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Characterization of auditory brainstem response in newborns infected by Zika virus

Caracterização do potencial evocado auditivo de tronco encefálico em recém-nascidos infectados pelo Zika vírus

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ABSTRACT

Purpose: To characterize sequential assessments of auditory brainstem responses in newborns infected by zika virus, correlating with presence of microcephaly and with Zika virus symptoms in mothers during pregnancy. **Methods:** A descriptive, longitudinal and quantitative study, in which 20 newborns, children of mothers infected by Zika virus during pregnancy, participated. Medical records of these babies were analyzed, and they underwent two electrophysiological assessments, one in the first month of life and the other, after 6 months. Comparative data were tabulated and analyzed using descriptive and inferential statistics. **Results:** Seventy percent of babies had microcephaly and 55% of mothers had symptoms of Zika infection in the first trimester of pregnancy. There was no significant alteration in electrophysiological thresholds at any moments. There was a statistically significant change, mainly in the latencies of waves III and V, between the tests, characterizing maturation of the auditory pathway in babies. No correlation was found between microcephaly and changes in ABR latencies. **Conclusion:** Babies with Zika had normal electrophysiological thresholds and decreased absolute latencies of waves III and V and interpeaks, confirming the cytotoxic action of Zika. There were two cases of significant worsening of the electrophysiological threshold. There was no correlation between ABR results and time of onset of the symptoms during pregnancy, or presence of microcephaly.

Keywords: Hearing; Newborn; Zika virus; Microcephaly; Electrophysiology

RESUMO

Objetivo: Caracterizar as avaliações seqüenciais do potencial evocado auditivo de tronco encefálico em recém-nascidos infectados pelo Zika vírus, correlacionando com a presença de microcefalia e com os sintomas de Zika nas mães durante a gestação. **Métodos:** Estudo descritivo, longitudinal e quantitativo, do qual participaram 20 recém-nascidos, filhos de mães infectadas pelo Zika vírus no período gestacional. Foram analisados os prontuários desses bebês, que passaram por duas avaliações eletrofisiológicas, uma no primeiro mês de vida e outra, após 6 meses. Os dados comparativos foram tabulados e analisados por meio de estatística descritiva e inferencial. **Resultados:** Setenta por cento dos bebês apresentaram microcefalia e 55% das mães tiveram os sintomas da infecção pelo Zika no primeiro trimestre de gestação. Não houve alteração significativa dos limiares eletrofisiológicos em nenhum dos momentos. Houve mudança estatisticamente significativa, principalmente das latências das ondas III e V, entre os exames, caracterizando maturação da via auditiva nos bebês. Não foi encontrada correlação entre a microcefalia e alterações nas latências do PEATE. **Conclusão:** Bebês portadores de Zika apresentaram limiares eletrofisiológicos dentro da normalidade e diminuição das latências absolutas das ondas III e V e interpicos, confirmando a ação citotóxica do Zika. Houve dois casos de piora significativa do limiar eletrofisiológico. Não foi observada correlação entre resultados do PEATE e época de aparecimento dos sintomas durante a gestação, ou a presença de microcefalia.

Palavras-chave: Audição; Recém-nascido; Zika vírus; Microcefalia; Eletrofisiologia

Study carried out at Hospital Universitário Júlio Muller – HUJM, Universidade Federal de Mato Grosso – UFMT – Cuiabá (MT), Brasil, by the academics and professors from Fonoaudiologia, Centro Universitário de Várzea Grande – UNIVAG – Cuiabá (MT), Brasil.

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Conflict of interests: No.

Authors' contribution: RFAN reviewed the literature, contacted the mothers to collect data, analyzed the medical records and ABRs, discussed data and wrote the article; PAL study supervisor, collected data through ABRs, defined inclusion and exclusion criteria, followed the analysis and discussion of data and the article writing; RRC study co-supervise, corrected and formatted the text, supported the literature review, supported the translation into English, assisted in the analysis and discussion of data and article writing; GCPLD corrected and formatted the text, supported the literature review, assisted in the analysis and discussion of data and article writing; TMO provided assistance as a pediatric infectologist physician, requesting and analyzing Zika virus serology confirmation tests and referring patients to ABR.

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INTRODUCTION

The Zika virus (ZIKV) is an arbovirus transmitted in Brazil by the Aedes mosquito (i.e. *Aedes Aegypti and Aedes Albopictus*)[1].

Between October 2014 and March 2015, the nine states in the Northeast region of Brazil reported the occurrence of an exanthematic syndrome, which was related to ZIKV after its detection in blood samples of patients with symptoms similar to dengue. In May 2015, the autochthonous transmission of ZIKV in Brazil was confirmed, leading the country to the highest number of positive cases for the congenital syndrome associated with infection by the Zika virus (SCZ) in Latin America[1].

According to the Epidemiological Bulletin released by the Ministry of Health[2], between November 2015 and September 2019, in the state of Mato Grosso, 465 suspected cases were identified, which 80 were confirmed and 106 are still under investigation. In Brazil, in the same period, 3.483 confirmed cases of SCZ were recorded.

In October 2015, concomitant with the SCZ outbreak in Brazil, there were increased cases of microcephaly in newborns (NB's), based on reports by doctors in Rio Grande do Norte[1]. Such fact caused the Ministry of Health to raise suspicion of a possible relationship between SCZ and malformations of the Central Nervous System (CNS)[1]. After consolidating important evidence that supported this fact, in November 2015, the Ministry of Health recognized the relationship between presence of SCZ and occurrence of microcephaly, which is the measurement of cephalic circumference at birth, less than or equal to two standard deviations below the average for gestational age and child's sex[3,4].

Additionally to microcephaly, studies have pointed out other clinical manifestations attributed to SCZ, including hearing disorders, which may also occur as a result of microcephaly itself[4-6].

National and international organs[7,8] recommend microcephaly and SCZ as risk indicators for hearing loss (RIHL). Newborn Hearing Screening (NHS) aims at the early identification of hearing loss in newborns and infants. In children with RIHL, NHS is conducted through the auditory brainstem responses (ABR), due to higher prevalence of retrocochlear hearing losses, which are not identified by the otoacoustic emissions test (OAEs)[8].

Retrocochlear hearing losses are characterized by disorders of the auditory nerve, which means that information processed correctly by the inner ear is not properly transmitted through electrical impulses to the brain[9]. A recent study revealed the presence of hearing disorders in newborns (NB) who were exposed to ZIKV at some time during pregnancy, relating hearing loss to one of the sensorineural disorders that characterize the SZC[5].

Viral infections cause up to 40% of all congenital hearing losses, but they can also cause delayed-onset hearing losses. Typically, viral-induced hearing losses are sensorineural, with degrees ranging from moderate to profound. Individuals affected by the Herpes Zooster virus can have sensorineural hearing disorders of retrocochlear origin[10]. A study observed that children with toxoplasmosis, aged 1 to 3 months, are five times more likely to have abnormal ABRs, than children at the same age range, without the infection[11]. To date, some studies which have assessed hearing function of children with SCZ using ABR, have been published, but there is still no consensus regarding auditory sequelae of congenital Zika [4,12,13].

The existence of cases of NBs with SCZ at the University Hospital Julio Müller (HUJM) and the fact that ZIKV can cause neurological and auditory consequences to these babies, aroused the need to develop a study seeking to characterize ABR findings, which is an important tool in the diagnosis of retrocochlear hearing loss, in this population.

Thus, this study aimed to characterize ABR findings, between birth and six 6 months of life, in newborns with SCZ, correlating, or not, with presence of microcephaly and with symptoms of Zika in mothers, during pregnancy.

METHOD

This study was conducted after approval by the Research Ethics Committee (REC) of the HUJM, under number 2.112.529. Study participants were exempted from signing Informed Consent Form (ICF) by REC, since the collection was based on analysis of the results of exams in the medical records.

It was a descriptive, longitudinal and quantitative study, conducted in 2016 and 2017, at the Júlio Müller University Hospital *(Hospital Universitário Júlio Muller - HUJM)*, affiliated to the Federal University of Mato Grosso *(Universidade Federal de Mato Gosso - /UFMT)*, located in the city of Cuiabá (MT). The HUJMis one of the references in the state of Mato Grosso in diagnosing and monitoring patients infected by ZIKV, where mothers with suspicion of ZIKV infection, during pregnancy, are referred by the basic health network, so that, after childbirth, their newborns are submitted to a neuroimaging exam and evaluated by a multiprofessional team, composed of an infectious pediatrician, neuropediatrician, ophthalmologist and audiologist.

Newborns' (NBs') medical records from the state of Mato Grosso, both sexes, born at term, whose mothers reported suspicion of ZIKV infection during pregnancy, with positive ZIKV serology, with or without microcephaly, and who underwent audiological assessment were analyzed through ABR in the first 30 days of life (initial assessment) and at 6 months of age (sequential assessment).

The tests used to identify the virus in these NBs were established in protocols published by the Ministry of Health: PCR - polymerase chain reaction, indicated for detecting the virus in the first days of maternal disease, during pregnancy, if the mother has symptoms suggestive of ZIKV infection; serological test of immunoglobulin M (IgM), which identifies antibodies in the bloodstream, during initial phase of ZIKV infection, with an average duration from 5 to 5 weeks in the serum of the infected patient; and immunoglobulin G (IgG) test, to verify if the person has already had contact with Zika at some point in life[7]. The diagnosis of microcephaly was made by a pediatrician, or an infectious pediatrician or neuropediatrician, according to WHO guidelines[3].

Medical records of syndromic neonates were excluded from the study; from neonates with positive serology for other viruses such as syphilis, rubella, herpes, toxoplasmosis and HIV; premature neonates; NB`s with a family history of hearing loss; babies who have been exposed to ototoxic drugs; those who did not attend the second ABR and babies with conductive disorders in ABRs, an alteration characterized by the increase in absolute latencies of waves I, III and V, with normal or abnormal electrophysiological thresholds.

At first, 56 newborns` medical records whose mothers had a clinical suspicion of having contracted ZIKV during pregnancy

were analyzed. Then, 20 medical records of the selected babies were recruited within the eligibility criteria for this study. This analysis allowed to check the history of the selected NBs, as well as the results of two NHS performed, according to guidelines recommended by the Joint Committee on Infant Hearing (JCIH) [8], one in the first thirty days of life and another, 6 months later, for babies with RIHL.

The tests were carried out in a room at the JMUH, properly equipped and electrically prepared for the procedure, according to predefined criteria by the service. ABRs were obtained with NBs in natural sleep, the skin clean with Nuprep® abrasive paste and using the Pasta Ten 20® conductive gel to fix electrodes in the following order: active electrode on the upper part of the forehead, close to the hair implantation; reference electrodes on the right and left mastoids and earth electrode laterally, on the forehead, according to the International System 10-20. Impedance between the electrodes remained below 5 Ohms.

ABRs were recorded with the equipment MEB 9400K model, from Nihon Kohden® brand, starting the testing using click stimulus with rarefaction polarity at 80 dBnHL, seeking to verify the neurophysiological integrity of auditory pathway, for analysis of the absolute latencies of waves I, III, V and interpeak latencies I-III; III-V; I-V. Then, electrophysiological threshold search was conducted with click acoustic stimulus, initially, at an 80 dBnHL, gradually decreasing from 20 dB to 20 dB, until wave V is no longer visualized. After that, intensity was increased from 10 dB to 10 dB, until the lowest intensity which wave V appeared in the smallest amplitude was obtained, this point being considered the electrophysiological threshold. The stimulus presented was click with rarefaction polarity, at a presentation rate of 27.1 clicks per second and a recording window of 12 ms, with a 100 Hz and 3000 Hz bandpass filter. For analysis of the generated trace, a total of 1000 clicks was presented twice, so that reproducibility could be observed between the tracings. The marking of the wave peaks I, III and V were performed jointly and simultaneously, by two experienced examiners, who assessed the patients and the standard adopted for equipment used was obtained by Rosa et al.(14).

After collecting data from the two assessments, a comparison was made between results of the two tests of each baby. Comparative data were tabulated and analyzed using descriptive and inferential statistics. Tests used were the two proportion z-test, Wilcoxon, Chi-Square and Mann-Whitney, with a level of significance of 5% (p-value of 0.05).

RESULTS

In this study, 20 NB infected by ZIKV were assessed, 12 (60%) female and 8 (40%) male. Nonparametric statistical tests were used, as normality of quantitative variables of the main outcome was tested using the Kolmogorov-Smirnov (KS) test, concluding that there was no assured normality distribution.

Table 1 shows the results found for the time of onset of symptoms caused by ZIKV infection in mothers, presence or absence of microcephaly in the NB and gender distribution in the sample. The test for equality of two proportions revealed statistical significance in the distribution of variables "time of onset of the symptoms" (p=0.002) and "presence of microcephaly" (p=0.011).

Table 2 shows the descriptive analysis of variables analyzed in the auditory brainstem responses during initial and sequential assessments.

Table 3 refers to the quantitative comparison between absolute latencies, interpeak latencies, interaural difference in I-V interval and interaural difference in wave V latency, as well as the electrophysiological threshold between the initial and sequential assessments of the ABR in newborns. As the data are paired (when the same subject is his/ her research and control), cases in which there were no answers for both moments were excluded. This exclusion (only in this analysis) was made variable by variable. Thus, the Wilcoxon test was used.

Table 4 shows the comparison of percentages of the qualitative results for absolute latencies, interpeak latencies, interaural difference of the IV interval and the absolute latency of the ABR wave V, in the initial and sequential assessments, through the test for equality of two proportions. Latency results were named as absent, increased, decreased or preserved. It is worth noting that, as there is no control group in this study, values found were matched by age, using reference criteria established in the literature [14].

Table 5 shows the relationship between mean of electrophysiological thresholds in the initial and sequential tests with microcephaly and time of onset of the symptoms. The Mann-Whitney test revealed that there was no statistically significant relationship between variables.

For the relationship between absolute latencies, interpeaks, interaural difference of the I-V interval and absolute latency of wave V with microcephaly and the time of onset of symptoms in pregnant women, respectively, the Chi-Square test did not show a statistically significant relationship.

DISCUSSION

In May 2015, ZIKV was identified as an etiological agent of exanthematic disease in Brazil. In October at the same year, neuro pediatricians from the state of Pernambuco warned about a microcephaly epidemic, being registered on Live Birth Information System (SINASC) 739 cases of suspected microcephaly by 7 November 2015, in 190 municipalities from Brazil, which led the Ministry of Health to declare a state of emergency and investigate the possible relationship between microcephaly and ZIKV[15]. Likewise, a study conducted in this

Table 1. Sample characterization

Period of symptoms in the mother	N	%	P-value
1º Trim	11	55.00%	Ref.
2º Trim	2	10.00%	0.002
3º Trim	2	10.00%	0.002
NI	4	20.00%	0.022
NS	1	5.00%	<0.001
Microcephaly	**N**	**%**	**value**
No	6	30.00%	0.011
Yes	14	70.00%	
Sex	**N**	**%**	**P-value**
Female	12	60.00%	0.206
Male	8	40.00%	

Two proportion z-test

Subtitle: NS = no symptoms; NI = no information; N = number of subjects; % = percent; Trim = trimester; Ref = reference

Table 2. Descriptive analysis of absolute latencies, interpeaks and electrophysiological thresholds in the initial and sequential assessments

		Mean	Median	Standard deviation	N
Initial assessment	I (RE)	1.59	1.6	0.11	20
	I (LE)	1.57	1.6	0.13	19
	III (RE)	4.24	4.2	0.29	20
	III (LE)	4.22	4.15	0.27	20
	V (RE)	6.45	6.45	0.36	20
	V (LE)	6.48	6.5	0.45	20
	I-III (RE)	2.6	2.55	0.26	20
	I-III (LE)	2.57	2.5	0.29	19
	III-V (RE)	2.2	2.2	0.25	20
	III-V (LE)	2.23	2.15	0.29	20
	I-V (RE)	4.84	4.9	0.32	20
	I-V (LE)	4.8	4.9	0.35	19
	THRESHOLD (RE)	36.5	30	10.4	20
	THRESHOLD (LE)	37.5	30	14.1	20
	INTERAURAL DIFFERENCE - V	0.16	0.1	0.239	20
	INTERAURAL DIFFERENCE I-V	0.095	0.1	0.091	19
Sequential assessment	I (RE)	1.57	1.6	0.1	19
	I (LE)	1.55	1.6	0.12	18
	III (RE)	3.84	3.9	0.23	19
	III (LE)	3.83	3.9	0.2	18
	V (RE)	5.85	5.9	0.27	19
	V (LE)	5.81	5.85	0.25	18
	I-III (RE)	2.22	2.2	0.21	19
	I-III (LE)	2.22	2.3	0.23	18
	III-V (RE)	1.97	2	0.14	19
	III-V (LE)	1.95	1.9	0.13	18
	I-V (RE)	4.24	4.2	0.22	19
	I-V (LE)	4.23	4.2	0.25	18
	THRESHOLD (RE)	36.3	30	10.1	19
	THRESHOLD (LE)	33.9	30	5	18
	INTERAURAL DIFFERENCE - V	0.122	0.1	0.088	18
	INTERAURAL DIFFERENCE I-V	0,1	0.1	0.077	18

Wilcoxon test
Subtitle: RE= right ear; LE = left ear; N = number of subjects

Table 3. Quantitative comparison of absolute latencies, interpeak latencies, interaural difference between the I-V interval and the absolute latency of wave V and electrophysiological threshold in the initial and sequential assessments

		Mean	Median	Standard Deviation	N	P-value
I (RE)	IA	1.59	1.6	0.11	19	0.581
	SA	1.57	1.6	0.1	19	
I (LE)	IA	1.57	1.6	0.13	18	0.686
	SA	1.55	1.6	0.12	18	
III (RE)	IA	4.24	4.2	0.3	19	**<0.001**
	SA	3.84	3.9	0.23	19	
III (LE)	IA	4.21	4.1	0.28	18	**<0.001**
	SA	3.83	3.9	0.2	18	
V (RE)	IA	6.45	6.5	0.37	19	**<0.001**
	SA	5.85	5.9	0.27	19	
V (LE)	IA	6.41	6.4	0.38	18	**<0.001**
	SA	5.81	5.85	0.25	18	
I-III (RE)	IA	2.6	2.5	0.26	19	**<0.001**
	SA	2.22	2.2	0.21	19	
I-III (LE)	IA	2.58	2.5	0.3	18	**0.001**
	SA	2.22	2.3	0.23	18	
III-V (RE)	IA	2.19	2.2	0.25	19	**0.004**
	SA	1.97	2	0.14	19	
III-V (LE)	IA	2.17	2.1	0.19	18	**<0.001**
	SA	1.95	1.9	0.13	18	
I-V (RE)	IA	4.83	4.9	0.33	19	**<0.001**
	SA	4.24	4.2	0.22	19	

Two proportion z-test
Subtitle: RE = right ear; LE = left ear; IA = initial assessment; SA = sequential assessment; N = number of subjects

Table 3. Continued...

		Mean	Median	Standard Deviation	N	P-value
I-V (LE)	IA	4.79	4.8	0.36	18	**<0.001**
	SA	4.23	4.2	0.25	18	
THRESHOLD (RE)	IA	36.8	30	10.6	19	0.666
	SA	36.3	30	10.1	19	
THRESHOLD (LE)	IA	35	30	7.1	18	0.414
	SA	33.9	30	5	18	
INTERAURAL DIFFERENCE - V	IA	0.106	0.1	0.094	18	0.596
	SA	0.122	0.1	0.088	18	
INTERAURAL DIFFERENCE I-V	IA	0.1	0.1	0.091	18	0.951
	SA	0.1	0.1	0.077	18	

Two proportion z-test

Subtitle: RE = right ear; LE = left ear; IA = initial assessment; SA = sequential assessment; N = number of subjects

Table 4. Qualitative comparison of absolute latencies, interpeak latencies, interaural difference between the I-V interval and the absolute latency of wave V in the initial assessment and the sequential assessment

		IA		SA		P-value
		N	%	N	%	
I (RE)	\	0	0%	1	5%	0.311
	D	9	45%	0	0%	**<0.001**
	P	11	55%	19	95%	**0.003**
I (LE)	\	1	5%	2	10%	0.548
	D	8	40%	1	5%	**0.008**
	P	11	55%	17	85%	**0.038**
III (RE)	\	0	0%	1	5%	0.311
	D	4	20%	9	45%	0.091
	P	16	80%	10	50%	**0.047**
III (LE)	\	0	0%	2	10%	0.147
	D	4	20%	8	40%	0.168
	P	16	80%	10	50%	0.047
V (RE)	\	0	0%	1	5%	0.311
	D	3	15%	8	40%	0.077
	P	17	85%	11	55%	**0.038**
V (LE)	\	0	0%	2	10%	0.147
	I	1	5%	0	0%	0.311
	D	4	20%	9	45%	0.091
	P	15	75%	9	45%	0.053
I-III (RE)	\	0	0%	1	5%	0.311
	I	1	5%	0	0%	0.311
	D	2	10%	8	40%	**0.028**
	P	17	85%	11	55%	**0.038**
I-III (LE)	\	1	5%	2	10%	0.548
	I	1	5%	0	0%	0.311
	D	1	5%	6	30%	**0.037**
	P	17	85%	12	60%	0.077
III-V (RE)	\	0	0%	1	5%	0.311
	D	0	0%	1	5%	0.311
	P	20	100%	18	90%	0.147
III-V (LE)	\	0	0%	2	10%	0.147
	D	0	0%	1	5%	0.311
	P	20	100%	17	85%	0.072
I-V (RE)	\	0	0%	1	5%	0.311
	D	1	5%	7	35%	**0.018**
	P	19	95%	12	60%	**0.008**
I-V (LE)	\	1	5%	2	10%	0.548
	D	1	5%	8	40%	**0.008**
	P	18	90%	10	50%	**0.006**
INTERAURAL DIFFERENCE - V	\	1	5%	2	10%	0.548
	NL	19	95%	18	90%	0.548
INTERAURAL DIFFERENCE I-V	\	1	5%	2	10%	0.548
	NL	19	95%	18	90%	0.548

Two proportion z-test

Subtitle: RE = right ear; LE = left ear; IA = initial assessment; SA = sequential assessment; N = number of subjects ; \ = absent; D = decreased; P = preserved; I = increased; NL = normal; % = percent

Table 5. Relationship between mean of electrophysiological thresholds with microcephaly and time of onset of the symptoms in the initial assessment and sequential assessment

Microcephaly x threshold		Mean	Median	Standard Deviation	N	P-value
IA (RE)	No	41.7	40	11.7	6	0.081
	Yes	34.3	30	9.4	14	
IA (LE)	No	36.7	35	8.2	6	0.638
	Yes	37.9	30	16.3	14	
SA (RE)	No	36.7	35	8.2	6	0.575
	Yes	36.2	30	11.2	13	
SA (LE)	No	33.3	30	5.2	6	0.74
	Yes	34.2	30	5.1	12	
Time of onset of the symptoms x threshold		**Mean**	**Median**	**Standard Deviation**	**N**	**P-value**
IA (RE)	1st Sem.	35.5	30	10.4	11	0.533
	2nd/3rd Sem.	37.5	35	9.6	4	
IA (LE)	1st Sem.	34.5	30	6.9	11	0.503
	2nd /3rd Sem.	47.5	35	28.7	4	
SA (RE)	1st Sem.	35	30	9.7	10	0.115
	2nd /3rd Sem.	45	45	12.9	4	
SA (LE)	1st Sem.	35	35	5.3	10	0.626
	2nd /3rd Sem.	33.3	30	5.8	3	

Two proportion z-test

Subtitle: RE = right ear; LE = left ear; N = number of subjects; IA = initial assessment; SA = sequential assessment; Sem. = semester

population demonstrated the relationship between ZIKV and disorders in sensory functions, such as vision and hearing[16], and the Ministry of Health recently determined the presence of microcephaly as an RIHL[17]. In view of this panorama, this study assessed the auditory function of 20 newborns, children of mothers infected by ZIKV during pregnancy, in order to characterize the ABR findings, between birth and 6 months of life.

The data analysis obtained in (Table 1) confirmed the possible relationship between contamination by ZIKV and occurrence of microcephaly, since most patients in the sample had this sign. Therefore, it reinforced the causal relationship between ZIKV infection and occurrence of microcephaly, suggested in previous studies[17].

It was also observed in (Table 1) that most mothers reported the ZIKV symptoms in the first trimester of pregnancy. This data is similar to that from previous studies[18] and reaffirms the Ministry of Health's guideline that it is important to guide women and couples on the prevention of ZIKV infection throughout pregnancy, but mainly in the first trimester[7]. Agreeing with data showed in this research in (Table 1), a study published in 2018, containing a sample of 19 children with microcephaly due to ZIKV, showed that the most children were infected in the first trimester of pregnancy[13].

Results of this study (Table 2) demonstrated the descriptive data of variables analyzed in the ABR, in the initial and sequential assessments. It was noted that most variables had low variability, because the variation coefficient (VC) was less than 50%. Result shows that the data are homogeneous. It was noticed that there was a decrease in the values of absolute latencies (III and V) and interpeaks (I-III, III-V and I-V), in the sequential assessment. The data (Table 2) also showed that infection by ZIKV did not significantly change the electrophysiological thresholds of newborns at any moments. A study carried out in the state of Pernambuco, in 2015 and 2016, confirmed the possible relationships between congenital ZIKV infection and presence of hearing disorders. However, the authors reported results related only to the type of hearing loss, where of the 69 children with microcephaly, four had sensorineural hearing loss[5]. Another study reported the case of a newborn with microcephaly, whose click ABRs showed no response bilaterally and specific frequency ABRs with toneburst stimulus confirmed bilateral hearing loss, with the presence of response only at 99 dBHL at 2000 Hz, to the right ear[4]. A more recent study, carried out using ABR in children with microcephaly due to ZIKV, did not assess the electrophysiological thresholds, only integrity of the auditory pathway[13]. Although there is still no consensus regarding possible threshold changes in this population, research on this data is important, due to the need for auditory monitoring recommended by the Multiprofessional Committee on Hearing Health *(Comitê Multiprofissional em Saúde Auditiva - COMUSA)* and the Joint Committee on Infant Hearing (JCIH), which in the 2019 Position Statement, considered that exposure to ZIKV during pregnancy, or with consistent SZC results, should be tested with neonatal hearing screening at birth, preferably through ABR.

It was found that quantitative differences of the variables analyzed in the ABR, in the two moments of assessment, were significant in relation to the absolute latency of waves III, V and interpeak intervals, for both ears (Table 3). There was no significant difference in wave I, interaural difference in wave V and interpeak I-V, or in the electrophysiological threshold.

According to literature, wave I is generated in the distal portion of the cochlear nerve, informs the speed of peripheral conduction and is practically mature at birth[19]. This indicates that the maturation of auditory pathways involves different mechanisms in the central and peripheral areas, since the conduction of stimulus depends on changes in speed associated with myelination and changes in the synaptic efficiency of various nuclei of the auditory pathway[20]. Thus, the stability in relation to latency of wave I found in this study is justified by this fact and confirms the results of previous studies[19-22].

In contrast, there was a significant difference for the occurrence of waves III and V and all interpeaks (Table 3). Comparing the tests in the two moments, it was possible to notice a decrease in latencies of these waves between the initial and sequential tests. Wave III is formed in the region of upper olive complex (pons) and wave V, at the level of lateral lemniscus (lower midbrain). The literature clearly establishes that the maturation process of auditory pathway occurs in the caudal-rostral order, that is, the more rostral the structure, the longer it takes to reach full maturation [23]. The development process of the auditory system occurs through the increase of neuronal myelination and greater synchronization of electrical conduction, which in the prenatal phase, is directed by intrinsic biological factors to the individual. In this phase, development can be altered by genetic factors or changes in metabolic control. In the perinatal and postnatal phases, a priori, it is sensory deprivation that has a negative impact on auditory development[24].

Thus, it can be interpreted that the difference observed in latency of waves III and V in most babies is explained by the occurrence of auditory maturation between the tests at birth and 6 months of age. Regarding the analysis of interpeak intervals I-III, III-V and IV (Table 3), there was a significant difference between the moments, determined by the decrease in latency of intervals I-III, III-V and IV, confirming the occurrence of auditory maturation between the initial and sequential tests. This data converges with literature, which establishes that, as the auditory pathways maturation, there is also a shortening of absolute wave latencies and interpeak intervals, with latency of wave V being the last to decrease [23,25].

Still in relation to these data, there was a relevant aspect that was not evidenced in the inferential analysis, but was observed in the descriptive analysis. For two babies in the sample, both with microcephaly, waves I, III and V were present in the initial ABR and absent in the sequential ABR. For the first baby, this occurred to both ears and, and for the second only to the left ear. This evidence reveals the possibility that SCZ may cause delayed-onset disorders in the auditory pathway, but other studies will be needed to elucidate this issue. However, an example of another type of congenital viral infection, cytomegalovirus, which can cause hearing loss of delayed-onset, which cannot be identified at birth[10], these data already demonstrate the need for audiological monitoring of babies affected by ZIKV until at least 30 months of life, as recommended by the JCIH[8].

The maturation level reveals the conduction speed and effectiveness of synapses along the auditory nerve to the brainstem in neonates[21]. According to literature, ABR responses in neonates and infants are influenced by the maturation process of the auditory system[25]. In the case of premature neonates, the effect of maturation is even more evident and thus, the pattern of response of these children is different from those born at term[19,26]. The sample of this study included only newborns born at term. Since most of them showed signs of auditory pathway maturation, with decreased latencies, compared to normal values, a relationship between ZIKV contamination and abnormal maturation pattern of brainstem auditory structures is assumed. Studies already conducted regarding toxicity power of ZIKV showed that the virus works by damaging cells that give rise to neurons, thereby impairing neural communication, causing a decreased cortex and even hypoplasia at the brainstem level[18].

Regarding the analysis of interaural differences in the interpeak interval IV, or the absolute latency of wave V (Table 3), no statistically significant difference was observed between the moments, since changes observed in the absolute latencies of waves III and V, between the two tests, resulting from the observed maturation process, did not significantly change the values of interpeak intervals.

For analysis of what is shown in Table 4, it is noteworthy that, as there is no control group in this study, values found were matched by age group, using reference criteria established in the literature[14]. Therefore, in general, wave I tended to achieve preserved latency values over time. In contrast, waves III and V and interpeaks had their latency values decreased over time, compared to normal values (control). The hypothesis for such finding is due to the cytotoxic power of ZIKV. The literature on neurobiology of the ZIKV states that infection by the virus in humans leads to decreased neurological growth, due to direct suppression of neurogenesis by the action of non-structural ZIKV proteins (NS4A and NS4B) and cell apoptosis. It is suggested that there is a specific vulnerability of the neural precursor cells to infection, leading to cell death. Moreover, there are receptors on the cell membrane of neuronal stem cells that facilitate viral endocytosis and are capable of promoting cell signaling that alters the neurogenesis and cell survival[27]. Thus, it is suggested that latencies observed in the ABR were reduced due to structural shortening of the brainstem, caused by ZIKV, when compared to the normal standards established in the literature for neurologically normal children.

There was no correlation between the electrophysiological threshold and microcephaly, or the time of onset of the mother's symptoms (Table 5). Future studies, with larger samples, may better elucidate the existence, or not, of this relationship.

Also, it was not possible to observe a statistically significant correlation between ABR latencies, both in the initial and sequential tests, with microcephaly and the moment of onset of the mother's symptoms during pregnancy. Studies have shown that, regarding viral infections, the risk of brain damage, as well as the severity and extent of disorders, have a direct relationship with the gestational period in which the fetus was infected. Neurological sequelae tend to be more severe and extensive when infection occurs during the first trimester of pregnancy and milder when it occurs in the third trimester[28]. Thus, it is believed that future analyses, with larger samples, will be necessary to better evidence the existence, or not, of this relationship, in the case of SCZ.

Confirming the finding highlighted in Table 5, a study by Marques Abramov et al.[13] also found no significant correlation between ABR wave latencies and head circumference of the children assessed, concluding that there is no dependence between microcephaly and changes in the auditory pathway at brainstem level. On the other hand, a study published in 2010[29], showed that the absolute latency of wave V and interpeak latencies III-V and I-V were significantly larger in microcephalic children without ZIKV, than in typical children. This study concluded that hearing impairment in microcephaly is a common neurological deficit, which can be authentically assessed using ABR, and that hearing impairment in microcephalics is mainly due to insufficiency of central components of the auditory pathway at brainstem level. In this context, analyses of the wave amplitudes would be interesting to elucidate this question, since the amplitude of ABR tracing reveals the amount of fibers activated in response to the auditory stimulus.

Finally, it is emphasized that current studies are not able to guarantee the ZIKV activity in the auditory pathway. Hypotheses are postulated, but they are not able to confirm the cause of

hearing loss in these cases, whether it is peripheral or central, permanent or temporary, progressive, or not.

This study had as main limitation the number of babies followed longitudinally. More representative samples would be needed to generalize the results. Moreover, auditory monitoring up to three years of age would be important, in an attempt to verify the cytotoxic potential of ZIKV to cause delayed-onset hearing loss.

Future studies following animal models will allow seeking for answers regarding the pathogenesis of ZIKV. Studies on patients with SCZ, through long-term follow-up, are needed to answer about the characteristics of hearing impairments in these patients and to determine the guidelines for monitoring these cases[30].

CONCLUSION

The comparison between initial and sequential assessments showed that babies with Zika had electrophysiological thresholds within normal limits and decreased absolute latencies of waves III and V and interpeaks, during semi annual monitoring, if compared with the reference values (control) for the age group, confirming the cytotoxic action of ZIKV and not related to the presence or absence of microcephaly. There were two cases of significant worsening of the electrophysiological threshold, referring to the possibility of delayed-onset hearing disorders. There was no correlation between ABR results and the time of onset of symptoms during pregnancy, or presence of microcephaly.

REFERENCES

1. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Secretaria de Atenção à Saúde. Zika virus in Brazil: the SUS role. Brasília: Ministério da Saúde; 2017. 136 p.
2. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Síndrome congênita associada à infecção pelo vírus Zika: situação epidemiológica, ações desenvolvidas e desafios, 2015 a 2019. Bol Epidemiol. 2019;50(N. esp.):1-31.
3. World Health Organization. Screening, assessment and management of neonates and infants with complications associated with Zika virus exposure in utero. Geneva: WHO; 2016.
4. Leal MC, Muniz LF, Caldas SS No, Van Der Linden V, Ramos RC. Sensorineural hearing loss in a case of congenital Zika virus. Braz J Otorhinolaryngol. 2020;86(4):513-5. https://doi.org/10.1016/j.bjorl.2016.06.001.
5. Leal MC, Muniz LF, Ferreira TSA, Santos CM, Almeida LC, Van Der Linden V, et al. Hearing loss in infants with microcephaly and evidence of congenital Zika virus infection: Brazil, November 2015–May 2016. MMWR Morb Mortal Wkly Rep. 2016;65(34):917-9. http://dx.doi.org/10.15585/mmwr.mm6534e3. PMid:27585248.
6. Ashwal S, Michelson D, Plawner L, Dobyns W, Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Practice parameter: evaluation of the child with microcephaly (an evidence-based review). Neurology. 2009;73(11):887-97. http://dx.doi.org/10.1212/WNL.0b013e3181b783f7. PMid:19752457.
7. Brasil. Protocolo de atenção à saúde e resposta à ocorrência de microcefalia. Brasília: Ministério da Saúde; 2016.
8. Joint Committee on Infant Hearing. Year 2019 position statement: principles and guidelines for early hearing detection and intervention programs. J Early Hear Detect Interv. 2019;4(2):1-44.
9. Stach BA, Ramachandran VS. Hearing disorders in childrren. In: Madell, J.R., Flexer C, editors. Pediatric audiology: diagnosis, technology, and management. 2nd ed. New York: Thieme; 2008. p. 3-12.
10. Cohen BE, Durstenfeld A, Roehm PC. Viral causes of hearing loss: a review for hearing health professionals. Trends Hear. 2014;18:1-17. http://dx.doi.org/10.1177/2331216514541361. PMid:25080364.
11. Fontes AA, Carellos EV, Romanelli RC. Study of brainstem auditory evoked potentials in early diagnosis of congenital toxoplasmosis. Braz J Otorhinolaryngol. 2019;85(4):447-55. https://doi.org/10.1016/j.bjorl.2018.03.012.
12. Borja A, Loiola AG. Triagem auditiva em crianças expostas ao zika vírus durante a gestação. Rev. Ciênc. Méd. Biol. 2017;16(3):271-6. http://dx.doi.org/10.9771/cmbio.v16i3.24387.
13. Marques Abramov D, Saad T, Gomes-Junior SC, de Souza E Silva D, Araújo I, Lopes Moreira ME, et al. Auditory brainstem function in microcephaly related to Zika virus infection. Neurology. 2018;90(7):e606-14. http://dx.doi.org/10.1212/WNL.0000000000004974. PMid:29352094.
14. Rosa LAC, Suzuki MR, Angrisani RG, Azevedo MF. Potencial evocado auditivo de tronco encefálico: valores de referência em relação à idade. CoDAS. 2014;26(2):117-21. https://doi.org/10.1590/2317-1782/2014469IN.
15. Brasil. Ministério da Saúde. Secretaria de Vigilância da Saúde. Protocolo de vigilância e resposta à microcefalia relacionada à infecção pelo vírus Zika. Versão 12 - 09/12/2015. Brasília: Ministério da Saúde; 2015.
16. Ribeiro BNF, Muniz BC, Gasparetto EL, Ventura N, Marchiori E. Congenital Zika syndrome and neuroimaging findings: what do we know so far? Radiol Bras. 2017 Set-Out;50(5):314-22. PMid:29085165.
17. Fantinato FF, Araújo EL, Ribeiro IG, Andrade MR, Dantas AL, Rios JM, et al. Descrição dos primeiros casos de febre pelo vírus Zika investigados em municípios da região Nordeste do Brasil, 2015. Epidemiol Serv Saude. 2016;25(4):683-90. http://dx.doi.org/10.5123/S1679-49742016000400002. PMid:27869981.
18. Zare Mehrjardi M, Poretti A, Huisman TAGM, Werner H, Keshavarz E, Araujo Júnior E. Neuroimaging findings of congenital Zika virus infection: a pictorial essay. Jpn J Radiol. 2017;35(3):89-94. http://dx.doi.org/10.1007/s11604-016-0609-4. PMid:28074379.
19. Schuler-Faccini L, Ribeiro EM, Feitosa IML, Horovitz DDG, Cavalcanti DP, Pessoa A, et al. Possível associação entre a infecção pelo vírus zika e a microcefalia: Brasil, 2015. Weekly. 2016;65(3):59-62. PMid:26820244.
20. Amorim RB, Agostinho-Pesse RS, Alvarenga KF. The maturational process of the auditory system in the first year of life characterized by brainstem auditory evoked potentials. J Appl Oral Sci. 2009;17(N. spe.):57-62. https://doi.org/10.1590/S1678-77572009000700010.
21. Guilhoto LMFF, Quintal VS, da Costa MTZ. Brainstem auditory evoked response in normal term neonates. Arq Neuropsiquiatr. 2003;61(4):906-8. http://dx.doi.org/10.1590/S0004-282X2003000600003. PMid:14762588.
22. Ken-Dror A, Pratt H, Zeltzer M, Sujov P, Katzir J, Benderley A. Auditory brain-stem evoked potentials to clicks at different presentation rates: estimating maturation of pre-term and full-term neonates. Electroencephalogr Clin Neurophysio. 1987 Maio 1;68(3):209-18. http://dx.doi.org/10.1016/0168-5597(87)90028-1. PMid:2436880.

23. Robertson D. Functional significance of dendritic swelling after loud sounds in the guinea pig cochlea. Hear Res. 1983;9(3):263-78. http://dx.doi.org/10.1016/0378-5955(83)90031-X. PMid:6841283.

24. Angrisani RG, Diniz EM, Guinsburg R, Ferraro AA, Azevedo MF, Matas CG. Auditory pathway maturational study in small for gestational age preterm infants. CoDAS. 2014;26(4):286-93. http://dx.doi.org/10.1590/2317-1782/201420130078. PMid:25211687.

25. Morgan DE, Zimmerman MC, Dubno JR. Auditory brain stem evoked response characteristics in the full-term newborn infant. Ann Otol Rhinol Laryngol. 1987 Mar 29;96(2 Pt 1):142-51. http://dx.doi.org/10.1177/000348948709600202. PMid:3566056.

26. Moore JK, Linthicum FH Jr. The human auditory system: a timeline of development. Int J Audiol. 2007;46(9):460-78. http://dx.doi.org/10.1080/14992020701383019. PMid:17828663.

27. Sleifer P, da Costa SS, Cóser PL, Goldani MZ, Dornelles C, Weiss K. Auditory brainstem response in premature and full-term children. Int J Pediatr Otorhinolaryngol. 2007;71(9):1449-56. http://dx.doi.org/10.1016/j.ijporl.2007.05.029. PMid:17629955.

28. Li H, Saucedo-Cuevas L, Shresta S, Gleeson JG. The neurobiology of Zika Virus. Neuron. 2016;92(5):949-58. http://dx.doi.org/10.1016/j.neuron.2016.11.031. PMid:27930910.

29. Das P, Bandyopadhyay M, Ghugare BW, Ghate J, Singh R. Auditory evaluation of the microcephalic children with brain stem evoked response audiometry (BERA). Indian J Physiol Pharmacol. 2010;54(4):376-80. PMid:21675037.

30. Barbosa MHM, Magalhães-Barbosa MC, Robaina JR, Prata-Barbosa A, Lima MAMT, Cunha AJLA. Auditory findings associated with Zika virus infection: an integrative review. Rev Bras Otorrinolaringol (Engl Ed). 2019;85(5):642-63. http://dx.doi.org/10.1016/j.bjorl.2019.05.002.