

Presumed Zika virus-related congenital brain malformations: the spectrum of CT and MRI findings in fetuses and newborns

Malformações congênitas do encéfalo presumivelmente relacionadas ao Zika vírus: espectro de achados de RM e TC em fetos e neonatos

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ABSTRACT

The new epidemic of Zika virus infection raises grave concerns, especially with the increasingly-recognized link between emerging cases of microcephaly and this infectious disease. Besides small cranial dimensions, there are striking morphologic anomalies in the fetal brain. Key anomalies include cortical developmental malformations and a peculiar distribution of pathologic calcifications. These potentially indicate a new pattern of congenital central nervous system infection. **Methods:** Eight women underwent fetal MRI. Four infants also underwent postnatal CT. Five of the women underwent amniocentesis. **Results:** All neonates were born with microcephaly. On fetal MRI, ventriculomegaly, marked reduction of white matter thickness, severe sylvian fissure simplification, abnormal sulcation, and diffuse volumetric loss of cerebellar hemispheres were consistently seen. On postnatal CT, diffuse subcortical and basal ganglia calcifications were observed. The Zika virus was detected in two amniocenteses by polymerase chain reaction assays. **Conclusion:** We hope to assist the medical community in recognizing the spectrum of encephalic changes related to congenital Zika virus infection.

Keywords: Zika virus; microcephaly; tomography, X-ray computed; magnetic resonance imaging.

RESUMO

Os novos casos epidêmicos de infecção pelo vírus Zika suscitam grande preocupação, sobretudo com o crescente reconhecimento da ligação entre casos emergentes de microcefalia e esta doença infecciosa. Além da cabeça de pequenas dimensões, existem profundas alterações morfológicas no encéfalo fetal. Anomalias mais típicas incluem malformações do desenvolvimento cortical e uma distribuição peculiar de calcificações patológicas. Estes dados potencialmente indicam um novo padrão de infecção congênita do sistema nervoso central. **Métodos:** Oito mulheres foram submetidas a RM fetal. Quatro crianças também realizaram TC pós-natal. Cinco mulheres foram submetidas a amniocentese. **Resultados:** Todos os neonatos nasceram com microcefalia. Na RM fetal, ventriculomegalia, acentuada redução da espessura da substância branca, acentuada simplificação da fissura sylviana, sulcação anormal e redução volumétrica difusa dos hemisférios cerebelares foram constantes. Na TC pós-natal, calcificações difusas subcorticais e nos núcleos da base foram observadas. O vírus Zika foi detectado por PCR em duas amniocenteses. **Conclusão:** Esperamos dar suporte à comunidade médica em reconhecer este padrão de imagem potencialmente específico.

Palavras-chave: Zika vírus; microcefalia; tomografia computadorizada por raios X; imagem por ressonância magnética.

The Zika virus (ZIKV), a RNA virus of the flavivirus family¹, was first isolated in 1947 in Rhesus monkeys in a forest called Zika, in Uganda, Africa². Up until 2007, only a few cases of human infection with this virus were seen in Africa and Asia. Before that, its circulation was limited to monkeys. In Brazil, its transmission is by the *Aedes aegypti* and *Aedes albopictus*³ mosquito. Brazil has coexisted with the *Aedes aegypti* mosquito, brought in from African ships, since the country's

colonization. This species easily breeds in standing water. The tropical climate conditions, poor sanitation, and low education level provide an excellent means through which this virus perpetuates in Brazil⁴.

The first report of ZIKV infection in Brazil was in April 2015⁵. Clinically, the infection is similar to Dengue virus infection, a disease that has been present in the country for a long time. Headache, fever, body ache, fatigue, and skin spot

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lesions are some of the representative signs and symptoms. In ZIKV infection, conjunctivitis can also occur⁶. Parallel to the appearance of ZIKV infection in Brazil, there was also an explosion of cases of microcephaly associated with congenital brain malformations, which also occurred in French Polynesia. In addition, the virus was found in the amniotic fluid, tissues, and blood of a baby with microcephaly who died in the Brazilian state of Ceará⁷.

Although yet to be proven, both the Brazilian Ministry of Health and the World Health Organization admit the relationship between ZIKV and microcephaly because of the strong available epidemiological data. In 2014, there were 147 cases of microcephaly in Brazil⁸. From mid 2015 to late January 2016 there were 4, 783 suspected cases of microcephaly in the country⁹. Unfortunately, these numbers increase everyday. On February 1, 2016, the World Health Organization declared microcephaly, and other neurological disorders possibly associated with the ZIKV, an international public health emergency¹.

At the birth of affected children in Brazil, microcephaly was defined as a head circumference measuring less than 32 cm (12.5 inches) in term newborns, or below two standard deviations (SD) on the obstetric ultrasound¹⁰. Microcephaly can be divided into primary, when the brain is never formed normally, and secondary, in which the brain had its normal development halted at some point. Among secondary causes are infections caused by rubella, syphilis, cytomegalo virus, and herpes virus (STORCH)¹¹. It is part of the prenatal program in Brazil to perform serological tests (IGG and IGM) in order to rule out these diseases during pregnancy (except for herpes virus).

Magnetic resonance imaging (MRI) is an excellent imaging method for assessing the central nervous system (CNS) of fetuses with suspected defects. The absence of ionizing radiation and the ability for greater tissue differentiation allows for a better study of the CNS, without the limitations caused by skull artifact or fetal position, making it an adjuvant method for ultrasound^{12,13}.

The aim of this study is to describe the MRI findings of fetuses and newborns with microcephaly presumably caused by congenital ZIKV infection.

METHODS

This retrospective study was approved by our institutional review board and patient/parental consent was obtained. Patient characteristics are shown in Table 1. Eight women (mean age, 29.4 years; range, 19–41 years), mean gestational age, 31.2 weeks (28–38), with suspected fetal malformations on ultrasound possibly associated with the ZIKV, underwent fetal MRI at the University Hospital of the Universidade Federal do Ceará, during the Brazilian epidemic, between November 2015 and February 2016.

All patients reported a rash suggestive of a ZIKV infection in the first trimester. They were living in an epidemic area for the virus and/or had contact with family members or neighbors with the disease symptoms. They all had negative serology for STORCH. Amniocentesis was performed in five of the patients. All fetuses met the clinical-radiological criteria for presumed congenital ZIKV infection, as established by the protocols defined by the Brazilian Ministry of Health¹², even without being tested for IgM antibodies to the virus, since the test was not yet routinely available. These criteria were also used in recently-published studies^{15, 16, 17, 18, 19}. Four of the infants underwent a postnatal CT scan.

The following fetal biometric parameters were assessed on ultrasound: biparietal diameter and head circumference, according to the guidelines proposed by the International Society of Ultrasound in Obstetrics and Gynecology²⁰. Mild microcephaly was defined as a fetal head circumference between 2 and 3 SD below the expected mean for gestational age. Severe microcephaly was defined as a fetal head circumference of 3 SD below the expected mean value for gestational age²¹. If microcephaly was detected, a thorough ultrasound scan was performed to identify brain pathology such as midline-echo anomalies, intracranial calcifications, ventriculomegaly and/or extracranial malformations.

All the pregnant women had consultations with fetal medicine specialists and geneticists to investigate other possible causes of the CNS malformations, including detailed morphological ultrasound research for other congenital infections, previous use of teratogens, and family history of hereditary diseases. They were followed up in the institution through out the entire prenatal period.

MR and CT Imaging

Fetal MRI was performed with a 1.5 – T system (GE Healthcare, Chalfont St Giles, United Kingdom), using an abdomen coil. The MR protocol included fast imaging employing steady-state acquisition, repetition time msec/echo times msec, 3.8/1.7, section thickness 4 mm, intersection gap 4.4 mm, field of view 600–700 mm, matrix 512 x 512), T2-weighted single-shot fast spin-echo images (1240. 54/87. 8, thickness 4 mm, intersection gap 4.4 mm, matrix 512 x 512), T2* gradient-echo (467/15, thickness 4 mm, intersection gap 4.4 mm, matrix 256 x 256) and T1-weighted fast spoiled gradient-recalled acquisition in the steady state (100/2.5, thickness 4 mm, intersection gap 4.4 mm, matrix 512 x 512). We considered parenchymal calcifications at fetal MRI when marked T2-hypointense and T1-hyperintense and/or T2*-hypointense foci were seen at the cortex, basal ganglia, periventricular regions and/or cerebellum. Non-enhanced brain CT scans were performed on a 64-section scanner (SOMATON Definition AS; Siemens Healthcare, Forchheim, Germany), using the spiral technique (total collimation width, 9.2; slice thickness, 1.0 mm; pitch, 0.65). Brain parenchymal thickness was assessed with both CT and MRI at the level of the frontal horns of the lateral ventricles in the frontal regions.

Table 1. Patient characteristics.

Patient N°	Mother's age	GA at viral infection (weeks, referred symptoms)	GA at MRI diagnosis* (weeks, days)	HC (cm) at US; SD	GA at birth (weeks, days)	HC (cm) at birth	Baby's serology (IgM)			ZIKA PCR (Amniocentesis)
							TORCH	Chikungunya	Dengue	
1	19	7–10 (fever)	28	21.1; < 4	37.5	27	(-)	(-)	(-)	Detected***
2	29	8 (fever, rash)	31	24.0; < 4	40	24	No data	No data	No data	No data
3	31	8–10 (fever, rash)	31.5	20.0; < 5	38	20	(-)	(-)	(-)	Not detected
4	41	8 (fever, rash)	37	27.7; < 4	38	27	No data	No data	No data	No data
5	29	1st trimester; exact period unknown (rash)	38	27.5; < 4	38	29	(-)	(-)	(-)	Not detected
6	26	1st trimester; exact period unknown (fever, rash, joint pain)	28.2	22.8; < 5	37.1	22.8	(-)	(-)	(-)	Not detected
7	20	Asymptomatic	38	27.3; < 4/5	38.6	27.3	(-)	(-)	(-)	Detected***
8	35	8 (rash)	28	23.4; < 2**	39	24.6	No data	No data	No data	No data

GA: gestational age; HC: head circumference; US: ultrasound; MRI: magnetic resonance imaging; SD: standard deviation; PCR: polymerase chain reaction; TORCH: Toxoplasmosis, Rubella, Cytomegalovirus, and Herpes Simplex; * Microcephaly on US; ** At 37 weeks, HC was 27.4 cm; < 4 SD; *** Not detected in mother's serum or urine.

RESULTS

The ZIKV was detected in both amniocenteses by polymerase chain reaction test (PCR). All the neonates were born with microcephaly. The measurements of head circumference were on average < -3 SD from the mean for estimated gestational age (mean, 25.8 ranging from 20 to 29 cm) (Table 1). The deliveries occurred at a mean gestational age of 38 weeks (38–40); newborns had a mean weight of 2,695 g and a first minute Apgar of 8.

Ultrasound demonstrated reduced brain volume and ventriculomegaly in all cases; supratentorial parenchymal calcifications were depicted in five patients and cerebellar hypoplasia was seen in two patients. Extracranial abnormalities such as clubfoot, nuchal skin wrinkling, and intrauterine growth restriction were seen in two patients (Table 2). The earliest abnormalities depicted on ultrasound in our series were ventriculomegaly at 22 weeks and microcephaly at 26 weeks (Patient 8) – all other abnormalities were seen after the 28th week of gestation.

On fetal MRI, there was ventriculomegaly, marked reduction of white matter thickness, severe sylvian fissure simplification (grade II, Knaap et al.¹³ classification) and abnormal sulcation, with predominance of pachygyria (Figures 1, 2, 3 and 4). There was also diffuse volumetric loss of cerebellar hemispheres (Figures 1D, 2B and 2C), brainstem hypoplasia (Figure 1A), subependymal occipital pseudocysts (Figure 5) and hypogenesis of the corpus callosum (Figure 6A). Focal calcifications were depicted on T2* and T1 in two patients (Figure 4). Occipital pseudocysts were seen in four patients (Table 2).

The CT studies performed on four newborns showed decreased cerebral volume with ventriculomegaly and reduced parenchymal thickness, calcifications seen predominantly in the basal ganglia and the junction between cortical

and subcortical white matter, and cerebral development malformations, including opercular dysplasia, pachygyria, lissencephaly and polymicrogyria (Table 3; Figure 6).

DISCUSSION

We have described the MRI findings of brain abnormalities in eight fetuses with presumed ZIKV-related congenital infection, of whom two tested positive for the virus, confirmed by PCR in the amniotic fluid. Even though not all cases were serologically confirmed, they met the protocol criteria for congenital infection presumably associated with the ZIKV as proposed by the Brazilian Ministry of Health¹⁴, which included 1) reported symptoms of the disease; 2) mothers who were living in an epidemic region; and 3) mothers who had negative serology for other CNS infections (TORCH). Incomplete serological confirmation was due to the unavailability of serological tests at the time of the diagnosis of the reported cases herein, as well as the difficulty of performing PCR and virus detection by this technique during the short period of viremia²². Other authors have also considered the same criteria in the presentation of their results^{15, 16, 19}.

Brain changes caused by infectious agents during pregnancy differ from those that occur in children and adults because they occur during nervous tissue development. Manifestations of these infections will differ depending on the time when they occur and are less related to the virulence of the agent. Thus, in general, infections during the first two trimesters will result in congenital malformations, while those occurring in the third trimester will result in destructive lesions²³.

The term TORCH is an acronym created to denote *Toxoplasma gondii*, rubeolla, cytomegalovirus and herpes viruses, and emphasizes these agents as a major cause of

Table 2. Imaging findings on fetal MRI and obstetric ultrasound.

Patient N°	GA at imaging (weeks, days)*	Fetal MRI (Other than decreased brain volume, ventriculomegaly, simplified gyral pattern, and corpus callosum hypogenesis)							US (other than reduced brain volume and ventriculomegaly)			
		MCD†	Calcifications††	Brainstem hypoplasia	Cerebellar hypoplasia	Enlarged cisterna magna	Transverse cerebellar diameter (cm), percentile	Frontal parenchymal thickness (mm)	Occipital pseudocysts	Parenchymal calcifications	Other CNS findings	Extracranial findings
1	28	Yes	No	No	No	No	3.5, 50th	7.4	No	Yes	Oligoamnios	-
2	31	Yes	No data	No	No	No	3.8, 50th	9.1	Yes	Yes	-	-
3	31,5	Yes	No data	Yes	Yes	Yes	2, < 5th	3.2	Yes	Yes	Cerebellar hypoplasia	Right clubfoot, nuchal skin wrinkling
4	37	Yes	No data	No	No	No	4.2, 50th	10**	No	Yes	-	-
5	33	Yes	Yes	Yes	Yes	Yes	3.6, < 5th	9.5	Yes	Yes	-	IGR
6	28,2	Yes	No data	Yes	Yes	Yes	2.8, < 5th	4.9	No	No	Cerebellar hypoplasia, enlarged cisterna magna	-
7	38	Yes	No data	Yes	No	No	4.5, 50th	6.1**	No	Yes	-	-
8	28	Yes	Yes	Yes	Yes	Yes	2.3, < 5th	4.0**	Yes	Yes	Cerebellar hypoplasia	-

GA: gestational age; MCD: malformation of cortical development; IGR: intrauterine growth restriction; US: ultrasound; MRI: magnetic resonance imaging; CNS: central nervous system; *US and MRI were performed at same GA; **Asymmetrical; † Opercular dysplasia, pachygyria and / or polymicrogyria; †† calcifications were not assessed because T1-weighted and susceptibility magnetic-weighted imaging were not performed.

perinatal and congenital infections in humans. These agents remain the main causative factor of permanent damage to CNS, despite advances in vaccines and antiviral therapies. In addition to these agents, other less common viruses include lymphocytic choriomeningitis virus, parechovirus, and varicella-zoster virus²³. All of these agents can cause irreversible damage to the CNS, the aspects of which can be very similar to each other, often making impossible the etiological diagnosis exclusively by imaging. The most common changes are microcephaly, ventriculomegaly, neuronal migration abnormalities, white matter changes, and abnormal CNS calcifications.

The alterations found in the fetuses and infants with suspected congenital ZIKV infection described herein demonstrated very similar patterns of change to those described in the TORCH syndrome with lissencephaly, sulcation abnormalities (including polymicrogyria and pachygyria), ventriculomegaly, and cerebral calcifications. The distribution of calcifications (basal ganglia, periventricular, and in the cortical and subcortical white matter junctions) seen in our patients is similar to the pattern described in cytomegalovirus infection, although the latter more commonly affects the periventricular region and the former the cortical and subcortical white matter junctions^{15, 24}. The only available methodology at the time of examination for a presumptive diagnosis of ZIKV infection was the negativity of serology for those agents already known, besides the positive epidemiological history of rash during pregnancy.

Our findings were consistent with the recently-published case series by Aragao et al.¹⁵, who described postnatal CT and MR findings in 23 children with presumed ZIKV infection. Besides the similar pattern of distribution of calcifications described here, malformation of cortical development with predominance of pachygyria or polymicrogyria in the frontal lobes and delayed myelination were seen. As well, Guillemette-Arturet et al.²⁵ recently reported very similar findings in a three-patient series using fetal MRI, including subependymal pseudocysts.

Although ocular findings were described in infants with microcephaly due to vertical ZIKV infection, particularly affecting the macular region, these ophthalmic alterations were not expressed on MRI or CT²⁶.

Mlakaret al.²⁷ recently published a case report describing the termination of pregnancy due to similarly severe brain malformations described herein. The ZIKV was isolated in brain parenchyma with absence in other fetal tissues, thereby demonstrating the neurotropism of this virus. This author also performed genetic sequencing of the virus and found similarity of the circulating viral strains in Brazil to those in Asia.

The possible relationship of ZIKV infection during pregnancy and the aforementioned CNS changes gives rise to great concern for global public health, as this virus presently does not have any effective treatment. Moreover, the spread of the mosquito vector in all parts of Brazil, and other countries in Latin America, Africa, Asia and Oceania, raises the possibility

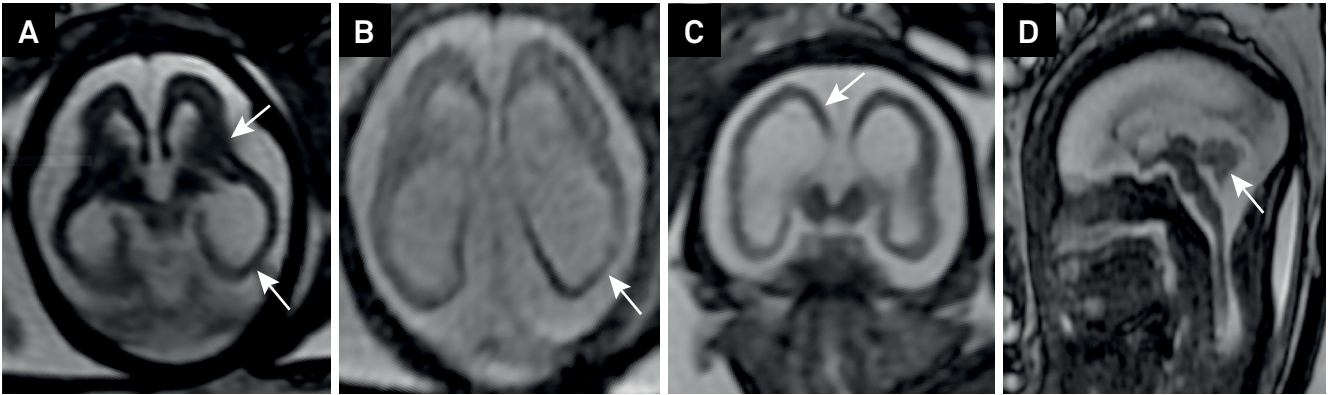


Figure 1. MR images of Patient 6 at 28.2 weeks of gestation (A, C, and D, fast imaging employing steady-state acquisition on axial, coronal and sagittal planes; B, T2-weighted single-shot fast spin-echo image on axial plane) demonstrating microcephaly and bilateral ventriculomegaly with marked reduction of white matter thickness, simplified gyri with shallow sylvian fissures (arrows in A–C) and cerebellar hypoplasia (arrow in D). Enlarged cisterna magna and brainstem hypoplasia are also noticed.

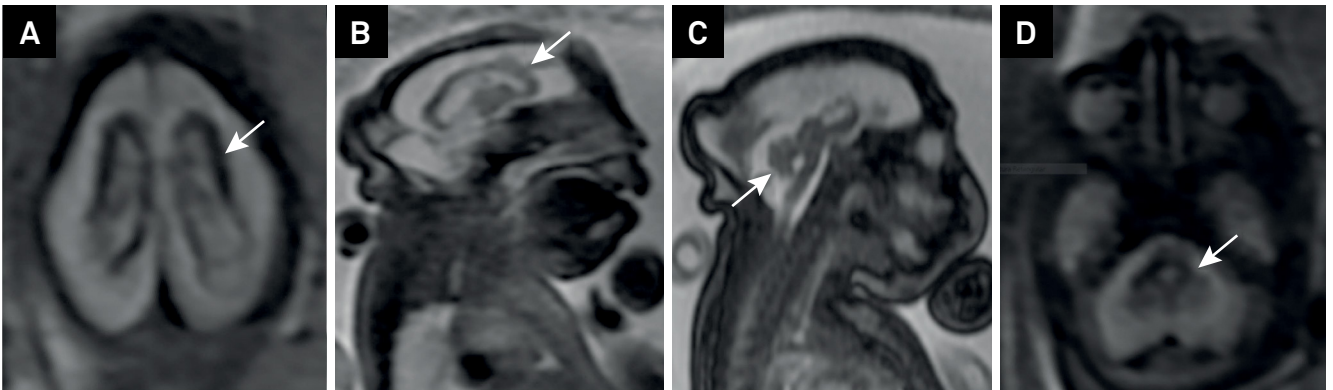


Figure 2. MR images of Patient 3 at 31.5 weeks of gestation (A, axial T2-weighted single-shot fast spin-echo image and fast imaging employing steady-state acquisition on axial, B, and sagittal planes, C and D). There is severe microcephaly, ventriculomegaly, and simplified gyral pattern with pachygyria (arrows in A and B). Brainstem hypoplasia, enlarged cisterna magna, and cerebellar hypoplasia (arrows in C and D) can also be seen.

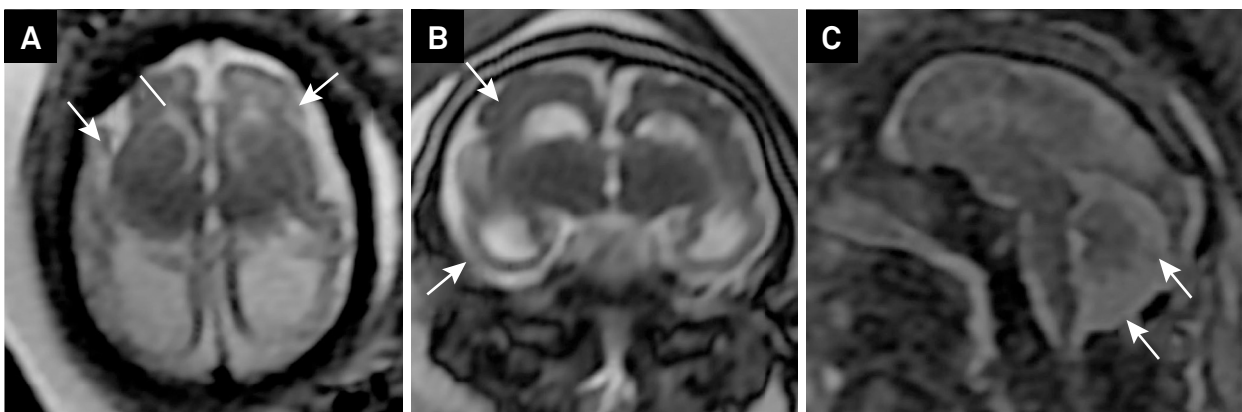


Figure 3. Patients with ZIKV detected by PCR in the amniotic fluid. MR images of Patient 7 at 38 weeks of gestation (A, T2-weighted single-shot fast spin-echo image on axial and B, fast imaging employing steady-state acquisition on axial plane) and of Patient 1 at 28 weeks of gestation (C, T2-weighted single-shot fast spin-echo image on coronal plane). Reduced parenchymal thickness, ventriculomegaly, opercular dysplasia, and simplified gyral pattern with pachygyria are consistently seen (arrows in A and B). The vermian size is within the normal range for the gestational age; enlarged cisterna magna is noticed (arrows in C). Figure A shows the location where the frontal parenchymal thickness was measured, at the level of the frontal horn of the lateral ventricle.

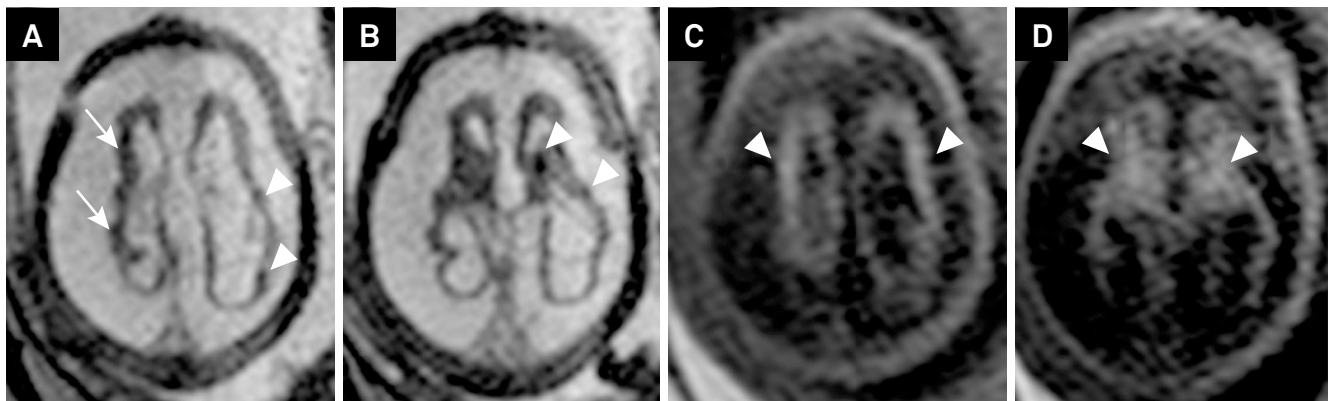


Figure 4. MR images of Patient 8 at 28 weeks of gestation (A and B, T2-weighted single-shot fast spin-echo images on axial and sagittal planes, C and D, T1-weighted images on axial plane) demonstrating microcephaly and bilateral asymmetrical ventriculomegaly. The cortex is irregular (arrows in A) suggesting polymicrogyria. The marked T2-hypointense and T1-hyperintense foci (arrowheads) of the cortex and basal ganglia are consistent with calcifications.

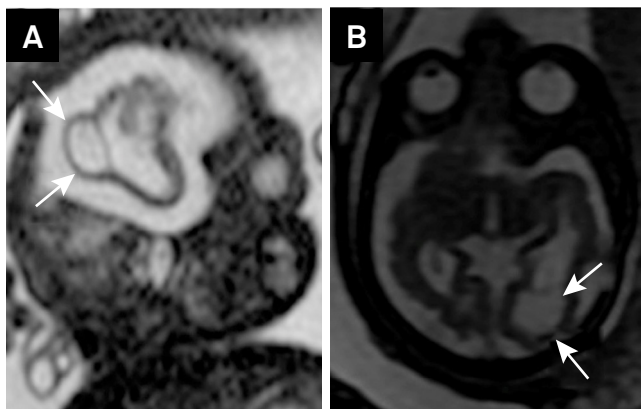


Figure 5. MR images of Patient 8 (A, T2-weighted single-shot fast spin-echo images on sagittal plane) and Patient 2 (B, fast imaging employing steady-state acquisition on axial plane) at 28 and 31 weeks of gestation, respectively, demonstrating the subependymal occipital pseudocysts (arrows).

of a pandemic of congenital brain malformations and microcephaly with incalculable social and economic consequences.

While evidence of the association between the ZIKV and fetal brain abnormalities continues to grow, the period of infection during fetal development that may be susceptible to viral pathogenesis, and how it affects the appearance of brain abnormalities on imaging, are currently poorly understood. Reported evidence suggests a predilection of the ZIKV strain for human neural lineage cells. Histopathological findings in a mid gestational fetus included loss of intermediately-differentiated postmigratory neurons through an apoptotic mechanism, with preservation of more differentiated neurons in the basal ganglia, limbic region, dorsal spinal cord and germinal matrix²⁸. A recent study has also shown a high rate of ZIKV infection in cortical neural progenitor cells, but not in embryonic or pluripotent stem cells²⁹.

When the outbreak of the Brazilian epidemic occurred, ZIKV laboratory assays were not routinely available; thus,

unfortunately, they were not performed in our patients. However, even at present, the clinical presentation of ZIKV infection can be misleading – it is estimated that 80% of ZIKV infections are asymptomatic²⁸, being difficult to diagnose and distinguish from other viral infections³⁰. The gold standard for identification of viral infections is the isolation of the etiological agent in culture, but it requires a high viral load and the right timing for sample collection, since the ZIKV remains circulating in blood for up to five days after the onset of symptoms. A precise and fast diagnosis of an ongoing infection can be achieved by performing a reverse transcription polymerase chain reaction. ZIKV RNA and viable viruses have also been detected in urine, saliva, semen and vaginal secretion for up to 30 days, making these specimens an alternative for late diagnosis^{30, 31}.

We have demonstrated fetal MRI brain abnormalities of the recently-named congenital Zika syndrome, providing an association of the presumed affected cases with the traditional findings of CNS infections. The association of microcephaly, ventriculomegaly, a simplified gyral pattern, and calcification distributed predominantly in the cortical and subcortical white matter can be demonstrated in fetal MRI, which shows better details than the findings from an ultrasound. These abnormalities are highly suggestive of ZIKV infection, when epidemiological criteria are met, thus allowing a more accurate diagnosis of CNS damage. In addition, because it does not use ionizing radiation and provides a greater tissue differentiation than ultrasound, MRI is preferably used to better evaluate CNS disorders. Future research should focus on the description of serologically-confirmed cases in which changes can be specifically linked to ZIKV. Moreover, there likely will be differences described in individual patients that may be associated with possible changes in clinical presentations and development of these children, most of whom are very seriously affected. We also need to know how these changes will evolve over the long term.

Table 3. Imaging Findings on Postnatal CT (Other than simplified gyral pattern and corpus callosum hypogenesis).

Patient n°	Age at imaging	Decreased brain volume	Ventriculomegaly	MCD	Location of calcifications					Brainstem hypoplasia	Cerebellum	Enlarged cisterna magna	Frontal parenchymal thickness (mm)
					Periventricular	Basal ganglia	Junction between cortical and subcortical white matter	Brainstem	Cerebellum				
1	3m11d	Yes (mild)	Yes*	Yes**	(+)	(+++)	No	No	No	No	No	9.2	
3	2m11d	Yes (severe)	Yes	Yes**	(++)	(+++)	(+)	No	Yes	Yes	Yes	4.5	
5	5d	Yes (mild)	Yes	Yes***	(+++)	(++)	No	No	Yes	Yes	Yes	9.7	
6	5d	Yes (severe)	Yes	Yes**	(+++)	(+)	No	(+)	Yes	Yes	Yes	8.9	

CT: computed tomography; MCD: malformation of cortical development; *Asymmetrical; **Opercular dysplasia, pachygyria and polymicrogyria; ***Opercular dysplasia, lissencephaly, polymicrogyria and pachygyria.

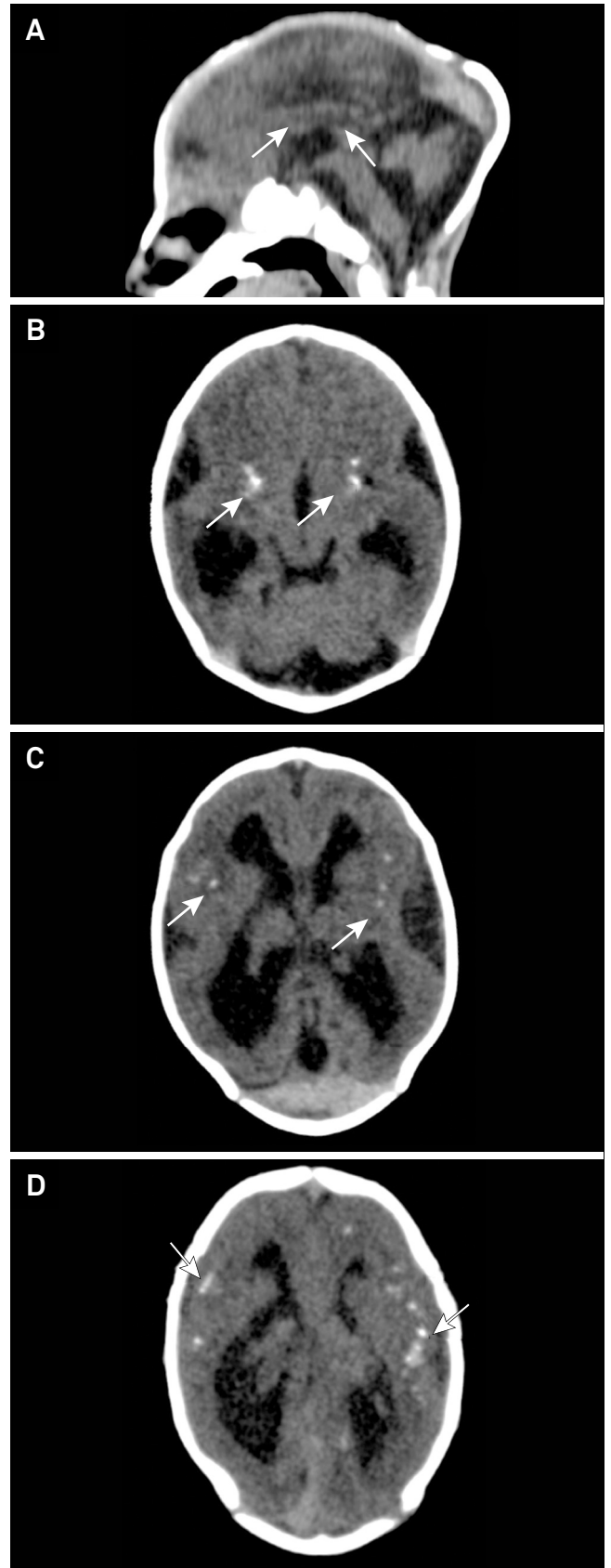


Figure 6. Postnatal CT of Patient 5 at five days (A–C, sagittal and axial planes) and Patient 1 (D, axial plane) showing microcephaly, corpus callosum hypogenesis (arrow in A) and the distribution of calcifications, which are predominantly in the cortical and subcortical white matter junction (arrows in C and D) and basal ganglia (arrow in B).

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