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Neurological and neuropsychological sequelae of Zika virus infection in children in León, Nicaragua

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ABSTRACT

Objectives. To describe the presence and persistence of neurological and neuropsychological sequelae among children with acquired Zika virus infection and assess whether those sequelae were more common in children infected with Zika virus compared to uninfected children.

Methods. We conducted a prospective cohort study of children with and without Zika virus infection in León, Nicaragua, using a standard clinical assessment tool and questionnaire to collect data on symptoms at three visits, about 6 months apart, and a battery of standardized instruments to evaluate neurocognitive function, behavior, depression, and anxiety at the last two visits.

Results. Sixty-two children were enrolled, with no significant differences in demographics by infection group. Children infected with Zika virus had a range of neurological symptoms, some of which persisted for 6 to 12 months; however, no consistent pattern of symptoms was observed. At baseline a small percentage of children infected with Zika virus had an abnormal finger-to-nose test (13%), cold touch response (13%), and vibration response (15%) versus 0% in the uninfected group. Neurocognitive deficits and behavioral problems were common in both groups, with no significant differences between the groups. Children infected with Zika virus had lower cognitive efficiency scores at the 6-month visit. Anxiety and depression were infrequent in both groups.

Conclusions. Larger studies are needed to definitively investigate the relationship between Zika virus infection and neurological symptoms and neurocognitive problems, with adjustment for factors affecting cognition and behavior, including mood and sleep disorders, home learning environment, history of neuroinvasive infections, and detailed family history of neuropsychological problems.

Keywords Zika virus infection; child; nervous system diseases; neuropsychological tests; Nicaragua.

Zika virus (ZIKV) is a mosquito-borne flavivirus that infected millions of people in the Americas and across the globe in 2015 and 2016 (1). Although ZIKV infection typically presents with mild symptoms, the World Health Organization declared ZIKV infection a Public Health Emergency of International Concern because of its apparent association with severe central nervous system anomalies in fetuses and neonates with congenital exposure to ZIKV (2, 3). Although studies have evaluated the biological pathways and outcomes of congenital ZIKV infection, research into postnatally acquired ZIKV infection in children is limited (4, 5).

While symptomatic ZIKV infection in postnatally exposed children appears to be mild (6), severe neurological complications have been described in case reports (7), epidemiological

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studies (8–10), and in postnatally exposed animals (11, 12). However, information about neurological complications is limited. The only study to date to evaluate neurocognitive functioning among children with acquired ZIKV infection assessed 37 people infected with ZIKV, aged 12 years and older, and found that they had lower memory scores compared to a dengue-infected group (13).

Like many Latin American countries, Nicaragua experienced epidemic spread of ZIKV throughout the country during 2016 (13, 14). To better understand the potential impacts of ZIKV infection, we conducted a prospective cohort study of neurological symptoms and neuropsychological functioning in ZIKV-infected children and uninfected children in León, Nicaragua.

METHODS

Study population

Nicaragua, a Central American nation with a population of 6.1 million in 2016, is among the lowest-income countries in Latin America (14). The department of León is an economically and geographically diverse region with a high intensity of *Aedes aegypti* mosquito-transmitted arboviruses. Between January 2016 and August 2017, researchers at the University of North Carolina, Chapel Hill and the Universidad Nacional Autónoma de Nicaragua, León conducted a ZIKV transmission study that included patients aged 2 years and older seeking care at the Perla Maria de Nori Health Center for fever, maculopapular rash, or non-suppurative conjunctivitis of less than 1 week's duration and asymptomatic household members (15). Thirty-five per cent of those enrolled in the study were children.

Between November 2016 and March 2018, children aged 2–17 years from the transmission study were recruited to participate in a study of neurological symptoms and neuropsychological functioning. Eligibility criteria included: complete data on age, sex, and ZIKV status; and parental permission/assent to participate in three study visits, conducted about 6 months apart.

Definition of Zika-infected and uninfected children

Convalescent serum specimens collected from participants at the second and third visits were stored temporarily in the laboratory of the Universidad Nacional Autónoma de Nicaragua, León before shipment to the University of North Carolina for testing. ZIKV infection was defined as a positive result in one or both specimens using a modified version of a validated serological assay based on Z-EDIII antigen (16). The assay has been validated with a larger panel of samples shown to be highly specific for ZIKV infection in convalescent samples collected after 2 months. Based on well characterized samples, an optical density cut-off of 0.34 was used to indicate prior ZIKV infection. Participants without convalescent specimens were tested in the transmission study by a reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) test using a Roche LightCycler 96 at the Universidad Nacional Autónoma de Nicaragua, León laboratory, using previously described methods (15, 17). Participants with positive PCR results were also included in the ZIKV-infected group. Participants with a negative RT-qPCR but no serology were excluded due to uncertainty about their ZIKV status; all other participants were characterized as Zika-uninfected.

Study assessments

A study physician was trained by a neurology professor from University of North Carolina-Chapel Hill to conduct the neurological examination. At each visit, the physician conducted a clinical assessment of a standard set of neurological signs and symptoms and administered a questionnaire to the participant or the participant's parent/guardian about recent changes in function that could be associated with neurological deficits. The clinical assessment included evaluation of: awareness level; general speech/language; visual acuity; ocular movement; facial and upper and lower extremity strength; bicep, knee, and ankle reflexes; a finger-to-nose test; cold and vibration touch response; and gait. The questionnaire assessed: current energy level (fatigue); interest in social activities; experience of memory loss or confusion; problems with speed of thought or concentration; headache or seizure in the previous 4 weeks; recent problems with hearing, vision, walking, use of hands and arms, bladder control, or numbness, tingling, or loss of feeling in any part of the body. For any indicated abnormality, additional questions sought information about the severity of the problem and timing (i.e., started 1 week, 2 weeks, 1 month, 1–6 months, or more than 6 months before).

At the second and third visits, about 6 and 12 months, respectively, after the baseline visit, a trained psychologist or physician conducted a battery of age-appropriate neuropsychological assessments – Woodcock Muñoz III Cognitive Battery (18, 19), Test of Nonverbal Intelligence 4 (20), the Child Behavior Checklist (21), and depression and anxiety questionnaires as appropriate for age group, i.e., Children's Depression Inventory (22), Beck Depression Inventory (23), Spence Child Anxiety Scale (24), and Beck Anxiety Scale (25). For the Woodcock Muñoz III Cognitive Battery, raw scores from all reported subtests and composite variables were converted to age-based standard scores. Two composite scores are reported: cognitive efficiency (visual matching and numbers reversed) and working memory (numbers reversed and auditory working memory). Overall raw scores of the Test of Nonverbal Intelligence 4 were converted to age-based standard scores. The standard score scale for the Woodcock Muñoz III Cognitive Battery and Test of Nonverbal Intelligence 4 is based on a mean of 100 and standard deviation (SD) of 15. For the Child Behavior Checklist, raw scores for all reported items and composite domains were converted to age-based t-scores. The standard score scale is based on a mean of 50 and SD of 10. The t-tests of parent response score distributions across age group (2–5 years and 6–18 years) indicated that compilation of form data into a single dataset was appropriate. For all other instruments, we examined the total summed score.

Children are considered at risk for diagnosable cognitive, behavioral, and/or mood disorders if their score falls above or below a certain threshold. Additional details about these instruments, including the definition of at-risk scores, are outlined in Table 1. To ensure that terminology was locally appropriate to the Nicaraguan context, we pilot tested all instruments among a small number of children and their parents.

TABLE 1. Description of assessments included in the neuropsychological assessment battery

Assessment	Administration method (age range of administration)	Measured constructs and outcomes	At-risk score defined as:
Woodcock-Muñoz III Cognitive Battery	Direct assessment by evaluator (2–18 years)	Comprehension-knowledge, visual-spatial thinking, auditory processing, processing speed, short-term memory, long-term retrieval, working memory, broad attention, and executive processes.	≤ 1.5 SDs from the mean (i.e., a score of ≤ 78)
Test of Nonverbal Intelligence-4	Direct assessment by evaluator (6–18 years)	Assessment of intelligence, aptitude, abstract reasoning, and problem solving. Language-free, thus ideal for evaluating those with limited language ability.	≤ 1.5 SDs from the mean (i.e., a score of ≤ 78)
Child Behavior Checklist	Caregiver report (2–18 years)	Ratings of internalizing, externalizing, and total behavior problems, and specific clinical scales (e.g., attention problems, anxiety, and withdrawal) which vary depending on age group.	≥ 1.5 SDs from the mean (i.e., a score of ≥ 65)
Child Depression Inventory	Self-report (7–12 years)	Evaluates dysphoria (e.g., depressive mood, sadness, and worry) and negative self-esteem (e.g., inefficacy and wickedness).	≥ 19
Beck Depression Inventory	Self-report (13–18 years)	Intensity of depression.	≥ 31
Spence Child Anxiety Questionnaire	Self-report (8–12 years)	Frequency of occurrence of experiences of anxiety, fears, and self-rating of positive aspects.	Ages 8–11 years, total scores ≥ 40 (males), ≥ 50 (females) Ages 12–15 years, total scores ≥ 33 (males), ≥ 39 (females)
Beck Anxiety Scale	Self-report (13–18 years)	Subjective, somatic, or panic-related anxiety	≥ 36

SD, standard deviation.
Source: Prepared by the authors based on published data. Woodcock Muñoz III Cognitive Battery (18, 19); Test of Nonverbal Intelligence 4 (20); Child Behavior Checklist (21); Children's Depression Inventory (22); Beck Depression Inventory (23); Spence Child Anxiety Scale (24); Beck Anxiety Scale (25).

Statistical analyses

We calculated descriptive summary statistics overall and by ZIKV infection for baseline characteristics of interest and performed chi-squared tests to determine if there were any differences between groups.

To investigate the long-term sequelae in children with ZIKV infection, we describe self-reported and physician-observed neurological symptoms and neuropsychiatric deficits in children who had their baseline visit within 60 days of enrollment into the transmission study.

Because all participants were asked to provide information about the estimated timing of the start of neurological symptoms, we compared that to the timing of infection to determine whether the reported problem began after or before ZIKV infection, or whether the timing was unknown. Only post-infection symptoms are reported for the baseline, 6-month, and 12-month visits in this descriptive analysis.

Finally, we examined neuropsychological functioning at the 6- and 12-month visits by ZIKV infection. For each test and select subtests, we generated descriptive summary statistics and the prevalence of children at risk. We calculated difference in prevalence at risk between the infected and uninfected groups, including 95% confidence intervals (CIs), using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Ethics statement

The Institutional Review Board of RTI International reviewed and approved this study. Study staff read consent forms to parents or guardians accompanying minors who participated in this study, and read assent forms to minors aged 7–17 years. Parents/guardians and minors aged 7–17 years had the opportunity to ask questions about the study before signing the consent and assent forms or declining to participate. Copies of the consent and assent forms were provided to participants. Parents or guardians of participating minors were provided with a monetary reimbursement to cover their time and transportation. All participant information was kept confidential by securing paper records locked in the study coordinator's office and entering data into a password-protected web-based REDCap electronic database hosted by RTI International (26).

RESULTS

Sample characteristics

Of 77 children enrolled in the transmission study at the time of suspected ZIKV infection, 71 consented to enroll in our study. Nine participants were excluded from the analysis because of unconfirmed age (one child) or ZIKV testing results (seven children), and a preexisting neurological condition, focal seizures (one child). There was a delay between enrollment into the transmission study and subsequent enrollment in our follow-up study: the first assessment (baseline visit) occurred 2–217 days (median 32; interquartile range (IQR) 16–146 days) after enrollment in the transmission study; the last assessment occurred 220–761 days (median 408; IQR 378–561 days) after enrollment in the follow-up study. All 62 children for whom baseline visit data were collected participated in a follow-up visit about 6 months later (6-month visit) and 60 participated in a second follow-up visit (12-month visit). Laboratory results confirmed that 40 children had ZIKV infection and 22 had not.

No significant demographic differences were seen between the ZIKV-infected and -uninfected groups (Table 2). In children aged 13 years and older, no drug use was reported and no children were at risk of alcohol abuse (data not shown).

TABLE 2. Baseline characteristics of the participants, by Zika virus infection, León, Nicaragua

Characteristic	ZIKV (*n* = 40)	No ZIKV (*n* = 22)	Total (*n* = 62)	*p*-value[a]
	n (%)	n (%)	n (%)	
Age group at baseline (years)				
2–5	5 (12)	3 (14)	8 (13)	0.4697
6–12	16 (40)	12 (54)	28 (45)	
13–17	19 (48)	7 (32)	26 (42)	
Sex				
Male	18 (45)	10 (46)	28 (45)	0.9725
Female	22 (55)	12 (54)	34 (55)	
Poverty[b]				
Not poor	11 (28)	8 (36)	19 (31)	0.2173
Poor	18 (45)	5 (23)	23 (37)	
Extremely poor	11 (28)	9 (41)	20 (32)	
Family history of neurological disorder				
Yes	8 (20)	3 (14)	11 (18)	0.5303
No	32 (80)	19 (86)	51 (82)	

ZIKV = had Zika virus infection; no ZIKV = did not have Zika virus infection.
[a] Chi-squared test.
[b] Poverty definitions. Not poor if there are no problems with sanitary services, home conditions, home density, education, or economic problems; poor if one of these problems exists; extremely poor if two or more of these problems exist. Sanitary services problem: if water source is a community well, river, bottled water or gifted, OR there is no toilet, OR there is no electricity. Home conditions problem: if walls are constructed from wood, palm leaves, or cardboard/plastic/metal, OR there is a dirt floor, OR the roof is constructed from cardboard/plastic or palm leaves. Home density problem: if the number of people living in the home divided by the number of rooms used for sleeping is > 2.5. Education problem: if one or more children age 7–14 years living in the home does not attend school. Economic problem: if number of dependents living in home plus the number of unemployed persons (age 15-65 years) living in the home divided by the number of employed persons living in the home is > 2 OR there are one or more dependents living in the home OR one or more unemployed persons (age 15–65 years) living in the home AND no one living in the home is employed.
Source: Prepared by the authors from study data.

Neurological observations

Twenty ZIKV-infected children had their baseline visit within 60 days of enrollment in the transmission study. These children displayed a range of neurological symptoms, some persisting for 6 to 12 months after infection, although no consistent pattern of symptoms was observed. Some of the more severe or persistent problems experienced included: a 13-year-old male with persistent memory difficulty and cognitive slowness accompanied by sensory loss; a 7-year-old female exhibiting abnormal awareness at all three visits; a 13-year-old female with severe headache and memory difficulty at two consecutive visits; a 16-year-old male with persistent headache and sensory loss; and a 13-year-old male with moderate sensory loss at baseline and persistent self-reported forgetfulness and cognitive slowness. Full descriptive information about the neurological outcomes of these children is available upon request to the authors.

When comparing the ZIKV-infected and -uninfected cohort, hallmark signs of neurological problems (e.g., fatigue, memory loss/confusion, difficulty concentrating/reduced speed of thought, and sensory problems) were self-reported by children or their parents in both groups (Table 3). At baseline, the most commonly reported symptoms were headache, lethargy, myalgia, and arthralgia; these symptoms were more frequently reported in ZIKV-infected children at the baseline and 6-month visits.

Clinically observed abnormal awareness and speech/language abnormalities were observed in both groups and varied over time (data not shown). At baseline, a small percentage of children with ZIKV infection had an abnormal finger-to-nose test (13%), cold touch response (13%), and vibration response (15%) versus 0% in the uninfected group (data not shown). No seizures, paralysis, or walking problems were reported (data not shown). At all time points, ZIKV-infected children were more likely to experience more than two strength or reflex problems compared to children who were not exposed – baseline: 15% versus 0%; 6-month: 7.5% versus 0%; 12-month: 10% versus 4.5%. Detailed data are available upon request.

Neuropsychological and behavioral findings

Descriptive statistics for the cognitive, behavior, anxiety, and depression assessments are shown in Table 4. Low scores on the neurocognitive tests were common in both groups. The 95% CIs of the difference in the at-risk prevalence were wide, with the CIs crossing zero for most domains (Figure 1). The proportion of children with an at-risk cognitive efficiency composite score was significantly greater in the infected group than the uninfected group at 6 months, but this difference was smaller at the 12-month visit. This observed difference in the cognitive efficiency composite score is driven mainly by the fact that 100% of infected children at 6 months had an at-risk score for the visual matching subtest (data not shown). At the 6-month visit, a larger proportion of children in the uninfected group had caregiver-reported behavior problems as assessed by the Child Behavior Checklist, compared to the infected group. This pattern was also seen at the 12-month visit, although CIs were wide due to fewer participant responses. We did not see any difference in depression or anxiety by infection. In general, the small sample sizes precluded drawing any solid conclusions about differences in neuropsychological deficits and behavioral problems between ZIKV-infected and -uninfected children.

DISCUSSION

We conducted an evaluation of neurological, neurocognitive, and neuropsychiatric outcomes following ZIKV infection in children and included children without ZIKV infection for comparison. Although our study is small, it builds on emergent findings suggesting possible neurological involvement of ZIKV in children postnatally exposed to the virus, with long-term impacts in some cases. Neurocognitive and behavioral problems, some reflecting clear deficits, were common in both groups, while anxiety and depression were observed infrequently in both groups.

Although case reports have described central nervous system and peripheral nervous system sequelae following ZIKV infection, characterization of neurological symptoms of acquired ZIKV infection among children is limited (7, 27). The largest pediatric case series to date included 18 756 suspected pediatric cases of ZIKV infection reported to Colombia's national surveillance system. Ninety-six instances of neurological manifestations secondary to ZIKV were reported, including peripheral nervous system disorders and degenerative and inflammatory diseases of the central nervous system (9). However, only 25 of the 96 suspected cases were tested for ZIKV, and only 12 of those had laboratory evidence of ZIKV. In a recent systematic review, six of 34 pediatric ZIKV case reports included descriptions of neurological complications, including

TABLE 3. Self-reported symptoms (by the children or their parents) experienced by Zika virus infection at baseline, 6-month, and 12-month visits, León, Nicaragua

Self-reported symptoms that started after Zika virus test	Baseline		6-month visit		12-month visit	
	ZIKV (*n* = 40)	No ZIKV (*n* = 22)	ZIKV (*n* = 40)	No ZIKV (*n* = 22)	ZIKV (*n* = 40)	No ZIKV (*n* = 20)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Fatigue	5 (13)	2 (9)	7 (18)	3 (14)	5 (13)	3 (15)
Mild fatigue, limits most demanding activities	2	0	4	3	3	1
Moderate or severe fatigue	3	2	3	0	2	2
Memory loss/confusion	5 (13)	3 (14)	4 (10)	0 (0)	5 (13)	5 (25)
Mildly more forgetful than usual	2	1	2	0	4	3
Definite memory difficulty or confused/ persistently confused	3	2	2	0	1	2
Difficulty concentrating/reduced speed of thought	5 (13)	2 (9)	4 (10)	1 (5)	5 (13)	5 (25)
Needs longer time to accomplish normal tasks, but able to manage nearly all of daily affairs	4	0	4	1	4	5
Loses track of conversations or tasks, or marked/severe slowing or loss of train of thought	1	2	0	0	1	0
Mildly impaired vision, retains normal activities	1 (3)	0 (0)	2 (5)	0 (0)	0 (0)	0 (0)
Mildly impaired hearing, retains normal activities	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Reduced interest in social activities	3 (8)	1 (5)	0 (0)	1 (5)	0 (0)	2 (10)
Mild limitation because of reduced interest	3	1	0	1	0	1
Moderate or severe reduction in social activities because of lack of interest or initiative	0	0	0	0	0	1
Sensory problems	7 (18)	3 (14)	9 (23)	3 (14)	3 (8)	3 (15)
Mild sensory loss or paresthesia – intermittent or nonintrusive	4	3	8	3	2	3
Moderate or severe sensory loss or paresthesia always present	3	0	1	0	1	0
Headaches within the past 4 weeks	12 (30)	11 (50)	11 (28)	8 (36)	15 (38)	4 (20)
Mild, occasional (about one a week)	4	6	7	6	6	2
Moderate, interferes with activities or sleep (several a week)	4	2	3	2	6	1
Severe, debilitating (daily or almost every day)	4	3	1	0	3	1
Self-reported neurological symptoms in the past 30 days						
Any	16 (40)	2 (9)	11 (28)	3 (14)	2 (5)	2 (10)
Lethargy (extreme tiredness)	12 (30)	1 (5)	9 (23)	2 (9)	0 (0)	1 (5)
Myalgia (muscle pain)	7 (18)	1 (5)	4 (10)	0 (0)	1 (3)	0 (0)
Arthralgia (joint pain)	5 (13)	2 (9)	3 (8)	0 (0)	0 (0)	1 (5)
Weakness in arms or legs	4 (10)	0 (0)	4 (10)	1 (5)	1 (3)	0 (0)

Note: ZIKV = had Zika virus infection; no ZIKV = did not have Zika virus infection.
Source: Prepared by the authors from study data

acute myelitis, encephalitis, peripheral neuropathy, left middle cerebral artery infarct with right hemiparesis, seizures and diffuse neurological manifestations, and Alice in Wonderland syndrome (27).

The most comprehensive study of neurological manifestations associated with ZIKV infection to date identified 87 laboratory-confirmed ZIKV virus patients during the 2016 outbreak in the French West Indies with diverse clinical manifestations in the central nervous system and peripheral nervous system, and associated findings in magnetic resonance imaging (10). This study only included six children, with diagnoses of Guillain–Barré syndrome, meningoencephalitis, stroke, and myeloradiculitis and neurological symptoms including headaches, myalgia, ataxia, bilateral motor weakness, areflexia, sensory problems, and seizures. Although our study physicians conducted a detailed assessment of neurological signs and symptoms, diagnosis of neurological conditions (e.g., myelitis and encephalitis) was beyond the scope of our study. Similar to Lannuzel et al. (10), our study observed headache, myalgia, ataxia, strength and reflex abnormalities, and sensory

TABLE 4. Neuropsychological and behavioral functioning at 6-month and 12-month visits by Zika virus infection, León, Nicaragua

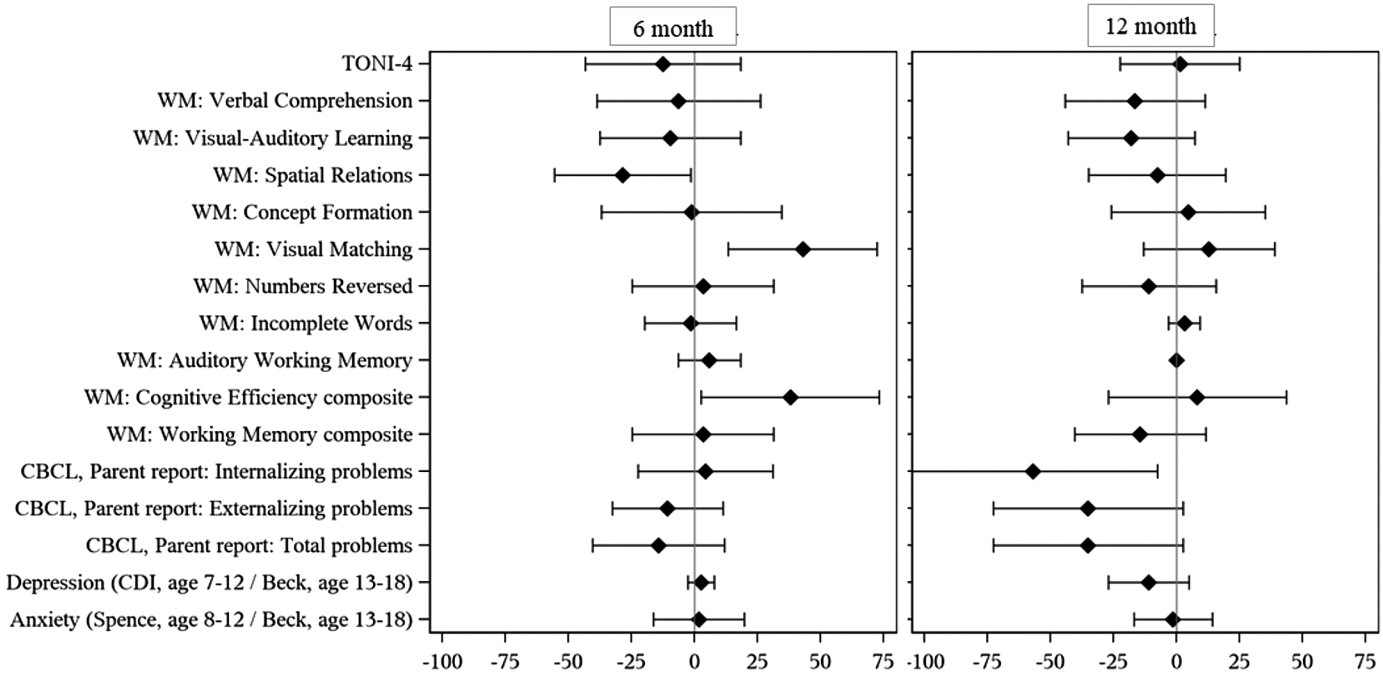
Test	Test output	6-month visit				12-month visit			
		ZIKV		No ZIKV		ZIKV		No ZIKV	
		n	Mean score (SD)	n	Mean score (SD)	n	Mean score (SD)	n	Mean score (SD)
Test of Nonverbal Intelligence, fourth edition	Non-verbal IQ	16	85.3 (11.1)	12	87.9 (10.9)	32	89.4 (12.8)	14	88.1 (10.7)
Woodcock–Muñoz III Cognitive Battery									
Verbal comprehension	Vocabulary and word usage	18	90.9 (12.2)	14	89.6 (14.0)	34	92.7 (16.6)	16	86.5 (20.9)
Visual–auditory learning	Multimodal learning	17	93.9 (11.6)	14	88.6 (20.2)	29	95.8 (11.0)	14	89.6 (18.0)
Spatial relations	Visual-spatial	16	91.2 (7.8)	14	86.0 (21.6)	29	88.1 (15.6)	14	88.6 (22.1)
Concept formation	Problem solving	17	87.9 (15.1)	13	87.8 (16.1)	29	86.2 (12.8)	13	93.9 (16.5)
Visual matching	Processing speed	18	55.7 (15.7)	14	73.6 (20.2)	33	61.6 (16.7)	16	67.3 (20.1)
Numbers reversed	Verbal working memory	17	91.0 (11.6)	14	98.0 (22.7)	29	91.7 (10.8)	14	97.6 (23.5)
Incomplete words	Phonological processing	18	109.6 (18.8)	14	114.6 (25.6)	33	111.1 (20.2)	15	112.1 (24.1)
Auditory working memory	Verbal working memory	17	97.1 (16.5)	14	102.8 (12.3)	29	100.4 (11.9)	14	99.1 (12.0)
Woodcock–Muñoz III Cognitive Battery composite scores									
Cognitive efficiency	Visual matching and numbers reversed	17	69.6 (11.6)	13	82.4 (16.4)	29	73.0 (11.7)	13	77.2 (17.4)
Working memory	Numbers reversed and auditory working memory	17	91.9 (12.7)	14	99.7 (20.8)	29	94.2 (9.9)	14	97.5 (21.2)
Child Behavior Checklist (parent report)									
Internalizing problems	Mood disturbance, including anxiety, depression, and social withdrawal	35	57.4 (12.7)	20	57.4 (12.6)	13	50.1 (11.5)	7	65.3 (19.1)
Externalizing problems	Conflict with others and violation of social norms	35	54.1 (11.3)	20	54.7 (13.9)	13	52.1 (8.9)	7	65.0 (13.7)
Total problems	Combination of internalizing and externalizing	35	56.6 (11.7)	20	57.4 (13.8)	13	52.2 (9.1)	7	66.6 (16.5)
Child Depression Inventory (age 7–12 years)	Symptoms of depression	15	7.7 (5.1)	10	9.8 (4.2)	13	9.7 (6.2)	10	9.3 (8.7)
Beck Depression Inventory (age 13–18 years)	Symptoms of depression	24	6.4 (10.1)	9	7.4 (6.9)	26	6.0 (7.0)	8	5.0 (9.4)
Spence Anxiety Scale (age 8–12 years)	Anxiety disorder symptoms	14	26.1 (20.3)	9	27.6 (14.7)	13	20.7 (20.6)	10	32.2 (23.8)
Beck Anxiety Inventory (age 13–18 years)	Anxiety severity and level	20	10.8 (9.2)	8	9.3 (10.2)	22	8.6 (7.4)	7	5.9 (8.4)

SD, standard deviation; IQ, intelligence quotient.
Notes: ZIKV= had Zika virus infection; no ZIKV = did not have Zika virus infection.
Test of Nonverbal Intelligence, fourth edition: higher scores indicate higher intellectual functioning.
Woodcock-Muñoz III Cognitive Battery subtests/composites: higher scores indicate higher achievement.
Child Behavior Checklist: higher scores indicate greater degree of behavioral and emotional problems.
Child Depression Inventory: higher scores indicate a more depressive state; scores ≥ 19 indicate likely depression.
Beck Depression Inventory: higher scores indicate a more depressive state; scores ≥ 31 indicate likely depression.
Spence Child Anxiety Scale: higher scores indicate more anxiety; scores ≥ 40 indicate elevated total anxiety for males aged 8–11 years; scores ≥ 50 indicate elevated total anxiety for females aged 8–11 years; scores ≥ 33 indicate elevated total anxiety for males aged 12–15 years; scores ≥ 39 indicate elevated total anxiety for females aged 12–15 years.
Beck Anxiety Inventory: higher scores indicate more anxiety; scores ≥ 36 indicate elevated total anxiety.
Source: Prepared by the authors from study data.

problems, but most of these symptoms were reported by both infected and uninfected children.

It is also important to understand the longer-term consequences of acquired ZIKV infection. A study by Salgado et al. (8) included 20 pediatric patients with suspected encephalitis, six of whom were confirmed to have ZIKV infection. Compared to children with other causes of encephalitis, children with ZIKV-associated encephalitis experienced shorter duration of hospitalization, with a maximum recovery time of 10 days. No sequelae were found during clinical follow-up of unknown duration (8). Of the six children followed for over a year in the French West Indies study, only the one child with myeloradiculitis had persistent neurological problems (10). In our study, the reflex problems and limb weakness observed at baseline mostly resolved over time, although for several children reflex issues were reported at all visits. Other symptoms reported at multiple visits were headache and fatigue.

FIGURE 1. Difference in at-risk prevalence and 95% confidence interval of neuropsychological problems at 6-month and 12-month visits between children with Zika virus infection and uninfected children, León, Nicaragua



TONI-4, Test of Nonverbal Intelligence, fourth edition; WM, Woodcock-Muñoz III test; CBCL, Child Behavior Checklist; CDI, Child Depression Inventory.
Source: Prepared by the authors from study data.

Our study found an array of neurocognitive concerns. To our knowledge, the only other study to include any measure of neurocognition among children infected with ZIKV postnatally was conducted in Chiapas, Mexico using the Montreal Cognitive Assessment (13). Investigators saw no improvement in cognitive scores between 0 to 7 days but did see improved cognitive scores from 7 to 28 days, likely related to better overall health and ability to function. No significant changes in function overall or in any cognitive subdomains were observed long term, between 28 and 108 days postinfection (13). In cognitive functioning assessments, we observed a higher prevalence of scores in the at-risk range among children infected with ZIKV compared to uninfected children on the visual matching subtest, which is a measure of processing speed. This finding may be explained by the damaging effects of ZIKV on myelination processes and axonal functioning in the white matter structures of humans, areas of the brain thought to be foundational to processing speed across the lifespan (28–30).

Although behavioral problems have been reported in infants and toddlers exposed to ZIKV in utero, to our knowledge, no behavioral assessments of children with postnatal ZIKV infection have been conducted. The only study contributing evidence to the hypothesized link between postnatal ZIKV infection and behavioral outcomes was conducted among six rhesus macaques infected with ZIKV postnatally (31). Neuroimaging revealed virus neuroinvasion, with a pattern of astrogliosis also seen in in utero ZIKV infection in humans and mice (32, 33). Magnetic resonance imaging conducted at 3 and 6 months of age revealed enlargement of lateral ventricles and blunted increases in hippocampal volume. Altered functional connectivity between brain regions that regulate emotional behavior and arousal functions was also seen (e.g., between amygdala and hippocampus) and corresponded with observed abnormal emotional behavior during intruder threat testing. In our study, the prevalence of behavioral issues measured by the Child Behavior Checklist was not significantly greater in the ZIKV-infected group. Longitudinal follow-up of the aforementioned rhesus macaques observed persistent structural and functional changes of the hippocampus at 12 months of age associated with memory deficits in ZIKV-infected macaques compared to controls (34). We did not see significant differences in memory between infection groups in our small study.

The high prevalence of at-risk scores for cognitive and behavioral issues may be the result of high rates of poverty in León, a general lack of access to remedial resources for learning deficits or disabilities, and the environment of violent civil and social unrest occurring during the course of study follow-up (35, 36). About 15–20% of children aged 7–12 years had elevated anxiety levels, a finding that deserves further attention, particularly with respect to studying the impact of anxiety on cognition and behavior.

Our study had some limitations. Although our goal was to include a group of children unaffected by ZIKV against which to compare outcomes in the ZIKV-infected group, the small number of children recruited precluded statistical comparisons and stratification by age. Furthermore, because children in the study only had mild ZIKV infections, our study was underpowered to detect significant differences in neurological and neuropsychological sequelae between groups. We also lacked neurocognitive and neuropsychiatric data on the children before infection to help determine whether observed deficits existed before the Zika epidemic. Some neurological symptoms

are difficult to assess in small children; we may therefore have missed important neurological sequelae, but the inclusion of both parent-reported observations and a clinician evaluation attempted to reduce the effect of this potential limitation.

The strength of this study is that we were able to implement for research purposes a standardized clinical neurological evaluation and a standardized neurocognitive and neuropsychiatric assessment battery in this resource-constrained Spanish-speaking population. We selected measures that minimized the potential influence of cultural and language factors on the standardized scores. For example, we used the Woodcock Muñoz III Cognitive Battery, a Spanish version of the Woodcock Johnson III. Norms were calibrated based on validation testing among Spanish-speaking children within and outside of the United States, including from Central America (19). The Test of Nonverbal Intelligence 4 uses shapes that are not culturally specific and avoids any need for verbal communication. The Child Behavior Checklist has been used in previous behavior studies in Nicaragua, and we selected the Children's Depression Inventory, Beck Depression Inventory, and Spence Child Anxiety Scale depression and anxiety instruments for this study because they were already in use in the family clinic at the study site (37, 38). The feasibility of implementing such a comprehensive testing battery is particularly important in the context of Nicaragua, where a shortage of medical specialists including neurologists has been further worsened by the recent political crisis (39, 40). The fact that general physicians and psychologists can be successfully trained to implement pediatric neurological, neurocognitive, and neuropsychiatric assessments is encouraging for supporting research in underserved populations in Nicaragua.

Conclusion

Although we observed neurological symptoms and neurocognitive problems among children exposed to ZIKV, these outcomes were not significantly more common in these children compared to uninfected individuals. While we developed a successful protocol for evaluating neurocognitive and neurobehavioral sequelae of ZIKV, larger studies are needed to definitively investigate this relationship to allow for stratification by age and adjustment for factors affecting neurocognition, e.g., mood and sleep disorders, child's home learning environment, history of other neuroinvasive infections such as dengue and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and a detailed family history of neurological and neurocognitive problems. Nevertheless, our study protocol can inform larger studies of neurological complications of ZIKV in the still-developing brains of children, particularly in resource-constrained settings where sequelae may be obscured by the effects of other sociopolitical and health-system challenges.

Author contributions. All authors conceived the original idea and designed the research project. JFL, SRH, AHR, and TJAR selected the study assessments. TC, AHR, and TJAR collected the data. RJ and NP analyzed the data. JFL, SRH, NMB, SBD, and PL interpreted results and drafted the paper. LB, SRH, and PDMM edited and commented on drafts. All authors reviewed and approved the final version.

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Conflicts of interest. None declared.

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Secuelas neurológicas y neuropsicológicas de la infección por el virus del Zika en niños y niñas en León, Nicaragua

RESUMEN

Objetivos. Describir la presencia y persistencia de secuelas neurológicas y neuropsicológicas en pacientes pediátricos que contrajeron la infección por el virus del Zika y evaluar si dichas secuelas fueron más comunes en los infectados con el virus del Zika en comparación con los no infectados.

Métodos. Se realizó un estudio de cohorte prospectivo en pacientes pediátricos con y sin infección por el virus del Zika en León (Nicaragua), con una herramienta de evaluación clínica estándar y un cuestionario para recopilar datos sobre los síntomas en tres visitas, con aproximadamente seis meses de diferencia, y un conjunto de instrumentos estandarizados para evaluar la función neurocognitiva, el comportamiento, la depresión y la ansiedad en las últimas dos visitas.

Resultados. Participaron 62 niños y niñas sin diferencias significativas en la demografía por grupo de infección. Los participantes infectados con el virus del Zika mostraron una variedad de síntomas neurológicos, algunos de los cuales persistieron entre 6 y 12 meses; no obstante, no se observó un patrón sistemático en los síntomas. Al inicio del estudio, un pequeño porcentaje de participantes infectados con el virus del Zika mostró resultados anormales a las pruebas dedo-nariz (13%), respuesta al tacto (frío) (13%) y respuesta a la vibración (15%), frente a un 0% en el grupo no infectado. Los déficits neurocognitivos y los problemas de comportamiento fueron comunes en ambos grupos, sin diferencias significativas entre los grupos. Los participantes infectados con el virus del Zika mostraron puntuaciones de eficiencia cognitiva más bajas en la visita a los 6 meses. La ansiedad y la depresión fueron poco frecuentes en ambos grupos.

Conclusiones. Son necesarios estudios más amplios para investigar definitivamente la relación entre la infección por el virus del Zika y los síntomas neurológicos y los problemas neurocognitivos, haciendo ajustes para los factores relacionados con la cognición y el comportamiento, incluidos los trastornos del estado de ánimo y el sueño, el entorno de aprendizaje en el hogar, los antecedentes de infecciones neuroinvasivas y los antecedentes familiares detallados de problemas neuropsicológicos.

Palabras clave Infección por el virus Zika; niño; enfermedades del sistema nervioso; pruebas neuropsicológicas; Nicaragua.

Sequelas neurológicas e neuropsicológicas da infecção pelo vírus zika em crianças em León, Nicarágua

RESUMO

Objetivos. Descrever a presença e a persistência de sequelas neurológicas e neuropsicológicas em crianças com infecção pelo vírus zika e avaliar se essas sequelas foram mais comuns em crianças infectadas pelo vírus zika em comparação com crianças não infectadas.

Métodos. Realizamos um estudo de coorte prospectivo em crianças com e sem infecção pelo vírus zika em León, Nicarágua, usando uma ferramenta de avaliação clínica padrão e um questionário para coletar dados de sintomas em três consultas, com cerca de 6 meses de intervalo, além de um conjunto de ferramentas padronizadas para avaliar função neurocognitiva, comportamento, depressão e ansiedade nas duas últimas consultas.

Resultados. Foram incluídas 62 crianças, sem diferenças significativas nas características demográficas por grupo de infecção. As crianças infectadas pelo vírus zika tinham uma gama de sintomas neurológicos, alguns dos quais persistiram por 6 a 12 meses. Entretanto, não se observou nenhum padrão consistente de sintomas. No início do estudo, uma pequena porcentagem de crianças infectadas com o vírus zika apresentou resultado anormal na prova índex-nariz (13%), resposta ao toque frio (13%) e sensibilidade vibratória (15%), em comparação a 0% no grupo não infectado. Déficits neurocognitivos e problemas comportamentais foram frequentes em ambos os grupos, mas sem diferenças significativas entre eles. As crianças infectadas com o vírus zika tiveram resultados mais baixos de eficiência cognitiva na consulta de 6 meses. Ansiedade e depressão não foram observadas com frequência em ambos os grupos.

Conclusões. São necessários estudos mais amplos para investigar a relação exata entre a infecção pelo vírus zika e sintomas neurológicos e problemas neurocognitivos, com ajuste para fatores que afetam a cognição e o comportamento, incluindo distúrbios do humor e do sono, ambiente de aprendizagem em casa, história de infecções neuroinvasivas e história familiar detalhada de problemas neuropsicológicos.

Palavras-chave Infecção por Zika virus; criança; doenças do sistema nervoso; testes neuropsicológicos; Nicarágua.