Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Pediatr Infect Dis J. Author manuscript.

Second-Trimester Ultrasound and Neuropathologic Findings in Congenital Zika Virus Infection

Cheng-Ying Ho, MD, PhD[1,*,¶], **Nicolas Castillo, MD**[2,¶], **Liliana Encinales, MD**[3], **Alexandra Porras, PhD**[4], **Alejandro Rico Mendoza, PhD**[4], **Rebecca Lynch, PhD**[5], **Amy Nemirovsky, BA**[1], **Grace Mantus, BS**[5], **Roberta L. DeBiasi, MD, MS**[6,7,8], **Jeffrey M. Bethony, PhD**[5], **Gary L. Simon, MD, PhD**[8], and **Aileen Y. Chang, MD, MSPH**[8,*]

[1]Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland, USA

[2]Department of Maternal Fetal Health, Allied Research Society LLC, Baranquilla, Atlántico, Colombia

[3]Department of Medicine, Allied Research Society LLC, Baranquilla, Atlántico, Colombia

[4]Grupo de Medicina Comunitaria, Universidad El Bosque, Bogotá, Colombia

[5]Department of Microbiology, Immunology, and Tropical Medicine, The George Washington University, Washington, D.C., USA

[6]Division of Pediatric Infectious Diseases, Children's National Health System, Washington DC, USA

[7]Department of Pediatrics, The George Washington University, Washington DC, USA

[8]Department of Medicine, The George Washington University, Washington, D.C., USA

Abstract

Zika virus (ZIKV) is a mosquito-borne virus that causes congenital Zika syndrome, characterized by microcephaly and other fetal brain anomalies. This case report presents a case of ZIKV-related fetal brain anomalies including pathologic evidence of cerebral neuronal apoptosis and macrophage infiltrates and intracerebral calcification, ventriculomegaly and corpus callosum dysgenesis detected by ultrasound at 18 weeks of pregnancy.

Keywords

Zika; microcephaly; fetal brain anomalies; congenital infection; neuronal apoptosis

ZIKV is a single-stranded RNA virus of the family Flaviviridae.[1] The virus is transmitted to humans primarily by the *Aedes* mosquito.[2] Although the majority of the individuals infected with ZIKV are asymptomatic, some individuals may experience fever, rash and myalgias. Conjunctival injection may also be present in ZIKV as well as dengue infection. Guillian-

[*]Corresponding author: Aileen Chang, George Washington University, 2150 Pennsylvania Ave 5-416, Washington, DC 20037, 202-741-2200 (Phone), 202-741-2185 (Fax), chang@email.gwu.edu.
[¶]Contributed equally to this work

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Barré syndrome may follow ZIKV infection in a small number of patients.[3] The most concerning complication of ZIKV infection is the congenital Zika syndrome characterized by microcephaly and/or an expanding list of fetal brain defects including intracranial calcifications, ventriculomegaly, corpus callosum abnormalities and cerebellar hypoplasia.[4,5] This is the earliest ultrasound-diagnosed case of Zika-associated microcephaly, which is also the first ZIKV-associated neuropathology report from Colombia. The mother gave consent for her case details to be published.

A 23-year-old woman from Barranquilla, Colombia, with no significant past medical history or prior pregnancy complications presented to clinic in her second trimester of pregnancy for routine prenatal ultrasound. She did not report a history of dengue or chikungunya virus infection. During her 8th week of pregnancy she developed fever, bilateral conjunctivitis, peripheral edema, and arthralgia.

Prenatal ultrasound performed at 18-weeks-and-2-days gestation revealed abnormal findings in the central nervous system, including microcephaly, ventriculomegaly with asymmetric anterior horns of lateral ventricles (Figure 1A and 1B), dysgenesis of corpus callosum (Figure 1C), multiple cerebellar (Figure 1D) and periventricular calcifications (Figure 1E), and hypoplasia of cerebellar vermis. Limb abnormalities, including claw-like appearances of the hands and hyperextension of the lower extremities (Figure 1F), were also detected. The placenta, amniotic fluid, umbilical artery Doppler, and uterine artery Doppler were normal.

Given these abnormal ultrasound findings, the patient elected to terminate her pregnancy at 19 weeks. A brief gross examination was performed on the fetus. The gender of the fetus was undetermined. The crown-heel length was normal (22 cm; reference range 18.8–21.4 cm). The head was disproportionally small compared with the trunk and extremities. There were no abnormal facies or other gross abnormalities. Other organ systems including the placenta were grossly unremarkable. The brain weight was not available, but the hemispheres appeared appropriately formed. Assessment of the gross brain anatomy was difficult due to extreme softening of the structures. Further laboratory testing confirmed that the amniotic fluid was positive for anti-Zika virus (ZIKV) IgM and IgG antibodies, and ZIKV RNA by RT-PCR. The mother's plasma was positive for anti-ZIKV IgM and IgG antibody binding to the ZIKV envelope protein and indicated high IgG titers with a reciprocal EC50 titer of 5697.

Histologic sections of the cerebral cortical plate showed abundant cellular apoptosis and macrophage infiltrates (Figure 2A and 1C), whereas the ventricular and subventricular zone germinal matrix appeared morphologically intact (Figure 2B and 2D). ZIKV infection was confirmed in both cortical plate and germinal matrix by immunofluorescent staining for flavivirus envelope protein (Figure 2C and 2F).

Discussion

Since the outbreak in 2015 and 2016, there have been >8,000 reported cases of microcephaly from Brazil,[6] greater than 500 cases from Colombia,[7] and a variable numbers of cases from other countries in the Americas. The rapid spread of the virus and its

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

complications prompted the World Health Organization (WHO) to declare a global public health emergency between February and November 2016.[8]

According to recent studies, symptomatic maternal ZIKV infection during all trimesters can be associated with adverse pregnancy outcomes.[9,10] The incidence of fetal brain abnormalities is highest when infection occurs in the first trimester.[10–13] However, the period between the end of the first trimester and the beginning of the second trimester is when neuronal proliferation and migration peak. This may be a period of unique vulnerability, because mild perturbations in the maternal-fetal environment can be amplified during this period and significantly alter the structure and function of the brain.[14] Given the critical nature of this time window, studies of infected fetuses at this gestational age may provide insight into the pathogenesis of ZIKV-associated microcephaly.

Although a number of congenital Zika syndrome cases have been reported, most are neuroimaging studies.[4, 15,16] To date there have been only a handful of neuropathologic studies.[5, 17–19, 21] In addition, all but one neuropathologic report described findings in the third trimester, when neuronal proliferation and migration has slowed or ceased.[17,21] The overall pathologic findings identified in the third trimester, e.g. intraparenchymal calcifications, cortical thinning, and migrational disturbances, are likely a manifestation of the neural injuries that may have occurred earlier in gestation.

We present the earliest ultrasound-diagnosed case of Zika-associated microcephaly, which is also the first ZIKV-associated neuropathology report from Colombia. Microcephaly is usually diagnosed at a mean gestational age of 28 weeks[18] and often is not clinically evident at the early stage that was seen in this patient. Notably, the neuropathology of the current case shares many similarities to another fetus who was infected by the Guatemalan variant of virus.[17,21] The most important finding of these two cases is the presence of cellular apoptosis and macrophage infiltrates in the cerebral cortex but not in the germinal matrix, despite the detection of ZIKV in both brain regions. These findings suggest that ZIKV-associated injuries may preferentially affect post-migratory immature neurons, which are most abundant in the second trimester. Furthermore, similarities in the second-trimester pathology of cases from Guatemala and Colombia suggests that a common pathogenic mechanism of microcephaly exists among Asian-lineage strains.[22]

Acknowledgments

This study was made possible by Allied Research Society, the Universidad El Bosque, and La Fundación Santa Fe. We would also like to thank Dr. Raul Garcia Tolosa for his role preparation of the specimen.

Sources of support: This work was supported by the National Institutes of Health U19 AI131130 (awarded to CYH), and UL1TR001876 and KL2TR001877 from the NIH National Center for Advancing Translational Sciences (awarded to AYC). Its contents are solely the responsibility of the authors and do not necessary represent the official views of the National Center for Advancing Translational Science or the National Institute of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, et al. Biology of Zika Virus Infection in Human Skin Cells. J Virol. 2015; 89(17):8880–96. [PubMed: 26085147]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2. Petersen EE, Staples JE, Meaney-Delman D, et al. Interim Guidelines for Pregnant Women During a Zika Virus Outbreak--United States, 2016. MMWR Morb Mortal Wkly Rep. 2016; 65(2):30–3. [PubMed: 26796813]

3. Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, et al. Guillain-Barré Syndrome Associated with Zika Virus Infection in Colombia. N Engl J Med. 2016; 375(16):1513–23. [PubMed: 27705091]

4. Melo AS, Aguiar RS, Amorim MM, et al. Congenital Zika Virus Infection: Beyond Neonatal Microcephaly. JAMA Neurol. 2016; 73(12):1407–16. [PubMed: 27695855]

5. Mlakar J, Korva M, Tul N, et al. Zika Virus Associated with Microcephaly. N Engl J Med. 2016; 374(10):951–8. [PubMed: 26862926]

6. Magalhães-Barbosa MC, Prata-Barbosa A, Robaina JR, et al. Trends of the microcephaly and Zika virus outbreak in Brazil, January-July 2016. Travel Med Infect Dis. 2016; 14(5):458–63. [PubMed: 27702683]

7. Collucci C. Colombia sees fourfold increase in microcephaly cases in a year. BMJ. 2016; 355:i6716. [PubMed: 27974296]

8. Ikejezie J, Shapiro CN, Kim J, et al. Zika Virus Transmission – Region of the Americas, May 15, 2015–December 15, 2016. MMWR Morb Mortal Wkly Rep. 2017; 66(12):329–34. [PubMed: 28358795]

9. Brasil P, Pereira JP, Moreira ME, et al. Zika Virus Infection in Pregnant Women in Rio de Janeiro. N Engl J Med. 2016; 375(24):2321–34. [PubMed: 26943629]

10. Johansson MA, Mier-y-Teran-Romero L, Reefhuis J, et al. Zika and the Risk of Microcephaly. New England Journal of Medicine. 2016; 375(1):1–4. [PubMed: 27222919]

11. Faria NR, Azevedo RoS, Kraemer MU, et al. Zika virus in the Americas: Early epidemiological and genetic findings. Science. 2016; 352(6283):345–9. [PubMed: 27013429]

12. Honein MA, Dawson AL, Petersen EE, et al. Birth Defects Among Fetuses and Infants of US Women With Evidence of Possible Zika Virus Infection During Pregnancy. JAMA. 2017; 317(1): 59–68. [PubMed: 27960197]

13. Reynolds MR, Jones AM, Petersen EE, et al. Vital Signs: Update on Zika Virus–Associated Birth Defects and Evaluation of All U.S. Infants with Congenital Zika Virus Exposure — U.S. Zika Pregnancy Registry, 2016. MMWR. 2017; 66(13):366–373. [PubMed: 28384133]

14. Miranda R. MicroRNAs and Fetal Brain Development: Implications for Ethanol Teratology during the Second Trimester Period of Neurogenesis. Frontiers in Genetics. 2012; 3(77)

15. Aragao MD, van der Linden V, Brainer-Lima AM, et al. Clinical features and neuroimaging (CT and MRI) findings in presumed Zika virus related congenital infection and microcephaly: retrospective case series study. BMJ. 2016; 353:i1901.

16. Soares de Oliveira-Szejnfeld P, Levine D, Melo AS, et al. Congenital brain abnormalities and Zika virus: what the radiologist can expect to see prenatally and postnatally. Radiology. 2016 Aug; 281(1):23. 203–18.

17. Driggers RW, Ho CY, Korhonen EM, et al. Zika Virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities. N Engl J Med. 2016; 374(22):2142–51. [PubMed: 27028667]

18. Martines RB, Bhatnagar J, de Oliveira Ramos AM, et al. Pathology of congenital Zika syndrome in Brazil: a case series. Lancet. 2016; 388(10047):898–904. [PubMed: 27372395]

19. Chimelli L, Melo ASO, Avvad-Portari E, et al. The spectrum of neuropathological changes associated with congenital Zika virus infection. Acta Neuropathologica. 2017; 133(6):983–99. [PubMed: 28332092]

20. Den Hollander NS, Wessels MW, Los FJ, et al. Congenital microcephaly detected by prenatal ultrasound: genetic aspects and clinical significance. Ultrasound in Obstetrics and Gynecology. 2000; 15(4):282–7. [PubMed: 10895445]

21. Ho C-Y, Ames HM, Tipton A, et al. Differential Neuronal Susceptibility and Apoptosis in Congenital ZIKV Infection. Annals of Neurology. 2017; doi: 10.1002/ana.24968 [PubMed: 28556287]

22. Metsky HC, Matranga CB, Wohl S, Schaffner SF, et al. Zika virus evolution and spread in the Americas. Nature. 2017; 546:411–415. [PubMed: 28538734]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

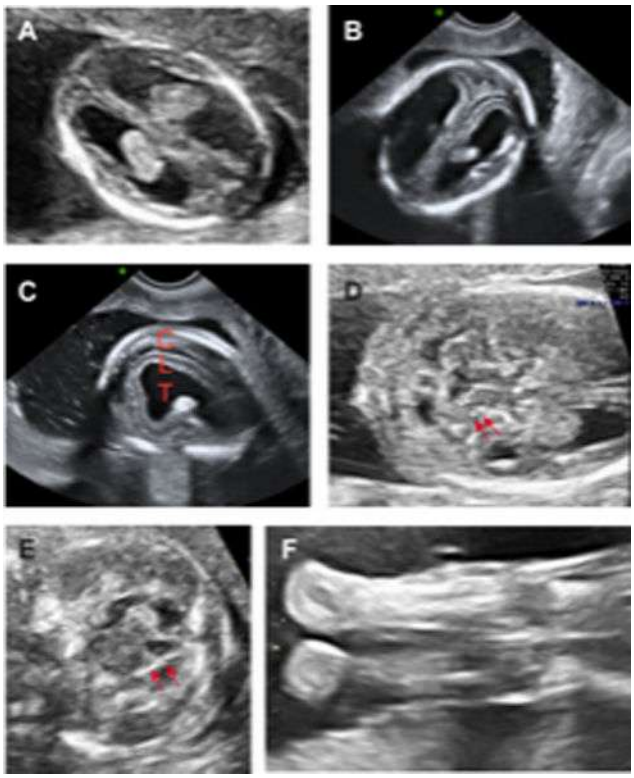

Figure 1. Fetal brain and limb abnormalities detected by prenatal ultrasound

Panel A and panel B showing thinning of cortical mantles and ventriculomegaly. Panel C shows dysgenesis of the corpus callosum (C) and calcifications in the genu of the corpus callosum. The third (T) and lateral (L) ventricles are annotated. Panel D shows transverse views of periventricular calcifications and panel E shows transverse views of cerebellar calcifications (red arrows). Panel F Coronal view shows hyperextension of the lower extremities.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

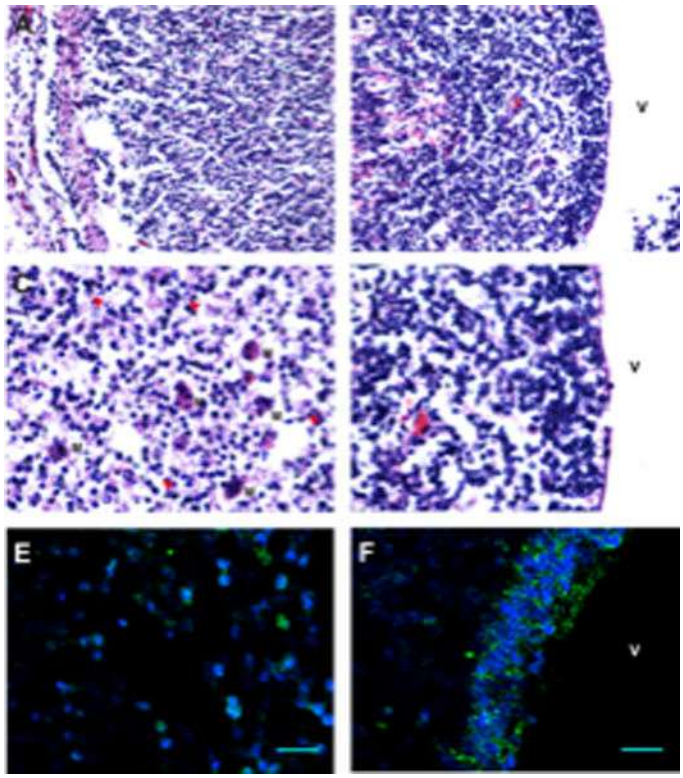

Figure 2. Neuropathological findings of intrauterine Zika virus (ZIKV) infection

Panels A, C and E show cortical plate demonstrating cell apoptosis (red arrowheads) and macrophage infiltrates (M) resulting from ZIKV infection. Panels B, D and F show ventricular and subventricular zone germinal matrix demonstrating evidence of ZIKV infection without apoptosis or inflammatory infiltrates. Lateral ventricles are designated with “V”. (A) and (B) Hematoxylin and eosin (H&E) staining, 200×. (C) and (D) H&E staining, 400×. (E) and (F) Immunofluorescent staining of flavivirus envelope protein (green). Nuclei stained with DAPI (blue). Scale bars: 20 μm.