Fetal central nervous system anomalies according to RT-PCR and trimester of maternal infection with Zika virus: A prospective cohort study

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Abstract

Introduction: In October 2015, an epidemic of Zika began in Colombia’s geographic areas with a high population of mosquitoes of the genus Aedes. We aimed to describe the fetal brain ultrasound findings in pregnant women with active symptoms or a history of symptoms suggestive of Zika virus (ZIKV) infection.

Material and methods: Eligible pregnant women were tested with reverse transcriptase-polymerase chain reaction (RT-PCR) for ZIKV and followed prospectively using detailed anatomic ultrasound and transvaginal neurosonography to detect structural anomalies of the fetal central nervous system (CNS).

Results: A total of 115 symptomatic women with a positive ZIKV RT-PCR and 55 with a negative ZIKV RT-PCR were enrolled in the study; CNS compromise of the fetus occurred in 22% and 17%, respectively \((p = 0.255)\). Callosal dysgenesis (14.5%) was the most frequent anomaly of the CNS, followed by microcephaly (13.6%) and neuronal migration disorders (8.3%). When symptomatic ZIKV RT-PCR-positive women were categorized by trimester of infection, CNS anomalies were present in 40% of first-trimester infections, compared with 21% and 7% in second- and third-trimester infections \((p = 0.002)\). CNS anomalies were also more severe in first-trimester-infected fetuses than in second- and third-trimester-infected fetuses. The high prevalence of CNS anomalies in fetuses of symptomatic ZIKV RT-PCR negative women suggests a high rate of false-negative cases and an even higher prevalence of CNS anomalies than observed in this study.

Conclusions: The prevalence of fetal CNS anomalies was higher than previously reported in the literature for both symptomatic RT-PCR-positive and -negative pregnant women. Corpus callosum anomalies, microcephaly, neuronal migration disorders, and brain parenchymal hyperechogenicities were the most frequent CNS anomalies detected. In addition, CNS anomalies were more frequent and severe in infected fetuses during the first trimester of pregnancy than during the second or third trimester.
1 | INTRODUCTION

The Zika virus (ZIKV), genus Flavivirus, was isolated in 1947 in Uganda, and in 1964, human transmission of the virus was confirmed. The distribution of the virus before 2007 was well established in tropical Africa and Asia, but only 13 cases of human infection were reported in the literature. In 2007, the first epidemic of ZIKV was documented in the Yap Island of Micronesia, with a second epidemic in 2013 in French Polynesia; during this epidemic, ZIKV was found to be associated with an increase in Guillain–Barré syndrome, suggesting a neurotropic nature in humans. In March of 2015, concurrent with a new epidemic of ZIKV in Brazil’s northeastern region, epidemiologists observed an abnormal increase in the frequency of neonatal microcephaly, which was later confirmed to be associated with prenatal exposure of the fetus to ZIKV. Retrospective and prospective studies have shown that central nervous system (CNS) compromise occurs in 6%–12% of fetuses exposed to the virus; the most frequent anomalies described today include microcephaly, callosal dysgenesis, white and gray matter calcifications, ventriculomegaly and posterior fossa anomalies. The disruptive mechanism has not been identified. However, it may be linked to the virus’s structural and functional characteristics, maternal nutrition, maturation of the maternal-fetal placenta barrier, maternal/fetal immunology and genetic factors.

The first case of human ZIKV infection in Colombia was identified in October 2015. The beginning of the epidemic that infected more than 100,000 people ended in January 2017. As part of a public health epidemiologic strategy, cases of suspected ZIKV infection were identified using pre-established clinical criteria. Based on these criteria, an operational definition of suspected cases was created, and mandatory testing for ZIKV was required in high-risk populations, including pregnant women and children younger than a year of age. Maternal exposure to ZIKV was confirmed using reverse transcriptase-polymerase chain reaction (RT-PCR) from blood, urine or amniotic fluid samples collected during the first week of pregnancy. Testing included blood, urine and amniocentesis samples for ZIKV RT-PCR. Government-mandated prenatal screening for syphilis and toxoplasmosis was followed; according to physician criteria, tests were also ordered to rule out other viral infections with similar symptomatology (Cytomegalovirus [CMV], Rubella, dengue and Chikungunya). We intended to collect cord blood, placental tissue and cerebral spinal fluid at the time of birth, as indicated by public health and government directives.

The ZEN initiative (Zika en Embarazadas y Neonatos) was created by the maternal-fetal medicine and the neonatal group at the Universidad Industrial de Santander to address questions related to clinical characteristics of maternal exposure, differences in CNS anomalies between symptomatic ZIKV RT-PCR-positive and -negative pregnant women, frequency of fetal compromise with trimester of infection, and neurodevelopment outcome of surviving infants. This study aimed to prospectively describe the outcome of fetuses of pregnant women with symptomatology suggestive of ZIKV infection tested with RT-PCR and categorized according to trimester of infection following detailed anatomic ultrasound and neurosonography (www.clinicaltrials.gov, NCT02943304).

Key message

Fetal CNS anomalies were found to be more prevalent than previously reported due to dedicated neurosonography in symptomatic pregnant women with ZIKV infection. CNS anomalies were more frequent in first-trimester ZIKV infections. Dedicated neurosonography is an essential diagnostic tool for pregnant women with suspected ZIKV infection.

2 | MATERIAL AND METHODS

2.1 | Study design and participants

This is a prospective cohort study that enrolled pregnant women from October 2015 to January 2017 during the ZIKV epidemic in Colombia. Participating women were recruited from the obstetric services of three medical centers in Bucaramanga, Santander: Hospital Universitario de Santander, Fundación Oftalmológica de Santander Clínica Carlos Ardila Lülle, and Clínica Materno Infantil San Luis.

2.2 | Research methods

Clinically relevant data were obtained from interviews and data extracted from pregnant women’s corresponding medical records at enrollment. Pregnant women were included if there was: the presence of an acute clinical condition 7 days before the prenatal visit with one or more of the following signs or symptoms: fever, skin rash (exanthema) with or without itching, non-purulent conjunctivitis, arthralgia, myalgia or general malaise without a specifically identified etiology. Pregnant women were also included if they had a history of symptomatology suggestive of ZIKV infection outside the 7-day window. Only pregnant women who were tested with RT-PCR to confirm or reject the diagnosis of ZIKV infection were included in the final analyses. Testing included blood, urine and amniocentesis samples for ZIKV RT-PCR. Government-mandated prenatal screening for syphilis and toxoplasmosis was followed; according to physician criteria, tests were also ordered to rule out other viral infections with similar symptomatology (Cytomegalovirus [CMV], Rubella, dengue and Chikungunya). We intended to collect cord blood, placental tissue and cerebral spinal fluid at the time of birth, as indicated by public health and government directives.

All pregnant women enrolled in the study were evaluated with fetal ultrasound at least once by an obstetrician and a maternal-fetal medicine specialist in 90% of cases. The ultrasound evaluation included a detailed anatomic investigation for fetal anomalies,
independent of gestational age; 51% of these women had an additional abdominal or vaginal neurosonography scan performed at the Hospital Universitario de Santander with a preestablished protocol using ISUOG guidelines. Microcephaly was categorized as mild or severe. Mild microcephaly was defined as a fetal ultrasound measurement of head circumference between −2.99 and −2 SD from the mean for gestational age; severe microcephaly was defined as ≤ −3 SD.

Relevant clinical data on maternal morbidity and fetal loss were captured prospectively, including perinatal and postpartum information such as gestational age at birth, sex, Apgar score at 5 minutes of life, and neonatal anthropometric measurements. Neonatal microcephaly was defined as a head circumference ≤ −2 SD from the mean for age.

### 2.3 | Statistical analyses

Clinical data was stored in a password-protected, web-based electronic database, REDCap, with the de-identification capability to protect patient privacy. Study population description is shown with median and interquartile ranges (IQR) for continuous variables and absolute numbers and proportions for nominal and ordinal variables. Differences in ultrasonographic and perinatal outcomes between symptomatic ZIKV RT-PCR-positive and symptomatic ZIKV RT-PCR-negative women were estimated using Wilcoxon or chi-square tests. Differences by trimester of ZIKV infection were estimated with the use of analysis of variance (ANOVA) or chi-square of linear tendency test. An α < 0.05 was considered significant. All data analysis was done in STATA/IC 16.1 (StataCorp LLC, 2020).

| TABLE 1 | Demographic characteristics of symptomatic pregnant women according to ZIKV RT-PCR |
|----------|-------------------------------------------------------------------------------------|
| **ZIKV RT-PCR (+)** | **ZIKV RT-PCR (−)** | **p-value** |
| **n** | **%** | **n** | **%** |  |
| Pregnant women evaluated | 115 | 55 |  |  |  |
| Age (years) |  |  |  |  |  |
| Up to 16 | 4 | 3.5 | 1 | 1.8 | 0.577 |
| 17–25 | 57 | 50.0 | 32 | 58.2 |  |
| 26–34 | 39 | 33.9 | 18 | 32.7 |  |
| 35 or more | 15 | 13.2 | 4 | 7.3 |  |
| Age (median, IQR) | 24 (21–30) | 23 (19–26) | 0.062 |  |
| Origin |  |  |  |  |  |
| Metropolitan area | 97 | 84.4 | 40 | 72.7 | 0.182 |
| Other areas of Santander | 6 | 5.2 | 4 | 7.3 |  |
| Outside Santander | 12 | 10.4 | 11 | 20.0 |  |
| Subsidized healthcare | 79 | 66.7 | 46 | 83.6 | 0.028 |
| Low socioeconomic status | 89 | 77.4 | 47 | 85.5 | 0.219 |
| Education level |  |  |  |  |  |
| Primary | 10 | 8.7 | 8 | 14.5 | 0.640 |
| High school | 54 | 47.0 | 22 | 40.0 |  |
| Technical | 32 | 27.8 | 13 | 23.6 |  |
| University | 6 | 5.2 | 4 | 7.3 |  |
| Unknown | 13 | 11.3 | 8 | 14.6 |  |
| Lives with a partner | 68 | 59.1 | 37 | 67.3 |  |

Abbreviations: IQR, interquartile range; RT-PCR, reverse transcriptase polymerase chain reaction; ZIKV, Zika virus.

*Bucaramanga.
2.4 | Ethical approval

The Committee on Ethics and Scientific Research (CEINCI) from the Universidad Industrial de Santander approved the study on 13 May 2016 (Acta N° 09). Signed consent was obtained from all participating pregnant women.

3 | RESULTS

A total of 230 pregnant women with symptoms suggestive of Zika virus (ZIKV) infection were eligible participants; signed informed consent was obtained in 209 women, seven with twin pregnancies (Figure 1). In all, 115 symptomatic pregnant women had a positive ZIKV RT-PCR test, three with twin pregnancies, and 55 symptomatic pregnant women had a negative ZIKV RT-PCR test, two of them with twin pregnancies. Thirty-nine symptomatic women that had never had a ZIKV RT-PCR test were excluded from this analysis.

The presence of exanthema/rash, arthralgia and fever was the most frequent maternal clinical manifestation. Unfortunately, not all symptomatic pregnant women had a complete work-up for TORCH due to our inability to access prenatal histories of infants born outside the participating centers. In all, 89 pregnant women were screened for the diagnosis of toxoplasmosis: all were negative. Two of 90 (2%) tested positive for syphilis, both from the ZIKV RT-PCR-positive group, but no cases of congenital syphilis were identified. Two of 28 women (9%) had a positive IgM for Rubella, and all belonged to the ZIKV RT-PCR-negative group; both cases were reviewed and showed no evidence of microcephaly or brain anomalies. One of 13 women tested positive for CMV in the ZIKV RT-PCR-positive group but the fetus had no CNS anomalies. Clinical evidence of herpes simplex lesions was confirmed in 9/79 (11%) pregnant women, 5/50 (10%) in the ZIKV RT-PCR-positive group, and 4/29 (14%) in the ZIKV RT-PCR-negative group; no cases of congenital herpes simplex were identified. Additionally, five infants were diagnosed with dengue among 10 pregnant women tested, three of which were also RT-PCR-positive for ZIKV. Finally, a total of 15 amniocenteses were done in symptomatic ZIKV RT-PCR-positive women to confirm the diagnosis of fetal infection in women with a gestational age >20 weeks; eight amniocenteses had a positive RT-PCR for ZIKV, and seven were negative.

Among the 115 pregnant women who were RT-PCR-positive for ZIKV infection, we confirmed the trimester of infection in 108 (94%). Tables 1 and 2 compare the demographic and clinical outcomes between symptomatic ZIKV RT-PCR-positive and ZIKV RT-PCR-negative pregnant women. Pregnant women with a positive ZIKV

| ZIKV RT-PCR (+) | ZIKV RT-PCR (−) | p value |
|----------------|----------------|---------|
| n | % | n | % |
| Pregnant women evaluated | 115 | 55 |
| Symptoms | | | |
| Eruption | 100 | 87.0 | 39 | 70.9 | 0.011 |
| Arthralgia | 36 | 31.3 | 14 | 25.5 | 0.434 |
| Fever | 60 | 52.2 | 37 | 67.3 | 0.063 |
| Headache | 57 | 49.6 | 36 | 65.5 | 0.052 |
| Pruritus | 52 | 45.2 | 20 | 36.4 | 0.274 |
| Myalgia | 48 | 41.7 | 57 | 49.1 | 0.366 |
| Conjunctivitis | 36 | 31.3 | 14 | 25.5 | 0.434 |
| Periarticular edema | 15 | 13.0 | 3 | 5.5 | 0.132 |
| Lymphadenopathy | 5 | 4.4 | 3 | 5.4 | 0.750 |
| Trimester of infection | | | |
| I | 27 | 23.5 | 19 | 34.5 | 0.303 |
| II | 45 | 48.7 | 26 | 47.3 |
| III | 30 | 26.1 | 10 | 18.2 |
| Not determined | 2 | 1.7 | 0 | — |
| Previous pregnancies | | | |
| None | 40 | 34.8 | 24 | 43.6 | 0.299 |
| 1-3 | 68 | 59.1 | 25 | 45.4 |
| 4+ | 6 | 5.2 | 3 | 5.4 |
| Not registered | 1 | 0.9 | 2 | 3.6 |
| Amniocentesis indication | | | |
| Genetics | 3 | 5.5 | 5 | 4.4 | 0.750 |
| ZIKV differential Dx | 15 | 13.0 | 4 | 7.3 | 0.264 |
| Chorioamnionitis | 1 | 0.9 | 1 | 1.8 | 0.592 |

Abbreviations: RT-PCR, reverse transcriptase polymerase chain reaction; ZIKV, Zika virus.
RT-PCR were slightly older, were less likely to have health insurance, and were predominantly from Bucaramanga. No other differences were observed concerning other demographic, perinatal or neonatal outcome variables (Tables 3 and 4). A large proportion of pregnant women were infected in the second trimester (49%). Table 5 presents the ultrasonographic findings between ZIKV-positive and -negative women; no significant differences were observed in prevalence percentages of any CNS anomaly between the two groups. In aggregate, the prevalence of any CNS anomalies was 22% in pregnant women with a positive ZIKV RT-PCR and 17% in those with a negative ZIKV RT-PCR. Callosal dysgenesis, microcephaly, neuronal migration disorders, cerebral parenchymal and periventricular hyperechogenicities, and ventriculomegaly were, in that order, the most frequent CNS anomalies observed in pregnant women with a positive ZIKV RT-PCR (Figure 2). Table 6 shows the ultrasound findings of fetuses according to the trimester of infection in symptomatic RT-PCR-positive women. The percentage of any CNS anomalies was higher during the first trimester (40%), dropping to 21% and 7% in the second and third trimester of pregnancy, respectively. Severe microcephaly was 12% in the first trimester vs 1.9% and 0.0% in second and third trimester. Similarly, callosal dysgenesis was observed in 28% of ZIKV RT-PCR-positive women during the first trimester, compared with 13% and 3% in the second and third trimester of pregnancy. Microcephaly was associated with other parenchymal anomalies of the brain in 64% of all cases. Isolated brain parenchymal anomalies (without microcephaly) were observed in 4% of cases during the first trimester, 13% in the second and 10% in the third trimester. Table 7 depicts the presence of fetal CNS anomalies according to trimester of symptomatology in pregnant women with a negative ZIKV RT-PCR; the presence of any CNS anomaly was 26% in the first trimester of pregnancy compared with 17% and 0.0% in the second and third trimester. Callosal dysgenesis, cerebral parenchymal and periventricular hyperechogenicities, neuronal migration disorders and severe microcephaly were, in that order, the most frequent CNS anomalies in first-trimester infections compared with other trimesters of pregnancy.

### TABLE 3 Perinatal characteristics of symptomatic pregnant women according to ZIKV RT-PCR

|                                      | ZIKV RT-PCR (+) | ZIKV RT-PCR (−) | p value |
|--------------------------------------|-----------------|-----------------|---------|
| Pregnant women evaluated             | 115             | 55              |         |
| Morbidity in pregnancy               |                 |                 |         |
| UTI                                  | 29/109 26.1     | 20/5 40.0       | 0.089   |
| IUGR                                 | 14/110 12.7     | 5/50 10.0       | 0.621   |
| HDP                                  | 13/110 11.8     | 9/50 18.0       | 0.293   |
| Diabetes                             | 8/110 7.3       | 3/50 6.0        | 0.768   |
| Multiple pregnancy                   | 4/115 3.5       | 2/55 3.6        | 0.958   |
| Chorioamnionitis                     | 3/110 2.7       | 1/50 2.0        | 0.785   |
| Placental abruption                  | 0/110 0.0       | 1/50 2.0        | 0.137   |
| Reason for end of pregnancy          |                 |                 |         |
| EPI                                  | 2/110 1.8       | 0/50 1.0        | 0.214   |
| Elective delivery                    | 22/110 20.0     | 5/50 10.0       | 10.0    |
| Emergency delivery                   | 38/110 34.5     | 16/5 32.0       | 32.0    |
| Spontaneous delivery                 | 48/110 43.6     | 29/5 58.0       |         |
| Vaginal delivery                     | 47/110 42.7     | 27/5 54.0       | 0.124   |
| GA at birth                          |                 |                 |         |
| 24+6 to 27+7 weeks                   | 2 1.7           | 0 0.0           | 0.136   |
| 28+6 to 31+7 weeks                   | 0 0.0           | 2 3.6           |         |
| 32+6 to 33+7 weeks                   | 3 2.6           | 0 0.0           |         |
| 34+6 to 36+7 weeks                   | 16 13.9         | 9 16.4          |         |
| 37+6 to 41+7 weeks                   | 94 81.7         | 42 76.4         |         |
| Not registered                       | 0 0.0           | 2 3.6           |         |
| Premature                            | 21 18.3         | 22/53 41.5      | 0.002   |
| GA at birth (median, IQR)            | 38.0 (37.0–39.2)| 38.0 (37.0–39.0)| 0.569   |

Abbreviations: EPI, elective pregnancy interruption; GA, gestational age; HDP, hypertensive disorder of pregnancy; IQR, interquartile range; IUGR, intrauterine growth restriction; UTI, urinary tract infection.
TABLE 4 Demographic and clinical characteristics of newborn infants according to ZIKV RT-PCR

|                        | ZIKV RT-PCR (+) n (%) | ZIKV RT-PCR (-) n (%) | p value |
|------------------------|------------------------|------------------------|---------|
| Newborns               | 118/226 (51.8)         | 57/226 (25)            |         |
| Female gender          | 49/109 (45)            | 25/50 (50)             | 0.554   |
| Intrauterine growth    |                        |                        |         |
| AGA                    | 89/107 (83.2)          | 46/47 (97.9)           | 0.251   |
| SGA                    | 5/107 (4.7)            | 1/47 (2.1)             |         |
| LGA                    | 4/107 (3.7)            | 0/45 (0.0)             |         |
| Apgar <7               |                        |                        |         |
| 1 min                  | 6/92 (6.5)             | 3/44 (6.8)             | 0.045   |
| 5 min                  | 5/92 (5.4)             | 2/44 (4.5)             | 0.376   |
| Microcephaly at birth  | 9/102 (8.8)            | 4/45 (8.9)             | 0.606   |
| Neontal RT-PCR         |                        |                        |         |
| Negative               | 11 (9.3)               | 27 (47.4)              | <0.001  |
| Positive               | 32 (27.1)              | 0                      |         |
| Not studied/available  | 75 (63.6)              | 30 (52.6)              |         |
| Chromosome anomalies   | 1 (2q23.1)             | 0                      |         |

Abbreviations: AGA, appropriate for gestational age; LGA, large for gestational age; RT-PCR, reverse transcriptase polymerase chain reaction; SGA, small for gestational age; ZIKV, Zika virus.

4 | DISCUSSION

The prevalence of any fetal CNS anomaly in symptomatic ZIKV RT-PCR-positive pregnant women was much higher in this study (22%) than previously reported in other similar population-based prospective studies.\(^5,6,19\) Dysgenesis of the corpus callosum was the most frequent anomaly observed in this population, followed by microcephaly, cerebral hyperechogenicities, ventriculomegaly, and posterior fossa anomalies. Although first-trimester maternal infections with ZIKV were less frequent than second- and third-trimester infections, the rates and severity of anomalies of the brain were more significant in infected women in the first trimester than in other trimesters. Third-trimester CNS anomalies were rare, supporting the results of a preliminary study in Colombia by Pacheco et al.\(^20\) that found fewer CNS anomalies in fetuses of women infected during the third trimester of pregnancy. These studies support a definite correlation between infection timing and the frequency and severity of fetal CNS anomalies.\(^21\)

To our knowledge, only two studies have followed prospectively pregnant women and their fetuses with suspected ZIKV infection to determine fetal and neonatal outcomes. The first study by Brasil et al.\(^6\) evaluated fetal anthropometric measures to determine growth restriction and the presence of microcephaly (>2 SD below the mean); the focus of their study was on the postnatal evaluation of infants born to symptomatic ZIKV RT-PCR-positive women; the incidence of microcephaly in their ZIKV RT-PCR-positive group was 3.4%, a lower percentage than the 4.5% observed in our study. A follow-up study by the same author identified CNS anomalies in 13.0% of symptomatic pregnant women who were ZIKV RT-PCR-positive compared with 22% in our study.\(^22\) Differences in the observed prevalence percentages of fetal CNS anomalies between our study and that of Brasil et al. may be explained in part by the fact that in the Brasil et al. study (a) 56% of the ZIKV RT-PCR-positive women refused fetal ultrasound, (b) the remaining 44% of the women who accepted the ultrasound study were evaluated using the abdominal technique as opposed to the transvaginal technique to identify CNS anomalies, and (c) in their supplementary information about the results, the authors did not describe corpus callosum anomalies, which is better identified by a transvaginal approach.

The second study, an observational prospective cohort study by Pomar et al.,\(^5\) recruited women from a population-based prevalence study who attended their perinatal clinic after ZIKV RT-PCR or serologic IgM testing for ZIKV; recruitment was not based on the presence of symptomatology compatible with ZIKV infection. Of the 301 ZIKV RT-PCR- or IgM-positive participants, only 17% were identified as symptomatic compared with our study, where 100% of the pregnant women recruited were symptomatic. The Pomar et al.\(^5\) study found that corpus callosum dysgenesis (5.0%) and cerebral hyperechogenicities (5.0%) were the most frequent anomalies of the CNS observed with preestablished neurosonography protocols. Our results support their finding, but the prevalence of these anomalies was much higher (corpus callosum dysgenesis 14.5%; cerebral hyperechogenicities 7.4%) in our study. Similarly, severe microcephaly was observed in 4.5% of our cohort of infants born to symptomatic RT-PCR-positive women compared with the 0.3% observed in their ZIKV-infected cohort. These differences may be partly explained because symptomatology suggestive of ZIKV infection may be more sensitive in capturing fetuses at higher risk for CNS anomalies due to higher maternal viral load than in asymptomatic women.\(^25,26\) The small percentage of symptomatic RT-PCR- or IgM-positive pregnant women (17%) and the high percentage of asymptomatic pregnant women (83%) with lower viral loads in the Pomar et al. study, may partly explain the lower prevalence of these CNS anomalies.\(^23–25\) Additionally, 72% of the symptomatic pregnant women in our study were ZIKV RT-PCR-positive in the first and second trimester of pregnancy instead of the 58% in ZIKV-infected women in Pomar et al.’s study.

In our study, the presence of any fetal CNS anomaly was consistently observed to be more frequent and severe in the first trimester of pregnancy than in the second and third trimester. This finding is supported by other published studies, although our findings reveal a higher prevalence than previously documented.\(^5,6,25,27\) First and second trimester fetal CNS anomalies accounted for 88% of all CNS anomalies. Factors that may explain this observation include higher viral load, maternal and fetal immunologic factors, the maturity of the maternal-fetal placental barrier, and factors related to the pathogenicity and neurotropic nature of ZIKV, which individually or as a group may play a fundamental role in exposing the fetus to ZIKV during pregnancy.\(^23,28\)
Finally, when we compared symptomatic ZIKV RT-PCR-positive and -negative pregnant women, we did not find any significant differences in fetal CNS anomalies between groups (22.3% vs 17.0%; \( p = 0.255 \)), including corpus callosal anomalies. Also, the persistence of a pattern of higher rates of CNS anomalies found by ultrasound in the first trimester than in the second and third trimester of pregnancy in asymptomatic RT-PCR-negative pregnant women suggests a high rate of false-negative ZIKV RT-PCR results, especially in pregnant women with symptomatology outside the 7-day window. The previous observation would imply an even higher prevalence of any CNS anomaly in symptomatic pregnant women than the one reported in this study. Unfortunately, we cannot confirm or rule out this possibility.

Our study has several limitations. First, it aimed to characterize fetal CNS anomalies according to trimester of infection, which could not be determined in asymptomatic women. From a public health perspective, during the ZIKV epidemic in Colombia, only symptomatic mothers were tested due to limited resources for diagnostic confirmation and limited understanding of the epidemiology of the disease. Based on seroprevalence studies, one of every four infected persons are symptomatic; therefore, testing symptomatic pregnant women captures only 25% of the population of infected pregnant women.\(^{2,29}\) The use of RT-PCR to diagnose ZIKV infection is a limitation shared by all studies published to date; this observation is supported by studies evaluating the sensitivity and specificity of RT-PCR for ZIKV where sensitivity has been shown to drop when the viral RNA load is \(<10^6\)/mL. Unfortunately, infected humans have an average range of viral loads that fluctuate between \(10^7\)–\(10^9\) mL, which explains the potential for a high percentage of false negatives, especially during outbreak-patient studies when caseloads are high.\(^{25}\) The use of quantitative RT-PCR improves the sensitivity and specificity of the test but it is too expensive and impractical in an epidemic or pandemic setting.\(^{26}\) Another limitation was our inability to screen all pregnant women enrolled in our study for TORCH; this limitation may have led to misclassification bias. In our cohort, no ZIKV

| TABLE 5 Characteristics of the prenatal ultrasound images of the fetuses according to ZIKV RT-PCR |
|---------------------------------------------------------------|
| Fetuses evaluated | 118 | 57 |
| Ultrasound | | |
| Not done | 7 | 5.9 | 4 | 7.0 | 0.772 |
| Basic | 11 | 9.3 | 3 | 5.3 | 0.508 |
| Detailed anatomic | 46 | 39.0 | 21 | 36.8 | 0.008 |
| Neurosonography (Transvaginal) | 54 | 45.8 | 59 | 50.9 | 0.508 |
| Ultrasound was done | 111 | 94.1 | 53 | 93.0 | 0.008 |
| Fetal growth | | |
| AGA | 98/107 | 91.6 | 46/47 | 87.9 | 0.296 |
| LGA | 4/107 | 3.7 | 0/47 | 0.0 | 0.008 |
| IUGR | 5/107 | 4.7 | 1/47 | 2.1 | 0.008 |
| Any CNS anomaly | 25/109 | 22.3 | 9/53 | 17.0 | 0.255 |
| Microcephaly | 15/110 | 13.6 | 5/53 | 9.4 | 0.311 |
| Mild microcephaly | 10/110 | 9.1 | 1/53 | 1.9 | 0.098 |
| Severe microcephaly | 5/110 | 4.5 | 4/53 | 7.5 | 0.508 |
| Macrocrania | 1/110 | 0.9 | 1/53 | 1.9 | 0.546 |
| Hydranencephaly | 1/110 | 0.9 | 0/53 | 0.0 | 0.675 |
| CC dysgenesis | 16/110 | 14.5 | 5/53 | 9.4 | 0.258 |
| CPH | 8/108 | 7.4 | 5/51 | 9.8 | 0.407 |
| Ventriculomegaly | 8/108 | 7.4 | 4/51 | 7.8 | 0.575 |
| NMD | 9/108 | 8.3 | 4/51 | 7.8 | 0.593 |
| PVH | 6/108 | 5.6 | 3/51 | 5.9 | 0.596 |
| Vermis anomaly | 3/109 | 2.8 | 2/51 | 3.9 | 0.512 |
| Cerebellum hypoplasia | 3/108 | 2.8 | 3/50 | 6.0 | 0.284 |
| Mega Cisterna magna | 3/109 | 2.8 | 2/51 | 3.9 | 0.512 |
| Microphthalmia | 1/109 | 0.9 | 0/51 | 0.0 | 0.681 |
| Fetal akinesia | 1/109 | 0.9 | 1/51 | 2.0 | 0.537 |

Abbreviations: AGA, appropriate for gestational age; CC, corpus callosum; CNS, central nervous system; CPH, cerebral parenchymal hyperechogenicities; IUGR, intrauterine growth restriction; LGA, large for gestational age; NMD, neuronal migration disorder; PVH, periventricular hyperechogenicities; RT-PCR, reverse transcriptase polymerase chain reaction; ZIKV, Zika virus.
**FIGURE 2** Neurosonographic images of fetuses exposed to Zika virus with malformations of cortical development. Coronal view of fetuses at 34 weeks. (A) Pachygyria frontal lobe, Sylvian fissure with abnormal angle for gestational age. (B) Pachygyria frontal lobe with microcephaly, other findings: subcortical coalescing microcalcifications. (C) Normal fetus at 29 weeks. (D) Axial view: Periventricular heterotopia and severe asymmetric ventriculomegaly. (E) Parasagittal view: Pachygyria occipital lobe and abnormal sulcation with increased extra-axial space. (F) Normal fetus at 36+4 weeks.

**TABLE 6** Ultrasound findings of the fetuses of pregnant women RT-PCR(+) for ZIKV

| Fetuses evaluated | I trimester | II trimester | III trimester | p value |
|-------------------|-------------|--------------|---------------|---------|
| GA (median, IQR)  | 28.3 (24.8–35.0) | 32.6 (29.0–36.1) | 33.6 (29.0–31.6) | <0.001 |

**Fetal growth**

|               | I trimester | II trimester | III trimester | p value |
|---------------|-------------|--------------|---------------|---------|
| AGA           | 18          | 48/52        | 29            | 0.010   |
| LGA           | 2           | 0/52         | 0             | 0.0     |
| IUGR          | 5           | 4/52         | 1             | 3.3     |
| Any CNS anomaly | 10         | 11/52        | 2             | 0.002   |
| Microcephaly  | 9           | 5/53         | 0             | <0.001  |
| Mild microcephaly | 6         | 4/53         | 0             | 0.703   |
| Severe microcephaly | 3        | 1/53         | 0             | 0.0     |
| CC dysgenesis | 7           | 7/53         | 1             | 3.3     |
| CPH           | 4           | 4/53         | 1             | 0.012   |
| Ventriculomegaly | 4         | 3/53         | 0             | 0.008   |
| NMD           | 5           | 20/53        | 0             | 0.004   |
| PVH           | 3           | 12/52        | 0             | 0.027   |
| Vermis anomaly | 3          | 12/52        | 0             | 0.003   |
| Cerebellum hypoplasia | 4         | 1/52         | 0             | 0.003   |
| Mega Cisterna magna | 1       | 1/52         | 1             | 3.3     |
| Microphthalmia | 1           | 4/52         | 0             | 0.556   |
| Fetal Akinesia | 1           | 4/52         | 0             | 0.556   |

**Abbreviations:** AGA, appropriate for gestational age; CC dysgenesis, corpus callosum dysgenesis; CNS, central nervous system; CPH, cerebral parenchymal hyperechogenicities; GA, gestational age; IUGR, intrauterine growth restriction; LGA, large for gestational age; NMD, neuronal migration disorder; PVH, periventricular hyperechogenicities; RT-PCR, reverse transcriptase polymerase chain reaction; ZIKV, Zika virus.
symptomatic pregnant women tested positive for toxoplasmosis, and none of the women who tested positive for CMV or rubella, or had clinical evidence of HSV, demonstrated fetal evidence of microcephaly or other CNS anomalies.

5 | CONCLUSION

In summary, we found a higher prevalence of CNS anomalies in fetuses of symptomatic RT-PCR ZIKV-positive and -negative pregnant women than previously reported in the literature. Corpus callosal dysgenesis and microcephaly were the most frequent CNS anomalies observed in ZIKV RT-PCR-positive women, followed by neuronal migration disorders, cerebral hyperechogenicities, ventriculomegaly and posterior fossa anomalies. Fetuses of pregnant women infected during the first trimester of pregnancy are the most frequently and severely affected. The high prevalence of CNS anomalies in symptomatic ZICV RT-PCR-negative pregnant women suggests a high rate of false-negative cases with this test, which implies a higher overall prevalence of CNS anomalies than observed in this study in pregnant women with symptoms suggestive of ZIKV infection. The results of our study support the use of detailed anatomic ultrasound and neurosonography protocols for the evaluation of fetuses of pregnant women in high-risk areas of ZIKV infection. Symptomatology compatible with ZIKV infection may be a risk factor for more frequent and severe fetal CNS anomalies.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

MAR, LAPV, GACG, LSPG, LADM, LAGS and CHBM participated in the design of the protocol and data collection tools. LAGS and CHBM were involved in patient recruitment, neurosonography evaluation of pregnant women and supporting data analysis. MAR managed the electronic database for quality assurance, participated in analyzing and interpreting the data, and wrote the first draft of the manuscript.
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