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Neurodevelopmental assessment of normocephalic children born to Zika virus exposed and unexposed pregnant people

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AUTHOR CONTRIBUTIONS

P.B., V.T.T., J.A., M.L.C., E.W.H., S.M.G. and D.W. contributed to the conception and design of the research. R.L. examined the children and performed BSID-III and ASQ:SE-2 assessments. L.W., Allison C., M.C., J.F., F.R., Diana V., Douglas V., Z.A., M.B., H.B., C.B., Alejandra C., Alvaro C., J.G.A., K.G., L.G., S.M.G., E.W.H., G.H., W.L., M.T.L., Carlos M., Cynthia M., C.O., K.P., H.R., A.P.C., C.S., D.W., C.Z. and A.F.T. contributed to the acquisition, analysis and/or interpretation of data. K.G., I.L., L.P. and D.W. contributed to the performance and analysis of the laboratory tests. J.A., M.L.C., V.T.T., P.B., L.W., Diana V., S.M.G. and E.W.H. drafted and revised the manuscript. All authors reviewed and approved the submitted version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Tulane University, New Orleans, USA and the Faculty of Medical Sciences, Universidad Nacional Autónoma de Honduras (UNAH), Tegucigalpa, Honduras, institutional review boards. Informed consent was obtained from parents or caretakers of all participating children. As described, all methods and procedures were performed in accordance with the relevant guidelines and regulations.

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Abstract

BACKGROUND: Studies examining the association between in utero Zika virus (ZIKV) exposure and child neurodevelopmental outcomes have produced varied results.

METHODS: We aimed to assess neurodevelopmental outcomes among normocephalic children born from pregnant people enrolled in the Zika in Pregnancy in Honduras (ZIPH) cohort study, July–December 2016. Enrollment occurred during the first prenatal visit. Exposure was defined as prenatal ZIKV IgM and/or ZIKV RNA result at enrollment. Normocephalic children, >6 months old, were selected for longitudinal follow-up using the Bayley Scales of Infant and Toddler Development (BSID-III) and the Ages & Stages Questionnaires: Social-Emotional (ASQ:SE-2).

RESULTS: One hundred fifty-two children were assessed;after exclusion, 60 were exposed and 72 were unexposed to ZIKV during pregnancy. Twenty children in the exposed group and 21 children in the unexposed group had a composite score <85 in any of the BSID-III domains. Although exposed children had lower cognitive and language scores, differences were not statistically significant. For ASQ:SE-2 assessment, there were not statistically significant differences between groups.

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CONCLUSIONS: This study found no statistically significant differences in the neurodevelopment of normocephalic children between in utero ZIKV exposed and unexposed. Nevertheless, long-term monitoring of children with in utero ZIKV exposure is warranted.

INTRODUCTION

Fetal exposure to maternal infections and inflammation may have long-term consequences into early to middle childhood.[1] The World Health Organization (WHO) Zika Causality Working Group's systematic review reported that Zika virus (ZIKV) is a cause of congenital abnormalities, concluding that there is a need for cohort studies, preferably from different geographic regions, to determine risks of pregnancies affected by asymptomatic and symptomatic ZIKV infection and to define the full range of physical and developmental abnormalities that comprise the congenital Zika syndrome (CZS).[2] Although microcephaly has been associated with severe central nervous system outcomes and has become a distinctive characteristic of CZS, a recent systematic review and meta-analysis assessed the effect of ZIKV exposure in utero on the neurodevelopment of children with normal head circumferences born to women with ZIKV infection in pregnancy.[3] The analysis of nine articles including data from 476 children 9–39 months of age found 6.5%, 29.7% and 11.5% of infants and children to have delays in the non-language cognitive, language and motor domains, respectively, although the authors recommended caution in the interpretation of these results due to high level of heterogeneity in the pooled estimates.[3]

In fact, affected developmental domains differ across reports. For instance, some prospective cohort studies found that between 36 and 40% of ZIKV-exposed children aged 6–42 months had one or more developmental delays with language function being most affected[4–6] and found declines on coefficients for communication, social cognition and mobility with increasing age.[7] Yet these studies were limited by the absence of a comparison group. A cross-sectional study of children 3–12 months of age found that those with prenatal ZIKV exposure had poorer language abilities but similar motor and visual reception abilities than those without prenatal ZIKV exposure.[8] In contrast, another study of children 22–30 months of age found that children with prenatal ZIKV exposure had poorer visual acuity and contrast sensitivity but similar cognitive, language, or motor abilities than those without prenatal ZIKV exposure.[9]

Children for this study were part of a prospective pregnancy cohort study in Tegucigalpa, Honduras,[10] the Zika in Pregnancy in Honduras (ZIPH) study. Enrollment into the cohort started in July 2016 and is ongoing. In a previous analysis, we presented microcephaly outcomes among the initial cohort of pregnant people enrolled between July and December 2016.[11]

In 2018, ZIPH expanded to include longitudinal follow-up of children selected from the existing cohort: (1) children with documented in utero exposure with positive ZIKV IgM and/or positive ZIKV PCR, and (2) a group of children born to pregnant people who did not have confirmed ZIKV infection in pregnancy. The objective of the current analysis was to assess the clinical and neurodevelopmental outcomes, including cognitive, language, motor, and social-emotional outcomes, among the cohort of children born with a normal head

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circumference, enrolled in ZIPH between July and December 2016 who were recruited into the follow-up study between May 2018 and March 2020.

METHODS

Setting

Hospital Escuela and Hospital de Especialidades San Felipe, Tegucigalpa, Honduras.

Study population and recruitment

Participants were selected from the ZIPH prospective study.[10,11] Participants were enrolled at any gestational age, during their first prenatal visit at the Alonso Suazo Health Center between July and December 2016 during the peak of the ZIKV epidemic in Tegucigalpa, Honduras. Venous blood was collected once at enrollment for pregnant participants (Supplementary Fig. S1).

We conducted ELISA to detect anti-Zika IgM antibodies (ZIKV Detect 2.0, InBios, Seattle, WA) according to the manufacturer's instructions, and molecular testing for ZIKV, DENV, and CHIKV on maternal serum samples using the Trioplex rRT-PCR assay, which detects RNA from all three viruses simultaneously, following CDC protocol.[12,13]

For the follow-up study, inclusion criteria included the following: children with normal head circumference at birth (occipital-frontal circumference within 2 standard deviations (SD) for sex and gestational age, using the INTERGROWTH-21 reference standard),[14] any age between 6 to 42 months, born between July and December 2016, during the peak of the ZIKV epidemic, at the Hospital Escuela and Hospital de Especialidades San Felipe in Tegucigalpa, Honduras.

The children were selected in chronological order of their birth date. Once a child was selected, one of the team members contacted the child's parent by cell phone. All the relevant information about the study and the process was provided to the parents, who were invited to participate in the neurodevelopmental study. If the parents were interested in participating, they were invited to attend Hospital de Especialidades San Felipe with their child for screening, and if eligible, informed consent was obtained. At the hospital, the study staff administered an eligibility screening questionnaire to participate in the study. If at least one parent was a Spanish speaker and lived with the child, then the parent was eligible to enroll the child in the follow-up study. Study staff administered the consent for the child's neurodevelopmental follow-up to eligible parents. With parental consent a clinical examination and neurodevelopmental assessment were performed (Supplementary Fig. S1).

ZIKV exposed children were defined from a positive ZIKV IgM and/or positive ZIKV RNA result, regardless of symptoms, at the study enrollment during the first prenatal visit. ZIKV unexposed children were defined from a negative ZIKV IgM with or without negative ZIKV RNA result. PCR testing was not performed in participants that not consented long-term storage of their blood sample. Children born to people without ZIKV testing or only negative ZIKV RNA result during pregnancy were considered undefined ZIKV exposure. This group was excluded from the analysis.

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Procedures

A parent-child follow-up questionnaire was administered, and standardized tools to assess multiple domains of the child's development, including achievement of developmental milestones and tools to assess qualities of the family environment were administered by trained study staff. The clinical examination included biometry: head circumference, height, and weight. The developmental evaluation included Spanish language versions of the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) that assesses key developmental domains: cognition, language (receptive and expressive communication), and motor (gross and fine)[15,16] and the Ages & Stages Questionnaires: Social-Emotional, Second Edition (ASQ: SE-2) screens for psychosocial abilities.[17] BSID-III and ASQ: SE-2 were performed at the same visit. Specific standard operating procedures were developed, and clinic and study staff were trained to use these instruments. Children with any neurodevelopmental delay or risk of delay were referred for specialized evaluation (psychology and/or neurology).

Data collection

Information from the clinical examination was recorded on study-specific forms. Data entry of BSID-III were limited to the summary score in the first stage. BSID-III examination forms were not copied, scanned, or photographed. Data about the ASQ: SE-2 Questionnaire were collected in the data form provided by the instrument. Data about family environment characteristics were obtained by interviewing the parent or a caretaker at the time of enrollment and recorded in a specific data form.

Variables collected

Maternal sociodemographic and clinical data during pregnancy were collected at enrollment and at birth. At enrollment the information collected included maternal age, gestational age (completed weeks) based on the last menstrual period (LMP) or a clinical estimate by the physician, and maternal Zika symptoms. At birth the following information was collected: gestational age, neonatal sex, birthweight, length and head circumference and maternal Zika symptoms. Anthropometry at neurodevelopmental visit: weight-for-age, height-for-age, and head circumference-for-age were described according to WHO growth curves.[18] Length was measured in the recumbent position for children under the age of two using a marked platform with a sliding footboard; for children over the age of 2 years, a length board (stadiometer) mounted at a right angle between a level floor and against a wall was used. An insertion tape, Seca 212 (Medical Measuring Systems and Scales, Hamburg, Germany), was used for measuring head circumference.

Neurodevelopmental assessment was performed using BSID-III and the ASQ: SE-2 by a trained psychologist. The BSID-III is a standardized assessment used to detect developmental delays in children between one and 42 months of age. The BSID-III yields composite scores in several developmental domains ($M = 100$; SD = 15): cognitive, language, and motor domains were used in this study. A score 70–84 (−1 to −2 SD) in any functional domain indicates risk of developmental delay, and a score <70 (<−2 SD) indicates moderate/severe developmental delay.[19] The BSID also yields scaled scores in five sub-domains ($M = 10$; SD = 3): cognitive, expressive language, receptive language, fine

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motor, and gross motor. Both composite and scaled scores compare a child's performance to other children of the same age.[15]

The ASQ: SE-2 is a developmental screener used to identify children who may be at-risk for social-emotional delays. Children who score above the cut-off score may need additional social-emotional assessment, and the cut-off scores vary by age. Each questionnaire yields information on whether there are no concerns or additional monitoring is required, or if the child exceeded the cut-off score, and, therefore, social-emotional concerns are noted[20] We did not use the social-emotional questionnaire that is part of Bayley because there is no Spanish translation. We used the ASQ:SE to assess the social-emotional domain, and although a screener, it is validated in Spanish.

Data management and quality

The data forms were entered in REDCap,[21] an open-source software for clinical research studies using distributed data entry. Digital pictures (with date and time) of each data form, other than BSID-III examination forms, were taken and were regularly sent to the data center. After the data were uploaded to the database, discrepancies were checked to ensure completeness, consistency, and accuracy.

Statistical analysis

The characteristics of the pregnant people and children at enrollment and at follow-up were summarized. For categorical variables, frequencies and proportions were reported. For continuous variables, mean and standard deviation (SD) were used.

The absolute and relative frequency of BSID-III composite risk of delay and delays (i.e., cognitive, language and motor domain scores 70–84 and <70, respectively) were reported by ZIKV exposure. To evaluate the risk of having a delay due to ZIKV exposure, the relative risk and the confidence interval were calculated. We also present crude and adjusted BSID-III composite and scaled scores (mean and SD) by ZIKV exposure, according to the gestational age of exposure and between children at 24 months of age and older compared to younger than 24 months of age. A t-test and a regression model adjusting for age and the education of the pregnant person were used for the comparison. We repeated the analysis after excluding children born preterm or low birthweight. The ASQ: SE-2 scores were analyzed according to the cut-off point established for each questionnaire according to age. The relative risk and the confidence interval were calculated. We also reported the proportion of children who had no concerns or needed developmental monitoring. Cases with missing data in the variable being reported were not considered in the analyses. R version 4.0.3 was used for data analysis.[22]

RESULTS

During July to December 2016, the ZIPH cohort study enrolled 668 pregnant people, among them 151 were invited and agreed to participate in the follow up study between May 23, 2018 and March 11, 2020 with a total of 152 children. Recruitment in the follow up study ended due to the COVID-19 pandemic (Supplementary Fig. S1).

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Clinical and sociodemographic data of pregnant people and their children at time of enrollment and at birth according to the exposure are shown in Table 1. Sixty children were exposed and 72 unexposed to ZIKV during pregnancy; for 20 children, the exposure was undefined, and they were excluded from the analysis (Supplementary Fig. S1 and Table 1). About 59% and 70%, exposed and unexposed, respectively, were pregnant people in the first trimester of their pregnancy at time of enrollment; 12% and 17%, exposed and unexposed, respectively, of the children were born preterm (<37 weeks). Three pregnant people were positive for ZIKV RNA. PCR testing was not performed in 17/60 exposed and 22/72 unexposed pregnant participants that not consented long-term storage of their blood sample (Table 1).

Children's clinical data at follow-up are shown in Table 2. The mean age was 1.9 +/− 0.5 years, for both, exposed and unexposed children. Most children (93–97%) had adequate anthropometric growth, with similar distribution between exposed and unexposed groups. BSID-III and ASQ: SE-2 were performed in 132 children.

Table 3 shows the proportions of the risk of delay and delay in the three BSID-III composite domains: cognitive, language, and motor. Thirty-six children had risk of delay (70–84 composite score) in any of the BSID-III domains: 7 cognitive, 21 language, and 8 motor domains. In regard to delay (<70 composite score), 3 children had language delay and 2 motor delay. The total number of children with risk of delay in at least one domain was 32 (data not shown). The proportion of children in the exposed group was higher than in the unexposed group for the metric of cognitive risk of delay/delay and language risk of delay/delay, but these findings were not statistically significant. The proportion of children in the unexposed group was higher than in the exposed group for the metric of motor risk of delay/delay, but this was not statistically significant.

Table 4 shows the BSID-III mean composite scores in the three composite domains and five sub-domains. There were no statistically significant differences between groups. The exposed group had lower cognitive and language scores, but this result was not statistically significant. This tendency persisted after excluding children born preterm and low birthweight from the analysis, although it disappeared for the motor scores (Supplementary Table S1).

There was not enough information to compare the scores obtained among ZIKV exposed children according to the gestational age of exposure because there was no information on gestational age at the first prenatal visit for 9 cases, and there were only 4 cases exposed during the third trimester. The scores obtained among cases exposed during the first trimester compared to the second trimester were similar, but there were less than 30 cases for each group (Supplementary Table S2). There were no statistically significant differences between the scaled scores among children at 24 months of age and older compared to younger than 24 months of age, among ZIKV exposed and unexposed children. However, in those younger than 24 months of age, the exposed group had lower scores in scalar and composite cognitive domains (Supplementary Table S3). The three children born to people who were positive for ZIKV RNA during pregnancy showed no risk of delay/delay in any of the domains (data not shown).

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Socioemotional development screener, ASQ: SE-2, showed that 92% of children had adequate development with a score equal to or below the cut-off point. There were no statistically significant differences between exposed and unexposed children (Supplementary Table S4).

DISCUSSION

This study shows the neurodevelopmental outcomes in children born with normal head circumference from ZIKV exposed and unexposed pregnant people enrolled at the first prenatal visit in Tegucigalpa, Honduras. To our knowledge, this is the first study about neurodevelopmental aspects in children born from pregnant people exposed and unexposed to ZIKV conducted in Honduras. Twenty exposed and 21 unexposed children presented some type of neurodevelopmental risk of delay or delay (Table 3). We found no evidence of statistically significant differences in the three BSID-III composite domains (cognitive, language and motor) in normocephalic ZIKV-exposed children compared to unexposed children at mean age of 1.9 years. These results are consistent with other studies.[9,23,24] Differences in cognitive and language scores between exposed and unexposed groups were not statistically significant, even though Zika exposed children scored lower, similar to other studies that reported these differences, although they did not all have the same study design.[5–7,25,26] The lack of significance could be a function of small sample size.

Our study did not find differences in socio-emotional development between exposed and unexposed children, similar to the findings of Grant et al. in children 24 months of age[23] (Table S4). Although there is lack of a normative Honduran population for both BSID-III and ASQ:SE-2 assessment tools, we used Spanish language versions of both tools that have been used in other Spanish-speaking populations.[27,28]

There are several studies that have described abnormal neurodevelopmental effects in children exposed to ZIKV during pregnancy but not including a comparison group.[3,5–7,29–34] At present, there are fewer studies regarding the effects of congenital ZIKV infection on the neurodevelopmental outcomes of children without apparent defects at birth.[3] These studies implemented a variety of neurodevelopmental assessments or screeners used to evaluate the children and applied at different ages. These screeners are different, and they are not interchangeable. One of the scales more frequently used is the BSID-III,[3,24–26,35] but there are several other scales used including Mullen Scales of Early Learning,[36] Ages and Stages Questionnaire,[23] General Movement Assessment,[35] INTERGROWTH-21st Neurodevelopment Assessment,[9] and Pediatric Evaluation of Disability Inventory (PEDICAT),[37] among others. In regard to the age of evaluation, these studies have reported neurodevelopmental assessments in children as young as 1 month up to 42 months of age.[3] Preschool children have also been evaluated.[37] All these factors may influence the results of the neurodevelopment evaluation in these studies, and such evaluations could be informed by longer term follow-up with validated assessments. As it was found in the systematic review and metanalysis,[3] the heterogeneity of neurodevelopmental assessment tools used limited the number of studies that could be included in the metanalysis. However, the authors concluded that ZIKV during pregnancy is a risk factor for early childhood neurodevelopmental delay in normocephalic children.

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They also mentioned that there could be different contributing factors, such as nutrition and socioeconomic status, and larger prospective studies including unexposed control groups are needed to confirm whether antenatal ZIKV exposure is associated with delayed child neurodevelopment.

This study presents some limitations. Only a single sample was obtained early during pregnancy. A positive IgM result might be measuring an infection before pregnancy given prolonged detection, although this might be mitigated because we initiated enrollment during the height of the ZIKV epidemic,[38] As Honduras is endemic for dengue, initiating enrollment during the height of the ZIKV epidemic,[11] increased the probability to detect ZIKV cases. We used the ZIKV IgM Detect 2.0 test (Inbios WA) which has a higher specificity than the original version of the kit.[39] We also assessed samples from the first pregnant persons enrolled who tested positive with Dengue virus ELISA, and we identified 69% (25/36) as a recent ZIKV infection using plaque reduction neutralization tests (PRNTs) for ZIKV and DENV-2 and using WHO criteria for interpretation.[13] On the other hand, we might have missed ZIKV infections later in pregnancy which may misclassify unexposed, so these findings are generalizable to infections in early pregnancy which may have the greatest impact on development of Zika associated birth defects and neurodevelopment. A group of participants had undefined ZIKV exposure, which was excluded from analysis. However, among these, there were only one child with cognitive risk of delay (0.1%), and one child with inadequate ASQ:SE-2 assessment (data not shown). Also, the sample size was small to achieve conclusive results. In addition, we did not perform eye (e.g., retinal examination), ear examinations, and neuroimaging in the enrolled children; therefore, some visual, hearing, or subtle central nervous system abnormalities may have not been detected. Among the strengths, our study provides information on a population-based prospective cohort including symptomatic and asymptomatic ZIKV exposure and a comparison population of children unexposed to ZIKV in utero; an unexposed comparison population has generally been unavailable in previous studies.

Children with neurodevelopment delays due to arboviral diseases or other causes may improve their outcomes with early interventions as was shown in a trial published recently that evaluated the impact of an intervention based on stimulation through home visits.[40] Because children born in the Zika virus epidemic of the Americas in 2016 are growing and getting close to school age, follow-up is important to be able to detect developmental abnormalities that might not be detected earlier in life.[37]

CONCLUSIONS

In conclusion, our overall results suggest that there were no statistically significant differences in the neurodevelopment in children with normal head circumference between those born to ZIKV-exposed and unexposed pregnant people. The exploration of cognitive and language differences in ZIKV exposed vs. unexposed children could be informed by additional research in larger samples. Children with prenatal ZIKV exposure, including those with normal head circumference and have no evidence of abnormalities at birth, should be monitored for cognitive and language delays and referred for interventions as needed.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. CDC.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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IMPACT:

- This study found no statistically significant differences in the neurodevelopment in normocephalic children with in utero Zika virus exposure compared to unexposed children, although the exposed group showed lower cognitive and language scores that persisted after adjustment by maternal age and education and after excluding children born preterm and low birth weight from the analysis.
- Children with prenatal Zika virus exposure, including those normocephalic and have no evidence of abnormalities at birth, should be monitored for neurodevelopmental delays. Follow-up is important to be able to detect developmental abnormalities that might not be detected earlier in life.

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Table 1.

Characteristics of the pregnant people and their children, by Zika virus exposure group.

	Exposed[C] (*N* = 60)	**Unexposed[C] (*N* = 72)**
Maternal age at enrollment (years)		
12–19	19/60 (31.7)	13/72 (18.1)
20–34	32/60 (53.3)	49/72 (68.1)
35–45	9/60 (15.0)	10/72 (13.9)
Gestational age at first prenatal visit (weeks)		
<14	30/51 (58.8)	46/65 (70.8)
14–27	17/51 (33.3)	12/65 (18.5)
28–39	4/51 (7.8)	7/65 (10.8)
Gestational age at birth (weeks)		
<33	0/60 (0.0)	0/72 (0.0)
33–34	1/60 (1.7)	5/72 (6.9)
35–36	6/60 (10.0)	7/72 (9.7)
37–42	53/60 (88.3)	60/72 (83.3)
Zika symptoms during pregnancy[a]		
Yes	2/60 (3.3)	0/72 (0.0)
No	58/60 (96.7)	72/72 (100.0)
Anthropometry at birth		
Birth weight in g		
<2500	6/60 (10.0)	11/72 (15.3)
≥2500	54/6 (90.0)	61/72 (84.7)
Length in cm[b]	49.3 (3.9)	49.5 (3.8)
Head circumference in cm[b]	34.4 (1.3)	34.1 (1.4)
Sex		
Female	32/60 (53.3)	32/72 (44.4)
Male	28/60 (46.7)	40/72 (55.6)
Status at birth		
Neonatal Intensive or Special Care Unit	3/60 (5.0)	5/72 (6.9)
Zika virus (ZIKV) IgM and RNA testing		
Both positive	1/60 (1.7)	0/72 (0.0)
IgM Positive and RNA Negative	40/60 (66.7)	0/72 (0.0)
IgM Negative and RNA Positive	2/60 (3.3)	0/72 (0.0)
IgM Positive and RNA Not done	17/60 (28.3)	0/72 (0.0)
IgM Negative and RNA Negative	0/60 (0.0)	50/72 (69.4)
IgM Negative and RNA Not done	0/60 (0.0)	22/72 (30.6)

[a]Arthralgia, arthritis, conjunctivitis, fever, rash.

[b]Mean and standard deviation.

[c] Zika virus (ZIKV) testing was conducted on maternal serum at first the prenatal visit. Exposed: positive ZIKV IgM and/or positive ZIKV RNA results at enrollment; unexposed: negative ZIKV IgM with/without negative ZIKV RNA results at enrollment.

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Table 2.

Clinical characteristics of children at the time of the follow-up assessment, by Zika virus exposure group.

	Exposed[c] (*N* = 60)	Unexposed[c] (*N* = 72)
Age at examination		
Age in years[a]	1.9 (0.5)	1.9 (0.4)
12–17 months	12/60 (20.0)	12/72 (16.7)
18–23 months	22/60 (36.7)	29/72 (40.3)
24–37 months	26/60 (43.3)	31/72 (43.0)
Anthropometric measures		
Weight		
z-score[b]		
<−2	0/60 (0.0)	2/72 (2.8)
≥ −2 and ≤2	57/60 (95.0)	67/72 (93.1)
>2	3/60 (5.0)	3/72 (4.2)
Length		
z-score[b]		
<−2	1/60 (1.7)	2/72 (2.8)
≥ −2 and ≤2	58/60 (96.7)	69/72 (95.8)
>2	1/60 (1.7)	1/72 (1.4)
Head circumference		
z-score[b]		
<−2	1/60 (1.7)	2/72 (2.8)
≥ −2 and ≤2	58/60 (96.7)	69/72 (95.8)
>2	1/60 (1.7)	1/72 (1.4)

[a] Mean and standard deviation.

[b] *Z*-score according to WHO growth curves by age and sex.

[c] Zika virus (ZIKV) testing conducted on maternal serum at first the prenatal visit. Exposed: positive ZIKV IgM and/or positive ZIKV RNA results at enrollment; unexposed: negative ZIKV IgM with/without negative ZIKV RNA results at enrollment

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Table 3.

Cognitive, language, and motor scores by Zika virus exposure groups, BSID-III (comparison by categories).

Dimension scores	Exposed[a] (*N* = 60)		Unexposed (*N* = 72)		Relative risk[b] (CI)	*p* value
	n/N	%	*n/N*	%		
Cognitive (<85)	4/60	6.7	3/72	4.2	1.6 (0.37; 6.87)	0.525
<70	0	0	0	0		
70–84	4	6.7	3	4.2		
Language (<85)	13/60	21.7	11/72	15.3	1.42 (0.69; 2.93)	0.345
<70	2	3.3	1	1.4		
70–84	11	18.3	10	13.9		
Motor (<85)	3/60	5.0	7/72	9.7	0.51 (0.14; 1.9)	0.309
<70	0	0	2	2.8		
70–84	3	5	5	6.9		

[a] Zika virus (ZIKV) testing was conducted on maternal serum at the first prenatal visit. Exposed: positive ZIKV IgM and/or positive ZIKV RNA results at enrollment; unexposed: negative ZIKV IgM with/without negative ZIKV RNA results at enrollment.

[b] Relative risk of exposed vs. unexposed.

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Table 4.

Cognitive, language, and motor scores by Zika virus exposure groups, BSID-III (comparison of continuous outcomes).

	All	Mean (standard deviation)		*p* value[b]	Adjusted Mean (standard error)		*p* value[c]
		Exposed[a] (*n* = 60)	Unexposed (*n* = 72)		Exposed (*n* = 60)	Unexposed (*n* = 72)	
Scalar cognitive	8.7 (1.7)	8.4 (1.5)	9.0 (1.8)	0.041	8.5 (0.2)	9.0 (0.2)	0.070
Cognitive composite	93.8 (8.1)	92.7 (6.9)	95.2 (9.0)	0.069	92.8 (1.1)	95.1 (1.0)	0.114
Receptive language	9.1 (1.9)	8.7 (2.1)	9.3 (1.9)	0.106	8.7 (0.3)	9.3 (0.2)	0.104
Expressive language	8.5 (2.1)	8.2 (2.1)	8.5 (2.2)	0.432	8.3 (0.3)	8.5 (0.3)	0.527
Language composite	93.0 (10.1)	91.2 (10.2)	93.7 (10.5)	0.168	91.3 (1.3)	93.6 (1.2)	0.199
Fine motor	9.5 (2.4)	9.6 (2.4)	9.6 (2.5)	1.000	9.6 (0.3)	9.6 (0.3)	0.919
Gross motor	9.5 (1.9)	9.5 (1.6)	9.5 (2.3)	0.936	9.5 (0.3)	9.5 (0.2)	0.992
Motor composite	97.0 (10.7)	97.4 (9.5)	97.3 (12.5)	0.950	97.4 (1.5)	97.2 (1.3)	0.937

[a] Zika virus (ZIKV) testing was conducted on maternal serum at the first prenatal visit. Exposed: positive ZIKV IgM and/or positive ZIKA RNA result at enrollment; unexposed: negative ZIKV testing.

[b] *T*-test was used to compare the mean scores between ZIKV exposed and unexposed groups.

[c] Regression model adjusted by age and education of the pregnant person was used to compare the mean scores between the ZIKV exposed and unexposed groups.