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Obstetric and perinatal outcomes in cases of congenital Zika syndrome

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Abstract

Objective: To describe obstetric and perinatal outcomes in cases of congenital Zika syndrome (CZS).

Methods: A dual prospective and retrospective cohort study involving 102 pairs of mothers and fetuses/children with CZS whose infection was confirmed by testing for the Zika virus in amniotic fluid, umbilical cord blood, and fragments from the placenta of the newborn infant (confirmed CZS), or by intrauterine imaging tests (neurosonography), and/or postnatal computed tomography (presumed CZS).

Results: Suspicion of CZS was investigated by ultrasonography during pregnancy in 52.9% of cases. The principal prenatal imaging findings were ventriculomegaly (43.1%) and microcephaly (42.2%). Median gestational age at delivery was 39 weeks, with 15.7% being premature. Mean head circumference at birth was 30.0 ± 2.3 cm, with 66% of cases being classified as having microcephaly. Arthrogryposis was found in 10 cases (9.8%). There were no fetal deaths; however, nine neonatal deaths were recorded, and three autopsies were performed.

Conclusion: Neonatal mortality was high, almost 10%. Regarding the abnormalities of CZS, microcephaly, although common, was not present in all cases and intracranial findings need to be taken into consideration for diagnosis. Therefore, ultrasound screening during pregnancy should be systematized and expanded in endemic zones.

1 | INTRODUCTION

At the end of 2015, Brazil faced a critical situation characterized by an alarming increase in the number of newborn infants with microcephaly that was first described in the state of Pernambuco.[1] Concomitantly, in the state of Paraiba, an increase was seen in the number of cases of fetuses with severe brain damage characterized by reduced brain volume, microcephaly, and calcifications, particularly subcortical, and in the basal nuclei and thalamus, in addition to ventriculomegaly and cerebellar hypoplasia, with the presence of Zika virus (ZKV) being detected in amniotic fluid.[2,3] In 2016, congenital Zika syndrome (CZS) was first described.[4]

Several studies were subsequently published in 2016 and 2017, characterizing CZS and describing other findings such as visual, auditory, and motor abnormalities, including arthrogryposis, as well as digestive abnormalities (dysphagia).[5-9] Nevertheless, the difficulty involved in obtaining a confirmatory laboratory diagnosis of the infection during pregnancy still obstructs the understanding of the extent of the epidemics and the complete characterization of the syndrome.[10]

Another aspect hampering the understanding of the magnitude of the disease was the rapid reduction in the number of cases.[11] The first epidemics occurred in Yap Island in 2007 and then in French Polynesia where it ended rapidly. The disease reached Brazil at the end of 2014,[3] then other countries of the Americas in 2016, the African continent and Asia in 2018,[12] and India in 2019.[13] Most studies have focused on describing clinical findings in fetuses and/or children, with little being discussed on the clinical implications of ZIKV infection for the pregnant woman or on fetal imaging abnormalities and immediate neonatal findings, including death and histopathology results.

The principal objective of the present study was to describe obstetric and perinatal outcomes in cases of CZS followed up at a referral center in northeastern Brazil.

What does this work add to what is already known?

- The high prevalence of unsuspected and undiagnosed cases of Zika virus infection during pregnancy in countries in which serology is not a valid option due to the cross-reaction with dengue, together with the fact that reverse transcription-polymerase chain reaction (RT-PCR) is not universally accessible and sonographers are not trained to identify intracranial abnormalities, may hamper confirmation of congenital Zika syndrome.

What are the clinical implications of this work?

- Intracranial findings constitute crucial data for improving intrauterine diagnosis of congenital Zika syndrome. Therefore, ultrasound screening during pregnancy should be systematized and expanded not only during epidemics but also in endemic areas.

2 | METHODS

This was a dual-design approach involving a prospective and a retrospective cohort. The prospective arm consisted of pregnant women exposed to the ZKV epidemic during pregnancy and whose fetuses had findings compatible with CZS. The retrospective arm consisted of women whose children with CZS were being followed up at the *Instituto de Pesquisa Professor Joaquim Amorim Neto* (IPESQ) between 2015 and 2018 in the city of Campina Grande, Paraiba, Brazil. The institute's internal review board approved the study protocol and all the women agreed to participate in the study and signed an informed consent form.

In the prospective arm, pregnant women with a clinical suspicion of ZIKV infection were referred to IPESQ. Urine and/or blood samples were taken from the mother to test for ZIKV, and a specialist in fetal medicine performed at least two ultrasound scans, the first at 20 to 24 weeks and the second at 28 to 34 weeks, using a Samsung WS80 Elite ultrasound scanner (Samsung Medison, Seoul, Republic of Korea). If ultrasound findings were suggestive of ZIKV infection (microcephaly, calcifications, ventriculomegaly, corpus callosum dysgenesis, and posterior fossa alterations), an amniotic fluid sample was then tested for the presence of the virus. Fragments of the placenta and samples of umbilical cord blood were taken following delivery.

The retrospective arm of the study was conducted at IPESQ. As a referral center, IPESQ had received and was providing care to children already diagnosed with CZS. Blood and urine samples were collected from the mother and child, and data on the pregnancy and birth were retrospectively collected from the mother's prenatal records and from the child's medical records. Hospital records were also consulted.

This non-probabilistic convenience study sample was obtained consecutively and consisted of pregnant women, receiving care at IPESQ, and their fetuses and newborn infants, as well as women whose children had CZS and who were attending the clinic following their birth. The inclusion criteria required a diagnosis by reverse transcription-polymerase chain reaction (RT-PCR) (confirmed CZS) or based on neuroimaging (fetal neurosonography/CT) plus exanthema (presumed CZS). Radiological diagnosis was based on calcifications at the gray-white matter junction and/or basal nuclei associated with some degree of delayed cortical development and severe microcephaly with a partially collapsed skull.[7,14] According to the current protocol at the Institute, all the children were submitted to computed tomography (CT) of the brain using a 16-channel CT scanner (Somatom Sensation 16; Siemens Healthcare, Germany). The exam was performed in the neonatal period to look for the radiological findings suggestive of CZS.[4,7]

Microcephaly at birth was defined in accordance with the Intergrowth-21st z-scores for head circumference (HC) and classified as mild (two SD from the mean for gestational age at birth and sex) or severe (more than three SD from the mean for gestational age at birth).[15]

Autopsies were performed in three of the nine cases of neonatal death. The methodology of the neuropathology study has already been published in detail elsewhere.[15] In summary, after removal of the brain, one hemisphere was frozen for molecular examination and the other was fixed in 10% formaldehyde for macro- and microscopic analyses. Samples of the intercostal muscle were also analyzed.

The mothers' biological, sociodemographic, obstetric, and lifestyle data (age, weight, height, presence of a partner, schooling, occupation, per capita family income, race, place of residence, parity, site of prenatal care, number of prenatal consultations, number of ultrasound scans, amniocentesis, intrauterine diagnosis of CZS, morbidities, smoking, alcohol use, and illicit drug use) were collected at interviews. Data on whether symptoms of ZIKV infection were present during pregnancy and gestational age at time of symptoms, whether the woman had been bitten by *Aedes aegypti*, and knowledge regarding ZIKV infection were also collected at interviews. TORCH screening tests were performed in all pregnant women and their newborn infants. In microcephaly cases, karyotype was performed.

Data on maternal morbidity and the child's birth were extracted from the mothers' health cards and medical records and from the children's medical records (gestational age at birth, weight, HC, first- and fifth-minute Apgar scores, need for resuscitation maneuvers, admission to intensive care unit, ICU, morbidities, associated malformations, and conditions at discharge). The occurrence of early neonatal death (≤7 days of life) and the presence of fetal akinesia deformation sequence/arthrogryposis were also investigated.

A specific database was created using Research Electronic Data Capture (REDCap) software. Statistical analysis was performed using MedCalc (MedCalc Software bvba, Ostend, Belgium), version 18.11.6. Frequency distributions were obtained for the categorical variables and measures of central tendency and dispersion for the numerical variables (means and SD for the continuous variables and medians and interquartile ranges, IQR for the discrete and ordinal variables).

3 | RESULTS

In this cohort of 102 pairs of mothers and fetuses/children, 28.4% of the cases had been monitored since pregnancy (prospective arm,

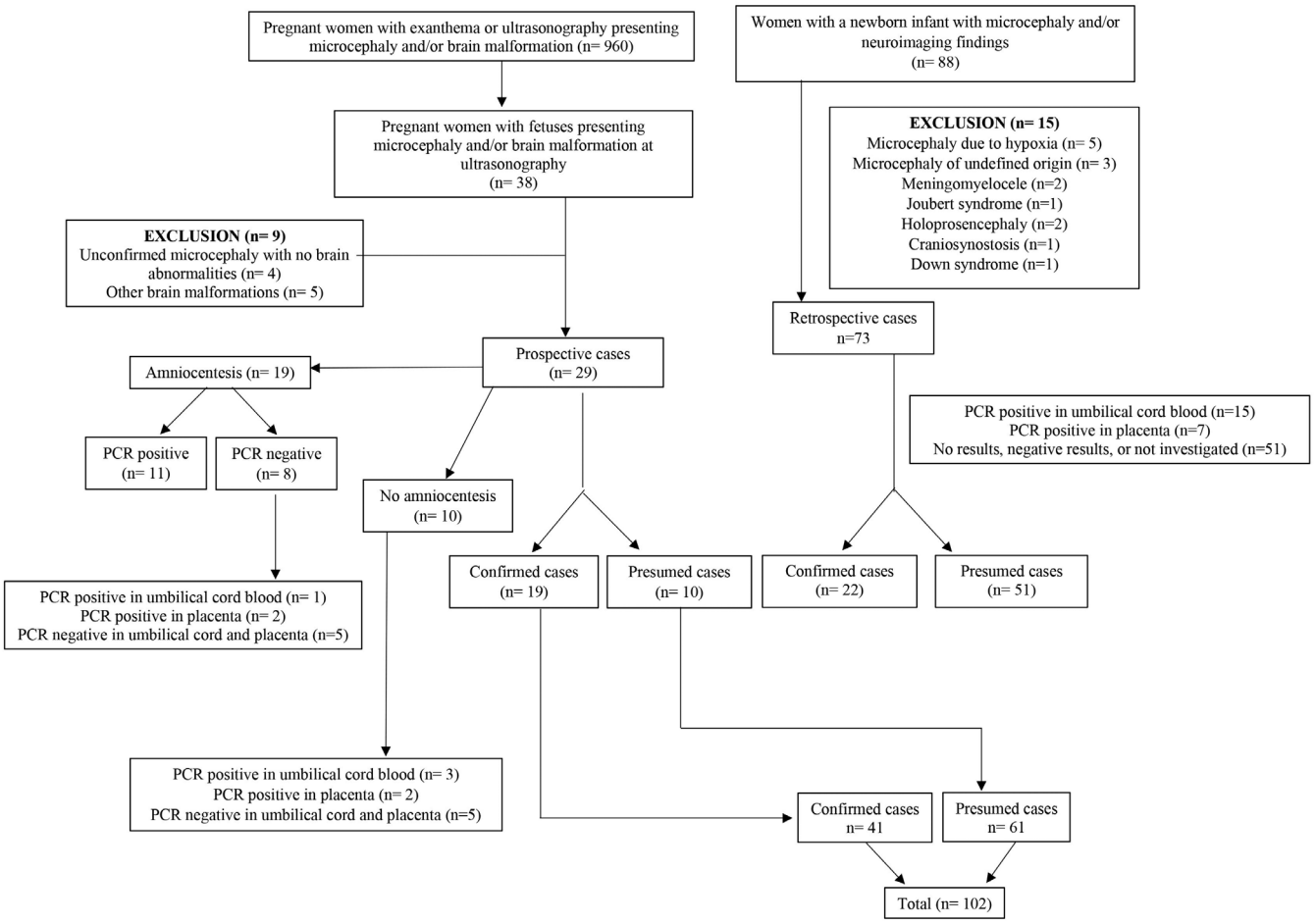

FIGURE 1 Flowchart of the study

n = 29) and 71.6% began follow-up after the child's birth (retrospective arm, n = 73) (Figure 1).

Mean maternal age was 26.5 ± 6.2 years and 5.9% of the participants were adolescents. Mean schooling was 10.3 ± 3.3 years (range 0-18 years), with seven women (6.9%) having graduated from university and two (2.0%) having no schooling. The majority (57%) had completed high school. Most of the women (69.6%) reported having a steady partner and 83.3% came from small provincial towns, with 85% living on the outskirts of an urban area. Mean per capita family income was US$ 93.9 ± 0.2 (range US$ 8.8-395.7), with 7.8% still employed at the time of the interview. Prior to becoming pregnant, 52% were employed, 18.6% in formal employment, and 33.4% in the informal job market. In relation to lifestyle habits, 4.9% of the women reported drinking alcohol and 1% smoking, while none reported using illicit drugs. Mean maternal weight was 63.8 ± 12.5 kg (range 35-99 kg) and mean height was 1.6 ± 0.96 m (range 1.3-1.7 m) (Table 1).

In most cases (89.2%), prenatal care was provided within the public healthcare system. The median number of pregnancies per woman was 2 (range 1-8), with 41.1% being primiparas. The mean number of prenatal consultations was 8 (range 1-17). Only 2.9% had heard of the ZKV before they became pregnant; however, 59.8% reported the presence of breeding sites of the *Aedes aegypti* mosquito at or around their home and 67.6% reported having been bitten by the mosquito. Use of insect repellent was reported by 14.7%. The presence of exanthema was reported by 90.2% of the women and the mean gestational age at which they experienced symptoms was 12 ± 0.5 weeks (range 6-34 weeks) (Table 2). Morbidities occurred during pregnancy in 63.7% of cases, with urinary infections (27.7%), anemia (10.8%), and hypertension (8.7%) being the most common.

CZS was suspected by obstetric ultrasonography in 52.9% of the cases. The graphs in Figure 1 show the variations in the growth of HC during pregnancy, beginning within the expected circumference for gestational age (prior to 20 weeks), with some cases then evolving to microcephaly, others remaining within the normal range, some evolving to microcephaly and then achieving HC compatible with normal, and even cases that resulted in a HC that was large for gestational age (Figure 2).

Ultrasound screening during pregnancy, either performed in a primary healthcare unit (retrospective arm) or in a tertiary healthcare unit (prospective arm), showed ventriculomegaly to be the most common finding (44/102; 43.1% of cases), followed by microcephaly (43/102; 42.2% of cases), subcortical calcifications and/or calcifications in the basal nuclei (31/102; 30.4% of cases), abnormalities in the structures of the posterior cranial fossa (23/102; 22.5% of cases), arthrogryposis (8/102; 7.8% of cases), and dysgenesis of the corpus callosum (6/102; 5.9% of cases). The median number of ultrasound scans performed was 4 (range 1-10). Amniocentesis was performed in 19 pregnant women (prospective arm), with positive RT-PCR in 11 cases (11%). Of the eight cases with negative results in amniotic fluid, three tested positive in the placenta and/or umbilical cord (RT-PCR). There were no cases of TORCH, fetal death, and any combination between CZS and other congenital infections, or aneuploidies, was observed.

TABLE 1 Biological and sociodemographic characteristics, obstetric history, and lifestyle habits of 102 women whose children had a diagnosis of congenital Zika syndrome (CZS)

Characteristics	n	%/Mean ± SD
Maternal age (y)	102	26.5 ± 6.2
Range		16-46
Number of pregnancies		
Primigravidas	42	41.2
Multigravidas	60	57.8
Parity		
Nulliparas	43	42.2
Multiparas	55	54
Grand Multiparas	13	3.8
Race		
Yellow	2	2
White	23	22.5
Black	12	11.8
Brown	65	63.7
Schooling		
No schooling	2	2
University	7	7
Elementary school	13	12.7
Middle school	22	21.6
High school	58	56.9
Years of study		10.4 ± 3.3
Place of residence		
Campina Grande	17	16.7
Another town	85	83.3
Zone of residence		
Rural	15	14.7
Urban	87	85.3
Alcohol consumption		
Yes	5	4.9
No	97	95.1
Smoking		
Yes	1	1
No	101	99
Employment		
Unpaid	94	92.2
Paid	8	7.8
Worked before pregnancy		
Yes, formal employment	19	18.6
Yes, informal employment	34	33.3
No	49	48
Steady partner		
Yes	71	69.6
No	31	30.4
Per capita income (US$)		93.9 ± 0.2
Maternal height (m)		1.58 ± 0.1
Maternal weight (kg)		63.8 ± 12.5
Body mass index		25.4 ± 4.5

TABLE 2 Prenatal care and Zika-related history in 102 women whose children had a diagnosis of CZS

Characteristics	n	%
Healthcare sector for prenatal care		
Public	91	82.2
Private	11	10.8
Number of prenatal consultations		
<6	20	19.6
≥6	82	80.4
Reported insect repellent use		
Yes	15	14.7
No	87	85.3
Knew about Zika virus before pregnancy		
Yes	3	2.9
No	99	97.1
Breeding sites of *Aedes aegypti* near home		
Yes	61	59.8
No	41	40.2
Had been bitten by *Aedes aegypti*		
Yes	69	67.6
No	33	32.4
Symptoms of Zika virus infection during pregnancy		
Yes	92	90.2
No	10	9.8
Trimester of pregnancy during which woman had symptoms		
First	63	68.5
Second	25	27.2
Third	4	4.3
Suspected diagnosis of intrauterine Zika virus infection		
Yes	59	57.2
No	43	42.2

The type of delivery was vaginal for 53.9% of the women, with early delivery being induced in 16.7% of cases, mostly due to hypertension. Median gestational age at delivery was 39 weeks (range 32-42 weeks), with 15.7% of infants being premature (Table 3).

Mean birth weight was 2721.0 ± 491.0 g, with 9% of infants being considered small for gestational age (SGA) and 2% large for gestational age (LGA). Mean length at birth was 45.7 ± 2.9 cm, with 31.3% being considered below and 1% considered above expected length for gestational age. Mean HC was 30.0 ± 2.3 cm (range 23-36.5 cm), with 66% being classified as having microcephaly. Of the newborn infants, 51% were female and 49% male (Table 4).

Median Apgar score was 8 at the first minute (range 1-9) and 9 (range 0-10) at the fifth minute, with five children having Apgar scores ≤3. Complications at birth were registered in 28.4% of cases, with respiratory distress being present in 23.3% of cases. Resuscitation maneuvers were required in 3.9% of cases and admission to an ICU in 15.7%. Arthrogryposis was registered in 10 cases (9.8%) and associated malformations in 17 cases (16.7%). Cryptorchidism was the most common associated malformation (64.7%). Nine neonatal deaths occurred (8.8%), and the mean number of days the newborn infants spent in hospital was 5.5 ± 5.3 (Table 4).

Autopsies were performed in three of the nine cases of neonatal death. Neuropathology in these three cases showed ventriculomegaly. In the first two cases, ventriculomegaly was severe and caused by obstruction due to the distortion and calcification of the brainstem, particularly of the mesencephalon, with occlusion of the cerebral aqueduct. In the third case, the only one in which microcephaly was present, ventricular dilatation, was less severe and was associated with intense reduction in the brain parenchyma (ex-vacuum), since the cerebral aqueduct was patent. In all cases, the reduction in brain parenchyma was severe, the gyri simplified or absent, with the brain surface being smooth in various areas. Neuronal migration disorders were present in all three cases, even in the cerebellum where cortical dysplasia was found. Foci of calcification were seen at all levels (cortex, white matter, deep gray nuclei, cerebellum, brainstem, and spinal cord); however, inflammation was sparse or absent. The cerebellum was hypoplastic, as was the pons, the bulbus, and the spine due to the lack of descending axons. Motor neurons were missing in the spinal cord and the skeletal muscle showed neurogenic atrophy (analysis of fragments of the intercostal muscle). Illustrations are shown in Figure 3.

4 | DISCUSSION

The women in this cohort had poor socioeconomic levels and were generally exposed to extremely poor sanitary conditions. Most (89.2%) were receiving care within the public healthcare system. Morbidity during pregnancy was high, as was the frequency of prematurity and of neonatal complications. Microcephaly was present in 66% of cases.

The presence of symptoms, represented here by exanthema, was much more common than reported in other studies with pregnant women. A recent review reported that the percentage of pregnant women with symptomatic ZIKV ranged from 17% to 56%.

Obstetric ultrasound can lead to a suspected diagnosis of CZS during pregnancy, generally from 20 to 24 weeks onward. In the present study, the main ultrasound findings were as follows: ventriculomegaly (43.1%), microcephaly (42.2%), calcifications (30.4%), and alteration of the posterior fossa (22.5%). The prevalence of these findings was lower than rates reported from some other studies in which the frequency of ventriculomegaly varied between 63.1% and 92%, the frequency of microcephaly from 33.3% to 64%, calcifications from 71% to 92%, and alterations in the posterior fossa from 21% to 82%. In the retrospective arm of the present study, the percentage of cases in which there was no suspicion of ZIKV infection during pregnancy was high (47.1%). In addition, even in cases in which ultrasound findings were suggestive of ZIKV, the extent of brain

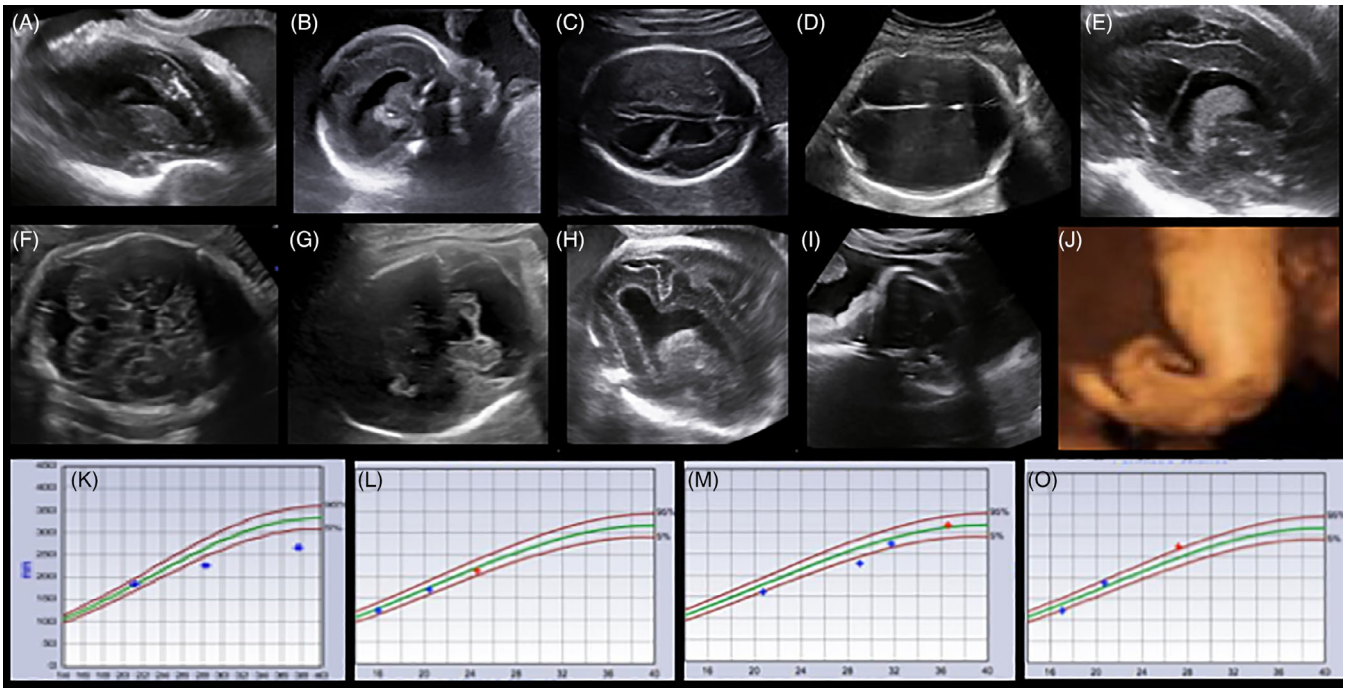

FIGURE 2 Neurosonography findings in cases of congenital Zika syndrome (CZS). A, Subcortical calcifications; B, calcifications in the basal nuclei; C, mild ventriculomegaly; D, severe ventriculomegaly; E, ventriculomegaly with intraventricular adhesion; F, normal cerebellum; G, cerebellar and vermis hypoplasia, widening of the posterior fossa; H, delay in cortical development, ventriculomegaly; I, collapse of the bones in the skull; J, arthrogryposis; from K-N, growth pattern of head circumference (HC) [Colour figure can be viewed at wileyonlinelibrary.com]

damage in most cases was not fully described, with ventriculomegaly and microcephaly being the most commonly reported findings.

There may be some explanations for these results.[16] First, there is the fact that this was a new disease, and, at the beginning, it was difficult to convince the medical community to investigate not only for microcephaly but also for intracranial findings, thus resulting in an extended learning curve. Secondly, access to ultrasound remains difficult in developing countries such as Brazil. At the time of the epidemic, only a single routine scan at around the 20th week of pregnancy was authorized within the public healthcare system. A third explanation may lie in the severe microcephaly, with collapse of the bones in the skull, and early closure of the cranial sutures and fontanels, hampering the passage of sound and consequently making adequate evaluation of the brain more difficult (Figure 2).

Nonetheless, this high percentage of cases that failed to be diagnosed during pregnancy must be discussed, particularly considering that calcifications and ventriculomegaly were found in only 30.4% and 43.1% of scans, respectively, during intrauterine life. Since these findings are present in other congenital infections, such as cytomegalovirus and toxoplasmosis,[14] these other conditions are probably also being underdiagnosed in primary healthcare services in Brazil.

Of the 29 pregnant women prospectively monitored and whose fetuses had neurological damage suggestive of ZIKV infection, 19 were submitted to amniocentesis; however, the virus was detected by RT-PCR in 11 cases. Of the eight cases that tested negative in amniotic fluid, three had a positive result in the placenta and/or cord blood. Schaub et al detected ZIKV in the amniotic fluid of eight cases evaluated; however, when they repeated amniocentesis in three of these cases at a more advanced gestational age, the result of PCR was negative. Unlike cytomegalovirus, in which a lasting viral persistence is found in the tissues and secretions of infected newborns, the persistence of the Zika virus in amniotic fluid and other fluids, such as blood, seems to be of a transient nature.[17] Factors such as gestational age at sample collection (the time between infection and amniocentesis), as well as the role of maternal and fetal immunity, deserve further investigation. Therefore, the absence of ZIKV in the amniotic fluid and/or in the blood of the neonate does not rule out CZS.

The fleeting persistence of ZIKV associated with the possibility of a cross-reaction in a serological test between ZIKV and other arboviruses, such as dengue, particularly in countries such as Brazil where dengue is endemic,[18,19] makes confirmation of the diagnosis difficult in many cases, with the majority being considered presumed cases of CZS based on clinical history and CT findings. Although this is not a pathognomonic sign of Zika, the presence of calcifications in the subcortical region associated with skull collapse is an extremely suggestive finding of CSZ, according to Levine et al.[14] Therefore, systematizing ultrasound as a screening test and providing adequate training for health professionals could facilitate the diagnosis of congenital infection during pregnancy.

The absence of microcephaly as an immediate neonatal outcome in around 34% of cases merits emphasis. At the beginning of the epidemic in Brazil, the term *microcephaly* became synonymous of the disease; however, the first paper published on CZS already stressed that microcephaly was only the tip of the iceberg.[2] Head growth generally

TABLE 3 Obstetric outcomes in 102 women whose children had a diagnosis of CZS

Characteristics		
Gestational age at delivery		
Median, interquartile range		39 (38-39)
Range		32-42
	n	**%**
Type of delivery		
Caesarian	47	46.1
Vaginal	55	53.9
Prematurity		
Yes	16	15.7
No	86	84.3
Medical indication for early delivery		
Yes	17	16.7
No	85	83.3
Indication for early delivery		
Hypertension	6	35.3
Amniorrhexis	4	23.5
Oligohydramnios	3	17.6
Polyhydramnios	2	11.8
Placental abruption	1	5.9
Fetal distress	1	5.9
Fetal death		
Yes	-	-
No	102	100

TABLE 4 Neonatal outcomes in the study sample of 102 patients with CZS

Characteristics	n	%/Mean ± SD
Neonatal death		
Yes	9	8.8
No	93	91.2
Sex		
Female	52	51
Male	50	49
Mean length at birth (cm)		45.7 ± 2.9
Classification of length		
Below	31	31.3
Normal	67	67.7
Above	1	1
Mean birth weight (g)		2721.9 ± 492
Classification of birth weight		
Small for gestational age	9	9
Appropriate for gestational age	89	89
Large for gestational age	2	2
Mean head circumference at birth		30.0 ± 2.3
Microcephaly at birth		
Yes	66	66
No	34	34
Classification of microcephaly at birth		
Mild	21	31.8
Severe	45	68.2
Fetal akinesia/Arthrogryposis		
Yes	10	9.8
No	92	90.2
Presence of associated malformations		
Yes	17	16.7
No	85	83.3
Associated malformations		
Cryptorchidism	11	64.7
Thyroglossal cyst	1	5.9
Imperforate anus	1	5.9
Hypospadias	1	5.9
Microphthalmia	1	5.9
Pectus carinatum	1	5.9
Overlapping toes	1	5.9
Presence of complications at birth		
Yes	29	28.4
No	73	71.6
Complication at birth		
Respiratory distress	16	53.3
Jaundice	6	20
Respiratory failure	8	26.6
Neonatal resuscitation		
Yes	4	3.9
No	98	96.1
Neonatal ICU		
Yes	16	15.7
No	86	84.3
	Range	**Median, IQR**
First minute Apgar score	1-9	8 (8-9)
Fifth minute Apgar score	9-9	9 (9-9)
Time in hospital (d)	1-31	3 (2-7)

Abbreviations: IQR, interquartile range.

starts to slow after 20 weeks of pregnancy.[20] However, in some fetuses, HC remains within normal limits throughout pregnancy, while in others, although a reduction is seen around the 28th week of pregnancy, HC progresses normally or may even increase to above normal for gestational age. This "normalization" of HC is probably due to an increase in ventriculomegaly, making microcephaly less evident at birth, even when the reduction in brain size is severe. Therefore,

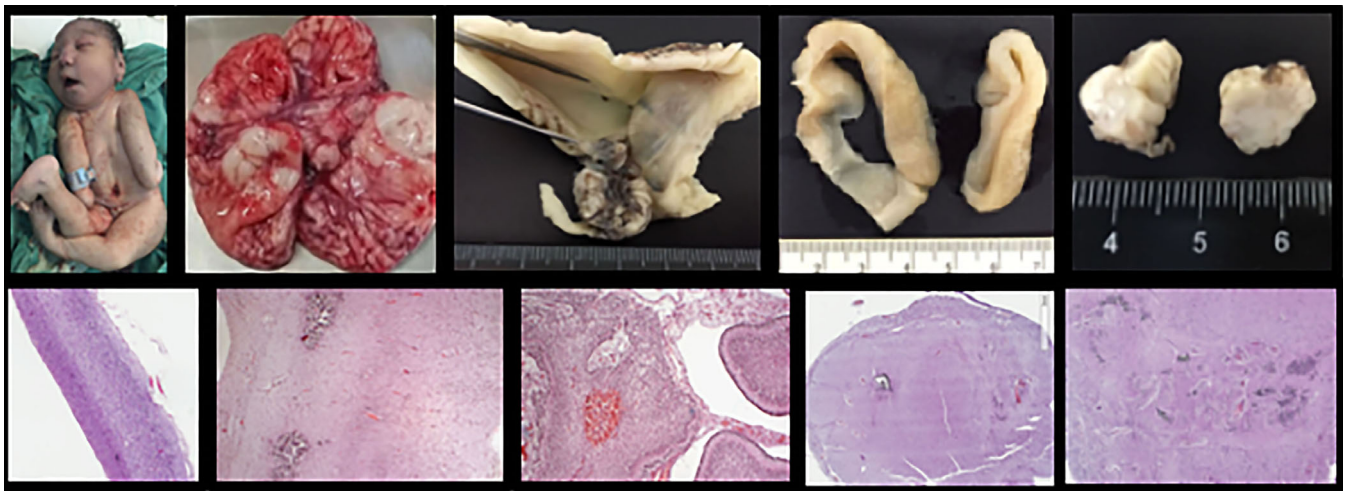

FIGURE 3 Histopathology findings in CZS. A, Newborn infant with microcephaly and arthrogryposis; B, brain with obstructive ventriculomegaly, collapse following removal of cranial cavity; C, very thin segment of the brain hemisphere and dilated ventricle; D, two segments of the hemisphere of the brain with very thin parenchyma and smooth surface; E, two levels of the distorted brainstem (mesencephalon), with no identification of the aqueduct. Note the yellowish areas of calcification in the fragment to the left; F, histological section of the entire thickness of the hemisphere of the brain; to the left the ventricular surface and to the right the meningeal surface. Hematoxylin and eosin (H&E), magnification 10×; G, detail of one of the hemispheres of the brain showing two foci of calcification and the cortical surface on the right outlining a gyrus. H&E; magnification 100×; H, histological section of the cerebellum showing cortical dysplasia on the left. H&E; magnification 100×; I, histological section of the distorted mesencephalon with no identification of the aqueduct. H&E; magnification 10×; J, detail of the mesencephalon with disorganized structures and various foci of calcification. H&E, magnification 20× [Colour figure can be viewed at wileyonlinelibrary.com]

measuring HC does not appear to be a good marker of Zika virus infection.

The cases without microcephaly in the present study are due to the inclusion of severe cases (characterized by severe hypoplasia of the brainstem, with obstruction of the cerebral aqueduct, and consequently severe ventriculomegaly or even hydrocephaly), as shown in the neuropathological study, as well as mild cases (characterized by discrete signs such as simplified gyral pattern and sparse foci of calcification). In these cases, diagnosis was only possible because women with exanthema had been monitored since pregnancy, and all the neonatal deaths included here involved cases that had been followed up since pregnancy.

Arthrogryposis and neonatal death, although less common outcomes, are findings that highlight the severity of the brain damage caused by ZIKV infection. Papers describing histopathology findings in neonates who died, some with arthrogryposis, showed hypoplasia of the brainstem and practically no segmentation. Another finding was a reduction in the ventral horns of the spinal cord.[21] These findings may explain death as the result of respiratory failure, and arthrogryposis from the reduction in fetal movements resulting in neurogenic muscle atrophy, present in the three cases of death evaluated here. We do not yet know the role of peripheral innervation in cases of arthrogryposis resulting from ZIKV infection; however, central nervous system lesions may in themselves justify this finding. Arthrogryposis was observed in about 10% of our sample, similar to that observed in other studies.

There are some limitations associated with this study. Most of the sample was evaluated retrospectively, making confirmation of diagnosis difficult in some cases. In addition, there may be a selection bias associated with the inclusion of prospective cases evaluated in a tertiary service where monitoring began at a late stage, hampering the evaluation of outcomes such as abortion and the non-inclusion of asymptomatic pregnant women. These limitations reflect the challenges of diagnostic research in developing countries such as Brazil, which was unprepared to face an epidemic such as that involving the Zika virus.

5 | CONCLUSION

Despite the fact, already known to the scientific community, that microcephaly is not the main radiological finding in CZS, this point needs to be reinforced in daily practice, particularly in developing countries. The data presented here reinforce the need to train sonographers in relation to intracranial findings in cases of congenital infection due to the high prevalence of undiagnosed cases. This is an important discussion regarding reaching diagnostic conclusion in cases of CZS, particularly in countries such as Brazil, in which dengue is endemic and where serology is not a good option due to cross-reactions.

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CONFLICT OF INTEREST

There are no conflicts of interest associated with this article.

DATA AVAILABILITY STATEMENT

Research data not shared.

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REFERENCES

1. Miranda-Filho D, Martelli C, Ximenes R, et al. Initial description of the presumed congenital Zika syndrome. *Am J Public Health*. 2016;106(4):598-600.
2. Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet Gynecol*. 2016;47(1):6-7.
3. Brito C. Zika virus: a new chapter in the history of medicine. *Acta Med Port*. 2015;28(6):679-680.
4. Melo AS, Aguiar RS, Amorim MM, et al. Congenital Zika virus infection: beyond neonatal microcephaly. *JAMA Neurol*. 2016;73(12):1407-1416.
5. Leal MC, Lilian FM, Ferreira TSA, et al. Hearing loss in infants with microcephaly and evidence of congenital Zika virus infection - Brazil, November 2015-May 2016. *MMWR Morb Mortal Wkly Rep*. 2016;65(34):917-919.
6. Ventura CV, Maia M, Travassos SB, et al. Risk factors associated with the ophthalmoscopic findings identified in infants with presumed Zika virus congenital infection. *JAMA Ophthalmol*. 2016;134(8):912-918.
7. Oliveira-Szejnfeld PS, Levine D, Melo AS, et al. Congenital brain abnormalities and Zika virus: what the radiologist can expect to see prenatally and postnatally. *Radiology*. 2016;281(1):203-218.
8. Leal MC, Van der Linden V, Bezerra TP, et al. Characteristics of dysphagia in infants with microcephaly caused by congenital Zika virus infection, Brazil, 2015. *Emerging Infect. Dis.* 2017;23(8):1253-1259.
9. Pessoa A, van der Linden V, Yeargin-Allsopp M, et al. Motor abnormalities and epilepsy in infants and children with evidence of congenital Zika virus infection. *Pediatrics*. 2018;141(Suppl 2):S167-S179.
10. Eppes C, Rac M, Dunn J, et al. Testing for Zika virus infection in pregnancy: key concepts to deal with an emerging epidemic. *Am J Obstet Gynecol*. 2017;216(3):209-225.
11. Brasil MdS. Boletim Epidemiológico. Monitoramento integrado de alterações no crescimento e desenvolvimento relacionadas à infecção pelo vírus Zika e outras etiologias infecciosas, até a Semana Epidemiológica 30 de 2018. [Integrated monitoring of growth and developmental changes related to Zika virus infection and other infectious etiologies by Epidemiological Week 30 of 2018]. Brazil, Ministry of Health. 2018. http://portalarquivos2.saude.gov.br/images/pdf/2018/setembro/11/2018-047.pdf.
12. Sassetti M, Ze-Ze L, Franco J, et al. First case of confirmed congenital Zika syndrome in continental Africa. *Trans R Soc Trop Med Hyg*. 2018;112(10):458-462.
13. Pomar L, Vouga M, Lambert V, et al. Maternal-fetal transmission and adverse perinatal outcomes in pregnant women infected with Zika virus: prospective cohort study in French Guiana. *BMJ*. 2018;363:k4431.
14. Levine D, Jani JC, Castro-Aragon I, Cannie M. How does imaging of congenital Zika compare with imaging of other TORCH infections? *Radiology*. 2017;285(3):744-761.
15. INTERGROWTH-21st. Standards/references. August 9, 2019. http://intergrowth21.ndog.ox.ac.uk/. Accessed January, 2020.
16. Malinger G. The Zika virus epidemic 3 years on: a personal perspective. *Ultrasound Obstet Gynecol*. 2019;53(4):436-437.
17. Schaub B, Vouga M, Najioullah F, et al. Analysis of blood from Zika virus-infected fetuses: a prospective case series. *Lancet Infect Dis*. 2017;17(5):520-527.
18. Farinelli EC, Baquero OS, Stephan C, Chiaravalloti-Neto F. Low socioeconomic condition and the risk of dengue fever: a direct relationship. *Acta Trop*. 2018;180:47-57.
19. Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill*. 2014;19(13):1–4.
20. Schaub B, Gueneret M, Jolivet E, et al. Ultrasound imaging for identification of cerebral damage in congenital Zika virus syndrome: a case series. *Lancet Child Adolesc Health*. 2017;1(1):45-55.
21. Chimelli L, Melo ASO, Avvad-Portari E, et al. The spectrum of neuropathological changes associated with congenital Zika virus infection. *Acta Neuropathol*. 2017;133(6):983-999.

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