

Neurodevelopmental Outcomes of Children Following In Utero Exposure to Zika in Nicaragua

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Background. Neurodevelopmental outcomes of asymptomatic children exposed to Zika virus (ZIKV) in utero are not well characterized.

Methods. We prospectively followed 129 newborns without evidence of congenital Zika syndrome (CZS) up to 24 months of age. Participants were classified as ZIKV exposed or ZIKV unexposed. The Mullen Scales of Early Learning (MSEL) was administered in the participants' homes at 6, 12, 15, 18, 21, and 24 months of age by trained psychologists. Sociodemographic data, medical history, and infant anthropometry at birth were collected at each home visit. Our primary outcome was the Mullen Early Learning Composite Score (ECL) at 24 months of age between our 2 exposure groups. Secondary outcomes were differences in MSEL subscales over time and at 24 months.

Results. Of 129 infants in whom exposure status could be ascertained, 32 (24.8%) met criteria for in utero ZIKV exposure and 97 (75.2%) did not. There were no differences in maternal age, maternal educational attainment, birthweight, or gestational age at birth between the 2 exposure groups. The adjusted means and standard errors (SEs) for the ELC score between the ZIKV-exposed children compared to ZIKV-unexposed children were 91.4 (SE, 3.1) vs 96.8 (SE, 2.4) at 12 months and 93.3 (SE, 2.9) vs 95.9 (SE, 2.3) at 24 months. In a longitudinal mixed model, infants born to mothers with an incident ZIKV infection ($P = .01$) and low-birthweight infants (<2500 g) ($P = .006$) had lower composite ECL scores.

Conclusions. In this prospective cohort of children without CZS, children with in utero ZIKV exposure had lower neurocognitive scores at 24 months.

Keywords. Zika; neurodevelopmental outcome; Nicaragua; children.

Although the Zika virus (ZIKV) was first isolated in 1947 in Uganda [1], it was not recognized as a risk to the developing fetus until 2015, when a large number of newborn cases of microcephaly were identified in Brazil [2–4]. Microcephaly is the hallmark of congenital Zika syndrome (CZS) [5–7], a constellation of findings that include brain, ocular, auditory, and musculoskeletal abnormalities [7–12]. While CZS is the most extreme

manifestation of congenital ZIKV infection, the majority of infants with in utero ZIKV exposure do not show signs at birth, and there is mounting evidence that unrecognized infection may cause neurologic sequelae in childhood [13, 14].

Scientific research around ZIKV and CZS has dramatically increased since the 2015–2016 ZIKV epidemic in the Americas. Yet, our understanding of the longer-term consequences of in utero and postnatal exposure to ZIKV on childhood neurodevelopment is only now emerging as children born during the ZIKV epidemic begin to reach toddler age. Evolving data from Brazil, Colombia, and other countries in South America suggest congenital ZIKV exposure may cause neurodevelopmental delay later in life [12, 14–16]. Most studies of ZIKV-exposed children have focused upon children with overt evidence of infection at birth because those are the easiest to identify. Unfortunately, very few studies have included groups of unexposed children as comparison. A controlled comparison is important because it is estimated that 30%–40% of children under the age of 5 in low- and

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middle-income countries may fail to meet developmental milestones for other reasons, such as poverty and malnutrition [17]. Also, very few studies have included mothers with asymptomatic ZIKV infections during pregnancy. As an estimated 80% of ZIKV infections are asymptomatic, examining only symptomatic maternal infections excludes the great majority of infections that occur in a population [18].

In Nicaragua, we had the opportunity to identify and follow women who were pregnant during the peak of the ZIKV epidemic. We hypothesized that children exposed to ZIKV in utero but without a neonatal diagnosis of CZS may still have adverse neurodevelopmental outcomes. Given the myriad of factors beyond congenital infection that can affect a child's cognitive and motor development [19–22], we assembled a cohort to study neurodevelopmental outcomes of ZIKV-exposed children with contemporaneous unexposed controls from the same community.

METHODS

Participants for this neurodevelopmental study were recruited from a prospective ZIKV pregnancy surveillance that coincided with the peak of the epidemic in León, Nicaragua. That study has been described in a prior publication [23], but we review elements that are germane to the present research. All participants received prenatal care at the Perla María Health Center in León between February 2015 and July 2017 and expressed intent to deliver at Hospital Escuela Oscar Danilo Rosales Argüello. We collected sociodemographic and clinical data and obtained a venous blood sample at each prenatal visit. At birth, we documented relevant clinical information, including gestational age, birthweight, sex, and head circumference. We also obtained an umbilical cord blood specimen and performed a physical examination assessment for birth defects. Anomalies consistent with CZS were defined by the presence of arthrogryposis, microcephaly, anencephaly, and/or hypertonía [17]. Microcephaly was defined as a head circumference more than 2 standard deviations below the mean for age and sex (consistent with the World Health Organization's definition).

Women who had participated in the pregnancy surveillance were offered enrollment of their children into the longitudinal neurodevelopmental study. We followed these children prospectively through home visits, at 6-month intervals. The household survey enumerated each person living in the home and collected relevant demographic and socioeconomic measures, including household wealth. Interval medical history and anthropometry were collected on all participants at each visit. Research staff were blinded to the ZIKV status of the mother-infant dyad.

Definition of Infant ZIKV Exposure Status

The pregnancy surveillance cohort categorized women as having experienced an incident ZIKV infection in pregnancy if

they met any of the following criteria: (1) high maternal serum titers for ZIKV-neutralizing antibody (focus reduction neutralization test [FRNT] >3000) at the time of birth; (2) a 4-fold or more increase in maternal serum 50% FRNT (FRNT₅₀) value between a prenatal and delivery samples; (3) detection of anti-ZIKV immunoglobulin M (IgM) in any prenatal maternal or an umbilical cord blood serum sample [23]. We were unable to confirm in utero ZIKV exposure among mother-infant pairs from whom a delivery specimen was not available. However, we were able to rule it out by measuring ZIKV neutralizing antibodies in the postpartum mother. Thus, children whose mother was ZIKV naïve by FRNT or who had evidence of infection prior to pregnancy were eligible for inclusion as unexposed controls. We used laboratory methods as previously described [23] and defined a ZIKV-neutralizing antibody titer of FRNT₅₀ >200 as evidence of prior maternal ZIKV infection.

Mullen Scales of Early Learning

The Mullen Scales of Early Learning (MSEL) [24] is a comprehensive performance-based test consisting of 124 items measuring specific developmental domains of gross motor, fine motor, visual reception, receptive language, and expressive language. Each item was scored and used to generate raw scores for each scale. These raw scores were then converted into a normative score (T score) for each of the 5 Mullen scales. The 4 cognitive subscales (visual reception, fine motor, receptive language, and expressive language) are combined to yield the MSEL Early Learning Composite score (ELC). The ELC serves as a general measure of fluid intelligence thought to underlie cognitive ability in general. Study staff including locally trained psychologists and research nurses administered the MSEL at 6, 12, 18, 21, and 24 months of age at the participant's home. Depending on the age and engagement of the child, MSEL testing took between 30 and 90 minutes to complete.

For administration of the MSEL, spoken item instructions to mother and/or child were given in the local language. Training of local psychologist occurred through weekly teleconferences and in-person trainings. Quality assurance measures were conducted throughout the entirety of the study.

Nicaraguan-Specific Poverty Index

We adapted an index created by the Center for Investigation of Demography and Health (CIDS) to assess household poverty in this study. The CIDS index has been validated for use in Nicaragua [25]. The composite poverty index evaluated housing quality, water supply, sanitation services, and economic dependence of family members, with each domain assigned a score of “1” if domain criteria are absent and “0” if present. Each home can receive a score ranging from 0 to a maximum of 4. In poverty was defined as having a score of >1.

Written informed consent was obtained by trained, Nicaraguan study staff, and all research was conducted under

approval of the Ethics Committee of the Universidad Nacional Autónoma de Nicaragua-León (UNAN) (Acta 93, 2016) and the Institutional Review Board of University of North Carolina (protocol number 16–1402). All research was conducted in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of the World Medical Association.

Statistical Analyses

Demographic and clinical characteristics of mothers and infants were summarized and compared between exposure groups with *t* tests for continuous variables and χ^2 tests for categorical variables. (Fisher exact test was used whenever table cells had <5 observations). Unadjusted means of the ELC were calculated and plotted by exposure status, time point, and subdomain. In these plots, children whose mothers had never experienced a ZIKV infection were combined with children whose mothers were infected prior to pregnancy.

We used longitudinal mixed models to assess the association of ZIKV exposure on ELC. The ELC outcome was standardized according to the distribution of the unexposed children's scores at each time point [26]. The models included fixed effects for ZIKV exposure, child age at time of assessment, a set of potential covariates (maternal age, maternal education, and poverty index), and a set of variables commonly adjusted for in cognitive development studies (infant sex, low birthweight, weight-for-age *z* score, and length-for-age *z* score). Random effects for infant and MSEL test administrator were included to account for repeated measures within each child and allow for correlation by administrator, respectively. Model-adjusted ELC means by exposure status and time were calculated and adjusted means for the 5 subdomains were calculated from similarly specified models using subdomain scores as the outcomes. Study data were managed using REDCap [27] and analyzed using R version 3.4 software; mixed models were fit using the lme4 package in R [28].

RESULTS

Between February 2017 and July 2017 (inclusive), 253 pregnant women were enrolled into the prospective ZIKV pregnancy cohort. Of these, 48 women were lost to follow-up before delivery, 12 refused to participate in the neurodevelopmental outcome study, 12 experienced an abortion or perinatal death, and 2 women delivered twins (Figure 1). Thus, 183 women gave consent for their infants to participate and their infants were immediately enrolled into the neurodevelopmental study. Of these 183, we were unable to determine ZIKV status in 32 women, leaving 151 with ZIKV status of pregnancy. Another 18 women were determined to have ambiguous ZIKV and were excluded from our primary analysis.

No measured maternal or infant characteristics differed substantially between the exposed and unexposed groups (Table 1).

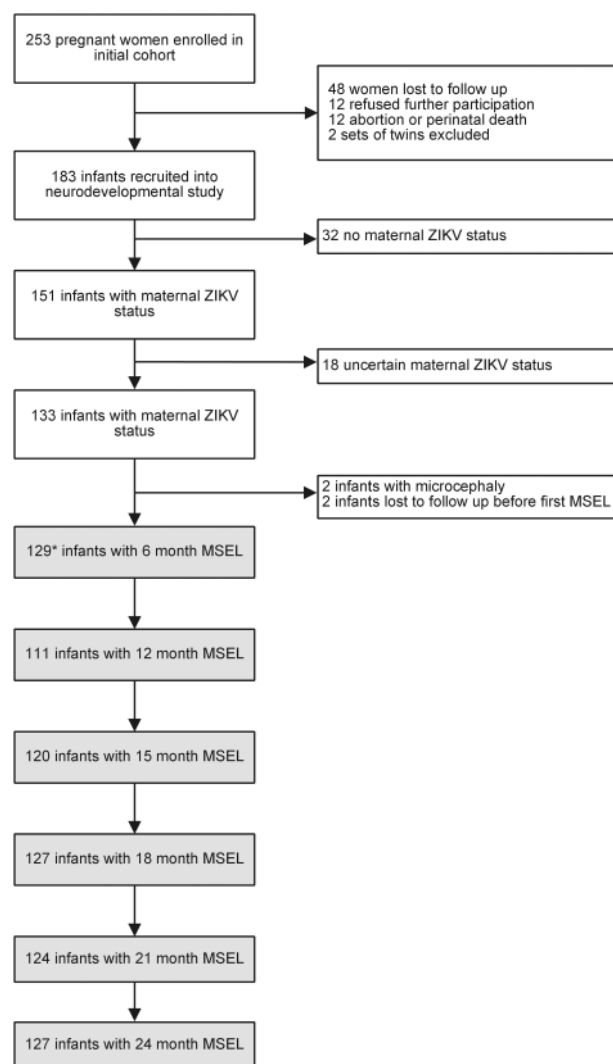


Figure 1. Study profile. *8 infants had 2 MSEL around 3–9 months yielding 137 assessments for 129 infants in the 6-month range. Abbreviations: MSEL, Mullen Scales of Early Learning; ZIKV, Zika virus.

Two microcephalic infants were excluded and an additional 2 infants were lost to follow-up before the first Mullen assessment.

Our primary analysis thus includes 129 children: 32 (24.8%) with in utero ZIKV exposure and 97 (75.2%) without in utero exposure. Only 2 of 129 (2%) participants were lost to follow-up after the initial Mullen assessment (Figure 1). Overall, 41 of 133 (27%) of participants' households met criteria for living in poverty, and 73 of 151 (48%) of infants were female. The mean birthweight of the infants was 3064 g (standard deviation [SD], 474 g) and the mean gestational age at birth was 38 weeks (SD, 1.6 weeks). Twelve of 133 (8%) were born prior to 37 weeks' gestation. The mean head circumference, mean weight, and mean length were similar at 12 months and 24 months between the 2 exposure groups.

In unadjusted longitudinal analysis, children with in utero ZIKV exposure had lower ECL scores at all time points (6, 12, 15, 18, 21, and 24 months) compared to unexposed children.

Table 1. Characteristics of Nicaraguan Mother-Child Pairs With and Without In Utero Zika Virus Exposure

Characteristic	All Participants (N = 133)	In Utero ZIKV Exposure (n = 33)	No In Utero ZIKV Ex- posure (n = 100)	Exposed vs Not Exposed, Mean Difference (95% CI)	Exposed vs Not Ex- posed, OR (95% CI)	P Value
Maternal age, y, mean (SD)	23.6 (5.7)	24.8 (6.6)	23.0 (5.3)	1.8 (−.7 to 4.3)16
Maternal education						
<8 y	43 (28)	8 (24)	33 (33)	...	Referent	.31
8–12 y	47 (31)	8 (24)	31 (31)	...	1.06 (.36–3.18)	
>12 y	61 (40)	17 (52)	36 (36)	...	1.95 (.74–5.11)	
In poverty	41 (27)	8 (24)	30 (30)	...	0.75 (.30–1.84)	.68
Infant sex (female)	73 (48)	11 (33)	54 (54)	...	0.43 (.19–.97)	.06
Infant birthweight, g, mean (SD)	3064 (474)	3179 (512)	3050 (462)	129 (−74.2 to 332)21
<2500 g	9 (6)	1 (3)	7 (8)	...	0.39 (.01–3.20)	.68
Gestational age at birth, wk, mean (SD)	38 (1.6)	38 (1.6)	38 (1.5)	0.1 (−.5 to .8)67
<37 wk	12 (8)	2 (6)	8 (9)	...	0.67 (.07–3.63)	1
Microcephalic ^a	2 (1)	1 (3)	1 (1)	...	2.85 (.04–228)	.46
Head circumference, cm, mean (SD)						
12 mo	46.0 (3.0)	45.9 (2.1)	46.2 (3.5)	−0.3 (−1.5 to .9)65
24 mo	47.6 (1.5)	47.7 (1.7)	47.6 (1.4)	0.1 (−.6 to .8)76
Child length, cm, mean (SD)						
12 mo	74.0 (5.1)	75.2 (3.9)	73.6 (5.8)	1.6 (−.5 to 3.7)14
24 mo	85.7 (3.9)	86.9 (3.9)	85.3 (4.0)	1.6 (−.1 to 3.2)06
Child length for age WHO z score, mean (SD)						
12 mo	−0.4 (1.9)	0.0 (1.5)	−0.5 (2.2)	0.5 (−.3 to 1.4)18
24 mo	−0.5 (1.2)	−0.1 (1.2)	−0.6 (1.3)	0.4 (−.1 to .9)09
Child weight, kg, mean (SD)						
12 mo	9.6 (1.2)	9.8 (1.8)	9.5 (1.2)	0.3 (−.3 to .9)28
24 mo	11.7 (1.9)	11.4 (1.6)	11.8 (2.0)	−0.4 (−1.1 to .3)27
Child weight for age WHO z score, mean (SD)						
12 mo	0.1 (1.1)	0.3 (1.1)	0.1 (1.0)	0.2 (−.3 to .7)46
24 mo	−0.2 (1.3)	−0.5 (1.2)	−0.1 (1.4)	−0.4 (−1.1 to .3)27

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CI, confidence interval; OR, odds ratio; SD, standard deviation; WHO, World Health Organization; ZIKV, Zika virus.

^aThese infants were excluded from subsequent analyses.

However, these differences only reached statistical significance at the 12- and 18-month time points (Figure 2; Supplementary Table 1). In both groups, the ELC mean score was lower at 12 and 18 months, but then showed gradual recovery by 24 months. The unexposed group was composed of both children with ZIKV-naïve mothers and those with ZIKV prior to pregnancy, who had similar ECL mean scores (Supplementary Figure 1). Receptive Language and Visual Reception scores were lower at all time points among exposed children and remained significantly different even at 24 months of age. Exposed children also had lower scores in the Expressive Language, Gross Motor, and Fine Motor domains compared to their unexposed counterparts at varying time points, but these deficits were no longer apparent at 24 months (Figure 2). The adjusted means and standard errors (SEs) for the ELC score between the ZIKV-exposed children compared to ZIKV-unexposed children were 91.4 (SE, 3.1) vs 96.8 (SE, 2.4) at 12 months and 93.3 (SE, 2.9) vs 95.9 (SE, 2.3) at 24 months (Table 2).

In adjusted longitudinal mixed models, children who were exposed to ZIKV in utero had significantly lower ECL scores

compared to unexposed children, with exposed children scoring on average −0.33 units lower on the transformed outcomes, equivalent to approximately 3.57 points (95% confidence interval [CI], .90–6.27) in the untransformed ELC outcome compared to unexposed children (Figure 3; Supplementary Table 2). In this same model, children with a birthweight <2500 g scored on average −0.74 units lower on the transformed outcome, equivalent to about 8.00 points (95% CI, 2.56–13.46) lower in the untransformed ELC outcome compared to those with a normal birthweight (Supplementary Table 2).

Children with the lowest ELC scores (n = 20) were referred to an Early Child Development program through UNAN; however, due to delays in staff training and clinic renovation, this started after 24 months of age.

DISCUSSION

Numerous studies have shown that ZIKV infection in pregnancy can cause severe disease in the fetus and newborn. While children born with overt CZS frequently experience significant

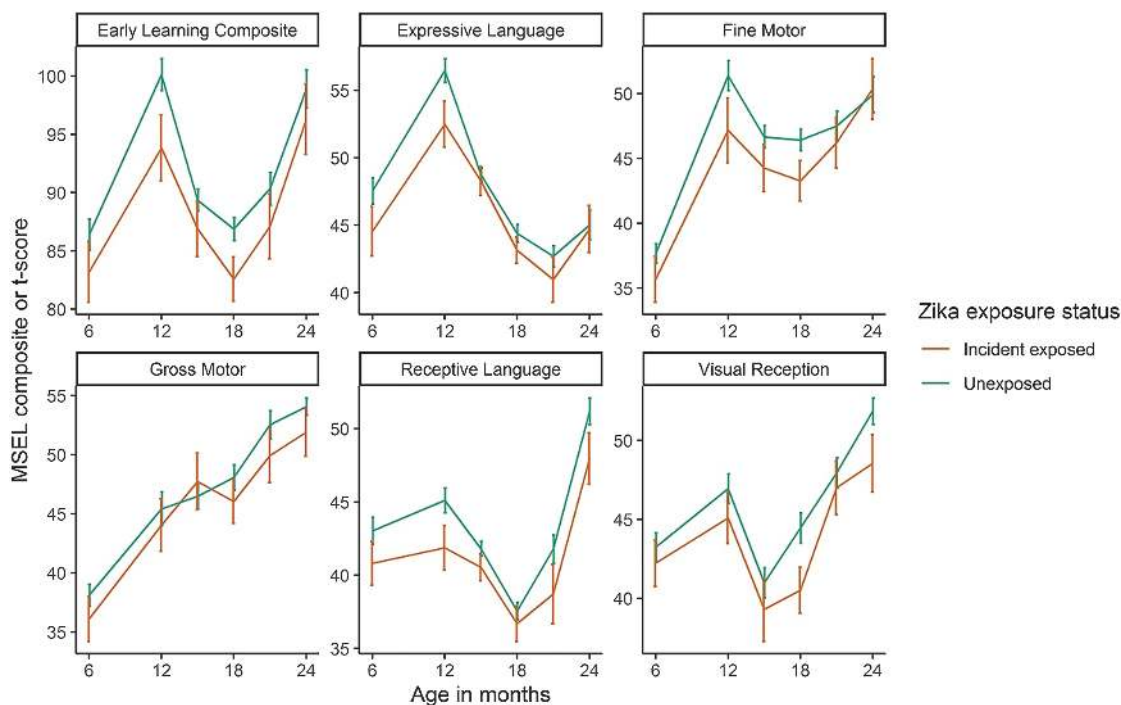


Figure 2. Adjusted longitudinal mixed model: Zika virus exposure status. Abbreviations: MSEL, Mullen Scales of Early Learning; ZIKV, Zika virus.

neurodevelopmental delays [29, 30], less is known about the outcomes of exposed infants who are asymptomatic at birth. Results from this longitudinal study in León, Nicaragua, suggest that the absence of CZS does not guarantee normal neurodevelopment among children exposed to ZIKV in utero and that ZIKV exposure and low birthweight (<2500 g) were associated with lower scores using the MSEL.

Research from Colombia [14] and Brazil [12] has suggested that asymptomatic infants whose mothers were infected with ZIKV in pregnancy may be at risk of delayed neurodevelopment. One of the main weaknesses of these 2 studies is that neither included a control group of children whose mothers were not infected with ZIKV in pregnancy. In Brazil, Nielsen-Saines and colleagues found that 35% of infants in their cohort had language development below average using the Bayley Scales of Infant and Toddler Development; however, the Bayley scale measures are standardized for American norms and may not be directly translatable to children in Brazil [12]. Forty-nine percent of infants in their study with abnormal findings in early infancy subsequently had normal assessments once they were toddlers. In Colombia, Mulkey and colleagues showed linear declines in communication; mobility; and social cognition using the Warner Initial Developmental Evaluation of Adaptive and Functional Skills [14] among ZIKV-exposed infants. Our findings also show a decline between 12 and 18 months, but then improvements in neurocognitive development through 24 months in the ELC, as well as the various subdomains of the MSEL. While we are uncertain as to why the decline in the

scores occurred at 12–15 months, it is unlikely artifact since it was observed across exposure groups. One explanation might be that the rebound in ELC scores at 18 months was driven by an accelerated development in language and processing skills that tends to occur after that age. In previous studies, the MSEL has proven to be a sensitive measure of change in language development in children younger than 5 years [31].

Many of the Central and South American countries where the 2015–2016 ZIKV epidemic occurred lack normative data against which to compare neurodevelopmental testing. Although the MSEL was originally developed in the United States, it has subsequently been used to monitor outcomes of children around the world including South America and Africa [32, 33]. We sought to overcome this limitation by including in our study a group of infants who were followed contemporaneously and not ZIKV exposed.

Strengths of our study include its robust ZIKV testing algorithm including serological testing that allowed us to reasonably assign infection timing in pregnant women without relying on polymerase chain reaction–based testing for ZIKV, which is limited by a high proportion of asymptomatic infections and the short time span when infected individuals were viremic during the outbreak [34]. Of note, only half of women in our cohort with an incident infection reported symptoms. Although serologic testing for flaviviruses can be complicated by cross-reactivity [35], particularly for IgM enzyme-linked immunosorbent assays, many women in our study were classified as having an incident ZIKV infection based on multiple criteria

Table 2. Adjusted^a Means and Standard Errors for Mullen Scales of Early Learning by Subdomain and Zika Virus Status Over Time

MSEL Subdomain	In Utero ZIKV Exposure (n = 32)	No In Utero ZIKV Exposure (n = 97)	Mean Difference (95% CI)
Early learning composite, mean (SE)			
6 mo	79.5 (3.1)	82.5 (2.3)	3.0 (−2.3 to 8.3)
12 mo	91.4 (3.1)	96.8 (2.4)	5.4 (−2 to 10.9)
15 mo	84.0 (3.0)	86.8 (2.3)	2.8 (−2.4 to 8.0)
18 mo	80.2 (2.9)	84.2 (2.3)	4.0 (−1.1 to 9.0)
21 mo	84.1 (2.9)	87.7 (2.3)	3.6 (−1.5 to 8.6)
24 mo	93.3 (2.9)	95.9 (2.3)	2.6 (−2.3 to 7.5)
Gross motor T score, mean (SE)			
6 mo	34.6 (3.0)	36.7 (2.6)	2.1 (−1.9 to 6.1)
12 mo	43.4 (3.1)	44.6 (2.7)	1.3 (−3.0 to 5.5)
15 mo	46.3 (3.0)	46.0 (2.6)	−0.3 (−4.4 to 3.7)
18 mo	44.9 (3.0)	46.8 (2.6)	1.9 (−2.0 to 5.8)
21 mo	46.5 (3.0)	49.8 (2.6)	3.3 (−.7 to 7.2)
24 mo	48.4 (2.9)	50.9 (2.6)	2.5 (−1.3 to 6.3)
Fine motor T score, mean (SE)			
6 mo	35.9 (2.7)	37.5 (2.2)	1.6 (−2.4 to 5.7)
12 mo	47.8 (2.7)	51.5 (2.2)	3.6 (−.8 to 8.0)
15 mo	44.3 (2.6)	47.0 (2.2)	2.7 (−1.4 to 6.8)
18 mo	43.6 (2.6)	46.4 (2.2)	2.8 (−.4 to 6.7)
21 mo	44.9 (2.6)	46.7 (2.2)	1.9 (−2.1 to 5.9)
24 mo	49.6 (2.6)	49.0 (2.2)	−0.7 (−4.5 to 3.2)
Visual reception T score, mean (SE)			
6 mo	38.4 (2.3)	39.1 (1.8)	0.7 (−2.9 to 4.3)
12 mo	41.3 (2.3)	43.1 (1.9)	1.9 (−2.0 to 5.7)
15 mo	35.8 (2.2)	38.0 (1.8)	2.3 (−1.4 to 5.9)
18 mo	37.4 (2.2)	42.3 (1.8)	4.9 (1.4–8.4)
21 mo	45.4 (2.2)	46.4 (1.8)	1.0 (−2.6 to 4.5)
24 mo	47.0 (2.2)	50.4 (1.8)	3.4 (0–6.8)
Expressive language T score, mean (SE)			
6 mo	42.5 (1.8)	45.4 (1.2)	2.9 (−.5 to 6.3)
12 mo	50.8 (1.8)	54.6 (1.3)	3.7 (1–7.3)
15 mo	46.8 (1.7)	47.0 (1.2)	0.1 (−3.2 to 3.5)
18 mo	41.5 (1.7)	42.3 (1.2)	0.8 (−2.5 to 4.1)
21 mo	39.1 (1.7)	40.8 (1.2)	1.7 (−1.6 to 4.9)
24 mo	42.1 (1.6)	42.8 (1.2)	0.7 (−2.4 to 3.8)
Receptive language T score, mean (SE)			
6 mo	38.9 (1.9)	41.0 (1.4)	2.0 (−1.2 to 5.3)
12 mo	40.5 (1.9)	43.3 (1.4)	2.7 (−.7 to 6.2)
15 mo	38.7 (1.8)	40.4 (1.4)	1.6 (−1.6 to 4.9)
18 mo	35.3 (1.8)	36.0 (1.4)	0.7 (−2.5 to 3.9)
21 mo	36.7 (1.8)	40.2 (1.4)	3.4 (1–6.6)
24 mo	46.3 (1.8)	49.5 (1.4)	3.2 (1–6.2)

Abbreviations: CI, confidence interval; MSLE, Mullen Scales of Early Learning; SE, standard error; ZIKV, Zika virus.

^aModels adjusted for maternal age, maternal education, infant sex, low infant birthweight (<2500 g at birth), length-for-age z score, weight-for-age z score, and a poverty index, as well as random effects for repeated measures and outcome test administrators.

in our algorithm, including neutralization testing, which is more specific for the infecting flavivirus [36]. Additionally, once infants were enrolled into our neurodevelopmental study, we had an intensive follow-up with multiple assessment time points, allowing us to account for individual testing variation that can occur if a child is ill on the day the assessment is performed. Another strength of our study was the high retention of participants, which was attributed to strong relationships between field staff and participants during household visits, the

use of an electronic tracking system, and small incentives provided to the families.

Our study is limited by its relatively small number of ZIKV-exposed infants. Because the Nicaraguan ZIKV epidemic occurred during a narrow time period, it was not feasible to expand the study to multiple sites. A larger study with a more intensive sampling scheme may also have been able to address the relationship, if any, between neurodevelopmental delays with the precise timing of infection during pregnancy. There

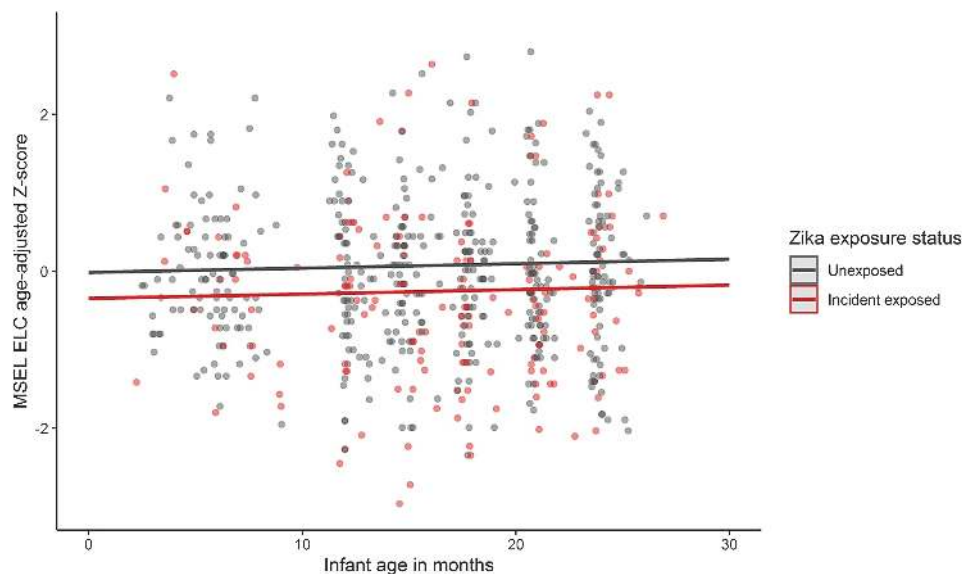


Figure 3. Adjusted longitudinal mixed model: Zika virus exposure status. Abbreviations: MSEL ELC, Mullen Scales of Early Learning Early Learning Composite; ZIKV, Zika virus.

is also the possibility of uncontrolled confounding; however, there is no imaginable randomized trial in this setting and thus high-quality observational studies must be considered the gold standard. Our study also did not perform brain imaging of the children because it was not standard of care, nor easily obtained.

Early childhood development interventions can be effective [37], but rely on knowing whom to test, having locally validated instrument(s), and the ability to refer infants who are not meeting milestones. While infants with known or suspected ZIKV exposure might benefit from access to brain magnetic resonance imaging or head ultrasounds, these technologies and qualified professionals to interpret them are lacking in many parts of the world. Yet, appropriate use of nonradiologic screening algorithms for children at risk of neurodevelopmental delay is feasible without expensive equipment. Our results suggest that maternal ZIKV infection at least merits enhanced clinical vigilance and dedicated neurodevelopmental testing for all infants, particularly those without abnormalities at birth and those who weigh <2500 g at birth. Our findings also suggest that in utero transmission may be occurring but missed due to limitations in testing infants and children.

The 2015–2016 Zika epidemic in the Americas laid bare the limited availability of early childhood interventions in many low- and middle-income countries and highlights a continuing need for such programs beyond the Zika epidemic [38]. Indeed, the United Nations Children's Fund is currently working to bolster these services in Nicaragua through a training program in early childhood development. Early interventions can have lifelong implications and are especially salient as countries work to meet the 2030 Sustainable Development Goals. Based on studies using

the MSEL in Africa, a 6-point difference in the ECL is considered to be a meaningful difference [32], which we did not observe, and thus our results may not be clinically relevant. Functional differences in more distal outcomes such as school readiness remain to be determined and our findings support recommendations that children with congenital ZIKV exposure warrant long-term follow-up; consequently, we plan to follow these children through school age.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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REFERENCES

- Haddow AJ, Williams MC, Woodall JP, Simpson DI, Goma LK. Twelve isolations of Zika virus from *Aedes (Stegomyia) africanus* (Theobald) taken in and above a Uganda forest. *Bull World Health Organ* **1964**; 31:57–69.
- Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet Gynecol* **2016**; 47:6–7.
- Schuler-Faccini L, Ribeiro EM, Feitosa IM, et al; Brazilian Medical Genetics Society–Zika Embryopathy Task Force. Possible association between Zika virus infection and microcephaly—Brazil, 2015. *MMWR Morb Mortal Wkly Rep* **2016**; 65:59–62.
- Malakar J, Korva M, Tul N, et al. Zika virus associated with microcephaly. *N Engl J Med* **2016**; 374:951–8.
- Gutiérrez-Bugallo G, Piedra LA, Rodriguez M, et al. Vector-borne transmission and evolution of Zika virus. *Nat Ecol Evol* **2019**; 3:561–9.
- Connery AK, Berrios-Siervo G, Arroyave P, et al. Responding to the Zika epidemic: preparation of a neurodevelopmental testing protocol to evaluate young children in rural Guatemala. *Am J Trop Med Hyg* **2019**; 100:438–44.
- Moore CA, Staples JE, Dobyns WB, et al. Characterizing the pattern of anomalies in congenital Zika syndrome for pediatric clinicians. *JAMA Pediatr* **2017**; 171:288–95.
- Guillemette-Artur P, Besnard M, Eyrolle-Guignot D, Jouannic JM, Garel C. Prenatal brain MRI of fetuses with Zika virus infection. *Pediatr Radiol* **2016**; 46:1032–9.
- Faria NR, Azevedo RDS, Kraemer MUG, et al. Zika virus in the Americas: early epidemiological and genetic findings. *Science* **2016**; 352:345–9.
- Lopes Moreira ME, Nielsen-Saines K, Brasil P, et al. Neurodevelopment in infants exposed to Zika virus in utero. *N Engl J Med* **2018**; 379:2377–9.
- Marques FJP, Teixeira MCS, Barra RR, et al. Children born with congenital Zika syndrome display atypical gross motor development and a higher risk for cerebral palsy. *J Child Neurol* **2019**; 34:81–5.
- Nielsen-Saines K, Brasil P, Kerin T, et al. Delayed childhood neurodevelopment and neurosensory alterations in the second year of life in a prospective cohort of ZIKV-exposed children. *Nat Med* **2019**; 25:1213–7.
- Gordon-Lipkin E, Peacock G. The spectrum of developmental disability with Zika exposure: what is known, what is unknown, and implications for clinicians. *J Dev Behav Pediatr* **2019**; 40:387–95.
- Mulkey SB, Arroyave-Wessel M, Peyton C, et al. Neurodevelopmental abnormalities in children with in utero Zika virus exposure without congenital Zika syndrome. *JAMA Pediatr* **2020**; 174:269–76.
- Faiçal AV, de Oliveira JC, Oliveira JVV, et al. Neurodevelopmental delay in normocephalic children with in utero exposure to Zika virus. *BMJ Paediatr Open* **2019**; 3:e000486.
- Wheeler AC, Toth D, Ridenour T, et al. Developmental outcomes among young children with congenital Zika syndrome in Brazil. *JAMA Netw Open* **2020**; 3:e204096.
- Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B; International Child Development Steering Group. Developmental potential in the first 5 years for children in developing countries. *Lancet* **2007**; 369:60–70.
- Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* **2009**; 360:2536–43.
- Nahar B, Hossain M, Mahfuz M, et al. Early childhood development and stunting: Findings from the MAL-ED birth cohort study in Bangladesh. *Matern Child Nutr* **2020**; 16:e12864.
- McCormick B, Caulfield L, Richard S, Pendergast L, Murray-Kolb L; MAL-ED Network Investigators. Nurturing environments and nutrient-rich diets may improve cognitive development: analysis of cognitive trajectories from six to sixty months from the MAL-ED Study (OR10-01-19). *Curr Dev Nutr* **2019**; 3(Suppl 1):733.
- McCormick BJJ, Richard SA, Caulfield LE, et al; MAL-ED Network Investigators. Early life child micronutrient status, maternal reasoning, and a nurturing household environment have persistent influences on child cognitive development at age 5 years: results from MAL-ED. *J Nutr* **2019**; 149:1460–9.
- Pendergast LL, Schaefer BA, Murray-Kolb LE, et al; MAL-ED Network Investigators. Assessing development across cultures: invariance of the Bayley-III Scales Across Seven International MAL-ED sites. *Sch Psychol Q* **2018**; 33:604–14.
- Collins MH, Zepeda O, Blette B, et al. Serologic surveillance of maternal Zika infection in a prospective cohort in Leon, Nicaragua during the peak of the Zika epidemic. *PLoS One* **2020**; 15:e0230692.
- Milosavljevic B, Vellekoop P, Maris H, et al. Adaptation of the Mullen Scales of Early Learning for use among infants aged 5- to 24-months in rural Gambia. *Dev Sci* **2019**; 22:e12808.
- Perez-Then E, Peña R, Tavares-Rojas M, et al; PMTCT Group. Preventing mother-to-child HIV transmission in a developing country: the Dominican Republic experience. *J Acquir Immune Defic Syndr* **2003**; 34:506–11.
- Bergemann TL, Bangirana P, Boivin MJ, Connett JE, Giordani BJ, John CC. Statistical approaches to assess the effects of disease on neurocognitive function over time. *J Biom Biostat* **2012**; Suppl 7:7310.
- Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* **2009**; 42:377–81.
- Bates D, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Software* **2015**; 67:1–48.
- Alves LV, Paredes CE, Silva GC, Mello JG, Alves JG. Neurodevelopment of 24 children born in Brazil with congenital Zika syndrome in 2015: a case series study. *BMJ Open* **2018**; 8:e021304.
- Carvalho A, Brites C, Mochida G, et al. Clinical and neurodevelopmental features in children with cerebral palsy and probable congenital Zika. *Brain Dev* **2019**; 41:587–94.
- Boivin MJ, Bangirana P, Nakasujja N, et al. A year-long caregiver training program improves cognition in preschool Ugandan children with human immunodeficiency virus. *J Pediatr* **2013**; 163:1409–16.e1–5.
- Boivin MJ, Maliwichi-Senganimalunje L, Ogwang LW, et al. Neurodevelopmental effects of ante-partum and post-partum antiretroviral exposure in HIV-exposed and uninfected children versus HIV-unexposed and uninfected children in Uganda and Malawi: a prospective cohort study. *Lancet HIV* **2019**; 6:e518–30.
- Boivin MJ, Sikorskii A, Familiar-Lopez I, et al. Malaria illness mediated by anaemia lessens cognitive development in younger Ugandan children. *Malar J* **2016**; 15:210.
- Donoso Mantke O, McCulloch E, Wallace PS, Yue C, Baylis SA, Niedrig M. External quality assessment (EQA) for molecular diagnostics of Zika virus: experiences from an international EQA programme, 2016–2018. *Viruses* **2018**; 10:491.
- Speer SD, Pierson TC. Virology. Diagnostics for Zika virus on the horizon. *Science* **2016**; 353:750–1.
- Collins MH, Waggoner JJ. Detecting vertical Zika transmission: emerging diagnostic approaches for an emerged flavivirus. *ACS Infect Dis* **2019**; 5:1055–69.
- Prado EL, Larson LM, Cox K, Bettencourt K, Kubes JN, Shankar AH. Do effects of early life interventions on linear growth correspond to effects on neurobehavioural development? A systematic review and meta-analysis. *Lancet Glob Health* **2019**; 7:e1398–413.
- Becker-Dreps S, Stringer EM, Bucardo F, Bowman NM, Boivin MJ. Is there a silver lining to the Zika virus epidemic in the Americas? *Lancet Infect Dis* **2020**; 20:14–5.