Title

A Novel Radiologic Finding to Predict Ophthalmic Abnormalities in Children with Congenital Zika

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A Novel Radiologic Finding to Predict Ophthalmic Abnormalities in Children with Congenital Zika

A thesis submitted in partial satisfaction

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in Epidemiology

by

Virginia Vilar Sampaio

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ABSTRACT OF THE THESIS

A Novel Radiologic Finding to Predict Ophthalmic Abnormalities

in Children with Congenital Zika

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Virginia Vilar Sampaio

Master of Science in Epidemiology

University of California, Los Angeles, 2019

Professor Anne W. Rimoin, Chair

Background: In northeastern Brazil, following the Zika virus (ZIKV) outbreak, we investigated whether a specific radiologic finding (i.e., infratentorial abnormalities) was associated with sight-threatening abnormalities in children with Congenital Zika Syndrome (CZS). We also investigated whether ophthalmic abnormalities correlated with head circumference (HC) and gestational age of infection.

Methods: Cohort Study from March 2016 to December 2018, in Paraiba, Brazil. We evaluated children born to mothers with laboratory confirmed ZIKV infection during pregnancy (ZIKV RT-PCR) and children born with clinical and radiologic features of CZS.

Results: A total of 102 infants were evaluated; 75 infants had both brain imaging and complete eye exams; 9 infants were excluded because of early death. 17 of 75 infants (22%) had infratentorial abnormalities; microcephaly was present in 71%; all had brain calcifications. Sixteen of 17 children (94%) with infratentorial abnormalities had ophthalmic abnormalities. Conversely, 28% without infratentorial abnormalities, had ophthalmic abnormalities (OR 42.0; 95%CI 5.1-342.9). Similar associations were observed when macular chorioretinal atrophy and optic nerve abnormalities were analyzed individually (OR 23.7; 95%CI 6.0-93.3, and OR 11.5; 95%CI 3.3-40.0, respectively). Infratentorial abnormalities were more frequently associated with ophthalmic abnormalities (94%) than microcephaly (43.4%) p=0.0002. Mean HC measurement was statistically different between groups with and without ophthalmic abnormalities (p=0.016). A statistically significant difference in gestational age between both groups was not noted (p=0.1233).

Conclusions: The presence of infratentorial abnormalities is a significant predictor of ophthalmic abnormalities. All neonates whose mothers had ZIKV exposure at any time during pregnancy should have ophthalmologic examination.

Keywords: Zika virus, ocular findings, congenital Zika syndrome, epidemic

The thesis of Virginia Vilar Sampaio is approved.

Anne L. Coleman

Robert J. Kim-Farley

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University of California, Los Angeles

2019

DEDICATION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

aOR Adjusted Odds Ratio

cOR Crude Odds Ratio

CZS Congenital Zika Syndrome

CNS Central Nervous System

CT Computerized Tomography

HC Head Circumference

IRB Institutional Review Board

MRI Magnetic Resonance Imaging

OR Odds Ratio

SD Standard Deviation

IQR Interquartile Range

US Ultrasound

ZIKV Zika Virus

LIST OF APPENDICES

INTRODUCTION

In early 2015, Latin America, especially Brazil, was afflicted by an epidemic of neonatal microcephaly due to an emerging infectious pathogen later identified as Zika virus (ZIKV), an arbovirus transmitted by *Aedes* mosquitoes. The epidemic first started in northeastern Brazil and then spread along the coast to the southeastern region of the country. The first cases in Brazil were detected in the states of Bahia, Pernambuco and Paraiba. [1–6]

Congenital Zika Syndrome (CZS), which was first identified during the Brazilian epidemic, is a spectrum of clinical findings observed in infants who have been exposed to ZIKV *in utero*.[7] It has a variable presentation, severity, and prognosis, which is dependent on several factors: the gestational age in which the infection occurred, head circumference (HC) at birth, and the presence of central nervous system (CNS) manifestations.[8] CZS, in its most severe form, has five features that are rarely seen with other congenital infections: 1) Severe microcephaly with partially collapsed skull; 2) Thin cerebral cortices with subcortical calcifications; 3) Macular scarring and focal pigmentary retinal mottling; 4) Congenital contractures; and 5) Marked early hypertonia and symptoms of extrapyramidal involvement.[9] Additionally, within the spectrum of ZIKV disease, there are infants who are asymptomatic at birth, but can develop clinical repercussions later in infancy.

Obtaining laboratory diagnosis of ZIKV during pregnancy and/or during infancy is challenging in endemic areas. A more accessible radiologic diagnosis with prenatal ultrasound performed by skilled radiologists has been a valuable tool in the recognition of at risk cases.[10] Although the radiologic finding of *in utero* microcephaly is the hallmark of Zika, in our experience, the presence of infratentorial abnormalities seems to be a more significant predictor

of disease severity. The association between microcephaly and ophthalmologic abnormalities has been described in recent literature[11–13] However, these abnormalities can be present with or without concurrent microcephaly or other central nervous system (CNS) abnormalities[11,14] Early identification of severe cases is critical for appropriate counseling of caregivers and prompt initiation of rehabilitation for improved long term outcomes especially neurodevelopment, which can lead to better motor and cognitive functions.[15,16]A radiologic finding with a strong predictive value for ophthalmologic abnormalities has yet to be elucidated in the literature. The aim of this study was to evaluate the association between radiologic findings, specifically infratentorial abnormalities, and ophthalmic abnormalities to provide additional means of early recognition and intervention. A secondary aim was to investigate previously described associations with ophthalmologic abnormalities such as head circumference and gestational age of infection. [11,12]

METHODS

Study Population, Place and Time

A total of 102 children were enrolled in this Cohort study. Infants were enrolled from two convenient sources 1) 24 infants came from a prospective cohort comprised by mother-infant pairs with positive RT-PCR for ZIKV infection during pregnancy in follow-up at IPESQ - Instituto de Pesquisa Professor Joaquim Amorim Neto, a non-profit organization located in Campina Grande, State of Paraíba, Brazil; 2) 78 infants who were referred to the same institution during maternal gestation or after birth because of radiologic abnormalities resembling ZIKV. Infants were enrolled between March 2016 and December 2018.

Newborn infants were evaluated according to a standard assessment protocol that included: history and physical examination, pediatric wellness, ophthalmologic examination and neurological assessment. The evaluations were repeated every three months or earlier if necessary. Imaging studies such as cerebral ultrasonography, Computerized Tomography (CT) and/or Magnetic Resonance Imaging (MRI) were included in the evaluation.

Inclusion Criteria

Two inclusion criteria were used for definition of Congenital Zika Infection 1) Laboratory based-diagnosis: positive RT-PCR for ZIKV in the newborns and/or their mothers during pregnancy 2) Clinical/ neuroimaging based-diagnosis: presence of calcifications in the gray-white matter junction[10,17] *Figure1*. associated with any degree of delayed cortical development ranging from mildly simplified gyral patterns to abnormalities such as lissencephaly, pachygyria or malformations of cortical development, visualized by transfontanel ultrasound and/or CT or MRI in newborn infants. This type of calcification has only been described in congenital ZIKV infection and was not observed in other TORCH conditions. Its location can be at the subcortical area, basal ganglia or cerebellum.

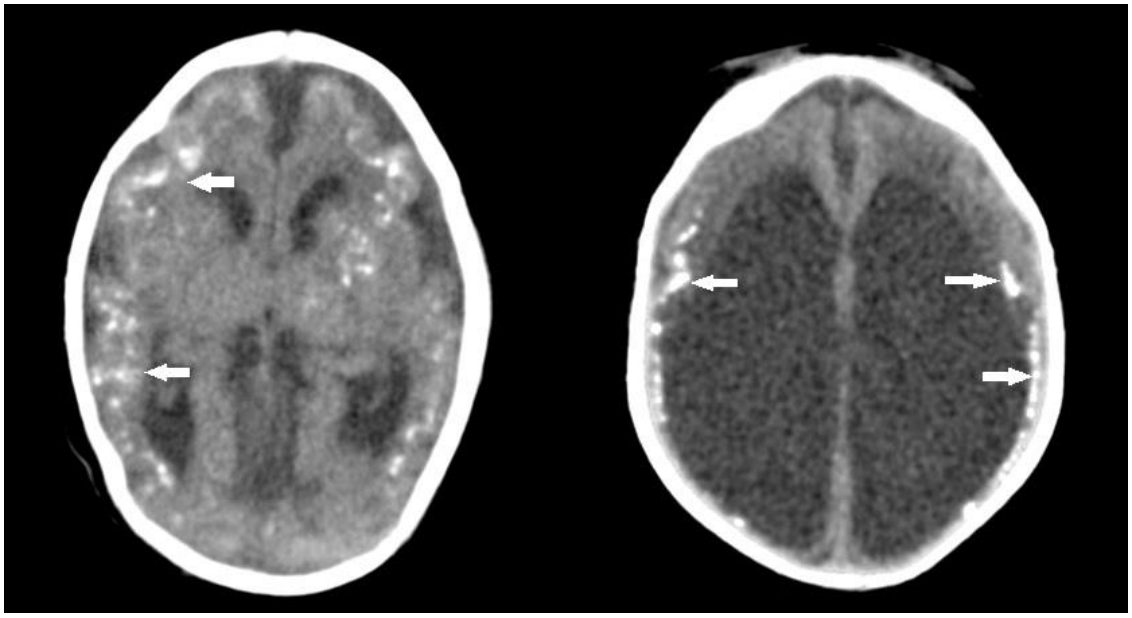

Figure 1: Calcifications in the gray-white matter junction

Pregnant women had ultrasonography performed with Samsung WS80 Elite and newborns infants were evaluated with MRI 1.5-T Espree unit (Siemens Healthcare). Infants were imaged via cranial CT with a 16-section CT scanner (Siemens Healthcare) and/or MRI 1.5-T Espree unit (Siemens Healthcare). Radiologic findings were interpreted by an experienced team of radiologists specialized in neuroimaging.

Exclusion Criteria

27 children were excluded from the analysis due to missing information (i.e., either radiologic or ophthalmologic results). Of note, nine of these children did not have all clinical information available because of early death but were included in a posterior isolated analysis of disease severity. *Figure 2*

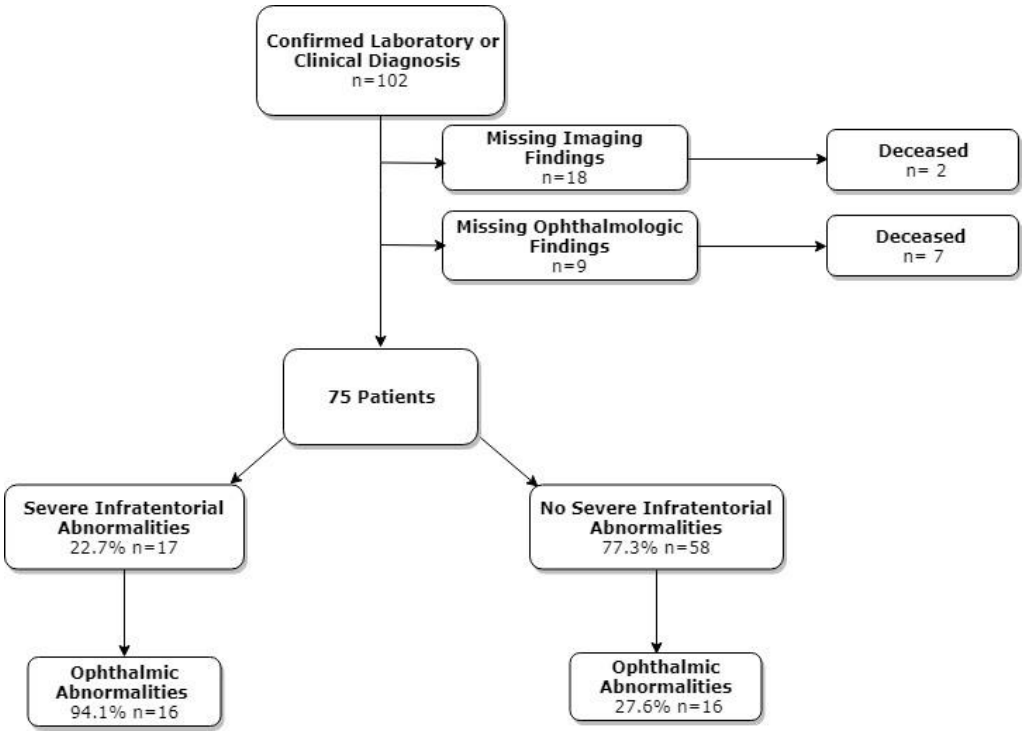

Figure 2: Patient Flowchart

Radiologic Classification

The main radiologic criteria used to differentiate the two groups was the occurrence of infratentorial abnormality which was defined by the presence of severe hypoplasia or dysmorphic cerebellum and vermis cerebelli associated with severe hypoplasia or absent segmentation of the brainstem. *Figure 3*

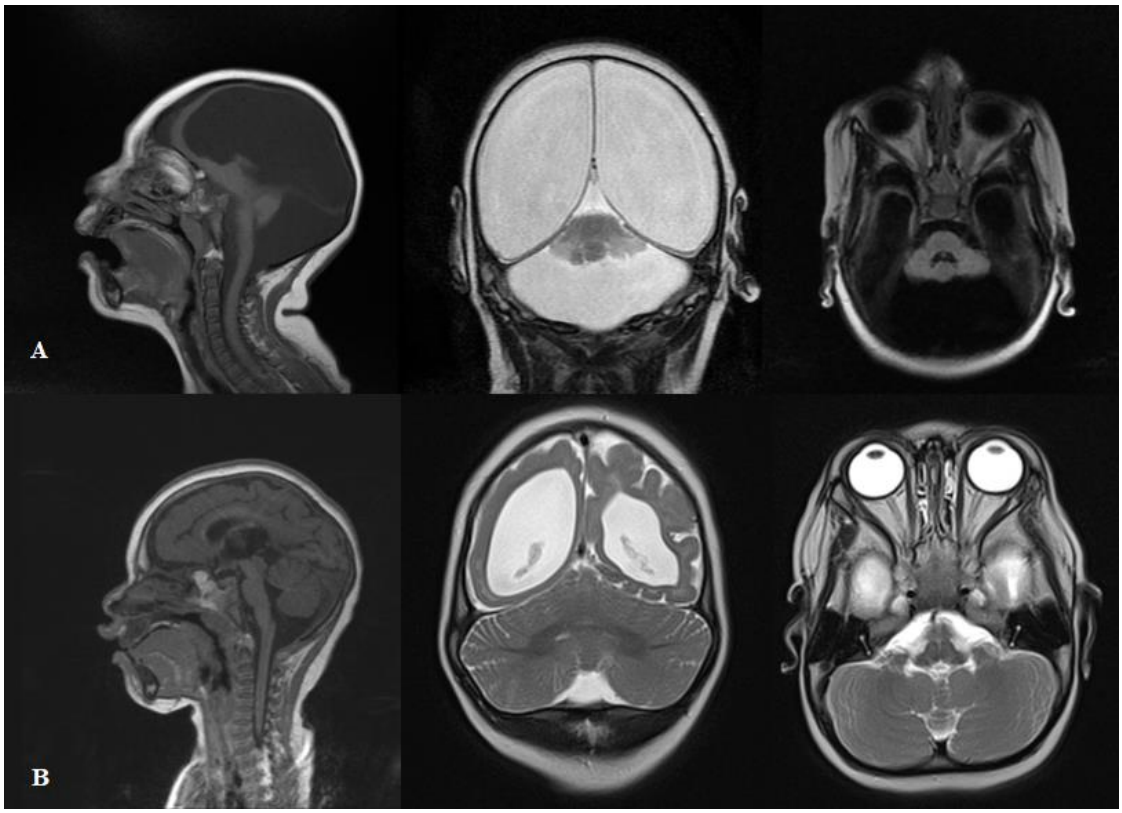

Figure 3: MRI of two children with CZS (from left to right sagittal, coronal and transversal views).A: Severe Infratentorial Abnormality (severe hypoplasia of cerebellum, vermis cerebelli and brainstem). B: Absence of severe infratentorial Abnormality.

Definition of Microcephaly and Classification

Microcephaly was defined according to head-circumference (HC) z-scores from Intergrowth-21st [18] classified as: "Mild Microcephaly", HC below two standard deviations [SD]), and "Severe Microcephaly", HC below 3SD for gestational age and sex. This measurement reflects brain size and brain development in primary microcephaly.[19]

Definition of Ophthalmic Abnormality

Infants were considered to have an abnormal ophthalmologic exam if they had macular chorioretinal atrophy and/or optic nerve alterations such as atrophy, hypoplasia or coloboma in at least one eye. Both abnormalities are recognized causes of vision impairment. *Figure 4*

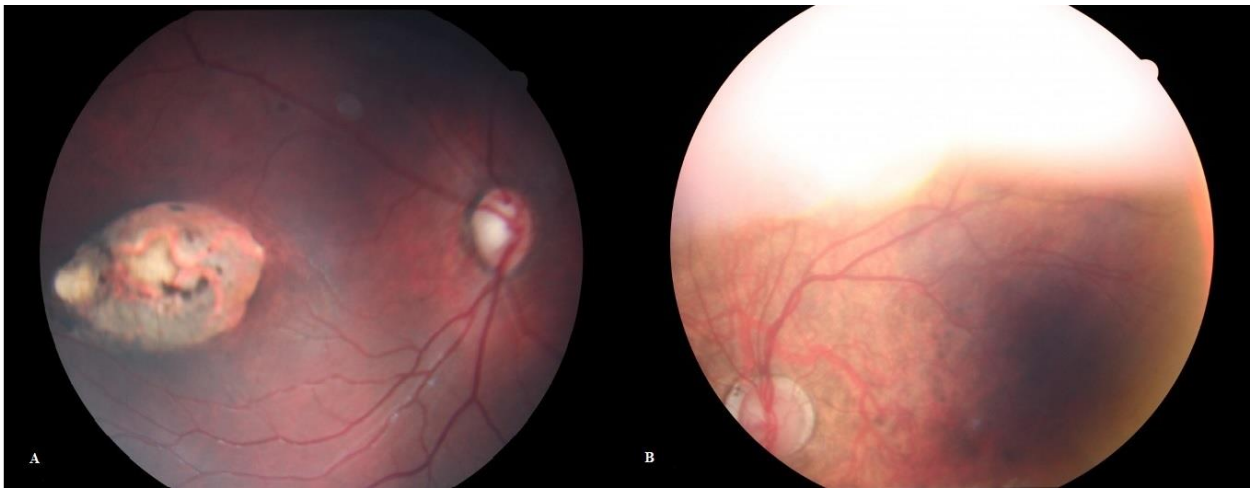

Figure 4 Chorioretinal Macular Atrophy B: Abnormal Optic Disk

Ophthalmologic Examination

Comprehensive examinations were performed as soon as possible after delivery and every three months thereafter until one year of age; After that, complete eye exam was performed every six months if no other abnormality requiring closer follow up was noted. A comprehensive eye examination consisted of the assessment of binocular vision acuity (*Teller Acuity* Cards™ II), ocular alignment and motility, biomicroscopy, pupillary reflex, cycloplegic refraction and indirect ophthalmoscopy to evaluate eye fundus. Children with any major fundus eye abnormalities were evaluated by Retinography (CF-1 Digital Mydriatic Retinal Cameras Canon, USA)

Other Potential Factors Associated with Ophthalmic Abnormalities

Other variables collected for possible confounder adjustment in the statistical analysis included mother's gestational age at infection, infant birthweight and length at delivery, pattern of calcification, presence of fetal akinesia, death (neonatal or during follow-up) and mother's socioeconomic status.

IRB and Informed Consent

The study was approved by the Institutional Review Board (IRB) for Local Research UEPB/PRPG- Universidade Estadual da Paraíba/Pró Reitoria de Pós-Graduação and by UCLA - University of California Los Angeles Institutional Review Board and conformed to all requirements of the US Health Insurance Portability and Accountability Act of 1996. All mothers or caretakers who fulfilled enrollment criteria were invited to participate in the study and provided written informed consent for study participation. All data were deidentified.

STATISTICAL ANALYSIS

Descriptive and exploratory statistics were described as means (±SD), medians (interquartile range [IQR]), and frequency distributions. The main outcome of interest was the presence of ophthalmic abnormality as a dichotomous variable (normal/abnormal) and the main predictor of interest was the presence or absence of infratentorial abnormality. Fisher's exact test was used for small sample analysis and Pearson's chi-squared test (χ^2) for categorical data. Logistic regression was used to assess the relationship between the dependent binary outcome variable and other independent variables. A two-sided p-value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using SAS statistical software (9.4 SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 102 infants whose mothers were exposed to ZIKV during pregnancy and were referred to IPESQ from March 2016 to December 2018 were eligible for study participation as seen in Figure 1. Sixteen 16 (21.3%) infants were identified because of a positive maternal ZIKV RT-PR during pregnancy; 59 (78.7%) newborns were referred because of clinical suspicion of ZIKV infection with a radiologic abnormality identified. Twenty-seven patients were excluded from this analysis because of missing data. Table 1 shows descriptive summary statistics.

Table 1. Descriptive summary statistics

Characteristics	Total n	Total %	With Ophthalmic Abnormality N=32			Without Ophthalmic Abnormality N=43		
			n	%	Mean±SD	n	%	Mean±SD
Diagnosis								
Laboratory	35	46.7	21	60	---	14	40	---
Clinical	40	50.3	11	27.5	---	29	72.5	---
Infratentorial Abnormality								
Yes	17	22.3	16	94	---	1	5.9	---
No	58	77.7	16	28	---	42	72.4	---
Microcephaly								
Yes	53	70.7	23	43.4	29.1 ± 2.4	30	56.6	30.3±1.5
No	22	29.3	9	40.1		13	59.1	
Trimester of maternal infection								
1st	47	68.1	24	51.1	11.5 ± 5.5	23	48.9	14.1 ± 8.2
2nd	19	27.5	6	31.6		13	68.4	
3rd	3	4.4	0	0		3	100	
Calcification Pattern								
1	24	32	5	20.8	---	19	79.2	---
2	48	64	24	50	---	24	50	---
3	3	4	3	100	---	0	0	---
Birthweight								
Adequate	68	90.7	29	42.6	2,651± 484.8	39	90.7	2,857± 457.9
Small for gestational age	5	6.7	2	40		3	7.0	
Big for gestational age	2	2.6	1	50		1	2.3	

*Means of HC in cm, trimester of maternal infection in weeks, birthweight in grams. Calcification Pattern 1:Subcortical 2: Subcortical and Basal ganglia 3: Subcortical, Basal ganglia and Cerebellum

The mean home income per capita was approximately 85 USD per month. The mean maternal age was 27 years (SD ± 6.7). More than 90% of mothers had ZIKV related symptoms during pregnancy such as a cutaneous rash at a mean gestational age of 13 weeks (SD ± 7.2). Almost half of the infants were male (49.3%).

Of 17 infants with infratentorial abnormalities, 16 (94.1%) had ophthalmic abnormalities (OR 42.0: 95%CI 5.1-342.9, p =0.0005), while among 58 patients without infratentorial abnormalities, 16 (27.6%) had ophthalmic abnormalities (*Figure1*). Similar associations were observed when the two types of ophthalmic abnormalities were analyzed individually. Patients with infratentorial abnormalities were almost 24 times more likely to have chorioretinal atrophy in the macular area when compared to those without infratentorial abnormalities (OR 23.7: 95% CI 6.0-93.3, $p < 0.0001$). Similarly, infants with infratentorial abnormalities were also very likely to have optic nerve abnormalities (OR 11.5; 95% CI 3.3-40.0 $p < 0.0001$) (Table 2). Infratentorial abnormalities were more frequently associated with ophthalmic abnormalities (94%) than microcephaly (43.4%) p=0.0002.

Table 2: Evaluation of variables potentially associated with Ophthalmic Abnormalities, (n=75).

Characteristic	Total		Crude Odds Ratio (cOR)		Adjusted Odds Ratio (aOR)[a]		t-test [b]
	n	%	cOR 95% CI	p-value	aOR 95% CI	p-value	
Infratentorial Abnormalities	17	100					
With Ophthalmic Abnormalities	16	94.1	42.0 5.1-342.9	0.0005	37.2 4.4-14.4	0.0028	---
Chorioretinal Atrophy	13	76.5	23.7 6.0-93.3	0.0001	---	---	---
Optic Nerve Abnormality	12	70.6	11.5 3.3-40.0	0.0001	---	---	---
Without Ophthalmic Abnormalities	1	5.9	1.0	----	1.0	---	---
Microcephaly (HC)	53	100					
With Ophthalmic Abnormalities	23	43.4	1.4 1.1– 1.8	0.0148	0.8 0.2-2.8	0.7192	0.0165
Without Ophthalmic Abnormalities	30	56.6	1.0	---	1.0	---	---
Gestational Age	69	100					
With Ophthalmic Abnormalities	30	43.5	2.8 0.9-8.3	0.0679	2.5 0.7-9.3	0.1748	0.1233
Without Ophthalmic Abnormalities	39	56.5	1.0	---	1.0	---	---

[a] aOR values were generated by the simultaneous entry of covariates in a logistic regression model. [b] Comparing results with and without ophthalmic abnormality.

The group of children with ophthalmic abnormalities (N=32) had a mean HC 29.1 cm (SD ± 2.4 [IQR 23-35]). By comparison, the group without ophthalmic abnormalities (N=43) had a mean HC measurement of 30.3 cm (SD ±1.5 [IQR 27-34]). There was a statistically significant difference in HC between the two groups (p =0.0165). For every centimeter decrease in HC, there was a 1.4 increase in the likelihood of ophthalmic abnormalities being present (OR 1.4; 95%CI 1.065 – 1.784, p=0.0148).

The group of children with ophthalmic abnormalities for whom information on gestational age was available (N=30) had a mean gestational age at infection of 11.5 weeks (SD ± 5.5 [IQR 6-28]). The group without ophthalmic abnormalities (N=39) had a mean gestational age at infection of 14.1 weeks (SD ± 8.2 [IQR 6-34]). A statistically significant difference in gestational age between both groups was not noted (p=0.1233). A comparison of ophthalmic outcomes between first trimester infections and combined second and third trimester infections also did not yield statistically significant results (OR 2.8; 95% CI 0.9-8.3). For every weekly increase in gestational age, there was a trend towards a decrease in the risk of ophthalmic abnormalities, (OR 0.95; 95%CI 0.9 – 1.0) however this finding was not statistically significant.

DISCUSSION

Undoubtedly, the presence of infratentorial abnormalities is strongly associated with ophthalmologic findings; these findings are unlikely to be due to bias or confounding. To the best of our knowledge, this is the first report of a radiologic finding predicting ophthalmic abnormalities in children with CZS. It is fundamental to point out, however, that the absence of infratentorial abnormalities was not necessarily associated with a normal ophthalmologic

outcome. In our group of patients, 50% of children with ophthalmic abnormalities did not have the radiologic predictor.

Although several ophthalmologic abnormalities have been described in patients with antenatal ZIKV exposure including cataracts, calcifications, microphthalmia, remnants of a pupillary membrane and glaucoma, the most prevalent and sight-threatening findings are observed in the retina and optic nerve, which are the ophthalmic abnormalities studied in this analysis'.[11,12,20,21] ZIKV is recognized for its neurotropism and ability to injure neural progenitor cells; this contributes to its devastating impact in the brain and other neurosensory organs such as the eye. [22] In addition to its pathophysiologic effects, there is in vivo evidence that ZIKV leads to depletion of ganglion cells, borderline inner nuclear layer thinning, and also less prominent photoreceptor loss which can be attributed to foveal maldevelopment and central chorioretinal atrophy. [23]

The positive predictive value of this association was 94.1%. In other words, there was 94% certainty in anticipating the ophthalmologic abnormality when the radiologic predictor was present. The negative predictive value for this association was 27.6%. Therefore, if no infratentorial abnormality was seen, there was still a high possibility of finding an ophthalmic abnormality. The sensitivity of the radiologic predictor was 50%, which implies that half of the neonates with ophthalmic abnormalities would not be identified if infratentorial abnormality was used as a radiologic screening test. On the other hand, the specificity of the radiologic predictor was 97.7%. The main public health advantage of identifying infratentorial abnormalities during prenatal ultrasound (US) would be the possibility of predicting low vision outcomes *in utero* which would allow infants to enter early rehabilitation programs soon after birth in order to curtail repercussions of visual impairment.

Prenatal US has been shown to have a sensitivity of 48.9% in predicting adverse neonatal outcomes in a study that evaluated mothers with ZIKV exposure during pregnancy.[24] There is no data available to demonstrate whether any specific ultrasonographic prenatal feature may be associated with ophthalmic abnormalities. It is also uncertain whether a prenatal US has adequate sensitivity to detect infratentorial abnormalities in comparison to postnatal imaging studies with CT or MRI as the gold standard. ZIKV infection is a relatively new disease that most affected individuals do not have any symptoms. For this reason the majority of cases were diagnosed after delivery.

In our patient sample, the prevalence of ophthalmic abnormalities had an overall higher prevalence of 42.7% when compared to other ZIKV infection ophthalmologic studies. Other cohorts reported frequencies of approximately 18.6% (Yepez et al) [25], 21.4% (Zin et al) [11] and 24.1% (Freitas et al).[13] *Appendix Table 1*. We did have a very heavily symptomatic group of children who were referred to our institution because of the presence of fetal abnormalities detected during prenatal US; i.e., 79% of all children referred to our cohort. Consequently, patients with varied radiologic abnormalities were overrepresented in our population and the association with ophthalmic abnormalities could thus be analyzed. All our patients had radiologic abnormalities including cerebral calcifications, ventriculomegaly, infratentorial abnormalities, pachygyria or lissencephaly, while in the other studies, those findings were not as prevalent [Zin et al (27.7%)][11]. The study with the most similar patient population was the one with the greatest geographic proximity to our site, which was the study in Pernambuco.[12] This study also reported a high prevalence of radiologic abnormalities (96.7%) and comparable ophthalmologic abnormalities (46.3%) *Appendix Table 1.*

Previous studies have suggested an association between the presence of lower HC and ophthalmologic findings[11,12]; this finding was also noted in our study when we compared the means of HC between children with and without ophthalmic abnormalities. Most of the patients in our sample had microcephaly (70.7%) while in a Rio de Janeiro eye study the prevalence was 17.9%[11]. The prevalence of microcephaly was 100% in other studies from northeastern Brazil, [12,13] Venezuela and Colombia.[25] Although this association seems to be true, we hypothesize that there might not be a linear relationship between HC and ophthalmic abnormalities or potentially such an association could be present except for the most severe cases. We noted that most children who died early did not have microcephaly (66.7%). A potential explanation is that a compensatory ventriculomegaly may develop with the intention to balance the lack of brain tissue. Thus, children may have a near normal or normal HC in this setting. Consequently, it is possible that ZIKV infection without microcephaly might be under notified. Therefore, instead of the HC measurement, other brain measurements should be considered as an alternative to HC for a more reliable correlation with CNS abnormalities and ophthalmic abnormalities. Although there is definitely an association, microcephaly is not always predictive of ophthalmologic abnormalities.

Contrary to other studies,[11–13] we did not see an association between first trimester infections and ophthalmic abnormalities. This is likely due to the fact that our patients were mainly infected in the first trimester and the comparator number in the other trimesters was low. Given the very high number of eye abnormalities in our patients, we can infer that infection in the first trimester was likely to result in newborns with ophthalmic abnormalities.

Visual impairment might not be the only consequence of viral pathogenesis in the retinal structures. Brain damage by ZIKV also has the potential to cause detrimental consequences to

visual acuity. Further studies determining which brain abnormalities correlate with low vision are essential in order to address this issue.

The major strength of our findings is that we were able to demonstrate a strong association between a radiologic risk factor (infratentorial abnormalities) and ophthalmic abnormalities. The sample size was adequate for the main purpose of the study as it included one of the largest groups of children reported to date who had a variety of radiologic abnormalities and significant disease severity with and without microcephaly. The findings have important public health repercussion since the radiologic parameters allows parents/caretakers to anticipate an abnormal ophthalmologic outcome in advance, thus avoiding delays in diagnosis and enabling prompt initiation of rehabilitation efforts.

One of our major study limitations was that we had a very limited number of children who were mildly symptomatic or asymptomatic, so we cannot make inferences regarding absence of radiologic findings and absence of ophthalmologic abnormalities. We also were not able to laboratory confirm the diagnosis of ZIKV infection in most pregnant women, nevertheless all children were born in the epicenter/ ground zero of the ZIKV epidemic in Brazil. Additional limitations are that similarly to other studies, pregnant women who did not have symptoms related to ZIKV but had the disease may not have been included given our entry criteria. As a result, the present report reflects findings observed in a symptomatic population of women only. Our population had extensive radiologic abnormalities which underscores inclusion of more severe cases than other studies. One can consider this a limitation, but to our main purpose of the study it was helpful having such affected population. Of note, our results do not reflect a representation of the general population of infants with antenatal ZIKV exposure as most of our infants were severely affected.

CONCLUSION

The presence of infratentorial abnormalities is without hesitation a significant predictor of ophthalmic abnormalities when identified during prenatal ultrasonography. We endorse the recommendation that all neonates whose mothers had possible ZIKV exposure at any time during pregnancy should have a complete ophthalmologic examination.

APPENDICES

Appendix Table 1: Population characteristics and ophthalmic abnormalities reported in different ZIKV studies

	Total	MC	Radiologic abnormalities[a]	Presence of Ophthalmic Abnormality (Sight-threatening lesions)					
				Total	Macular Atrophy	Optic Nerve	With MC	Without MC	Without CNS abnormality
IPESQ, Paraiba	75	53 (70.7%)	75 (100%)	32 (42.7%)	20 (62.5%)	22 (68.7%)	23 (71.9%)	9 (28.1%)	0 (0%)
Zin, Rio	112	20 (17.9%)	31 (27.7%)	24 (21.4%)	7 (29.2%)	19 (79.2%)	14 (58.3%)	10 (41.7%)	8 (33.3%)
Ventura, Pernambuco	40	40 (100%)	29/30 (96.7%)*	22 (46.3%)	7 (31.8%)	5 (22.7%)	22 (100%)	0	**
Yepez, Colombia/ Venezuela	43	43 (100%)	**	8 or less?	3 (7%)	5 (12%)	8 at least	0	**
Freitas, Bahia	29	29 (100%)	**	7 (24.1%)	3 ***	4	7	0	**

[a]At least one present: ventriculomegaly, calcifications, posterior fossa abnormality, pachygyria, lissencephaly
*30 had CT results, 29 had calcifications
** Not mentioned
*** Eyes

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