Normal Hearing Function in Children Prenatally Exposed to Zika Virus

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Abstract

Introduction The association between prenatal Zika virus infection and hearing alterations in offspring has been the object of some studies, although few have assessed children without microcephaly. However, a current trend to include prenatal Zika virus exposure in the group of risk indicators for hearing loss is noted.

Objective To present a series of 27 children prenatally exposed to the Zika virus submitted to multiple hearing assessments over time.

Methods A cohort of children born to symptomatic mothers with laboratorial Zika virus infection confirmation during pregnancy was submitted to an otoacoustic emission test, auditory brainstem response test (automated, neurodiagnostic and frequency-specific), audiometry, and imitanciometry over a period of 36 months since birth. The hearing assessment was performed independently of the presence of microcephaly or other apparent signs of congenital Zika syndrome.

Results The hearing tests presented predominantly normal results. Some children had signs of middle ear pathology. The only microcephalic child had normal electrophysiological tests, as well as preserved audiometric thresholds, but presented altered motor responses to sound.

Conclusion Prenatal exposure to Zika virus does not always determine hearing impairment. This risk seems to be more associated to the severity of the central nervous system damage. Hearing screening and follow-ups of the affected children are important, as well as further research in this area.

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**Introduction**

The relationship between congenital Zika virus (ZIKV) infection and hearing impairment has been studied, especially after the Latin American epidemic in 2015, with reports of sensorineural hearing loss in affected children with microcephaly.1,2 The occurrence of neurological and ocular alterations and multiple malformations has raised the hypothesis of a possible association of Zika infection with hearing impairments, as demonstrated in other congenital infections.3–5 However, few studies to date have assessed the hearing function of infected children without microcephaly.6–8 On the other hand, ocular findings in non-microcephalic children have been reported.9

The hearing screening of children with risk indicators for hearing loss must be conducted by the otoacoustic emission (OAE) and the auditory brainstem response (ABR) tests.9 Congenital infections such as syphilis, cytomegalovirus, toxoplasmosis, and rubella are hearing loss indicators.10 Based on existing reports, a trend to include children with congenital ZIKV infection in this group is noted.2,11–14 Furthermore, it is recommended that children in the risk group are followed-up by developmental milestone surveillance, hearing abilities, and middle ear conditions. At least one audiological evaluation should be performed between 24 and 30 months of age, due to the risk of progressive or late onset hearing loss.9

Although the incidence of new cases is currently declining, the risk of a new epidemic is constant, as the mosquito vector and favorable climatic conditions for its proliferation are present in many countries, in addition to increasing global mobility of people. Moreover, many infected individuals, notably children affected by congenital Zika syndrome (CZS), exhibit outcomes due to the infection and, therefore, require regular multidisciplinary follow-up. Further studies on the consequences of this infection in the human body are necessary to support screening, management and follow-up recommendations of individuals exposed to the ZIKV.

**Objective**

The aim of the present study is to present the results of the hearing assessments of 27 children born to mothers with laboratorial confirmation of ZIKV infection during pregnancy.

**Methods**

This is an observational, descriptive and longitudinal study of a cohort of 27 children followed from birth to ~36 months. The study was conducted in a public university maternity hospital, where 50% of the patients are high-risk pregnant women referred to the institution and the remaining are normal pregnancies from the surrounding neighborhood. Most of the mother and child study procedures were performed in the maternity hospital, such as information collection, blood sample collection for laboratorial tests, otoacoustic emission (OAE) hearing tests, neurodiagnostic auditory brainstem response (ABR) and frequency-specific ABR (FS-ABR). The automated ABR (a-ABR), imitanciometry and audiometry tests were performed in a quaternary public hospital belonging to the same university. Efforts to reduce missing tests in the follow-up phase were made, such as more than one telephone contact, transport reimbursement and more than one option of days to attend the reevaluation visits.

All infants born to mothers admitted to the maternity hospital from December 1st 2015 to June 30th 2017 with laboratorial confirmation of ZIKV infection during pregnancy were included. Exclusion criteria comprised refusal to sign the free and clarified consent term (FCCT) and the presence of infant malformations that precluded the performance of the auditory tests. Participants that withdrew consent after the start of the study and those that could not, for any reason, be submitted to the hearing tests were classified as losses.

Pregnant women presenting a clinical disease consistent with ZIKV infection gave a blood sample collected up to the 5th day of symptoms and a urine sample collected up to the 28th day from symptom onset to perform a reverse transcriptase polymerase chain reaction (RT-PCR) test. In addition, a blood sample was collected between the 7th and the 14th day for immunoglobulin M (IgM) and immunoglobulin G (IgG) tests. An umbilical cord blood sample was also collected for an IgM test to eventually confirm newborn infection. Simultaneously, serologies for dengue virus (DENV), chikungunya virus (CHIKV), and for the syphilis, toxoplasmosis, rubella, cytomegalovirus and herpes (STORCH) group were collected to discard infection by these agents. Some mothers with suspected or confirmed ZIKV infection gave urine samples throughout their gestation for ZIKV RT-PCR testing. Laboratorial evidence of maternal infection by ZIKV was considered as: 1) detection of ZIKV RNA by RT-PCR in blood and/or urine; 2) positive IgM serology for ZIKV, and negative IgM serology for DENV.

Pregnant women with laboratorial evidence of ZIKV infection were considered as confirmed cases. Therefore, their newborns were eligible for the study. Suspected cases of ZIKV infection with no laboratorial confirmation were not eligible.

The children included in the study were scheduled for hearing function exams comprising OAE, a-ABR, FS-ABR, neurodiagnostic ABR, imitanciometry (tymanometry + acoustic reflex test) and audiometry assessments. Regarding neonatal hearing screening, OEA exhibited sensitivity of 0.77 (confidence interval [CI] 95% 0.65–0.86) and specificity of 0.93 (CI 95% 0.92–0.93), and a-ABR exhibited sensitivity of 0.93 (CI 95% 0.87–0.96) and specificity of 0.97 (CI 95% 0.96–0.98), compared with ABR.15 Audiology is the gold standard for hearing loss diagnoses, but FS-ABR is considered an accurate test to estimate hearing thresholds in infants that cannot cooperate with audiometry.16,17 Neurodiagnostic ABR is used to verify the conductibility of the auditory pathways. There is evidence that the combination of these tests (imitanciometry + OAE) improves sensitivity without compromising specificity (sensitivity of 0.86 and specificity of 0.96).18

Right before each exam, the acoustic meatus and tympanic membranes of the infants were inspected with an otoscope. Most children performed the OAE test within 24 h to 48 h of life, and, in some cases, up to 25 days from birth. The hearing assessment by the OAE and ABR tests were performed according to the Care Guidelines for Newborn
Hearing Screening from the Brazilian Ministry of Health\textsuperscript{19} and following the BC Children’s Hospital practice guidance procedures.\textsuperscript{20} The Otoport Lite OEA DP + TE (Otodynamics, Hatfield, Hertfordshire, England) device,\textsuperscript{21} with a non-linear click stimulus comprising frequencies from 1 kHz to 5 kHz and equivalent peak of intensity around 84 dBHL, was used. A stimulus stability of 70% or higher was considered adequate for exam validity. The standard noise rejection value was 47 dBHL. For the test to be considered positive (indicating a present OEA), the signal-to-noise ratio (SNR) should be at 3 to 6 dB, and the reproducibility for each frequency band or general of over 50%. The a-ABR was performed in a silent sound isolated room, with the child under natural sleep, using the Intelligent Hearing Systems – IHS4754 (Intelligent Hearing Systems, Miami, FL, USA).\textsuperscript{22} A maximum individual impedance of 5 kΩ was admitted between the electrodes and the skin, and intraelectrode impedances were less than 3 kΩ. The test was performed using insert earphones, elicited by 100 µs rarefaction clicks, presented at a rate of 15.1/s and stimulus level of 80 dBHL, and with progressive decrease to establish a minimal auditory response level, which is the intensity of the stimulus at which the wave V could be identified and replicated. Responses from 2,000 to 4,000 stimulus presentations were averaged. The filters were set from 100 to 3,000 Hz, and a recording window of 15 ms was used. The neurodiagnostic ABR and the FS-ABR were performed using the Eclipse EP15 (Interacoustics) device\textsuperscript{23} with insert earphones. The impedance criteria and filters were the same used for the a-ABR. The recording window was of 20 ms. For the neurodiagnostic ABR, a click stimuli presentation of 100 µs at 80 dBHL at alternating polarities was set up, with a sound presentation rate of 21.1/s, for the auditory pathways integrity verification. The presence or absence of waves and their morphology and reproducibility were assessed, and the study of cochlear microphonic potentials was performed when necessary. The FS-ABR was performed with tone-burst stimuli at 0.5 kHz, 1 kHz, 2 kHz and 4 kHz to establish the minimal auditory response level for each frequency, using alternating polarities and a sound presentation rate of 39.1/s. At least 2,000 stimuli were presented to register the response. Responses ≤ 35 dBnHL for 0.5 kHz and ≤ 30 dBnHL for 1 kHz, 2 kHz, and 4 kHz were considered normal.\textsuperscript{17,20} As the children were already 1 to 2 years of age by the time they performed this test, they sedated, by the oral administration of 20% chloral hydrate performed by a trained pediatrician, acquainted with this procedure. The imitanciometry test was performed after 2 years of age, right before the audiometry assessment and after ear, nose, mouth and throat examinations. A tympanogram and the stapled reflex test were performed using a Kamplex A27-R imitanciometer (Interacoustics).\textsuperscript{23} Depending on the age and cooperation level of each child, a vocal and pure tone audiometry was performed with conditioned play and/or visual reinforcement, in an open field (to establish the minimal level of response) or with headsets (to establish the hearing threshold in each ear). Alternatively, a behavioral audiometry was performed. Responses ≤ 20 dB were considered as normal.

The sample size was determined by convenience, corresponding to the number of participants included while the study admitted new participants.

The study was submitted to the research ethics committee of the proponent institute and was approved under protocol number 1.516.904.

**Results**

Of the 31 eligible pregnant women and their 32 children (one twin case), 2 newborns were excluded because the parents refused to sign the FCCT. Three losses comprising discontinued follow-up were noted.

All included pregnant women presented laboratorial confirmation of ZIKV infection, 24 (92.3%) by means of a positive RT-PCR and 2 (7.7%) exclusively by positive IgM serology. Nine (34.6%) had the infection in the 1st trimester, 10 (38%) in the 2nd trimester, and 7 (26.9%) in the 3rd trimester.

Only one infant exposed to the virus during the first trimester presented microcephaly. No children tested positive for umbilical cord blood IgM serology, including the infant with microcephaly. We ruled out STORCH group infection in the mothers. The main clinical and sociodemographic characteristics of the mothers and children are displayed in the – Table 1.

Only three children were submitted to all hearing tests, mainly due to family difficulties in bringing their children to the visits. Seventeen children (63%) had OAE + any type of ABR = audiometry + imitanciometry testing and 20 (74%) were submitted to FS-ABR or audiometry + imitanciometry (– Table 2).

Most children presented normal hearing tests. Seven (38.9%) presented a hearing evaluation suggestive of middle ear alterations in at least one of the ears at the time of the audiometric/imitanciometric test. All of those children (7) presented congested upper airways at the time of the exam (i.e., congested mucosa, rhinorrhea, report of recent upper airway infection or allergic exacerbation). One of the children (n=2) had already presented this type of condition since the neurodiagnostic ABR test, at 23 months of life, in which delayed wave absolute latencies were identified. This child was referred to otolaryngologic evaluation and follow-up and was diagnosed with bilateral chronic otitis media with effusion (– Table 3).

The only microcephalic child in the cohort presented normal electrophysiological tests, with normal electrophysiological hearing thresholds for both ears. Her imitanciometry test was normal, presenting a bilateral “A” type curve in the tympanogram and present ipsilateral and contralateral stapedial reflex in every tested frequency. The blink reflex was present during the behavioral audiometry, and the patient reacted to every instrument at all intensities. However, her motor reactions were always directed to the left side, even for stimuli presented on the right side.

**Discussion**

This study demonstrates that a sample of children presenting prenatal exposure to ZIKV assisted in a public health service,
ever, only the infant with microcephaly could be considered present. Laboratorial confirmation of ZIKV infection was not affected by prenatal exposure to the ZIKV, neither at birth or after. Some of the children presented microcephaly. The authors also report a few cases of clinical involvement or even association to central impairment manifestations are related to a worse spectrum of clinical involvement or even association to central alterations.

Considering the accuracy of the combined tests, normal hearing evaluation results were observed in the present study, both related to neural auditory pathway conduction and hearing threshold, indicate that the assessed children’s hearing function was not affected by prenatal exposure to the ZIKV, neither at birth or after. Some of the children showed results suggesting middle ear pathology, but associated to a recent history of upper airway infection, chronic nasal obstruction and/or otitis media with effusion. These results are consistent with other reports, which suggests that hearing impairment manifestations are related to a worse spectrum of clinical involvement or even association to central alterations.

Although prenatal exposure to the ZIKV occurred predominantly in the first two trimesters in this cohort, when a greater risk of impairment of fetal development is noted, most children did not present apparent CZS signs, which can explain the lack of hearing impairment. Most studies identifying hearing loss in ZIKV infection were conducted with microcephalic children, which suggests that hearing impairment manifestations are related to a worse spectrum of clinical involvement or even association to central alterations.

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Abbreviations: DM, diabetes mellitus; IQR, interquartile range; NICU, neonatal intensive care unit; PIH, pregnancy-induced hypertension, STD, sexually transmitted disease.

\(^{a}\)one presenting congenital hearing loss (sister), one presenting presbyacusis (grandfather), one presenting hearing loss after craniocerephalic trauma (father), one presenting possible noise-induced hearing loss (father) and one with progressive hearing loss of unknown cause (great-aunt).

most of which with no apparent abnormalities suggestive of CZS, displayed predominantly normal hearing evaluations. According to the World Health Organization’s (WHO) interim guidance update (2016), all included pregnant women presented laboratorial confirmation of ZIKV infection. However, only the infant with microcephaly could be considered

### Table 1 Clinical and sociodemographic characteristics of the mothers and children assessed in the present study

| Clinical characteristics | Mothers (n = 26) |
|--------------------------|-----------------|
| Age at child birth       | median (IQR)    |
| 1st trimester            | 29 (23.5–33.5)  |
| 2nd trimester            | 3 (35.6)        |
| 3rd trimester            | 10 (38.5)       |
| Schooling level          | n (%)           |
| Elementary school        | 6 (23.1)        |
| Middle/High school       | 15 (57.7)       |
| Higher education         | 1 (4.4)         |
| Not known                | 1 (3.5)         |
| Comorbidity related to gestation (ex. PIH, gestational DM, etc) | 10 (38.5) |
| Pre-existing chronic diseases | 9 (34.6) |
| STD diagnostic/treatment before the pregnancy | 3 (11.5) |
| Children (n = 27)        |                 |
| Gestational age at birth | n (%)           |
| < 28 weeks               | 0 (0.00)        |
| 28–32 weeks              | 3 (11.1)        |
| 33–36 weeks              | 1 (3.7)         |
| 37–41 weeks              | 23 (85.2)       |
| ≥ 42 weeks               | 0 (0.00)        |
| Sex                      | n (%)           |
| Female                   | 20 (74.1)       |
| Male                     | 7 (25.9)        |
| Microcephaly             | 1 (3.7)         |
| Other congenital malformations | 0 (0.00) |
| NICU admission           | 3 (11.1)        |
| Use of ototoxic medications | 0 (0.0)       |
| Hyperbilirubinemia       | 4 (14.8)        |
| Familiar history of hearing loss | 5 (18.5) |

### Table 2 Child distribution for each hearing test

| Hearing tests | Type of test | n (%) of children submitted |
|---------------|--------------|-----------------------------|
| T-OAE         | 27 (100)     |
| a-ABR         | 14 (52)      |
| Neuro-ABR     | 11 (41)      |
| FS-ABR        | 10 (37)      |
| Audiometry    | 18 (67)      |
| Imitanciometry | 18 (67)       |

Abbreviations: a-ABR, automated auditory brainstem responses; FS-ABR, frequency specific auditory brainstem responses; neuro-ABR, neurodiagnostic (click) auditory brainstem responses; T-OAE, transient otoacoustic emissions.

\(^{a}\)of the total, three presented unreliable testing results in one of the ears.
| Patient | Date of birth | Auditory test (date) | T-OAE | a-ABR | Neuro-ABR | FS-ABR | Audio | Imitancio |
|---------|--------------|----------------------|-------|-------|-----------|--------|-------|-----------|
| 1       | 09/08/16     | pass Bi (09/08/16)   | normal Bi (12/23/16) | normal Bi (12/11/17) | normal Bi (12/11/17) | Behavioral – lateralization to all instruments (always turning the head to the left, even for sounds presented on the right), EBR present (01/16/2019) | Tymanogram type “A” Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (01/16/2019) |
| 2       | 03/09/16     | pass Bi (05/12/16)   | NP    | Absolute latency delay LE; normal RE (02/19/18) | NP | Conditioned in an open field, with MRL in 20 dB in 1,000 Hz (could not be conditioned to the other frequencies); SRT in 20 dB (02/06/2019) | Tymanogram type “B” Bi; absent ipsilateral and contralateral SR Bi (02/06/2019) |
| 3       | 07/14/16     | pass Bi (07/15/16)   | NR    | normal Bi (11/13/17) | normal Bi (11/13/17) | Conditioned in an open field (could not be conditioned to pure tone testing); SRT in 25 dB (02/06/2019) | Tymanogram type “A” Bi; present ipsilateral and contralateral SR Bi in 500, 1,000; 2,000 and 4,000 Hz (02/06/2019) |
| 4       | 05/05/16     | pass Bi (05/11/16)   | NP    | normal Bi (02/26/18) | normal Bi (02/26/18) | NP | NP |
| 5       | 04/19/16     | pass Bi (04/19/16)   | NP    | normal Bi (01/22/18) | normal Bi (01/22/18) | Conditioned in an open field, with MRL in 20 dB in 500; 1,000; 2,000 and 4,000 Hz; SRT in 20 dB (01/16/2019) | Tymanogram type “A” Bi; ipsilateral and contralateral SR RE in 2,000 and 4,000 Hz (did not cooperate for the rest of the test) (01/16/2019) |
| 6       | 06/17/16     | pass Bi (08/24/16)   | normal LE and NR RE (11/23/16) | normal Bi (03/05/18) | normal Bi (03/05/18) | Conditioned with headphones, with hearing threshold at 20 dB in 500; 1,000; 2,000 and 4,000 Hz; SRT in 20 dB (02/19/2019) | Tymanogram type “A” Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (02/19/2019) |
| 7       | 10/17/16     | pass Bi (10/17/16)   | normal Bi (01/04/17) | normal Bi (11/06/17) | normal Bi (11/06/17) | Conditioned in an open field, with MRL in 25 dB in 500; 1,000; 3,000 and 4,000 Hz and in 20 dB in 2,000 Hz; SRT in 20 dB (02/06/2019) | Tymanogram type “B” RE and type “A” LE; absent ipsilateral SR in RE and contralateral SR in LE; present ipsilateral SR in LE and contralateral SR in RE (tested in 500; 1,000; 2,000 e 4,000 Hz) (06/02/2019) |
| 8       | 10/05/16     | pass Bi (10/07/16)   | NP    | normal Bi (11/13/17) | normal Bi (11/13/17) | NP | NP |
| 9       | 06/29/16     | pass Bi (06/30/16)   | NP    | normal Bi (02/26/18) | normal Bi (02/26/18) | Conditioned in an open field, with MRL in 20 dB in 500; 1,000; 2,000 and 4,000 Hz; SRT in 20 dB (04/05/2019) | Tymanogram type “C” RE and type “A” LE; absent ipsilateral SR in RE; present contralateral SR in RE; present ipsilateral SR in LE and present contralateral SR only in 2,000 and 4,000 Hz in LE (04/05/2019) |

(Continued)
| Patient | Date of birth | Auditory test (date) | T-OAE | a-ABR | Neuro-ABR | FS-ABR | Audio | Tympanogram type | Imittance |
|---------|--------------|----------------------|-------|-------|-----------|-------|-------|------------------|-----------|
| 10      | 04/26/16     | pass Bi (04/26/16)   | NP    | normal Bi (03/05/18) | normal Bi (03/05/18) | Conditioned with headphones, with hearing threshold at 15 dB at 500; 1,000 and 2,000 Hz and 20 dB at 4,000 Hz in RE, and at 10 dB at 500; 1,000; 2,000 and 4,000 Hz in LE; SRT at 0 dB in RE and at 5 dB in LE | (01/30/2019) | Tymanogram type "A" Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz |
| 11      | 02/23/16     | pass Bi (02/23/16)   | NP    | normal Bi (12/18/17) | normal Bi (12/18/17) | Conditioned with headphones, with hearing threshold at 20 dB at 500; 1,000; 2,000 and 4,000 Hz Bi; SRT at 20 dB Bi | (02/20/2019) | Tymanogram type "A*" RE and type "A" LE; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (02/20/2019) |
| 12      | 05/16/16     | pass Bi (05/17/16)   | normal Bi (12/28/16) | NP | NP | Conditioned with headphones, with hearing threshold at 20 dB at 500; 1,000; 2,000; 3,000 and 4,000 Hz Bi; SRT at 20 dB Bi | (04/03/2019) | Tymanogram type "B" Bi; absent ipsilateral and contralateral SR Bi |
| 13      | 02/05/16     | pass Bi (02/11/16)   | normal Bi (06/28/16) | NP | NP | Conditioned with headphones, with hearing threshold at 25 dB at 500 Hz, 15 dB at 1,000; 2,000 and 4,000 Hz and 20 dB at 3,000 Hz Bi; SRT at 20 dB Bi | (04/03/2019) | Tymanogram type "A" Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (04/03/2019) |
| 14      | 07/17/16     | pass Bi (07/19/16)   | normal Bi (12/23/16) | NP | NP | NP | | |
| 15      | 06/25/16     | pass Bi (06/25/16)   | normal Bi (08/24/16) | NP | NP | Conditioned with headphones, with hearing threshold at 20 dB in 500; 2,000 and 4,000 Hz and 15 dB at 1,000 Hz in OD e 20 dB at 500; 1,000; 2,000; 3,000 and 4,000 Hz at LE; SRT at 20 dB Bi | (02/12/2019) | Tymanogram type "A" Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (02/12/2019) |
| 16      | 06/23/16     | pass Bi (06/23/16)   | normal Bi (08/24/16) | NP | NP | Conditioned with headphones, with hearing threshold at 20 dB at 500; 1,000; 2,000 and 4,000 Hz Bi; SRT at 20 dB Bi | (02/19/2019) | Tymanogram type "A" Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (02/19/2019) |
| 17      | 05/06/16     | pass Bi (05/25/16)   | normal Bi (06/07/17) | NP | NP | Conditioned with headphones, with hearing threshold at 20 dB at 500; 1,000; 2,000 and 4,000 Hz Bi; SRT at 20 dB Bi | (02/12/2019) | Tymanogram type "A" Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (02/12/2019) |
| 18      | 05/06/16     | pass Bi (05/25/16)   | normal Bi (06/07/17) | NP | NP | Conditioned with headphones, with hearing threshold at 20 dB at 1,000 and 2,000 Hz Bi (did not cooperate for testing at the other frequencies); SRT at 20 dB Bi | (02/12/2019) | Tymanogram type "A" Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (02/12/2019) |
| 19      | 04/25/16     | pass Bi (05/16/16)   | NP | NP | NP | NP | | |
| Patient | Date of birth | Auditory test (date) | T-OAE | a-ABR | Neuro-ABR | FS-ABR | Audio | Imitancio |
|---------|---------------|----------------------|-------|-------|-----------|--------|-------|-----------|
| 20      | 05/26/16      | pass Bi (05/27/16)   | normal Bi (06/17/16) | NP     | NP        | Conditioned in an open field, with MRL at 20 dB at 500, 1,000; 2,000 and 4,000 Hz; SRT at 25 dB (04/30/2019). | Tympanogram type “B” RE and type “Ar” LE; absent ipsilateral SR in RE and contralateral SR in LE; present ipsilateral SR in LE and contralateral SR in RE (tested in 500, 1,000; 2,000 e 4,000 Hz) (04/30/2019) |
| 21      | 05/30/16      | pass Bi (05/30/16)   | normal Bi (06/22/16) | NP     | NP        | NP     | NP     | NP        |
| 22      | 10/20/16      | pass Bi (10/21/16)   | NP     | NP     | NP        | NP     | NP     | NP        |
| 23      | 07/30/16      | pass Bi (07/30/16)   | NR     | NP     | NP        | NP     | NP     | NP        |
| 24      | 07/31/16      | pass Bi (07/31/16)   | NR     | NP     | NP        | Conditioned with headphones, with hearing threshold at 15 dB at 1,000 and 20 dB at 2,000 and 4,000 Hz in RE and at 20 dB in 1,000, 2,000 and 4,000 Hz in LE (did not cooperate for testing at 500 Hz); SRT in 20 dB Bi (02/20/2019) | Tympanogram type “A” Bi; present ipsilateral and contralateral SR Bi in 500; 1,000; 2,000 and 4,000 Hz (02/20/2019) |
| 25      | 09/04/16      | pass Bi (09/27/16)   | normal RE and NR LE (01/04/17) | NP     | NP        | Conditioned in an open field, with MRL at 20 dB in 500; 3,000 and 4,000 Hz, 15 dB at 1,000 Hz and 10 dB at 2,000 Hz; SRT (with headphones) at 20 dB Bi (04/05/2019) | Tympanogram type “A” RE and type “B” LE; absent ipsilateral SR in RE and LE and contralateral SR in LE, present contralateral SR in LE in 500; 1,000; 2,000 and 4,000 Hz (04/05/2019) |
| 26      | 04/12/16      | pass Bi (04/13/16)   | NR     | NR     | NP        | NP     | NP     | NP        |
| 27      | 01/07/17      | refer LE (10/01/17)  | normal LE e NR RE (01/18/17) | NR     | NP        | NP     | NP     | NP        |

Abbreviations: a-ABR, automated auditory brainstem responses; Audio, audiometry; Bi, bilateral; EBR, eye blink reflex; FS-ABR, frequency specific auditory brainstem responses; Imitancio, imitanciometry; LE, left ear; MRL, minimal response level; neuro-ABR, neurodiagnostic (click) auditory brainstem responses; NP, not performed; NR, not reliable; RE, right ear; SR, stapedial reflex; SRT, speech recognition threshold; T-OAE, transient otoacoustic emissions.

Note: the tests classified as NR were performed but, due to technical matters, did not provide a reliable result – most of the NR were a-ABR in which the child did not sleep and did not stay quiet during the exam, interfering in analyzed the waves.

a the frequency of 1,000 Hz could only be performed for the right ear.
b only performed at 500 and 2,000 Hz.
In a review, Leal et al\textsuperscript{11} mentioned a not yet published longitudinal study that assessed children with congenital ZIKV infection up to 18 months of life and seemed to rule out the possibility of a progressive or late onset hearing loss. Nevertheless, the authors recommend auditory follow-up even in children who passed the initial hearing screening. They also emphasize the association between microcephaly and hearing impairment, suggesting a possible central origin in a significant amount of cases, even when presenting normal electrophysiological tests. On the other hand, Marques-Abramov et al\textsuperscript{30} evaluated 19 children with congenital ZIKV infection and microcephaly and found no disturbances in the neural conductibility of the auditory pathways up to the brainstem. However, this finding does not rule out hearing threshold alterations (not tested in the aforementioned study) or a more central auditory path impairment.

In the present study, the only microcephalic child presented normal hearing tests, with positive behavioral audiometry responses at all intensities and frequencies. Motor responses to sound always comprised head lateralization to the left side, suggesting that the child presented preserved hearing function, but that her neuropsychomotor impairment determined this response pattern. Additionally, this does not rule out a possible limitation in certain auditory abilities that depend on the integrity of the auditory cortex, as previously highlighted in other studies.\textsuperscript{2,11}

A considerable frequency of maternal comorbidities was present, such as pregnancy-induced hypertension, gestational diabetes, and preexisting chronic diseases. However, most children presented no complications, with only three cases of prematurity and neonatal intensive care unit (NICU) admission and four cases of non-severe and self-limited neonatal jaundice, including two twins, who were among the premature infants admitted to the neonatal intensive care unit (NICU). If any hearing loss were to be identified in those infants, their neonatal complications would have been taken into consideration, as they are known risk indicators for hearing loss.\textsuperscript{10} Therefore, their families were instructed to closely follow neurepsychomotor and linguistic milestones acquisition and to stimulate oral language in the familiar environment.\textsuperscript{10}

Some study limitations should be noted, as follows. Confirmation of fetal infection by the ZIKV was not possible. All mothers underwent laboratory confirmation of the infection during pregnancy, either by a positive RT-PCR or a positive IgM serology. However, confirmation was more complex in the fetuses and newborns, since the risk associated to amniocentesis precluded this procedure as a routine for collecting organic fetus material. As most of the mothers presented the infection in the first two trimesters of pregnancy, newborn IgM serology was expected to be negative, since too much time until birth would have passed. On the other hand, as IgG crosses the transplacental barrier, it could persist in the infant’s blood during the first months of life\textsuperscript{31,32} even in non-infected individuals. The RT-PCR testing in newborns would only be useful if the birth occurred up to two weeks after the infection (window period), which was not the case in the present cohort. The IgG serology after the first year of life may strongly suggest congenital ZIKV infection, but postnatal infection cannot be ruled out, considering the epidemiological characteristics and the presence of the vector and the circulating virus in the region. In this scenario, as the hearing tests were normal, two situations may be considered: 1) the children did not contract intrauterine infection by ZIKV from their mothers. This has been reported for other viral infections, such as rubella, in which the frequency of transmission to the fetus diminishes according to the increased gestational age\textsuperscript{33}, 2) the children were infected by ZIKV, but the infection did not determine hearing loss to date. It is worth noting that a considerable portion of studies describing hearing loss in congenital ZIKV infection performed the tests only in microcephalic children, considered the worst clinical manifestation of CZS.\textsuperscript{1,2,11,27,28} On the other hand, the children in this cohort—comprising only one microcephalic individual—could represent a mild spectrum of manifestations, which do not include hearing loss. Another study limitation consists in the reduced number of children submitted to a complete auditory evaluation. In some cases, the caregivers faced difficulties to attend follow-up exams, despite the efforts of the research team to avoid this situation. In other cases, adequate sedation for the FS-ABR test was not possible. Nevertheless, 74% of the children performed the FS-ABR or audiometry + iImmitanometry, the most accurate tests for diagnosing hearing loss in infants, while 63% performed the combined tests (OAE + any type of ABR + audiometry + immitanometry), ensuring high sensitivity and specificity. Finally, the ZIKV epidemic progressively waned during the study period, which contributed to the limited number of study participants.

Conclusions

The results of this study indicate that intrauterine exposure to the ZIKV does not always determine hearing impairment. This risk seems to be more associated to the level of the central nervous system involvement, as demonstrated in other studies. Some cases of middle ear pathology were detected, which are common during the assessed age. The longitudinal and prospective assessments, observed in only a few studies, indicate no late or progressive hearing impairment. However, based on the knowledge of other congenital infections, the maintenance of auditory follow-up in children at risk for hearing loss is advised.

Conflict of Interests

The authors declare that have no conflict of interests.

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