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Auditory braistem evoked potential in infants with congenital cytomegalovirus: preliminary results of a case-control study

Potencial evocado auditivo de tronco encefálico em lactentes com citomegalovírus congênito: resultados preliminares de um estudo de caso-controle

Potencial evocado auditivo de tronco encefálico en lactentes con citomegalovirus congénito: resultados preliminares de un estudio de caso-control

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Abstract

Introduction: Congenital cytomegalovirus (cCMV) is the leading congenital infection associated with sensorineural hearing loss and is one of the infections encompassed by the TORCH acronym. It is therefore recommended that infants diagnosed with cCMV undergo audiological monitoring throughout the early years of life. The brainstem auditory pathway continues to mature during the first two years of life, and monitoring its responses serves as a neurophysiological marker. **Objective:** This study aimed to compare the neurophysiological integrity of the brainstem auditory pathway in infants with congenital cytomegalovirus to that of infants without the infection or other risk indicators for hearing loss. **Methodology:** This was a case-control study. The sample consisted of eight infants diagnosed with

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Authors' contributions:
JVMF: methodology, data collection and writing.
ADSNA: methodology and critical review.
SAB: study conception, methodology, critical review and orientation.

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congenital cytomegalovirus, without other risk indicators for hearing loss, matched with eight control infants based on sex, age, and maternal education. Auditory Brainstem Response was recorded using click stimuli at an intensity of 80 dB nHL. Latency and amplitude values of waves I, III, and V were analyzed, as well as the interpeak intervals I–III, III–V, and I–V. The groups were compared using the Mann-Whitney U test, with a significance level set at 5%. **Results:** No statistically significant differences were observed between groups in the absolute latencies and amplitudes of waves I, III, and V, nor in the interpeak intervals I–III, III–V, and I–V. **Conclusion:** These preliminary findings indicate no impairment in the brainstem auditory pathways of infants with congenital cytomegalovirus in the sample studied.

Keywords: Cytomegalovirus; Hearing; Audiology; Hearing Disorders; Speech therapy.

Resumo

Introdução: O citomegalovírus congênito é a infecção congênita apontada como principal indicador de risco para perdas auditivas do tipo sensorioneural, sendo uma das infecções do acrônimo TORCH. Recomenda-se, então, que lactentes com o diagnóstico, realizem o monitoramento audiológico ao longo dos primeiros anos de vida. A via auditiva do tronco encefálico encontra-se em processo maturacional nos dois primeiros anos de vida, sendo o acompanhamento das suas respostas um marcador neurofisiológico. **Objetivo:** O objetivo do estudo foi comparar a integridade neurofisiológica da via auditiva do tronco encefálico de lactentes com citomegalovírus congênito pareados com lactentes sem a infecção ou outros indicadores de risco para deficiência auditiva. **Metodologia:** Estudo de caso-controle. A amostra foi constituída por oito lactentes com citomegalovírus congênito sem outros indicadores de risco para deficiência auditiva pareados com oito lactentes do grupo controle, pelo sexo, idade e escolaridade maternas. Foi realizado o potencial evocado auditivo de tronco encefálico com estímulo clique na intensidade de 80 dB nNA e analisado os valores de latência e amplitude das ondas I, III e V; os intervalos interpicos I-III, III-V e I-V. Os grupos foram comparados quanto às variáveis estudadas com o Teste Mann-Whitney, sendo adotado significância de 5%. **Resultados:** Na comparação entre os grupos não foram observadas diferenças estatisticamente significativas na latência e amplitude absoluta das ondas I, III e V, bem como nos intervalos interpicos I-III, III-V e I-V. **Conclusão:** Estes resultados preliminares evidenciaram não haver comprometimento das vias auditivas do tronco encefálico em lactentes com citomegalovírus congênito na amostra estudada.

Palavras-chave: Citomegalovírus; Audição; Audiologia; Transtornos da Audição; Fonoaudiologia.

Resumen

Introducción: El citomegalovirus congénito es la infección congénita más relacionada con la pérdida auditiva neurosensorial, formando parte del grupo TORCH. Se recomienda que lactantes con este diagnóstico reciban seguimiento audiológico durante los primeros años de vida, ya que la vía auditiva del tronco encefálico está en maduración en este período, siendo sus respuestas un marcador neurofisiológico. **Objetivo:** Comparar la integridad neurofisiológica de la vía auditiva del tronco encefálico en lactantes con citomegalovirus congénito frente a lactantes sin la infección ni otros indicadores de riesgo para hipoacusia. **Metodología:** Estudio de casos y controles. La muestra incluyó ocho lactantes con citomegalovirus congénito sin otros factores de riesgo auditivo, emparejados por sexo, edad y escolaridad materna con ocho lactantes del grupo control. Se realizaron potenciales evocados auditivos del tronco encefálico con estímulo de clic a 80 dB nNA. Se analizaron las latencias y amplitudes de las ondas I, III y V, así como los intervalos interpicos I-III, III-V e I-V. Los grupos se compararon mediante la prueba de Mann-Whitney, con nivel de significancia del 5%. **Resultados:** En la comparación entre los grupos, no se observaron diferencias estadísticamente significativas en la latencia ni en la amplitud absoluta de las ondas I, III y V, ni en los intervalos interpicos I-III, III-V e I-V. **Conclusión:** Estos resultados preliminares evidencian que no hubo compromiso de las vías auditivas del tronco encefálico en los lactantes con citomegalovirus congénito en la muestra estudiada.

Palabras clave: Citomegalovirus; Audición; Desarrollo; Trastornos de la Audición; Terapia del habla.

Introduction

Congenital infections are risk indicators for hearing impairment and can also contribute to changes in neurodevelopment. Congenital cytomegalovirus is a congenital infection identified as the main risk indicator for sensorineural hearing loss and is one of the infections included in the acronym TORCH. Human cytomegalovirus belongs to the Herpesviridae family and can be transmitted vertically from pregnant women to infants[1]. Among congenital infections, cytomegalovirus can present symptoms or remain asymptomatic. Infants who show clinical signs from birth have symptomatic congenital cytomegalovirus, and these are the ones who are at greater risk of developing permanent sequelae. Approximately 10-15% of infants with congenital cytomegalovirus (cCMV) show clinical signs from birth, and approximately 50% of cases with symptomatic cCMV have hearing impairments, while in asymptomatic cases this rate drops to 7%[2]. In addition, cCMV is also identified as the congenital infection with the highest number of cases of congenital sequelae in the United States[3]. Clinical manifestations associated with infection may include microcephaly, intrauterine growth retardation, hepatosplenomegaly, jaundice, hyperbilirubinemia, seizures, and others[4].

The characteristic hearing impairment associated with congenital cytomegalovirus is late-onset sensorineural hearing loss[3], which occurs in both symptomatic and asymptomatic cases and is progressive. In symptomatic cases, bilateral sensorineural hearing loss with progression to profound hearing loss is most commonly found. Regarding asymptomatic cases, unilateral sensorineural hearing loss is more prevalent, also with progression of the degree[5]. Factors that call for the implementation of a rapid and safe diagnostic protocol for monitoring infants throughout their development.

Additionally, cCMV can affect the white matter present in the central nervous system (CNS), resulting in sensory, motor, and cognitive deficits[6]. Such deficits can result in delays in language development and learning difficulties. A study evaluating 192 infants with cCMV showed that infants with the infection performed worse in speech development at 24 and 36 months compared to infants without the infection[7].

As cCMV is an infection characterized by late-onset hearing loss with progressive severity, the *Joint Committee on Infant Hearing*[8], recommends that these infants undergo audiological monitoring every three months during their first year of life and every six months until the age of three. For children over three years of age, monitoring should be performed annually at least until the age of six.

Given that this infection increases the likelihood of hearing loss until at least 18 months of age, and considering that hearing is one of the most important systems for overall child development, we recognize the need for an audiological assessment protocol that can immediately identify any minimal changes in the auditory and neurodevelopmental responses of these children.

In addition, the importance of early diagnosis should be emphasized, given that the greatest period of neural plasticity occurs up to 24 months of age. That immediate intervention can minimize the damage caused by hearing loss or delayed neurodevelopment of the central auditory system, which is essential for a population at risk that is subject to many other complications related to infection.

Auditory Brainstem Response (ABR) is a test that assesses the functional integrity of the auditory pathway from the cochlear nerve to the brainstem nuclei. Neurodiagnostic ABR using clicks at an intensity of 80 dBnNA is not sufficient to determine the audiological diagnosis. However, by highlighting responses from the vestibulocochlear nerve, cochlear nuclei, and lateral lemniscus[9-10], through the presence of waves I, III, and V, respectively, with absolute latencies and inter-peaks I-III, I-V, and III-V within the expected range at each stage of development, it is possible to assess the maturational development of this central auditory pathway, which is a significant neurophysiological marker.

The literature does not provide details on the characteristics of auditory responses in infants with congenital cytomegalovirus using ABR with click stimulation in the neurodiagnostic protocol. This issue becomes important for scientific research, as the auditory pathway assessed in ABR may impact language development in infants and be caused by neurological changes present because of cCMV.

Based on the above, this study aimed to compare the neurophysiological integrity of the brainstem auditory pathway in infants with congenital cytomegalovirus matched with infants without cCMV and other Risk Indicators for Hearing Loss (RIHLs).

Metodology

This is a case-control study with preliminary data. This project was approved by the Research Ethics Committee of the Hospital Universitário Onofre Lopes (HUOL) (No. 5,389,138). All legal guardians signed the Informed Consent Form (ICF) approved by this Committee. Data collection was carried out in the hearing and language room of the Laboratory for Technological Innovation in Health (LAIS) at HUOL/UFRN.

This study targeted infants born in public maternity hospitals in the metropolitan region of Natal (RN). The sample was recruited for convenience, based on referrals made by the pediatric infectious disease specialist after diagnosis of cCMV. As this is a preliminary report, the sample size was not calculated.

Thus, the sample consisted of two groups: Group 1 (G1) infants exposed to congenital cytomegalovirus, who tested positive for PCR in the first days of life, diagnosed and referred by an infectious disease pediatrician; none of the infants included in this study showed clinical signs of the disease; Group 2 (G2) infants from the control group, with no risk indicators for hearing loss (RIHL), referred by a pediatrician specializing in child care. As this is a case-control study, the groups were matched according to gender, age, and maternal education level, with infants in the control group selected based on these characteristics for matching. In cases where more than one eligible infant was found in the control group, the infant included was chosen at random. The sample consisted of 16 infants, eight from each group.

The exclusion criteria for both groups were: medical diagnosis of syndromes and/or other congenital infections, congenital malformations, neurological diseases, gestational age less than 36 weeks, birth weight less than 1,500 grams, infants whose mothers have a history of alcohol and/or drug use during pregnancy, absence of responses in transient evoked otoacoustic emissions (TEOAE) unilaterally or bilaterally, tympanometry with results indicative of middle ear changes, and infants with risk indicators according to the *Joint Comitte of Infant Hearing*[8].

Initially, mothers or guardians were asked to complete a questionnaire about the baby's prenatal history and birth data. The purpose of this procedure was to classify and/or exclude infants from the study based on the information obtained if they did not meet the eligibility criteria.

Infants with a minimum signal-to-noise ratio (SNR) of ≥ 6 dB in at least three of the five bands of 1000, 1400, 2000, 2800, and 4000 Hz in transient evoked otoacoustic emissions and tympanometry indicating normal middle ear function with a 1000 Hz probe for infants up to 6 months of age and a 226 Hz probe for infants over 6 months of age presenting a positive peak or type A tympanometry.[11]. Both procedures were performed using *Interacoustic's Titan*® equipment.

The Auditory Brainstem Response test, which uses click stimuli to assess the integrity of the auditory pathway, was performed using *Intelligent Hearing System's Smart EP* equipment. A total of 2048 stimuli were presented with rarefied polarity at an intensity of 80 dB NA, lasting 100 μs, and delivered at a rate of 27.7/s. The rejection rate and artifacts did not exceed 10%, the analysis window was 24 ms, and the filter was 30-3000 Hz. In addition, electrophysiological threshold testing was performed at an intensity of 20 dBNA.

The ABR was performed with infants sleeping naturally in their mothers' arms, in a quiet room at a comfortable temperature. The air conditioning was turned off before the procedure began, and all infants were evaluated under the same conditions. Surface electrodes were placed on the infants, with the active electrode positioned at Fz, the ground electrode at Fpz, and the reference electrodes on the ipsilateral mastoid (M1/M2) and contralateral to the tested ear. Before placing the electrodes, the surface was cleaned with gauze and abrasive paste so that the impedance of the electrodes did not exceed 2 KΩ and 5 KΩ between the electrodes. Subsequently, the *Etymotic ER-3A* earplug was inserted into the ear that would be tested first, and the presentation of stimuli began.

Offline analysis of ABR was performed in two stages: (1) Preparation of the signal for analysis and (2) Identification and marking of the latency of waves I, III, and V, referring to the responses of the vestibulocochlear nerve, cochlear nuclei, and lateral lemniscus. The wave markings were performed by two independent speech therapists with experience in auditory electrophysiology. In case of discrepancies, a third specialist was called in.

In preparing the signal for analysis, the two averages in the rarefied polarity were added together. In cases where more than two averages were performed, those with the least artifact, highest SNR with the least residual noise, best reproducibility, and best wave morphology were selected. Afterwards, the latency of waves I, III, and V was identified and marked by visual identification and manual marking of waves I, III, and V.

The absolute latency of waves I, III, and V and the interpeaks I-III, III-V, and I-V were analyzed according to the groups (G1 and G2) and ears. Due to the non-normal distribution of data, as measured by the Shapiro-Wilk test, the Mann-Whitney test was used to compare the latencies and amplitudes of the waves and interpeaks between the two groups. A significance level of 5% was adopted.

Results

Figure 1 shows the recruitment and selection process for composing the sample of the congenital cytomegalovirus group (G1). Nine infants participated in the ABR click; however, one of them did not undergo the TEOAE. Thus, the final sample for G1 consisted of eight infants.

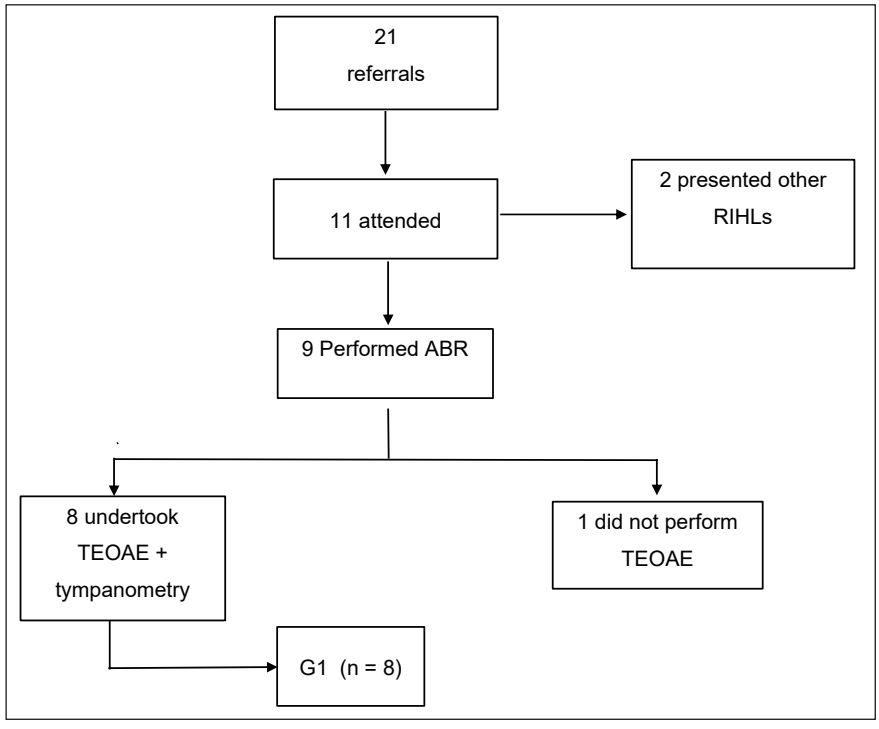

Figure 1. Recruitment and selection of the study sample from the cCMV group.

Table 1 shows the categorization of the sample groups that comprised the study.

Table 1. Characterization of the sample by group.

Features	G1 (n= 8)	G2 (n=8)
Sex		
Male	1 (12.50%)	1(12.50%)
Feminino	7 (87,50%)	7 (87,50%)
Age (days)	311 ± 193	252 ± 181
Maternal education level		
1	0	0
2	0	0
3	2 (25%)	2 (25%)
4	6 (75%)	6 (75%)

Key: n - sample; 1 - incomplete elementary education; 2 - complete elementary education; 3 - incomplete high school education; 4 - complete high school education. Age (days) - median.

Table 2 presents the descriptive statistical analysis of the absolute latencies of waves I, III, and V, as well as the interpeaks I-III, III-V, and I-V, for each ear in each group. The absolute latencies of ABR waves I, III, and V were within the expected parameters for the age group evaluated in G1. Based on the inferential analysis using the Mann-Whitney test, it was not possible to detect a statistically significant difference concerning the absolute latency of waves I, III, and V, and the interpeak intervals I-III, III-V, and I-V.

Table 2. Descriptive and inferential statistics of the absolute latency of waves I, III, and V and interpeaks I-III, III-V, and I-V at an intensity of 80 dB nNA.

	RE			LE			RE			LE		
	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3
Wave I	1.40	1.55	1.55	1.48	1.55	1.58	1.49	1.52	1.55	1.45	1.48	1.55
Wave III	4.00	4.28	4.53	4.00	4.15	4.70	3.93	4.10	4.29	3.98	4.13	4.29
Wave V	5.82	6.15	6.58	5.95	6.10	6.63	5.86	6.03	6.26	5.94	6.03	6.24
Interpeak I-III	2.45	2.85	3.06	2.53	2.60	3.10	2.41	2.63	2.81	2.48	2.67	2.75
Interpeak III-V	1.84	1.90	1.97	1.90	2.00	2.13	1.87	1.92	2.04	1.83	2.02	2.06
Interpeak I-V	4.27	4.75	5.10	4.42	4.65	5.20	4.35	4.55	4.80	4.45	4.55	4.76

Key: RE - right ear; LE - left ear; Q1 - percentile 25; med - median; Q3 - percentile 75.

Discussions

Preliminary results from this study indicate that infants with cCMV had similar responses to those without infection in auditory brainstem response (ABR) testing.

The absolute latencies of waves I, III, and V, as well as the interpeaks I–III, III–V, and I–V, were similar between the groups with and without cCMV and compatible with the normative values described in studies that adopted similar protocols[12-13]. Gorga et al[4] performed ABR in 535 children, and the values obtained for waves I, III, and V, as well as for the interpeak intervals I-III, III-V, and I-V, were similar to those in the present study. The variations between the values of the studies can be explained by the parameters adopted, the equipment used, and the practice, since ABR marking is done visually and manually.

In the current study, infants who had any other RIHL in addition to CMV were excluded. In the infants included, no changes were observed in the ABR responses, all of which were consistent with normative standards described in normal-hearing children[13-14], suggesting that cCMV, under the conditions evaluated, did not influence the electrophysiological responses evoked by the click stimulus. These findings differ from other longitudinal studies in which automatic ABR or click-stimulated procedure was used, since in those studies changes in auditory pathway responses were observed, showing progression of hearing loss[15-17].

Although no studies have yet been found that characterize auditory pathway responses through brainstem auditory evoked potentials in infants with cCMV, there are studies on other congenital infections that present results similar to those of the present study. Silva[18] used ABR click with a protocol and parameters identical to those used in this study in infants with congenital syphilis, observing no differences compared to infants without other RIHL. A study conducted by Sideri[19] using ABR click with a protocol and parameters similar to those of the present study evaluated the responses of infants with Zika virus compared to the normative standard of the equipment used, and no differences were observed with the normative standard adopted. However, in the follow-up conducted at 19 months, two of the participants in the Sideri[19] study showed lower than expected results for the development of auditory skills. These results may be related to the form of analysis, the evaluation period, or even the procedure used, since imaging tests could show different results.

Cytomegalovirus can negatively influence the maturation process of the auditory pathway due to neurological changes, causing sensorineural hearing loss, which may occur later in life due, for example, to viral reinfection or reactivation of the virus[20].

It was observed that all infants in this study presented waves I, III, and V at an intensity of 80 dB nNA, as well as wave V at an intensity of 20 dB nNA. Although this information does not allow for the establishment of an audiological report, it can be inferred following the Cross-check[21] principle, in which the result of one test must confirm the other independently. All infants included in the study underwent tympanometry and presented tympanometric curves of type "A" and TEOAEs with bilateral response. These findings allowed us to rule out involvement of the middle ear and cochlea, respectively. Based on this, the ABR-click test was performed, a neurodiagnostic test at an intensity of 80 dB nNA. Thus, considering the above procedures, it is possible to rule out the presence of moderate hearing loss (35 to 50 dB nNA)[22]. When using ABR-click with the presence of wave V at 20 dB nNA, a bilaterally normal psychoacoustic threshold can be inferred in the region of 2 to 4 KHZ.

Contrary to the findings of this study, Chung et al[23] related the neurological changes resulting from cCMV to the degree of sensorineural hearing loss found in infants. Another study[5] points to a correlation of 71.2% for bilateral hearing loss in children with symptomatic cCMV, 65.1% of whom developed severe to profound sensorineural hearing loss. In asymptomatic cases, this correspondence was 57%, with unilateral hearing loss, although most cases were also severe to profound. The study also points out that 9% of subjects had late-onset hearing loss, and 1 in 5 asymptomatic cases showed progression of hearing loss.

Although the most alarming cases of cCMV are symptomatic, most of which present with bilateral hearing loss, it is essential to identify asymptomatic cases. Given that cCMV is characterized by late-onset hearing loss and progressive severity, early diagnosis and audiological monitoring are important, as the literature indicates improvements in hearing with early treatment[17,24].

A study conducted by Morimoto et al.[17] using automatic ABR showed that a newborn who had no hearing impairment at birth presented impairment at 3 months of age, corroborating the findings of Dollard et al.[25] regarding the characteristic of late onset hearing loss, because in the third month, when performing a new evaluation, Morimoto et al.[17] found changes in the electrophysiological thresholds of this infant, the presence of wave V was found only at 50 dBnNA for the right ear and 70 dBnNA for the left ear, which would be equivalent to a psychoacoustic threshold of 35 and 55 dB, respectively, in the 2 to 4 kHz range.

In this regard, it is important to note a limitation of this study, as the audiological protocol adopted did not minimally test the 4 kHz ABR-FE frequency as suggested at 20 dB nNA in infants with cCMV by the *British Society of Audiology*[26] or even at all other frequencies as suggested by the

Joint Comittee of Infant Hearing[8]. The exclusion was partly because the objective of this study was to assess the neurophysiological integrity of the auditory pathway from the vestibulocochlear nerve to the brainstem. In addition to an initial technical limitation in the research process, the frequencies of 500, 2000, and 4000 Hz by air conduction and when altered by bone conduction have already been included in the FS-ABR protocol.

Yamada et al[16] demonstrated in their study that there is a higher incidence of late-onset hearing loss around 18 months of age. On the other hand, Fowler et al.[27] demonstrated that the average age at which infants with late hearing loss are diagnosed is 27 months, ranging from 25 to 65 months. The average age of the infants in this study is between 193 and 311 days (6 to 11 months), while Fowler[2] points out that late hearing loss occurred on average at 27 months (± 820 days), thus generating a need to continue the longitudinal follow-up of these infants and design new studies in which they are monitored from birth to at least 2/3 years of age, with a recommendation of up to 5 years.

Corroborating the findings of Yamada et al[16], Iwasaki et al[28] conducted a study with 18 infants in which audiological monitoring was performed until the subjects reached 4 years of age. Late hearing loss was detected in two of them, both with asymptomatic cCMV. One of them had passed the automatic ABR test at birth, but at 12 months, the family reported complaints, and upon performing click ABR, he was diagnosed with bilateral deafness. The other was diagnosed with mild bilateral hearing loss in the first month of life. Of the other participants, two did not present hearing loss, and all others had been previously diagnosed and monitored, also presenting progression of hearing loss, which corroborates the data presented in the literature regarding hearing deterioration[15-16, 27].

The absence of audiological changes in the audiological procedures performed in the present study may be directly related to the late onset of sequelae, since late hearing loss is one of the characteristics of the infection[25]. Therefore, the absence of audiological changes to date in the cases studied does not allow for the discontinuation of audiological monitoring of these infants.

Furthermore, considering the critical period in which auditory skills are in full development, it is necessary to observe the maturation process of these skills longitudinally, given their strong influence on language development[29]. Since cCMV affects not only hearing but also language development and cognition[6-7], the use of procedures that assess cortical and subcortical auditory pathways, rather than just the brainstem, can be an important tool for the early identification of neurodevelopmental changes.

Therefore, longitudinal studies with a more representative sample are needed to assess the late and progressive effects associated with cCMV. In addition to the need to use a complete audiological diagnostic protocol. It is also worth noting the relevance of other studies with medium- and long-latency potentials, speech-stimulated potentials such as frequency following response (FFR), as well as imaging tests such as functional near-infrared spectroscopy (fNIRS), considering the neurological changes that accompany the infection. In addition to monitoring language and cognitive development.

Conclusion

This study, which presents preliminary results, found that the functioning of the auditory pathways at the brainstem level in infants with cCMV is within the expected range for their age group. However, considering the progressive nature of hearing loss associated with this infection, the importance of continuous audiological monitoring of these infants throughout their development is reinforced.

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