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Epilepsy after congenital zika virus infection: EEG and neuroimaging features

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ABSTRACT

Purpose: To describe epilepsy after congenital Zika virus infection (ZIKV) and its relationship with structural neuroimaging findings.
Methods: This was a cross-sectional study in children (aged 13–42 months) who were born with microcephaly due to ZIKV infection between 2015–2017. Patients underwent a brain imaging scan (magnetic resonance) and a video-EEG study.
Results: Among the patients (n = 43), 55.8 % were male, 88.4 % were born at term, mean head circumference at the birth was 29.7 ± 1.8 cm, and 44.8 % were infected in the first trimester of pregnancy. Neuroimaging was moderately abnormal in 30.2 % and severely abnormal in 46.5 % of patients. Early seizures (<6 months of age) were observed in 41.9 %. EEG background was abnormal when asleep or awake in 72.1 % and during sleep in 62.8 %. The interictal epileptogenic activity was recorded on 41/43 of the EEGs and was predominantly multifocal (62.8 %). An ictal EEG was obtained in 22 patients and 31.8 % had more than one seizure type. Sleep EEG (background) patterns, interictal epileptogenic activity ($p = 0.046$), interictal discharge localization ($p = 0.015$), type of ictal epileptogenic activity ($p = 0.002$), and localization of ictal discharge ($p = 0.024$) were significantly different between neuroimaging groups. The mild neuroimaging group had a higher chance of having more frequently normal sleep EEG patterns, no interictal epileptogenic activity and a further increase in the probability of walking without limitations, and less neurodevelopment delay.
Conclusion: In patients with congenital Zika virus syndrome, epilepsy tended to be early and refractory. EEG features correlated with degree of neuroimaging abnormalities.

1. Introduction

The outbreak of zika virus infection (ZIKV) in Brazil was first reported in the northeast region of the country in 2015. This area is endemic for other arboviruses such as dengue and chikungunya. The possible association of congenital transmission of ZIKV and microcephaly was first suspected in Campina Grande (state of Paraíba) and Recife (state of Pernambuco), which are both states located in northeastern Brazil. By the end of 2015, physicians observed a sudden increase in neonates with head circumference below 32 cm associated to maternal reports of mild skin rashes during pregnancy [1–3].

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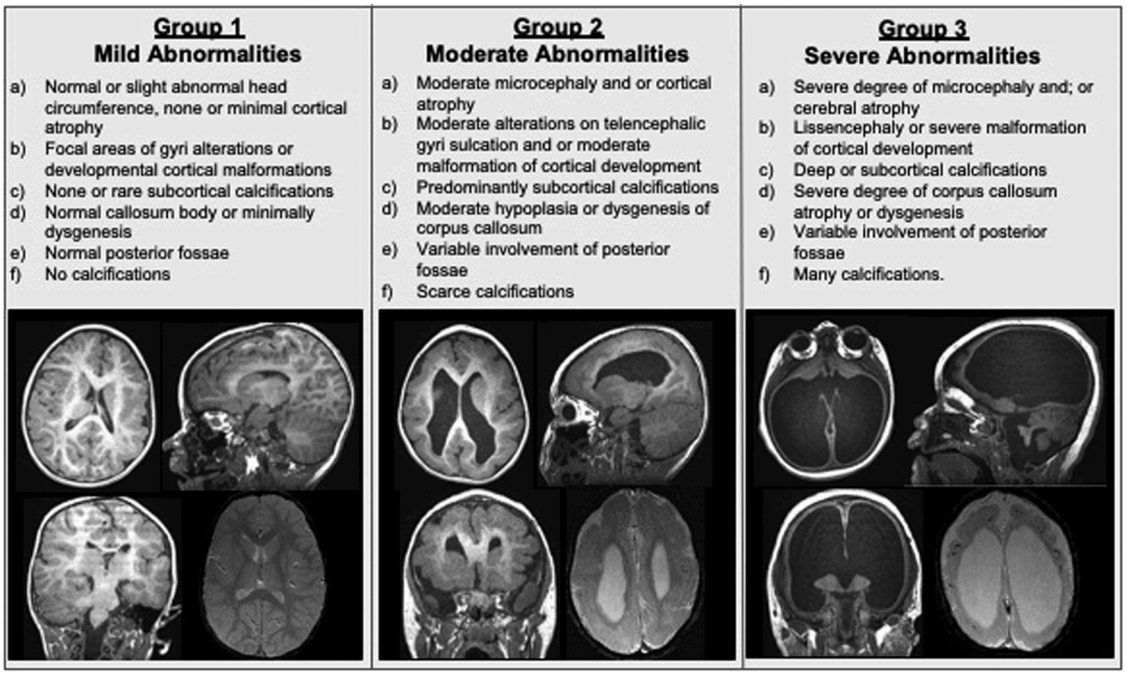

Fig. 1. The proposed classifications for neuroimage abnormalities.

November 2019, from 2015 to 2019, 3474 cases of maternal–fetal ZIKV transmission were already confirmed, with the vast majority in northeastern Brazil. The number of new cases has significantly decreased across the years, but there were still 55 cases reported in 2019 [4].

Previous studies have shown a variable prevalence of epilepsy (9%–50 %) after ZIKV infection [5–8]. However, in a more recent follow-up study, a higher prevalence of epilepsy (67 %) after confirmed congenital ZIKV infection was observed, with a mean onset of epilepsy at 4.9 months [9].

The expected burden of neurological disorders that are associated with ZIKV, such as epilepsy, developmental delay, and other deficits has reached all levels of Brazilian healthcare facilities, and unfortunately, rehabilitation programs and specific support centers to follow these children are not accessible to all patients [3].

Thus, this study aimed to describe epilepsy after congenital Zika virus infection (ZIKV) and its relationship with structural neuroimaging findings.

2. Methods

2.1. Design

This was a cross-sectional study of children who were identified from an ongoing cohort of pregnant women during a ZIKV outbreak in the State of Alagoas, northeast of Brazil, between 2015 and 2017. This study included only children aged 13–42 months. This evaluation consisted of a clinical appointment, a brain imaging scan (magnetic resonance), and a video-EEG study.

2.2. Ethical issues

The study was approved by the Research Ethics Committee of the *Pontifícia Universidade Católica do Rio Grande do Sul*, CAAE 61642016.6.1001.5336. Caregivers or guardians of the children signed a written informed consent before inclusion in the study.

2.3. Participants

The study population consisted of children registered at Health State Secretary (SESAU) of Alagoas, Brazil with suspected congenital ZIKV infection. The inclusion criterion was based on the Brazilian Health Ministry guidelines for ZIKV infection [10]. To identify vertical transmission, neonates born with microcephaly (head circumference equal or below 32 cm) should be tested using specific serological reactions and mothers should have a history of pruritic maculopapular exanthema and two or more of the following features: fever, pruritus, arthralgia of multiple joints, periarticular edema, and a serological reaction confirming ZIKV [10].

Children were included if they were born with microcephaly and tested positive for Zika, IgM or polymerase chain reaction (PCR), and if mothers had suspected (clinical history that was suggestive of ZIKV infection in pregnancy, negative screening for congenital infectious disorders and reactive immunoglobulin G [IgG] for ZikV) and/or confirmed ZIKV infection during pregnancy (positive immunoglobulin [IgM] serological reaction or positive PCR result). Between August 2017 and February 2019, patients were reevaluated at the Brain Institute of Rio Grande do Sul (BraIns) in Porto Alegre (n = 30) or at the Hospital Memorial Arthur Ramos – Radiology Division and University Hospital of Federal University of Alagoas (DIRAD) (n = 13). Before these evaluations, all children were retested using IgG and IgM for Zika and other congenital infectious disorders (syphilis, toxoplasmosis, rubella, cytomegalovirus, herpes simplex).

2.4. Clinical neurological evaluation

A Pediatric Neurologist (FKN) who was unaware of the neuroimaging and neurophysiological (EEG) findings clinically evaluated all patients who traveled to Porto Alegre. The evaluation consisted of a structured interview, information was collected regarding birth conditions, previous diseases, age at first seizures, seizure characteristics, antiepileptic drug use, seizure control, and developmental milestones. A complete physical/neurological examination was performed to characterize phenotype. Motor function was classified from I to V, I meaning

Table 1
Clinical characteristics and Neuroimage Findings.

Patients (n = 43) Variables	N	%
Sex		
Male	24	55.8
Female	19	44.2
Gestational age		
Term	38	88.4
Preterm	5	11.6
Birth weight (grams)		
Mean (SD)	2657.7 (±544.9)	
Minimum/Maximum	1230 – 3800	
Median	2720.0	
Interquartile Range	722.0	
Head circumference at birth(cm)		
Mean (SD)	29.7 (±1.8)	
Minimum/Maximum	25 – 33	
Median	30.0	
Interquartile Range	3.0	
*Percentile(median)**	<3	
Maternal infection		
1st trimester	21	44.8
2nd trimester	13	30.2
3rd trimester	5	11.6
Unknown	4	9.3
Maternal symptoms during pregnancy**		
Fever, Rash cutaneous, Myalgia	15	34.9
Fever and Myalgia	7	16.3
Rash cutaneous and/or fever	13	30.2
Other	2	4.7
Age of clinical evaluation (months)***		
Mean (SD)	25.5 (±6.01)	
Minimum/maximum	13 – 42	
Median	24.0	
Interquartile Range	9.0	
Head circumference at clinical evaluation (cm)		
Mean (SD) N=31	40.4 (±3.1)	
Minimum/maximum	34 – 49	
Median	40.0	
Interquartile Range	4.5	
*Percentile(median)**	<3	
Neuropsychomotor development		
Normal for age	2	4.7
Delayed for age	41	95.3
Language		
Normal for age	2	4.7
Delayed for age	2	4.7
Never acquired	39	90.7
GMFS****		
I	2	4.7
II	0	0.0
III	1	2.3
IV	4	9.3
V	33	76.7
Neuroimage		
Group 1 (normal or mild abnormalities)	10	23.2
Group 2 (moderate abnormalities)	13	30.2
Group 3 (severe abnormalities)	20	46.5

Data is presented as mean ± standard deviation or counts (percentages). *Percentile by the Intergrowth curve from WHO** 6 mothers have not reported symptoms suggestive of Zika infection during pregnancy, but all newborns tested positive for Zika. ***Age of clinical (neurodevelopmental), radiological and neurophysiological evaluation reported in this article. **** 3 patients were seen by a pediatric neurologist in Alagoas but the report of GMFC was not available. GMF = gross motor function scale.

"walk without limitations" and V "use of wheelchair", according to the gross motor function scales (GMFS) [11]. The primary clinical outcome measures analyzed were : (1) GMFS using an ordinal scale; (2) binary development based on Denver Developmental Screening Test [12]; and (3) binary language acquisition. Patients unable to come to Porto Alegre, had their clinical data collected by a neurologist (FTG) when they underwent the MRI and video-EEG exams in Maceio.

Table 2
Characteristics of Epilepsy and EEG recordings.

Patients (n = 43)	No.	%
Age of first seizure		
From 0–3 months	15	34.9
From 4–6 months	3	7.0
From 7–9 months	3	7.0
From 10–12 months	3	7.0
Information not available	19	44.2
Predominant seizure type at onset		
Generalized/motor		
Spasms	13	30.2
Myoclonic	1	2.3
Focal/motor		
Tonic	5	11.6
Focal/non motor		
Autonomic	2	4.7
Information not available	22	51.2
Treatment		
Monotherapy	11	25.6
Polytherapy	13	30.2
Do not use antiepileptic drugs	3	7.0
Information not available	16	37.2
Awake EEG – background*		
Normal	3	7.0
Slow (diffuse)	18	41.9
Asymmetric	1	2.3
Special patterns	7	16.3
Fast activity (drug impregnation)	5	11.6
No awake recording	9	20.9
Sleep EEG – background*		
Normal	6	14.0
No cycling or no recognizable sleep elements	21	48.8
Special patterns	6	14.0
No sleep recording	10	23.3
Interictal EEG (n = 43)		
Interictal epileptogenic activity		
Focal unilateral	3	7.0
Bilateral synchronous	5	11.6
Multifocal	27	62.8
Generalized	3	7.0
Burst-suppression	3	7.0
No interictal epileptogenic activity	2	4.7
Type of interictal discharge		
Spike	8	18.6
Spike wave	6	14.0
Polyspike-wave	13	30.2
Acute wave	1	2.3
Two or more types	13	30.2
No discharges	2	4.7
Interictal discharge localization		
Only left hemisphere	1	2.3
Only right hemisphere	4	9.3
Both hemispheres	36	83.7
No discharges	2	4.7
Ictal EEG (n = 22)		
Ictal epileptogenic activity		
Focal unilateral	1	4.5
Bilateral synchronous	8	36.4
Multifocal	0	0.0
Generalized	5	22.7
Burst-suppression	8	36.4
Localization of ictal discharges		
Only left hemisphere	1	4.5
Only right hemisphere	1	4.5
Both hemispheres	20	90.9
Ictal seizure type		
Clonic	1	4.5
Myoclonic	1	4.5
Tonic	2	9.1
Behavior arrest (impaired awareness)	4	18.2
More than 1 seizure type	7	31.8
*Electrographic only***	3	13.6
ESES	4	18.2

OBS: * All patients had video-EEG recording with at least 1 h duration. On 24 participants it was possible to have both sleep and awake epochs recorded. Classification of awake EEG: normal (background pattern adequate to age), slow diffuse (predominance of slow activity, no recognizable anterior-posterior

gradient), asymmetric (asymmetric slow background or focal slow), special patterns (inactivity, diffuse low voltage, burst-suppression pattern). Classification of sleep EEG: normal (cycling with physiological elements according to age), No sleep cycling and/or no clear physiological elements as spindles, K complexes), special patterns (low voltage, inactivity, burst suppression). **Electrographic seizures without visible clinical manifestations. ESES = electrical status epilepticus during sleep.

2.5. EEG evaluation

At both sites, video-EEGs were obtained using an EMSA digital recorder, with a sensitivity of 7 μV/mm, low frequency 0.6 Hz filters, and a high frequency of 70 Hz, and a speed of 1.5 cm/s. Electrodes were placed according to the 10–20 system that was modified for newborns (because of their small head circumference), and the bipolar montage (Fp1-C3, C3-01, Fp1-T3, T3-01, Fp2- C4, C4-02, Fp2-T4, T4-02, C3-Cz, Cz-C4) was used [13]. Recordings (at least 60 min and a maximum of 3 h in duration) were performed while the patient was awake and spontaneously asleep. Video analysis was used to characterize the behavioral sleep patterns and the semiology of seizures. EEG background and epileptiform activity were classified according to the scheme that was proposed by Lüders and Noachtar [14]. Background rhythm while awake was classified as normal, when adequate for age, or abnormal if in the presence of any of the following patterns (diffuse slow rhythm, asymmetry, low voltage, burst-suppression, or fast activity-due to drug impregnation). Background rhythm during sleep was classified as normal when cycling with physiological elements was according to age. It was considered abnormal when: a) there was no cycling and/or no clear or rudimentary physiological elements, and/or b) in the presence of special abnormal patterns such as low voltage, inactivity, or burst suppression. Morphology and location of interictal abnormalities and ictal discharges were also described. The interictal activity was considered when epileptiform patterns such as spikes, sharp waves, polispikes, or hypsarrhythmia either alone or in combination were identified [14]. The ictal activity was defined as repetitive EEG discharges with relatively abrupt onset and termination and a characteristic pattern of evolution lasting at least several seconds either accompanied or not by clinical manifestations [15]. Sudden desynchronization of electrical activity (electrodecremental seizures) was also considered to be an ictal pattern if it was associated with spasms. The ictal seizure semiology was classified as proposed by the International League against Epilepsy (ILAE) [16]. All video-EEGs were analyzed by the same researcher (MLN) who was blinded to neuroimaging data and clinical information.

2.6. Epilepsy evaluation

Epilepsy was diagnosed according to the ILAE 2017 classification [17], which is based on parents' information, when available and reliable, and on ictal EEG findings. Drug resistance epilepsy was defined by the ILAE criteria as using two antiepileptic drugs (AEDs) with adequate doses without achieving seizure control [18].

2.7. Neuroimaging evaluation

Forty-three patients underwent either a 1.5 T (at Maceio; GE Healthcare Optima MR450w) or 3.0 T (at Porto Alegre; GE Healthcare Signa HDxt) MRI examination. Patients were sedated to perform the imaging exams. At the Brain Institute, T1 structural scans were acquired using a BRAVO sequence, as follows: repetition time, 6.16 ms; echo time, 2.18 ms; flip angle, 8°; acquisition matrix, 240 × 240 × 196; and voxel size, 1.0 × 1.0 × 1.0 mm. At DIRAD, the T1 structural scans were acquired using a BRAVO sequence, as follows: repetition time, 8.7 ms; echo time, 3.224 ms; flip angle, 12°; acquisition matrix, 256 × 2256 × 100; and voxel size, 0.938 × 0.938 × 1.2 mm. Neurological findings of postnatal T1 volumetric images were analyzed by two experienced neuroradiologists (RBS and RB), who independently reviewed all scans and classified brain malformation severity based on the MRI images. Based on these images, patients were classified into the following three categories of brain morphological and structural involvement: mild, moderate, and severe. Visual analysis included evaluation of the degree of ventricular enlargement due to white matter hypoplasia; malformation of cortical development and sulcation; abnormalities of the corpus callosum (agenesis, hypogenesis, hypoplasia); evaluation of myelination (normal or delayed); presence and location of brain calcifications; decreased brainstem and cerebellum volume; enlarged cisterna magna; enlarged anterior supratentorial subarachnoid space; and presence of intraparenchymal cysts. Group 1 included normal or mild imaging abnormalities; group 2 included moderate imaging abnormalities; and group 3 included severe imaging abnormalities (Fig. 1). Further details regarding classification of the three categories of brain malformation can be seen in Esper et al. [19]. Interrater agreement between interpreting radiologists was assessed using Cohen's kappa.

2.8. Statistical analysis

The SPSS software version 23.0. was used to analyze data. Values observed were described by count and percentages. Continuous variables were expressed as mean (standard deviation), minimum/maximum count and median (interquartile range). Chi-square and post-hoc tests (Bonferroni residual analysis) were used to verify associations between groups and variables. Findings with $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Sample characteristics

Table 1 shows the general sample characteristics (43 children with congenital ZIKV infection, where 41 were born and lived in Maceio, capital of Alagoas state, or neighborhood cities. Two patients lived in southern Brazil. However, mothers were infected during pregnancy in Northeast Brazil). There was a predominance of males 55.8 % and infants born at term (88.4 %), with a mean head circumference at birth of 29.7 ± 1.8 cm, and most were infected in the first trimester of pregnancy (44.8 %). Mean age at follow-up was 25.5 ± 6.01 months. All patients with mothers who were infected in the first trimester of pregnancy were born at term.

3.2. Epilepsy and EEG

Characteristics of seizures/epilepsy and EEG findings are presented in Table 2, which is divided into clinical information, background EEG characteristics while awake and/or during sleep, interictal, and ictal findings. Clinical information was obtained from caregivers (the majority of historians for patients were the mothers) and roughly 50 % were able to inform the age of the first seizure or seizure type at onset of epilepsy. Only two patients did not have active epilepsy at the time the study evaluation and were not taking antiepileptic drugs. Both had normal sleep and awake EEG and were placed within group 1 of the neuroimaging analysis. Information regarding seizure control was difficult to obtain because caregivers were not able to recognize more

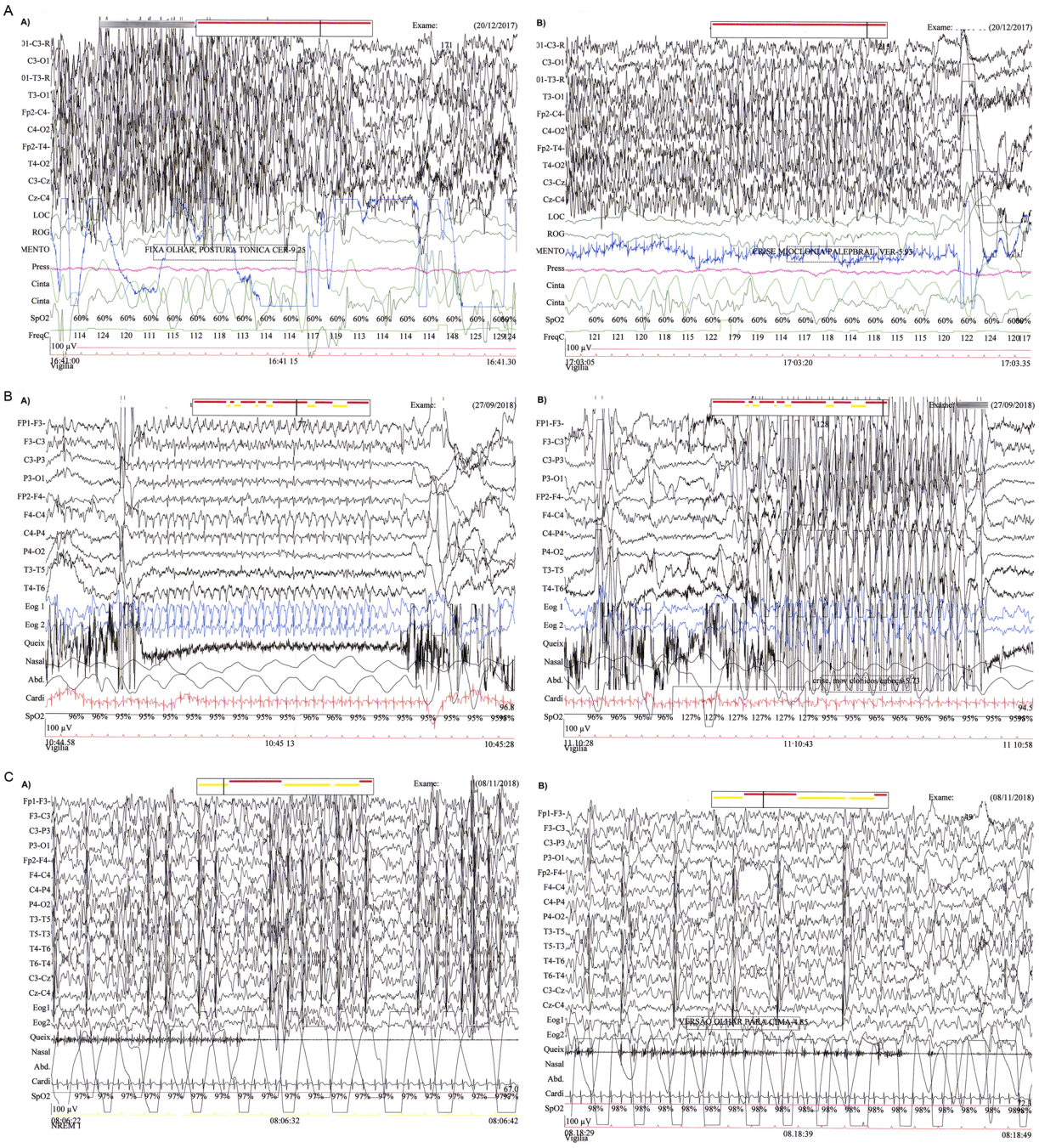

Fig. 2. Samples of EEGs from patients with varied interictal and ictal features. a) The patient's details are as follows: female, term, aged 18 months, trimester of mother infection unknown, MRI group 1, while awake two seizures with different semiology, at 16:41:00 behavioral arrest and staring tonic cervical posture, EEG generalized polispikes, at 17:03:20 eyelid myoclonic seizure, and spike-wave discharges mainly in the right hemisphere with some propagation to left rolandic-temporal region. b) The patient's details are as follows: male, preterm, aged 2 years 11 months, mother infected in the second trimester, MRI group 2, while awake at epoch 10:45 around 18 s of bilateral synchronous spike wave-discharges with higher amplitude in the frontal-rolandic region, behavioral arrest and staring, and at 11: 10 generalized slow spike-waves discharges (around 1 Hz) and clonic head/neck movements. c) The patient's details are as follows: male, preterm, aged 2 years 9 months, mother infected in the first trimester, MRI group 2, during sleep continuous polispikes and wave discharges, and while awake upward eye deviation with focal (high amplitude) right rolandic-temporal discharges. d) The patient's details are as follows: male, term, aged 2 years, mother infected in the first trimester, MRI group 3, during sleep bilateral synchronous slow spike-waves complexes at frontal-temporal region followed by background suppression, while awake slow flexor spams of four limbs associated with suppression of background rhythms, and bilateral frontal fast activity.

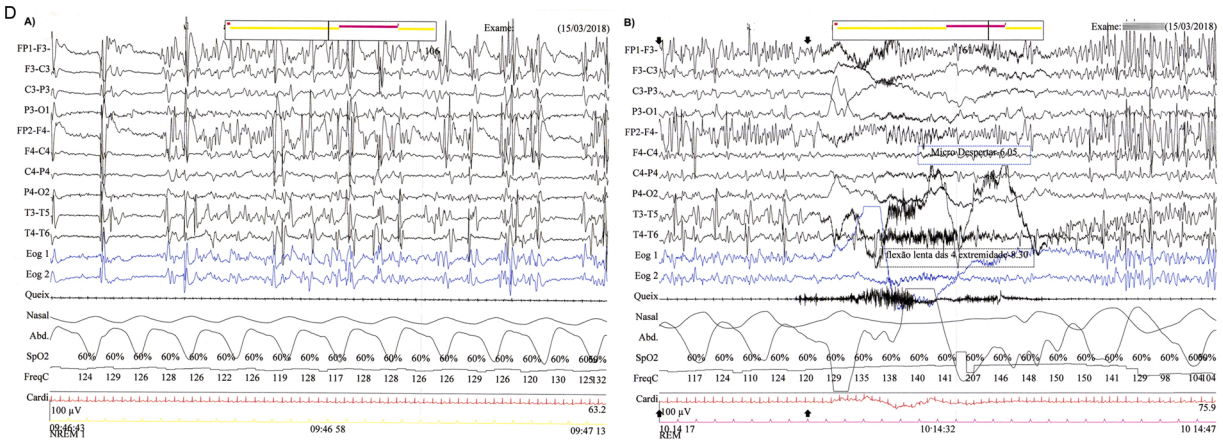

Fig. 2. (*continued*).

subtle or complex seizure types. As an example, in some patients for whom we obtained ictal recordings, caregivers did not recognize the event as a seizure. Drug-resistant epilepsy based on clinical information of lack of seizure control and/or ictal EEG plus the use of more than two AEDs made it possible to diagnose 13 patients (30.2 %).

All patients were recorded for at least 1 h with video-EEG; in 24 (55.8 %), we were able to register awake and sleep epochs, in nine (21 %), there were only sleep epochs, and in ten (23.2 %), there was only wakefulness. Abnormal awake and sleep background were identified in most patients (72.1 % and 62.8 % respectively). The Interictal epileptogenic activity was predominantly multifocal (62.8 %), and it was located in both hemispheres (83.7 %). Ictal EEGs were obtained in half of the sample (n = 22), and epileptogenic activity was recorded predominantly with bilateral synchronous discharges or as burst–suppression (36.4 % each), involving both hemispheres (90.9 %). Six out of 22 patients had more than one seizure type in the ictal event and among them, three had epileptic spasms that were associated with other seizure types. Fig. 2 shows examples of ictal events in four patients with different EEG patterns and the degree of brain malformation (Fig. 2).

Among the 22 patients with ictal recordings, eight were taking monotherapy, one had never used an AED, and in six, caregivers were not able to report the treatment regimen.

3.3. Neuroimaging findings

The kappa value for interobserver agreement on rating the scans among the three groups was excellent (0.96). Analysis of the structural MRIs showed that there were ten patients who were classified in group 1 (mild), 13 in group 2 (moderate), and 20 in group 3 (severe).

Table 3 shows the results from the Chi-square tests to evaluate if there are differences in EEG patterns between the neuroimaging groups. Results indicate that there are significant differences between neuroimaging groups in sleep EEG (background) patterns, interictal epileptogenic activity ($p = 0.046$), interictal discharge localization ($p = 0.015$), type of ictal epileptogenic activity ($p = 0.002$), and localization of ictal discharge ($p = 0.024$). Post-hoc tests revealed that the mild group, compared to the other groups, had a higher chance of having more frequently normal sleep EEG patterns, no interictal epileptogenic activity, and less interictal discharge in both hemispheres.

3.4. Clinical outcomes

Only two patients had a neurological exam and neurodevelopment evaluation that was normal for their age; their neuroimage was classified as group 1.

We evaluated the distribution between clinical outcomes with the EEG background abnormalities, presence of EEG ictal activity, and neuroimaging abnormality severity. The full list of Chi-square tests is shown in Table 4. Patients with mild neuroimaging abnormalities (group 1) showed a statistically significant increase in the probability of walking without limitations and also decreased neurodevelopment delay. Patients who have not acquired language skills also had moderate or severe neuroimaging abnormalities ($p = 0.030$). No significant associations were found between the grade of GMFS or the development evaluation related to the trimester of maternal infection or refractory epilepsy.

The age of first seizure was analyzed in relation to neuroimaging abnormalities, refractory epilepsy and EEG findings, and no significant differences were found (Table 5 Supplementary Data).

4. Discussion

In this study, we have described the characteristics of epilepsy that developed after congenital ZIKV syndrome, depicting the clinical developmental characteristics of the affected children together with ictal and interictal EEG features and its correlation to brain malformations. Different from previous studies [9,20], in this cohort, a high prevalence of active epilepsy was observed. This might be because seizures started early in life (<6 months) in around 40 % of the cohort, and they were also drug resistant (30 % under polytherapy). Furthermore, these patients have not been continuously followed as the Recife cohort, and many of them had more subtle or complex seizures that caregivers did not even recognize the ictal events as “true” epileptic seizures. We were able to obtain ictal recordings in half of the cases. In addition to more easily recognizable seizures such as motor clonic/tonic/myoclonic, there were some, such as behavioral arrest, more difficult to be identified by caregivers [15]. Epileptic spasms, which were the most frequently reported seizures at the beginning of epileptic manifestations, were seen in this latter evaluation in a reduced proportion and mixed with other manifestations. Although we have not performed continuous EEG recording, we were able to detect a significant amount of ictal

Table 3
Neuroimage and Epilepsy (n = 43).

Clinical and EEG Variables*	Neuroimage Group			p-Value
	Mild (N = 10) No. (%)	Moderate (N = 13) No. (%)	Severe (N = 20) No. (%)	
Seizure Type at onset **				
Generalized Motor				
Spasm	1 (33.3)	2 (50.0)	10 (71.4)	
Myoclonic	0 (0.0)	1 (25)	0 (0.0)	
Focal Motor				0.186
Tonic	2 (66.7)	2 (22.2)	1 (11.1)	
Focal non Motor				
Autonomic	0 (0.0)	0 (0.0)	2 (22.2)	
Refractory Epilepsy ***				
Yes	2 (40)	5 (71.4)	6 (40)	0.359
No	3 (60)	2 (28.6)	9 (60)	
Awake EEG (background)				
Normal	3 (37.5)	0 (0.0)	0 (0.0)	
Slow diffuse	2 (25)	7 (63.6)	9 (60)	
Asymmetric	0 (0.0)	0 (0.0)	1 (6.7)	0.071
Special patterns	1 (12.5)	2 (18.2)	4 (26.7)	
Fast activity (drug impregnation)	2 (25.0)	2 (18.2)	1 (6.7)	
Sleep EEG (background)				
Normal	5 (62.5) §	1 (10.0)	0 (0.0)	
No cycling or no recognizable sleep elements	2 (25)	8 (80)	11 (73.3)	**0.004**
Special patterns	1 (12.5)	1 (10)	4 (26.7)	
Interictal EEG (n = 43)				
Interictal Epileptogenic Activity				
Focal unilateral	2 (20)	0 (0.0)	1 (5)	
Bilateral synchronous	1 (10)	1 (7.7)	3 (15)	
Multifocal	5 (50)	9 (69.2)	13 (65)	**0.046**
Generalized	0 (0.0)	2 (15.4)	1 (5)	
Burst-suppression	0 (0.0)	1 (7.7)	2 (10)	
No interictal epileptogenic activity	2 (20) §	0 (0.0)	0 (0.0)	
Interictal Discharge Localization				
Only left hemisphere	0 (0.0)	0 (0.0)	1 (5.0)	
Only right hemisphere	3 (30.0)	0 (0.0)	1 (5.0)	**0.015**
Both hemispheres	5 (50.0) §	13 (100)	18 (90.0)	
No discharges	2 (20.0)	0 (0.0)	0 (0.0)	
Ictal EEG (n = 22)				
Type of Ictal Epileptogenic Activity				
Focal unilateral	1 (50.0) §	0 (0.0)	0 (0.0)	
Bilateral synchronous	1 (50.0)	6 (75.0)	1 (8.3) §	
Multifocal	0 (0.0)	0 (0.0)	0 (0.0)	**0.002**
Generalized	0 (0.0)	1 (12.5)	4 (33.3)	
Burst-suppression§	0 (0.0)	1 (12.5)	7 (58.3)	
Localization of Ictal Discharge				
Only left hemisphere	0 (0.0)	0 (0.0)	1 (8.3)	
Only right hemisphere	1 (50.0) §	0 (0.0)	0 (0.0)	**0.024**
Both hemispheres	1 (50.0)	8 (100)	11 (91.7)	
Ictal seizure type ****				
Clonic	0 (0.0)	1 (12.5)	0 (0.0)	
Myoclonic	0 (0.0)	0 (0.0)	1 (8.3)	
Tonic	0 (0.0)	1 (12.5)	1 (8.3)	
Behavior arrest (impaired awareness)	1 (50.0)	2 (25.0)	1 (8.3)	0.750
More than 1 seizure type	1 (50.0)	1 (12.5)	5 (41.7)	
Electrographic only	0 (0.0)	2 (25.0)	1 (8.3)	
ESES	0 (0.0)	1 (12.5)	3 (25.0)	

OBS: *Number of patients might differ in each category because they are either related to availability of information, as described in Table 1 or specifications regarding EEG as described on Table 2. **Predominant seizure type as described by mother/guardian. *** On 16 patients there is not enough available information to characterize either refractory or non-refractory epilepsy. ****According to ILAE classification (focal/generalized/motor/non motor). Chi-square test was applied, followed by a post-hoc test to verify significance. §Statistical significant after post-hoc test.

seizures, considering just a single ambulatory EEG (1–3 h duration).

There was a correlation between EEG and neuroimaging findings. Patients in the mild group of structural abnormalities had more frequently normal EEG. However, as the number of patients with a good outcome was low, it is not possible to state that the normal EEG is a definitive biomarker of a good outcome.

Besides microcephaly, which is a disorder of the neuronal proliferation phase and occurs early in pregnancy (between the third and the fourth month of pregnancy), the neuroimaging results obtained for our patients showed other malformations of fetal cortical development, such as neuronal migration disorders (3rd to 5th month of pregnancy) and diffuse calcifications (neuronal death). These findings might suggest long-term virus pathogenesis in the central nervous system because there was a clear susceptibility of more phases of cortical development [2,21].

In two available previous neuroimaging studies, most patients underwent only CT scans, and the features that were most commonly found were brain calcifications in the junction between cortical and subcortical white matter associated with malformations of cortical development, abnormalities of the corpus callosum, and ventriculomegaly [22,23]. In our study, because all patients underwent an MRI, we were able to detail aspects of brain malformation and correlate to clinical outcomes and types of epilepsy.

Limitations of this study are due to difficulty of caregivers to recognize seizures and report them. In addition, information on epilepsy treatment is lacking for almost half of the sample. Although patients were evaluated at two different centers, the same researchers analyzed all MRI studies and EEG. Finally, neuroimaging data for the sample were acquired using two MRIs with different field strength, 1.5 T and 3.0 T. Due to logistical constraints, neuroimaging exams needed to be performed in Porto Alegre (3.0 T) and also in Maceió (1.5 T). However, before starting the study, we performed preliminary tests using both MRIs to maximize the similarity in image quality.

In conclusion, data obtained in our study showed that in patients with congenital Zika virus syndrome, epilepsy tends to be early and refractory. EEG features correlated with degree of neuroimaging abnormalities.

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Author contributions

Drs Nunes and da Costa had full access to all of the data in the study and take responsibility for the integrity of the data and data analysis. Concept and design: Nunes, Soder, Neto, Franco, Portuguez, and da Costa. Acquisition, analysis, or interpretation of data: Soder, Bomfim,

Table 4
Interaction of EEG and Neuroimaging to clinical outcome (n = 43).

Clinical Variables*	EEG/Epilepsy							Neuroimaging Group				Trimester of Infection			
	Ictal No. (%)			Interictal** No. (%)			Refractory Epilepsy No. (%)			Mild	Moderate	Severe		First	Second	Third	
	Yes	No	p-value	Yes	No	p-value	Yes	No	p-value	No. (%)	No. (%)	No. (%)	p-value	No. (%)	No. (%)	No. (%)	p-value
GMFCS																	
Walk without limitations	0 (0)	2 (10)		2 (5.3)	0 (0)		0 (0)	1 (7.1)		2 (22) §	0 (0)	0 (0)		1 (4.7)	1 (7.7)	0 (0.0)	
Walk using a manual mobility device	0 (0)	1 (5)		0 (0)	1 (50)		0 (0)	1 (7.1)		1 (11.1)	0 (0)	0 (0)		0 (0.0)	1 (7.7)	0 (0.0)	
Self-mobility with limitations (can use a motorized mobility)	3 (15)	1 (5)	0.258	4 (10.5)	0 (0)	**<0.001**	1 (7.7)	2 (14.3)	0.479	0 (0)	3 (25)	1 (5.3)	**0.030**	3 (14.3)	1 (7.7)	0 (0.0)	0.771
Wheelchair	17 (85)	16 (80)		32 (84.2)	1 (50)		12 (92.3)	10 (71.4)		6 (66.7)	9 (75)	18 (94.7)		17 (81)	10 (76.9)	4 (100)	
Neuromotor development																	
Normal	0 (0)	2 (9.5)		2 (4.9)	0 (0)		0 (0)	1 (7.1)		2 (20) §	0 (0)	0 (0)		1 (4.8)	1 (7.7)	0 (0.0)	
Abnormal	22 (100)	19 (90.5)	0.138	39 (95.1)	2 (100)	0.749	13 (100)	13 (92.9)	0.326	8 (80) §	13 (100)	20 (100)	**0.031**	20 (95.2)	12 (92.3)	5 (100)	0.798
Language																	
Normal	0 (0)	2 (9.5)		2 (4.9)	0 (0)		0 (0)	1 (7.1)		2 (20) §	0 (0)	0 (0)		1 (4.8)	1 (7.7)	0 (0.0)	
Delay	1 (4.5)	1 (4.8)	0.331	1 (2.4)	1 (50)	**0.008**	0 (0)	1 (7.1)	0.367	1 (10)	1 (7.7)	0 (0)	**0.030**	1 (4.8)	1 (7.7)	0 (0.0)	0.917
Not acquired	21 (95.5)	18 (85.7)		38 (92.7)	1 (50)		13 (100)	12 (85.7)		7 (70) §	12 (92.3)	20 (100)		19 (90.5)	11 (84.6)	5 (100)	

* Number of patients might differ in each category because they are either related to availability of information, as described in Table 1 or specifications regarding EEG as described on Table 2. **Abnormal interictal EEG. Chi-square test was applied, followed by a post-hoc test to verify significance. § Statistically significant after post-hoc test.

Table 5
Relations between EEG and Neuroimaging with the age of first seizure.

	Age of first seizure			
	0–3 moths	4 to 12 months	Not available	p-value
Neuroimaging Group				
Mild	4 (26.7)	0 (0.0)	6 (31.6)	
Moderate	3 (20)	3 (33.3)	7 (36.8)	0.248
Severe	8 (53.3)	6 (66.7)	6 (31.6)	
Refractory Epilepsy				
Yes	7 (53.8)	6 (66.7)	0 (0.0)	0.094
No	6 (46.2)	3 (33.3)	5 (100)	
Awake EEG				
Normal	1 (10)	0 (0.0)	2 (12.5)	
Slow diffuse	3 (30)	8 (100)	7 (43.8)	
Asymmetric	1 (10)	0 (0.0)	0 (0.0)	0.163
Special patterns	3 (30)	0 (0.0)	4 (25)	
Fast activity (drug impregnation)	2 (20)	0 (0.0)	3 (18.8)	
Sleep EEG				
Normal	3 (27.3)	0 (0.0)	3 (23.1)	
No cycling or no recognizable sleep elements	6 (54.5)	8 (88.9)	7 (53.8)	0.410
Special patterns	2 (18.2)	1 (11.1)	3 (23.1)	

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Declaration of Competing Interest

Authors have no conflict of interest or disclosures to declare.

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