Factors associated with increased odds of sensorineural hearing loss in infants exposed to the Zika virus during pregnancy

Erika J Verján-Carrillo1,2, Efrén Murillo-Zamora3,4, Gabriel Ceja-Espíritu4, José Guzmán-Esquível4,5, Oliver Mendoza-Cano6

1 Master of Medical Science Program, Faculty of Medicine, University of Colima, Colima, Mexico
2 Department of Audiology, General Hospital No. 1, Mexican Institute of Social Security, Villa de Alvarez, Colima, Mexico
3 Department of Epidemiology, Family Medicine Unit No. 19, Mexican Institute of Social Security, Colima, Mexico
4 Faculty of Medicine, University of Colima, Colima, Mexico
5 Research Unit of Clinical Epidemiology, Colima, Mexico
6 Faculty of Civil Engineering, University of Colima, Coquimatlan, Mexico

Abstract

Introduction: The Zika virus (ZIKV) infection in pregnant women has been associated with an increased risk of birth defects. We aimed to estimate the prevalence of sensorineural hearing loss (SNHL) in infants exposed to the ZIKV during their gestation and evaluate the factors associated with its increased odds.

Methodology: A cross-sectional study was performed from July 2016 to June 2019 in a Western state of Mexico and data from 61 infants that presented with laboratory-positive (RT-qPCR) evidence of in utero exposure to ZIKV were analyzed. Brain stem auditory evoked potentials were used.

Results: Hearing loss was documented in 6 (9.8%) of infants. The prevalence of SNHL in children with microcephaly was 75.0%, as compared to 5.3% in those without anomalies (odds ratio, OR = 14.31, 95% CI = 2.54 – 19.12). Half of children with SNHL had no physical manifestations of gestational ZIKV exposure.

Conclusions: Hearing loss was a frequent event in ZIKV-exposed children, particularly among those with microcephaly. Our results highlight the relevance of systematic hearing screening.

Key words: Zika virus; pregnancy; brain stem; hearing loss; sensorineural.

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Introduction

Zika virus (ZIKV) infection in pregnant women has been associated with an increased risk of birth defects, defining the congenital Zika syndrome (CZS) that includes severe microcephaly, subcortical calcifications, and hypertonia [1]. An increased risk for sensorineural hearing loss (SNHL) has been documented in infants with microcephaly and in utero ZIKV exposure [2,3].

The ZIKV burden in Mexico has been high and, within the first nine months from the local outbreak start, the computed incidence rate among pregnant women was 66 per 100,000 pregnancy-months [4].

Published studies evaluating SNHL in Latin-American among Latin-American children that have no manifestations of gestational ZIKV exposure are scarce and results are heterogeneous [5,6]. The present study aimed to estimate the risk of SNHL in infants born to women with laboratory-confirmed ZIKV infection during pregnancy. The factors associated with the risk of hearing impairment were also evaluated.

Methodology

A cross-sectional study was conducted in Colima, a subtropical western state of Mexico, from July 2016 to June 2019. The state of Colima is a subtropical area located in the Western region of Mexico and high Aedes aegypti indices, and related vector-borne diseases, have been documented [7].

The eligible subjects were randomly selected from full-term infants, born to women with laboratory-confirmed exposure to ZIKV (RT-qPCR, reverse transcription quantitative polymerase chain reaction) (TaqMan™ Zika Virus Triplex Kit, catalogue code A31747, Applied Biosystems™, U.S.A) [8] during pregnancy. The Mexican Institute of Social Security
(Instituto Mexicano del Seguro Social - IMSS), where this study took place, has a Laboratory Network for Epidemiological Surveillance integrated by four highly specialized settings. Their analytical procedures are endorsed by the Institute for Epidemiological Diagnosis and Reference, the highest related-authority in Mexico. Eligible children were identified from data belonging to the National System for Epidemiological Surveillance (Sistema Nacional de Vigilancia Epidemiológica - SINAVE). Known infectious or traumatic causes (cranioencephalic trauma) of hearing loss were the exclusion criteria. The communicable diseases were evaluated by reviewing the laboratory results of prenatal tests (TORCHS screen) that are mandatory according to national normative standards guiding the prenatal and postnatal care to mother and child [9]. Subjects with documented neonatal jaundice or kernicterus were also excluded, as wells as those with reported intra-/extra-uterine exposure to ototoxic drugs.

After written statements of informed consent to participate in the study obtained from the parents/legal guardians of the infants, brain stem auditory evoked potential (BAEP) (Nihon Kohden Neuropack S1 MEB-9400, Japan) tests were performed by a trained health professional (doctor of audiology). Prior to be evaluated, all children were physically examined (otoscopy) by the same professional that performed the BAEP in order to discard causes of conductive hearing impairment (i.e. wax in the ear canal or middle ear pathology). Infants with persistent wave V after a 40 dB stimuli tested positive for SNHL.

Rate ratios with their 95% confidence intervals are presented (univariate analysis). Odds ratios (OR) with 95% confidence intervals (CIs) and estimated through logistic regression models were used to evaluate the association between the exposures analyzed and the odds for SNHL. A multivariate model was built by following a purposeful selection procedure [10].

### Table 1. Characteristics of the study sample for the selected variables, Mexico 2016 – 2019.

| Characteristics                              | Overall (n = 61) | SNHL Yes (n = 6) | RR (95% CI) |
|----------------------------------------------|-----------------|-----------------|-------------|
| **Sex**                                      |                 |                 |             |
| Female                                       | 38 (62.3%)     | 2 (5.3%)       | 1.00        |
| Male                                         | 23 (37.7%)     | 4 (17.4%)      | 3.28 (0.93 - 7.32) |
| **First-degree family history of hearing loss** |               |                 |             |
| No                                           | 58 (95.1%)     | 6 (10.3%)      | NC          |
| Yes                                          | 3 (4.9%)       | 0 (0%)         | NC          |
| **Trimester of pregnancy**                   |                 |                 |             |
| First                                        | 19 (31.2%)     | 1 (5.3%)       | 1.00        |
| Second                                       | 15 (24.5%)     | 3 (20.0%)      | 3.77 (0.82 - 9.07) |
| Third                                        | 27 (44.3%)     | 2 (7.4%)       | 1.40 (0.17 - 4.58) |
| **Folate intake during pregnancy**           |                 |                 |             |
| No                                           | 3 (4.9%)       | 1 (33.3%)      | 1.00        |
| Yes                                          | 58 (95.1%)     | 5 (8.6%)       | 0.26 (0.09 - 0.57) |
| **Delivery mechanism**                       |                 |                 |             |
| Vaginal                                      | 32 (52.5%)     | 3 (9.4%)       | 1.00        |
| Cesarean section                             | 29 (47.5%)     | 3 (10.3%)      | 1.10 (0.23 - 2.91) |
| **Birth weight for gestational age**         |                 |                 |             |
| Low                                          | 6 (9.8%)       | 1 (16.7%)      | 1.00        |
| Normal                                       | 55 (90.2%)     | 5 (9.1%)       | 0.54 (0.18 - 1.19) |
| **Clubfoot**                                 |                 |                 |             |
| No                                           | 59 (96.7%)     | 4 (6.8%)       | 1.00        |
| Yes                                          | 2 (3.3%)       | 2 (100%)       | 14.71 (2.33 - 27.09) |
| **Microcephaly**                             |                 |                 |             |
| No                                           | 57 (93.4%)     | 3 (5.3%)       | 1.00        |
| Yes                                          | 4 (6.6%)       | 3 (75.0%)      | 14.15 (3.66 - 18.75) |
| **Cleft lip/palate**                         |                 |                 |             |
| Yes                                          | 4 (6.6%)       | 0 (0%)         | NC          |
| No                                           | 57 (93.4%)     | 6 (10.5%)      | NC          |

1) The absolute and relative (%) frequencies are presented, as well as the p-value from the chi-square tests; 2) The brain stem auditory evoked potential (BAEP) Nihon Kohden Neuropack S1 MEB-9400 EMG EP test was used to determine the functional integrity of the auditory system; the absence of waves or the presence of wave V after 80 dB or higher stimuli were considered as positive for sensorineural hearing loss. * At the medical diagnosis of acute Zika virus disease; b More than 2 standard deviations below the mean for age and sex. SNHL: sensorineural hearing loss; RR: rate ratio; CI: confidence interval; NC: non-computable.
ORs were corrected through the method suggested by Zhang and Yu [11]. The present study was approved (R-2019-601-006) by the Local Health Research Ethics Committee of the IMSS.

**Results**

Data from 61 infants were analyzed and the overall prevalence of SNHL was 6 (9.8%). All mothers reported ZIKV-suggestive symptoms during pregnancy. Occurrence of hearing loss in children with microcephaly (> 2 standard deviations below the mean for age and sex) was 75%, as compared to 5.3% in those without anomalies. Half (n = 3/6; 50%) of children with SNHL had no physical manifestations of gestational ZIKV exposure. All children with hearing impairment recorded no waves or wave V after 80 dB or higher stimuli in BAEP testing. Mean age of participants upon the neurosensitive test was 4.9 ± 0.8 months. Table 1 shows the characteristics of the study sample for the selected variables.

None of the infants with hearing loss had a family history of hypoacusia neither the use of any ototoxic drugs during pregnancy or extra-uterine life. The prevalence of SNHL among children with clubfoot (n = 2) was 100%. Therefore and as it is presented in Table 1, a 14-fold increase in the rate of hearing impairment was documented in children with clubfoot (RR = 14.71, 95% CI = 2.33 – 27.09) or microcephaly (RR = 14.15, 95% CI = 3.66 – 18.75). In multivariate analysis (Table 2), infants with microcephaly were at increased odds of deafness (OR = 14.31, 95% CI = 2.54 – 19.12).

**Discussion**

Our findings suggest that hearing impairment is a frequent event among children exposed to ZIKV during pregnancy and SNHL was confirmed in about 1 out 10 infants analyzed. While microcephaly was clearly related to hearing dysfunction with a more than fourteen-fold prevalence than in those without microcephaly, hearing loss was also documented in infants that did not present with microcephaly or a CSZ-suggestive (namely clubfoot or microcephaly) phenotype, which highlights the importance of systematic hearing screening in all exposed newborns.

The mechanism of ZIKV-induced microcephaly has not been elucidated. Animal models have documented a large number of alterations in brain development after ZIKV infection and include abnormal gene expression and immune response, lysosome circuits among others [12].

The overall occurrence of SNHL observed in our study was lower than that estimated in a sample of Brazilian children (9.8% vs. 17.3%) [2]. Only children with microcephaly were included in the South American study. In our analysis, the prevalence SNHL among children with microcephaly was 75.0%.

No hearing loss was documented in Colombian children born to women exposed to ZIKV during pregnancy [13]. However, in that analysis, exposure to the pathogen was suggestive, with no confirmatory tests performed on the majority of the study subjects. In addition, evoked potentials were only utilized in a subset of infants in the Colombian research.

The pathogenic mechanism of hearing impairment after in utero exposure to ZIKV remains poorly understood. Animal models have evidenced that the virus can directly infect and damage the auditory and vestibular components of the embryonic chicken inner ear [14]. Besides, ZIKV infection induces maternal immune activation, which is associated with the occurrence of neuroanatomic and other fetal abnormalities [15]. Nearly 7% of infants in our study presented with cleft lip or cleft palate, but no association with hearing loss was found. That frequency is higher than the general incidence documented in Mexico (1:1,000 live births) [16]. The association between ZIKV infection and the risk of cleft lip/palate

**Table 2. Factors associated with the odds of sensorineural hearing loss in the study sample, Mexico 2016 – 2019.**

|                      | Univariate analysis |          |           | Multivariate analysis |          |           |
|----------------------|---------------------|----------|----------|-----------------------|----------|----------|
|                      | OR                  | 95% CI   | p        | OR                    | 95% CI   | p        |
| **Sex**              |                     |          |          |                       |          |          |
| Female               | 1.00                |          |          | 1.00                  |          |          |
| Male                 | 1.34 (0.84 - 1.49)  | 0.151    | 1.37 (0.82 - 1.50) | 0.134    |
| **Folate intake during pregnancy** |                     |          |          |                       |          |          |
| No                   | 1.00                |          |          | 1.00                  |          |          |
| Yes                  | 0.87 (0.22 - 1.02)  | 0.203    | 0.91 (0.22 - 1.03) | 0.481    |
| **Microcephaly**     |                     |          |          |                       |          |          |
| No                   | 1.00                |          |          | 1.00                  |          |          |
| Yes                  | 14.25 (2.53 - 18.88) | < 0.001 | 14.31 (2.54 - 19.12) | < 0.001 |

1) The odds ratios (OR) and 95% confidence intervals (CI) were estimated through logistic regression models. 2) The multiple analysis estimators were adjusted by the variables presented in the table. 3) The following were included: microcephaly (more than 2 standard deviations below the mean for age and sex).
has been previously described in previous studies, specifically in Brazilian children [17].

The inclusion of children with laboratory-conclusive evidence of in utero exposure to ZIKV, together with the performance of BAEPs in all the subjects analyzed, are major strengths of the present study. Its potential limitations include the limited sample size the fact that we lacked neuroimaging data that would have enabled the radiographic identification of CZS cases and that the sample size was relatively small. Nevertheless, only infants with laboratory-conclusive evidence of in utero exposure to ZIKV were enrolled and all the study subjects underwent BAEP testing.

Conclusions

Hearing impairment was a frequent event in the individuals analyzed in the present study, and those with microcephaly were at an 14-fold increased risk for SNHL. That neurosensory disorder was also documented in infants that did not present with phenotypic alterations, highlighting the benefits of systematic hearing screening of all exposed newborns.

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References

1. Moore CA, Staples JE, Dobyns WB, Pessoa A, Ventura CV, Fonseca EB, Ribeiro EM, Ventura LO, Neto NN, Arena JF, Rasmussen SA (2017) Characterizing the Pattern of Anomalies in Congenital Zika Syndrome for Pediatric Clinicians. JAMA Pediatr 171: 288-295.
2. C Lage ML, Carvalho AL, Ventura PA, Taguchi TB, Fernandes AS, Pinho SF, Santos-Junior OT, Ramos CL, Nascimento-Carvalho CM (2019) Clinical, Neuroimaging, and Neurophysiologic Findings in Children with Microcephaly Related to Congenital Zika Virus Infection. Int J Environ Res Public Health 16: 309.
3. Leal MC, Muniz LF, Ferreira TS, Santos CM, Almeida LC, Van Der Linden V, Ramos RC, Rodrigues LC, Neto SS (2016) Hearing Loss in Infants with Microcephaly and Evidence of Congenital Zika Virus Infection - Brazil, November 2015-May 2016. MMWR Morb Mortal Wkly Rep 65: 917-919.
4. Hernandez-Avila JE, Palaciu-Mejia LS, Lopez-Gatell H, Alpuche-Aranda CM, Molina-Velez D, Gonzalez-Gonzalez L, Hernández-Avila M (2018) Zika virus infection estimates, Mexico. Bull World Health Organ 96: 306-313.
5. Barbosa MHM, Garcia CFD, Magalhães- Barboza MC, Robaina JR, Prata-Barbosa A, Lima MAMT, Cunha AJLAD (2020) Normal Hearing Function in Children Prenatally Exposed to Zika Virus. Int Arch Otorhinolaryngol 24: e299-e307.
6. Faria AOP, Miterhof MEVDCR, Vianna RAO, Carvalho FR, Dalcastel LAB, Oliveira SA, Fonseca SC, Riley LW, Velarde LGC, Cardoso CAA (2020) Audiological Findings in Children Suspected to Have Been Exposed to the Zika Virus in the Intrauterine Period. Otol Neurotol 41: e848-e53.
7. Lopez-Sanchez EJ, Sanchez-Torres NY, Trenado C, Romero JM (2016) The dengue fever in four municipalities of the Colima state, Mexico, from 2008 to 2015 and its relation with some climatological normals. ArXiv Preprints. 1601.07212.
8. Barrera-Cruz A, Díaz-Ramos RD, López-Morales AB, Grajales-Muñiz C, Viniegra-Osorio A, Zaldívar-Cervera JA, Arriaga-Dávila JJ (2016) Technical guidelines for the prevention, diagnosis and treatment of Zika virus infection. Rev Med Inst Mex Seguro Soc 54: 211-224. [Article in Spanish]
9. Government of Mexico (2016) Official Mexican Normativity NOM-007-SSA2-2016, for the care of women during pregnancy, childbirth and the puerperium, and of the newborn. Available: https://www.dof.gob.mx/nota_detalle.php?codigo=5432289&fecha=07/04/2016;height=NORMA%20Oficial%20Mexicana%20NOM%20D007,de%20la%20persona%20recién%20nacida. Accessed: 27 October 2020 [document in Spanish].
10. Zhang Z (2016) Model building strategy for logistic regression: purposeful selection. Ann Transl Med 4: 111.
11. Zhang J, Yu KF (1998) What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. JAMA 280: 1690-1691.
12. Xu D, Li C, Qin C-F, Xu Z (2019) Update on the animal Models and underlying mechanisms for ZIKV-induced microcephaly. Annual review of virology. 6: 459-479.
13. Fandino-Cardenas M, Idrovo AJ, Velandia R, Molina-Franky J, Alvarado-Socarras JL (2019) Zika Virus Infection during Pregnancy and Sensorineural Hearing Loss among Children at 3 and 24 Months Post-Partum. J Trop Pediatr 65: 328-335.
14. Thawani A, Sammudin NH, Reygaerts HS, Wozniak AN, Munnamalai V, Kuhn RJ, Fekete DM (2020) Zika virus can
directly infect and damage the auditory and vestibular components of the embryonic chicken inner ear. Dev Dyn 249: 867-883.

15. Camargos VN, Foureaux G, Medeiros DC, da Silveira VT, Queiroz-Junior CM, Matosinhos ALB, Figueiredo AFA, Sousa CDF, Moreira TP, Queiroz VF, Dias ACF, Santana KTO, Passos I, Real ALCV, Silva LC, Mourão FAG, Wnuk NT, Oliveira MAP, Macari S, Silva T, Garlet GP, Jackman JA, Soriani FM, Moraes MFD, Mendes EMAM, Ribeiro FM, Costa GMJ, Teixeira AL, Cho NJ, Oliveira ACP, Teixeira MM, Costa VV, Souza DG (2019) In-depth characterization of congenital Zika syndrome in immunocompetent mice: Antibody-dependent enhancement and an antiviral peptide therapy. EBioMedicine 44: 516-529.

16. González-Osorio CA, Medina-Solis CE, Pontigo-Loyola AP, Casanova-Rosado JF, Escoffié-Ramírez M, Corona-Tabares MG, Maupomé G (2011) Ecologic study in Mexico (2003-2009) on cleft lip and/or palate and associated sociodemographic, socioeconomic and pollution factors. An Pediatr (Barc) 74: 377-387. [Article in Spanish]

17. Moura da Silva AA, Ganz JS, Sousa PD, Doriqui MJ, Ribeiro MR, Branco MD, Queiroz RC, Pacheco MJ, Vieira da Costa FR, Silva FS, Simões VM, Pacheco MA, Lamy-Filho F, Lamy ZC, Soares de Britto E Alves MT (2016) Early growth and neurologic outcomes of infants with probable congenital Zika Virus Syndrome. Emerg Infect Dis 22: 1953-1956.

**Corresponding authors**

Efrén Murillo-Zamora, PhD
Department of Epidemiology, Family Medicine Unit No. 19, Mexican Institute of Social Security, Av. Javier Mina 301, Col. Centro, C.P. 28000, Colima, Colima, Mexico
Phone: 0052 (312) 3163795
Email: efren.murilloza@imss.gob.mx

Oliver Mendoza-Cano, PhD
Faculty of Civil Engineer, University of Colima, km 9.0 highway Colima – Coquimatlan, Col. Jardines del Llano, Coquimatlan, Colima, Mexico
Phone: 0052(312) 3161167
Email: oliver@ucol.mx

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