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Discordant clinical outcomes of congenital Zika virus infection in twin pregnancies

Evolução clínica discordante da infecção congénita do vírus Zika em gestação gemelar

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ABSTRACT

Congenital Zika syndrome is an emergent cause of a congenital infectious disorder, resulting in severe damage to the central nervous system and microcephaly. Despite advances in understanding the pathophysiology of the disease, we still do not know all the mechanisms enrolled in the vertical transmission of the virus. As has already been reported in other types of congenital infectious disorders in dizygotic twin pregnancies, it is possible that the virus affects only one of the fetuses. In this article, we report on two cases of twin pregnancies exposed to the Zika virus, but with only one of the fetuses affected with microcephaly and brain damage. This indicates the urgent need for more studies regarding the pathophysiology of viral infection and the mechanisms involved in the natural protection against the virus.

Keywords: Zika virus; Zika virus infection; twins, dizygotic.

RESUMO

A síndrome congênita do Zika vírus é uma causa de infecção congênita emergente, resultando em graves danos ao sistema nervoso central e microcefalia. Apesar dos avanços na compreensão da fisiopatologia da doença, ainda não conhecemos todo o mecanismo envolvido na transmissão vertical do vírus. Como já foi relatado em outros tipos de infecções congênitas em gestações gemelares dizigóticas, é possível que apenas um dos fetos seja afetado pelo vírus. Este artigo descreve 2 casos de gestações gemelares expostas ao vírus Zika, onde apenas um dos fetos foi afetado, com microcefalia associado a graves danos no sistema nervoso central. Isso indica a necessidade urgente de mais estudos sobre a fisiopatologia da infecção viral e os mecanismo envolvidos na proteção natural contra o vírus.

Palavras-chave: Zika virus; infecção pelo Zika virus; gêmeos dizigóticos;

Gestational Zika virus (ZIKV) infection has been robustly associated with a well-delimited congenital syndrome including microcephaly and specific neuroradiological abnormalities, defining the congenital Zika syndrome (CZS)[1,2,3]. Other symptoms have also been described in association with the syndrome, such as ophthalmologic lesions, hearing loss and arthrogryposis[4,5,6]. Like other congenital viral infections, only a proportion of gestational ZIKV results in children affected by CZS. Reports of discordant clinical outcomes for twin pregnancies with gestational infections have been reported

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for cytomegalovirus and toxoplasmosis[7,8]. We describe two cases of twin pregnancy with gestational infection by ZIKV, in which only one fetus was born with CZS.

METHODS

Data collection

A standard form was used to collect demographic and clinical data, including the recollection of a rash during pregnancy.

All investigations described were conducted as part of the clinical protocol or clinical indication; no investigations were conducted for research reasons. Informed consent was obtained from the participants or their legally authorized representatives.

Laboratory tests

Serologic tests were performed on both mother and newborns to exclude the main differential diagnoses of CZS *(i.e.*, other congenital infections that lead to brain calcifications and microcephaly), which are cytomegalovirus, toxoplasmosis, rubella, syphilis and HIV. When cytomegalovirus IgG was present in both mother and child, real-time polymerase chain reaction was performed in urine or blood.

The cerebrospinal fluid (CSF) of patients was tested for IgM antibody capture enzyme-linked immunosorbent assay (ELISA) for ZIKV, following the Center for Disease Control and Prevention (CDC) protocol, as described by Martin et al.[9] Laboratory confirmation was considered as a positive ZIKV-specific IgM in CSF with capture ELISA, according to the CDC Emergency Use Authorization protocol with reagents by Robert Lanciotti (CDC, Fort Collins, CO, USA), as recently published by Cordeiro et al.[10]

Clinical evaluation

For clinical evaluation, microcephaly was defined as a head circumference two standard deviations below the mean for gestational age and sex, according to the Fetal International and Newborn Growth Consortium for the 21st Century (Intergrowth-21st) for newborns and the World Health Organization child growth standards for infants[11,12]. Birth weight was classified as appropriate, small or large for gestational age and sex according to the Intergrowth-21st curve[11].

All the infants underwent neurologic, orthopedic, ophthalmological, and hearing evaluations, including clinical examination and ancillary exams. The infant patients had brain imaging by non-contrasted computerized tomography (CT) and simple radiography of the hips. Clinical assessment of dysphagia was made by a speech therapist. Audiometric screening was carried out by auditory brainstem response audiometry, and confirmed by diagnostic tests (confirmatory frequency-specific auditory brainstem response with tone burst stimuli and behavioral audiometry) using the routine recommended by the Brazilian Heath Ministry and the American Academy of Pediatrics' Joint Committee on Infant Hearing[13].

CASE REPORTS

Patient 1

A boy, born in 2015 in Recife, State of Pernambuco, from a dizygotic twin pregnancy. His mother had an episode of skin rash associated with itching and fever in the first month of gestation. At the 25th week of pregnancy, microcephaly was diagnosed by obstetric ultrasonography, but no anomaly was detected by this method in the twin brother. Delivery occurred at a gestational age of 37 weeks, with the newborn weighing 2,100 g; the head circumference was 28 cm (3 SD from the gestational age and sex, classified as severe microcephaly). The patient also presented with craniofacial disproportion, closed anterior fontanelle, exuberant occipital protuberance, redundant scalp skin and a right clubfoot. Brain CT revealed diffuse bilateral reduction of cerebral parenchyma, ventriculomegaly, cortical underdevelopment, multiple calcifications predominantly in the basal ganglia and cortical/subcortical white matter regions, and hypoplasia of the brainstem and cerebellum (Figure 1).

First eye exam (13 days after birth): clear cornea, depth anterior chamber, phakic, isocoria, pathologic red reflex test both eyes. High myopia (-12.00 OD and -9.00 OS), vitreous haze, bilateral staphyloma chorioretinal lesions (sharply demarcated atrophy) involving the posterior pole and optic disc OD and only macula in OS. After three months of age, developed congenital glaucoma OD and underwent surgery.

Last exam (one year old): ocular pressure under control, clear cornea, stable myopia, fundus findings were the same, clear vitreous.

Auditory evaluation by frequency-specific auditory brainstem response and behavioral audiometry detected bilateral profound hearing loss. After an unsuccessful attempt at rehabilitation with hearing aids and speech therapy, a cochlear implant was scheduled for this child.

At seven months of age, he started presenting with spasms in clusters. The pattern of EEG was focal, with discharge in the frontal lobe at first, and at 12 months of age, the EEG presented with multifocal discharge. The seizures were controlled with valproate. At the age of 12 months he presented with neurodevelopmental arrest, with no interaction with the environment and no head control. An X-ray of the hips showed left hip dysplasia.

The development and neurological evaluation of his brother was normal at 10 months of age.

Patient 2

Dizygotic twins, a girl and a boy, were born in Recife, State of Pernambuco, with a history of maternal fever and rash associated with itching at three months of pregnancy. Microcephaly

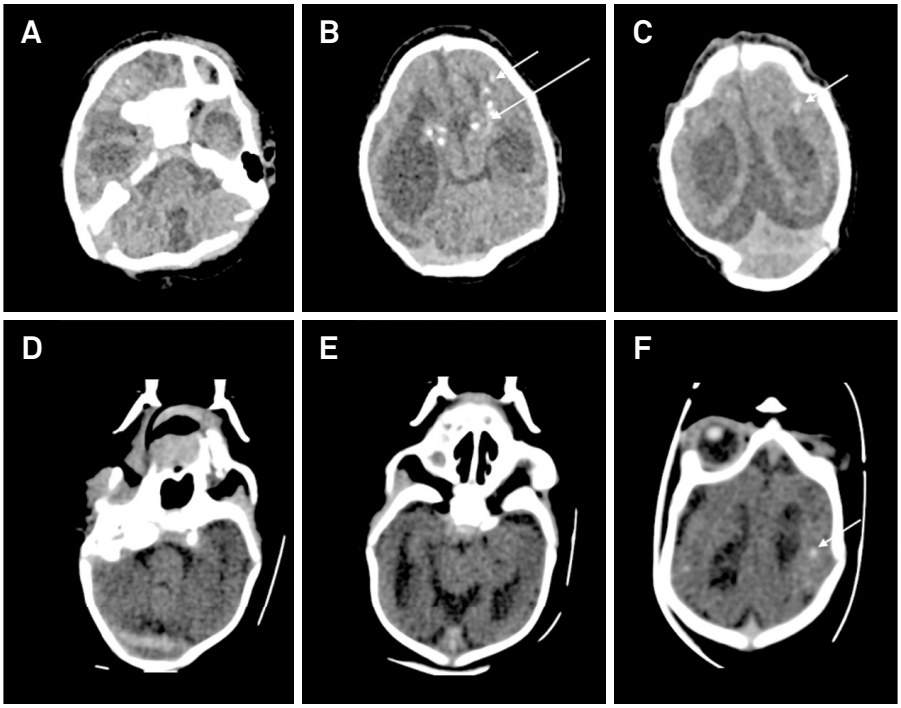

Figure 1. A, B and C are brain computerized tomography from Patient 1 and D, E and F are from Patient 2. A and D show the brainstem and cerebellum hypoplasia; B, C, E and F show diffuse bilateral reduction of cerebral parenchyma, ventriculomegaly and cortical underdevelopment; B, C and F show calcifications in the basal ganglia (long arrow) and cortical/subcortical white matter regions (short arrow).

was diagnosed at 32 weeks of pregnancy by ultrasonography in the female twin. After delivery at 35 weeks, the female newborn had a head circumference of 26 cm (below 3 SD for the gestational age and sex) and weighed 1,750 g. The same craniofacial findings observed in Patient 1 were present. Brain CT revealed diffuse bilateral reduction of cerebral parenchyma, ventriculomegaly, malformation of cortical development, multiple calcifications in cortical/subcortical white matter regions and mild hypoplasia of the brainstem and cerebellum (Figure 1). The anterior and posterior segments of the eye were normal in the ophthalmological assessment. The auditory evaluation was normal.

Epilepsy was diagnosed at six months of age, with the same seizure pattern, but we are still waiting for the EEG. Her seizures were reduced with levetiracetam. At the last evaluation, at seven months of age, she presented with severe neurodevelopmental delay, with no interaction with the environment and no head control. The X-ray of the hips was normal.

The development and neurological evaluation of her brother were normal at seven months of age.

In these two cases, screening tests for the most common causes of congenital infection (toxoplasmosis, cytomegalovirus, rubella, syphilis and HIV) were negative. The CSF sample was tested by IgM antibody capture ELISA for ZIKV and it was positive. And, in both cases, the unaffected twin was normal on clinical examination, and blood and CSF tests for ZIKV were negative.

Tables 1 and 2 summarize the main findings of the two newborns.

DISCUSSION

This article describes two twin siblings exposed to the Zika virus during pregnancy, but only one of the siblings presented with a typical picture of CZS. The craniofacial aspects of our patients (Figure 2) were described by Russel et al. as a fetal brain disruption sequence[14]. The fetal brain disruption sequence phenotype is hypothesized to be a result of loss in brain volume and decrease in intracranial pressure, and it is not specific to the etiologic agent. Moore et al. described the fetal brain disruption sequence phenotype related to CZS[15].

The brain imaging of the two patients, showing calcifications predominantly in the subcortical region, with abnormalities of cortical development, was consistent with the pattern fully described by Aragão et al. for CZS[3]. The diagnosis of this syndrome was based on the neuroimaging findings, exclusion of other congenital infections and the presence of positive IgM in the CSF of the two affected children.

Only one patient presented with ophthalmic and auditory abnormalities. Congenital infection due to presumed ZIKV exposure has been shown to be associated with vision-threatening findings, such as the bilateral macular and optic nerve abnormalities seen in the first patient of this study. Glaucoma has been described in one infant from Bahia, Brazil[5,16].

Congenital infection by ZIKV is a new condition and the real risk of mother-fetus transmission of this virus is still unknown. Nishiura et al. tried to estimate a theoretical risk of microcephaly occurrence from congenital

Table 1. Clinical findings

Variable	Case	
	1	2
Sex	M	F
Abnormalities gestational ultrasonography	yes, 25 w	yes, 32 w
ZIKV IgM antibody in CSF	positive	positive
Gestational age at birth	36 w	35 w
Weight at birth (g)	2100	1750
Birth weight for gestational age	Appropriate	Appropriate
Head circumference at birth (cm)	28	26
Microcephaly	yes, severe	yes, severe
Thoracic circumference at birth (cm)	no data	24
APGAR – 1 min / 5 min	9/10	9/10
Rash during pregnancy	yes	yes
Gestational age of cutaneous rash	1 month	3 months
Craniofacial disproportion	yes	yes
Exuberant occipital protuberance	yes	yes
Redundant scalp skin	yes	yes
Irritability during the first months of age	no	yes
Hyperexcitability	yes	yes
Hip dysplasia	yes	no
Other malformations	club foot	no

M: male; F: female; w: weeks; ZIKV: Zika virus; CSF: cerebrospinal fluid; min: minutes.

Table 2. Neurological findings.

Variable	Patient	
	1	2
Age of testing	12 mo	7 mo
Corrected age	11 months	6 months
Head circumference	38 cm	34 cm
Microcephaly	yes	yes
Weight	8020g	
Visual fixation and pursuit	no	no
Interaction with the environment	no interaction	no interaction
Strabismus	yes	yes
Nystagmus	yes	yes
Social smile	no	no
Head control	no	no
Sitting without support	no	no
Grasp	grasping reflex	grasping reflex
Asymmetric tonic neck reflex	present	present
Muscle tone	Limb hypertonia with pyramidal and extrapyramidal signs	Limb hypertonia with pyramidal and extrapyramidal signs
Dysphagia	yes, moderate	yes, moderate
Epilepsy	yes	yes

ZIKV and assumed that it could be of at least 14.0%[17] For Ellington et al.[18], following the Puerto Rico outbreak, the risk of microcephaly ranged from 1% to 13% for maternal infection in the first trimester, up to 0.7% in the second trimester, and up to 0.2% in the third trimester.

There are many reports in the literature about congenital infections in twin pregnancy affecting only one sibling, most of them dizygotic[7,8,19,20,21,22]. Maternal factors related to immunologic competency may explain why some mothers transmit the virus to the fetus, causing neurologic damage, and others do not. However, this cannot explain the differences in the outcomes of the children in twin pregnancies. One possible explanation for this is the differences in fetal susceptibility. Lazzarotto et al. showed that twin fetuses may react differently to primary maternal cytomegalovirus infection, in spite of being exposed to the same maternal influences[7]. A few reports

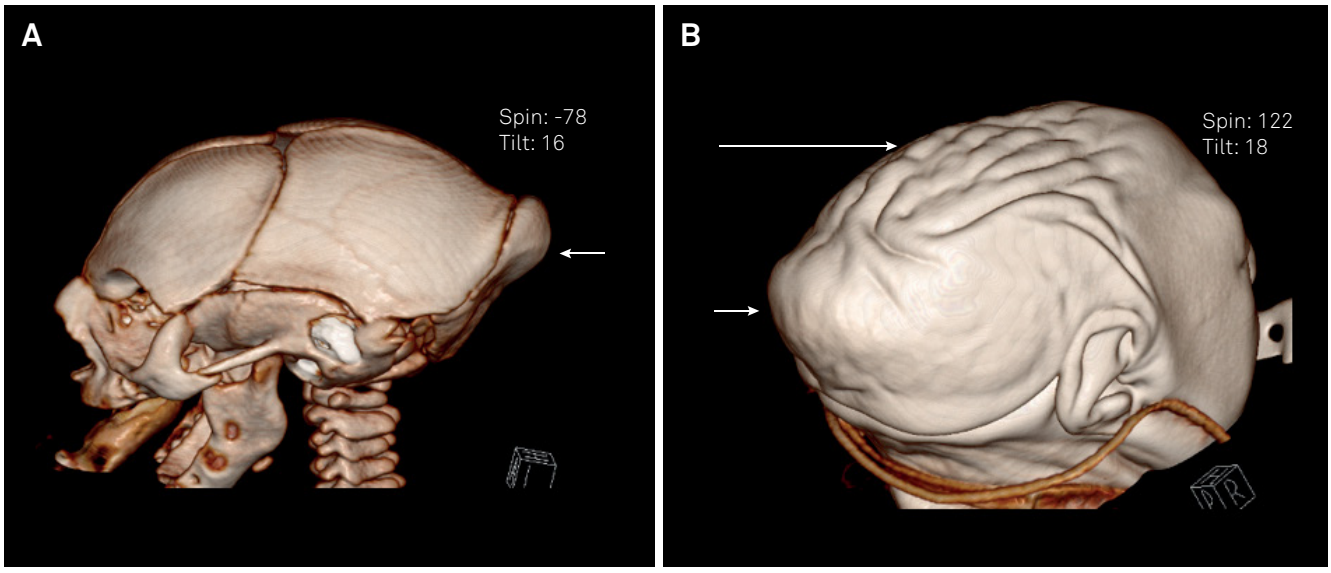

Figure 2. A and B show the computed tomography of the skull with reconstruction, of Patient 1, with the typical phenotype of fetal brain disruption sequence characterized by microcephaly with exuberant occipital protuberance (short arrow) and redundant skin on the scalp (long arrow).

of different outcomes in monozygotic twins in cytomegalovirus-affected pregnancies corroborate this theory[23,24].

Another explanation is the differences in placental function in dizygotic twin pregnancies. Although the placenta acts as a portal for mother-fetus transmission of viral diseases, it is not completely permeable, as demonstrated by Fowler et al., who found fetal contamination in only 40% of cases of gestational cytomegalovirus[25].This placental barrier function can be explained by several mechanisms, but it is still not completely understood how, in various cases of twin pregnancies with viral infection, only one of the fetuses is affected[7,26]. In the patients presented, the placentas were not studied. The comparison of the placentas from the normal and the affected child could throw some light onto the question of distinct outcomes.

Despite the advances in understanding the pathophysiology of the disease, we still do not know all the mechanisms enrolled in vertical transmission of the ZIKV virus. As has already been reported in other types of congenital infectious disorders in dizygotic twin pregnancies, it is possible for the virus to affect only one of the fetuses. This indicates the urgent need for more studies regarding the pathophysiology of the viral infection. Other possible variants, like genetic factors, viral tropism and the placenta barrier could influence the pathophysiology of the CZS and must be investigated in further studies.

There was no pathology study of the placentas in this patient series. This could be important to define whether the virus compromised the placenta of the non-affected child or not. This information is crucial to understand whether the placenta is the most important barrier against the virus invasion, or if intrinsic fetal factors are more important for this particular protection. There is lack of evidence on how exactly the virus disseminates through the pregnant body, how it reaches the fetus, and which type of barrier could influence this mechanism. Further genetic studies may elucidate if there are genes involved in specific protection against external agents.

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