Nile virus (WNV), with clinical symptoms manifesting for years after primary infection.[12-14] The extent to which postnatal ZIKV infection in humans can negatively affect the central and peripheral nervous systems and cause long-term neurological damage or cognitive effects is currently unknown.

To better understand the potential for neurological sequelae associated with ZIKV infection acquired during childhood, we reviewed the biological, clinical, and epidemiologic literature and summarized the evidence for this link. In this article, we first review biological mechanisms for neurological manifestations associated with ZIKV infection in experimental *in vitro* and *in vivo* studies. Second, we review observational studies of congenital ZIKV infection and case studies and surveillance reports of neurological sequelae secondary to ZIKV infection in adults and in children. Lastly, we discuss the challenges of conducting ZIKV-neurological sequela studies in children and future directions for pediatric ZIKV research.

Review of experimental studies

Neurotropism of ZIKV

In vitro *studies*

Evidence of ZIKV neurotropism is salient in the experimental literature. To identify the direct target cells of ZIKV, Tang et al. infected human-induced pluripotent stem cells in various stages of cortical neural development.[15] Tang et al. found that ZIKV had high efficiency for infecting forebrain-specific human NPCs and low efficiency for infecting human embryonic stem cells, human pluripotent stem cells, and more differentiated cortical neurons. Tang et al. further discovered that these forebrain-specific human NPCs had attenuated growth and 30% reduced viability three days after ZIKV infection.[15] Souza et al. observed similarly deleterious effects of ZIKV infection, that is, reduced NPC proliferation and cell death.[16] Cugola et al. showed that human cortical progenitor cells, but not fully differentiated neurons, continued to produce ZIKV RNA in the culture supernatant after ZIKV exposure.[3] Further, these investigators developed three-dimensional brain organoids derived from NPCs. ZIKV-infected brain organoids developed morphologic abnormalities and had higher rates of cell death compared with control organoids. Notably, these *in vitro* studies support the affinity of ZIKV for human NPCs, which may result in detrimental impact on developing brains.

Murine models

Since the 1970s, mouse models have provided important insights into the neurotropic properties of ZIKV. The tropism of ZIKV for immature neural cells was first demonstrated by Bell et al., who performed intracerebral inoculation of ZIKV into newborn and five-week-old mice. Bell et al. observed viral replication in neural and astroglial cells and marked enlargement of astroglial cells.[8] In another study of newborn mice, Manangeeswaran et al. infected one-day-old immunocompetent mice with two different strains of ZIKV and found that ZIKV localizes to the CNS, resulting in degeneration of Purkinje and granular cell layers of the mouse cerebellum and neurological manifestations (e.g., ataxia, tremors, and seizures) two weeks after infection.[17] In another study of newborn mice, Fernandes et al. reported that mice exposed to ZIKV via subcutaneous injection showed signs of CNS disease, including cortical lesions and myelopathy, with moderate neuronal death.[18] A related study by Lazear et al. showed that five- to six-week-old transgenic mice lacking innate antiviral immune responses sustained high viral loads of ZIKV in the brain and the spinal cord after ZIKV challenge.[19] These mice also experienced related morbidity, including muscle weakness and paralysis. These studies provide evidence of the tendency of ZIKV to infect the developing CNS and cause cell death and related neurological symptoms.

Other studies provide evidence of a less well-known but potentially serious effect of ZIKV on NPCs in older brains. For example, in a study of the impact of ZIKV on adult mouse brain neuropathology, Li et al. found that ZIKV infection reduced NPC proliferation and induced apoptotic cell death in the subgranular zone of the hippocampus and the subventricular zone of the anterior forebrain of infected mice.[10] Because neurogenesis occurs in the same regions of the brain in adult mice and in adult humans,[6,20] results from these murine studies reinforce the possible link between ZIKV infection and neuropathologic alterations in the mature human brain.

Nonhuman primates

Evidence of the potential for persistent ZIKV infection and its effect on the nervous system has been examined in nonhuman primates, who are natural hosts for the virus. ZIKV infection of adult rhesus monkeys resulted in persistence of ZIKV in cerebrospinal fluid (CSF) within the various body compartments examined for weeks after viremia was cleared.[21] Despite a robust humoral response detected in blood, ZIKV-specific antibodies were not found in CSF, suggesting a potential mechanism for viral persistence in the CSF. Furthermore, the investigators observed upregulation of the mechanistic target of rapamycin (mTOR), a major regulator of key processes of the developing and mature brain.[22] Simultaneous upregulation of mTOR and proinflammatory and antiapoptotic pathways was suggested as part of the mechanism for persistent ZIKV infection in the CNS. This viral persistence pathway may be relevant to other ZIKV-related neurological sequelae (e.g., encephalitis and Guillain-Barré syndrome [GBS]) in addition to neurodevelopmental disorders. In another study, Hirsch et al. found that infection of adult macaques with ZIKV resulted in persistence of the virus in the peripheral nerves and the spinal cord for up to five weeks post infection.[23]

The most compelling evidence to date of long-term neurological sequelae associated with ZIKV infection comes from a study of infant rhesus macaques (RMs) by Mavigner et al.[24] They studied six infant RMs who were postnatally inoculated with ZIKV PRVABC59 and two age-matched control RMs. In infected RMs, ZIKV persisted in peripheral and CNS tissues for 14 to 15 days after infection and histopathologic abnormalities were observed, including Wallerian degeneration and astrogliosis. In the same study, magnetic resonance imaging (MRI) and functional MRI conducted in two infected and two control RMs at ages three and six months showed enlarged lateral ventricles, attenuated hippocampal development, a reduction in white matter volume, and altered connectivity between the amygdala, hippocampus, and orbital frontal cortex in infected RMs compared with control RMs. The authors also reported an alteration in the emotional behavior response to acute stress at six months in infected RMs. Mavigner et al.'s study suggests the potential for longer term functional and structural neurological sequelae secondary to acquired ZIKV infection.

Flaviviruses and neurotropism

Animal and human studies have shown that, similar to ZIKV, other flaviviruses, such as WNV and Japanese encephalitis, are

neurotropic and can persist for months in the CNS.[25-27] In humans, it is well established that WNV and Japanese encephalitis virus can result in encephalitis and other severe consequences related to neuroinflammation.[28,29] Researchers have also observed neuroinflammation after ZIKV infection in animal models,[21,30] but little is known about the neuroinflammatory properties of ZIKV in humans. Although rare cases of ZIKV-related neuroinflammatory sequelae have been reported,[31] more research is needed to understand the mechanism by which ZIKV may result in encephalitis or meningitis, particularly in children.

Review of epidemiologic research and case studies of ZIKV infection

Congenital ZIKV exposure

ZIKV is the only flavivirus known to cause congenital brain abnormalities. During the Brazilian ZIKV outbreak, ZIKV was identified by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) in amniotic fluid[32] and in the fetal brain tissue of microcephalic infants.[33,34] ZIKV was also detected by RT-PCR in CSF and in brain tissue in seven autopsies conducted on neonates with congenital ZIKV infection who died within the first week of life. These autopsies revealed decreased brain weight with thinning parenchyma, ventriculomegaly, cerebellar hypoplasia, and both macroscopic and microscopic calcifications.[35] Studies of human fetal and neonatal CNS development following intrauterine exposure to ZIKV have identified some overlapping cognitive and sensorimotor disabilities often observed in other congenital neurological syndromes. However, those exposed to ZIKV also displayed some unique features leading to the development of a clinical definition for congenital Zika syndrome, that is, severe microcephaly with partially collapsed skull; malformation of cortical development; subcortical or basal ganglia calcifications; chorioretinal atrophy and focal pigmentary retinal mottling; congenital contractures not previously observed in fetal brain disruption sequence; and marked early hypertonia and symptoms of extrapyramidal involvement.[36,37] It is unknown whether the neurobiological pathways that cause the morphologies seen in congenital Zika syndrome also cause neurological effects in children who acquire ZIKV infection postnatally.

Acquired ZIKV infection among children

Only a few ZIKV cases with neurological involvement have been described among children. For example, travel-acquired ZIKV infection appeared to trigger neuropsychiatric and cognitive symptoms in a 16-year-old, although Epstein-Barr virus could not be ruled out. Symptoms included poor processing speed, short-term memory loss, and behavior suggestive of hypomania. ZIKV IgM antibodies were detected in the adolescent's CSF 15 days after symptom onset, and single-photon emission computed tomography showed focal moderate-to-severe hypoperfusion in the inferior left frontal region.[38] Another case of ZIKV infection in an adolescent resulted in left-sided hemiparesis, severe pain, and a bilateral loss of temperature sensation. Although the adolescent's brain MRI results were normal, the spinal MRI result showed myelitis. In this case, Epstein-Barr virus was ruled out and ZIKV RNA was detected in the CSF by RT-PCR.[39]

Epidemiologic studies of children and adults with acquired ZIKV infection indicate that children appear to experience mild symptoms similar to those experienced by adults, most commonly fever, maculopapular rash, arthralgia, or nonpurulent conjunctivitis. Few studies have reported age-specific ZIKV prevalence and incidence rates. Among children in Colombia, the highest rates were reported among children ages 15 to 18 years.[40] In Micronesia, children had lower reported rates compared with adults,[41] whereas in Puerto Rico, incidence among nonpregnant persons was highest among the 10- to 19-year-old age group.[42] Thirty-three percent of individuals enrolled in the Sentinel Enhanced Dengue Surveillance System in Puerto Rico were ZIKV infected, of which 26% were children.[43] These studies include only symptomatic children reported to the countries' surveillance systems and, except for Puerto Rico, are mainly based on probable rather than laboratory-confirmed case reports. Studies of children with asymptomatic ZIKV have not been published.

No studies have systematically evaluated risk of neurological sequelae associated with ZIKV acquisition during childhood, and only one study evaluated neurological sequelae secondary to ZIKV infection in children. Tolosa et al. reported detailed demographic, geographic, health outcome, and ZIKV incidence data for children throughout Colombia.[40] Of the 18,576 children ages 1 month to 18 years with suspected ZIKA infection identified through Colombia's National Health Surveillance System between August 2015 and May 2016, only 1655 (8.9%) were tested for ZIKV infection by RT-PCR, 72.9% of whom were ZIKV positive. At the time of the report, 96 (0.5%) of the 18,576 children with suspected ZIKV had an associated neurological diagnosis related to ZIKV infection, 29% of whom were aged one month to six years. However, only 25 of the 96 children were tested for ZIKV by RT-PCR, and only 12 (48%) of those were positive. Of the 96 children with suspected ZIKV with neurological complications, 40 had GBS, 24 had polyneuropathy, and 20 had encephalitis. The conditions observed among the 12 children with laboratory-confirmed ZIKV infection were not specifically reported. Tolosa et al.'s study also reported that 631 (3.4%) of the children with suspected ZIKV were hospitalized, but reasons for hospitalization were not reported. One child with laboratory-confirmed ZIKV infection died because of complications of GBS, secondary to ZIKV infection.

Acquired ZIKV infection among adults

Although the epidemiologic and clinical research on acquired ZIKV infection has focused mainly on adults, such studies can elucidate the possible range of severe central and peripheral nervous system manifestations that may present with acquired ZIKV infection in childhood. Several studies found an increased incidence of GBS during the ZIKV epidemic, and recent GBS case series have found evidence of ZIKV infection in many of these patients.[31,44-49] Other neurological manifestations in adults have included myelitis, encephalitis, meningoencephalitis, chronic and acute inflammatory demyelinating polyneuropathy, and transient hearing loss.[31,50-54] Acute neuromuscular disease has been observed among patients with ZIKV-related encephalitis and among patients with ZIKV-related GBS.[31,51] MRI results of adult ZIKV-infected patients have depicted cortical edema, changes in the lenticular nucleus, and brain abnormalities consistent with cerebral vasculitis, which ultimately resolved.[51,54] Although fully formed adult brains may be affected by and recover from ZIKV infection differently from children's brains, the fact that acquired ZIKV infection can cause an inflammatory response in the brain raises concern about the possible impact of ZIKV infection on the developing nervous system.

Discussion

Although many studies have investigated the risks of ZIKV during pregnancy and onset of GBS after ZIKV infection, few studies have evaluated ZIKV symptoms and associated health outcomes among children with acquired ZIKV infection. Symptoms appear to be similar among children and adults, but experimental studies illustrate the ability of ZIKV to spread through brain tissues, persist in CSF fluid, and disrupt important brain cell development when exposure occurs outside the womb. This body of evidence raises questions about the possibility of undetected neural cell damage among children with

acquired ZIKV infection and related subtle neurological impairment that may not be discovered unless scientists take a closer look.

Emerging evidence indicates that ZIKV is capable of disrupting the function of NPCs and causing neural stem cell death. NPCs differentiate into mature brain cells, and although much of this occurs during fetal brain development, the process of differentiation and maturation continues throughout childhood and into adulthood.[6] Furthermore, the areas of the brain that house these vulnerable NPCs are the hippocampus and the anterior forebrain, responsible for learning, memory, and behavior. Although many of the symptoms of ZIKV-associated neurological conditions (e.g., meningitis, encephalitis, and GBS) have known trajectories, the potential neurodevelopmental impact of more subtle damage is unknown and may ultimately have a broader public health impact.

Information is limited about disease characteristics (e.g., infection prevalence, incidence, severity, and transmission) and health outcomes among children with postnatally acquired ZIKV infection. Transmission risk specific to children has not been studied. ZIKV can be found in breast milk,[55] but it is unknown whether the presence of virus in breast milk can result in mother-to-infant transmission. More research is needed to understand the risk of ZIKV acquisition among children, particularly if the epidemiologic evidence reveals poor health outcomes associated with pediatric ZIKV infection. Better surveillance is needed to quantify the incidence and attack rates of ZIKV infection among children and to identify potential downstream neurological sequelae; geolocation of these cases is needed to anticipate future outbreaks. Children may be exposed to ZIKV in different settings compared with adults (e.g., in day care and schools), making it important to understand how and when case clustering occurs among children, ZIKV virulence in children, and the potential for herd immunity. Other factors that may increase risk of adverse outcomes among children have also not been studied in the context of ZIKV. For example, are children with existing neurological conditions or autoimmune disorders at risk of worsening clinical outcomes? Are there sensitive periods of neurodevelopment where ZIKV may have its worst impact? Additional studies are needed to evaluate both subclinical neurological damage (e.g., brain lesions and cell death) and neuropsychological outcomes (e.g., executive functioning, sensorimotor functioning, and memory) among healthy children and those with health vulnerabilities.

To address these research gaps, scientists, health-care providers, and public health practitioners must improve dissemination of ZIKV-related public health information to the public, develop noninvasive and accurate assays, and increase capacity building for neurological and neuropsychological evaluation. It is increasingly important to communicate ZIKV transmission risks and symptoms to health-care practitioners and the general public and to encourage families to bring their asymptomatic children in for ZIKV testing. We also recommend that children be included in epidemiologic ZIKV studies, when possible, and that results of those studies be stratified by age, sex, and presence of symptoms to permit a better understanding of the distribution of infections, clinical characteristics, and health sequelae among children. Development of more specific and sensitive assays is currently under way. A noninvasive urine or saliva collection procedure may facilitate testing of infants and young children in routine surveillance and research. In some children, ZIKV RNA can be detected in saliva in the absence of RNA detection in blood.[56] ZIKV RNA has been detected in urine at higher levels and for longer durations compared with serum, making urine an excellent specimen type for ZIKV testing, particularly in the acute period of infection.[57,58] Further development of assays for these fluid types is under way.[59-61]

Many of the well-established and normed neurocognitive, psychological, and developmental tests for this type of research were developed in the United States and have not been validated in low-resource, Spanish- and Portuguese-speaking settings. Validation of existing neuropsychological assessment instruments in regions with ZIKV transmission is needed to evaluate the cultural appropriateness of vocabulary and concepts and to develop norms for diverse contexts. In this regard, instruments should be selected for use and validation based on their ability to uncover focal and generalized dysfunctions; age appropriateness with tasks across the life span if possible; availability of age-based normative data; strong psychometric properties; ease of administration by nonpsychologists; and, if possible, availability or ease of translation into Spanish and Portuguese and other local (e.g., Quechua and Creole) dialects. Increased capacity building among health-care and school-based providers to administer these instruments is necessary to encourage their use in research and to ensure standardized implementation and common data elements across studies. In the context of ZIKV, robust neuropsychological assessment training and implementation will facilitate evaluation of the full impact of ZIKV on children's neurodevelopment in regions with ZIKV transmission.

Conclusion

Given the large public health impact of ZIKV, it is critically important to understand how this infection will impact neurobehavioral outcomes and neurodevelopmental trajectories for children at risk of ZIKV infection. Although experimental studies provide key insights into the biological mechanism of ZIKV infection, longitudinal epidemiologic studies in children with acquired infection are needed to better understand how ZIKV infection may impair short- and long-term neurodevelopment and to evaluate the persistence of neurological sequelae over time. This information will help determine intervention strategies to address the care and follow-up of ZIKV-infected children.

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