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Clinical follow-up of children with *in utero Zika virus* exposure in the Metropolitan Region of Belém, Pará State, Brazil

Seguimento de crianças expostas intraútero ao vírus Zika na Região Metropolitana de Belém, Pará, Brasil

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ABSTRACT

INTRODUCTION: The emergence of the *Zika virus* (ZIKV), which severely affected Northeastern Brazil, revealed the occurrence of congenital malformations during pregnancy. Currently, changes have been recorded from physical abnormalities, such as microcephaly, arthrogryposis, and brain abnormalities detected by neuroimaging tests, to behavioral changes as irritability and excitability. OBJECTIVE: From this perspective, a multidisciplinary approach was conducted by clinical follow up and lab and imaging tests, with emphasis on investigating the neurological and psychomotor development, vision and hearing in order to get information on maternal and fetal infection. MATERIALS AND METHODS: For that purpose, 92 children born to women infected by ZIKV during pregnancy were followed up from August 2017 to July 2018. RESULTS: Among the patients investigated, 55 (59.8%) children were male; 46 (50.0%) mothers were infected in the second trimester of pregnancy; two (2.2%) children had microcephaly at birth and one (1.1%) presented signs and symptoms associated with postnatal microcephaly. In addition, during clinical follow-up, behavioral changes that could affect child neurodevelopment were found, such as extreme irritability, with significant incidence ($p < 0.0001$), followed by aggressiveness and hyperexcitability, despite normal imaging tests. CONCLUSION: According to these results, must be enhanced the need for multi-professional follow up of children with *in utero* ZIKV exposure in order to identify early and late changes associated with ZIKV and to conduct psychomotor activities that can reduce the sequelae in child neurodevelopment.

Keywords: *Zika Virus*; Pregnancy; Infection; Child Development; Microcephaly.

RESUMO

INTRODUÇÃO: A emergência do vírus Zika (ZIKV), que afetou gravemente o nordeste do Brasil, revelou a ocorrência de malformações congênitas durante a gestação. Atualmente, têm sido registradas alterações que envolvem desde anormalidades físicas, como microcefalia, artrogripose e anomalias cerebrais detectadas por exames de neuroimagem, até alterações de comportamento, como irritabilidade e excitabilidade. OBJETIVO: Sob essa perspectiva, procurou-se, por meio do acompanhamento clínico e de exames laboratoriais e de imagem, uma abordagem multiprofissional com ênfase na investigação do desenvolvimento neuropsicomotor, da visão e da audição para a obtenção de informações sobre a infecção materno-fetal. MATERIAIS E MÉTODOS: Para tal, 92 crianças nascidas de mulheres infectadas durante a gravidez pelo ZIKV foram acompanhadas no período de agosto de 2017 a julho de 2018. RESULTADOS: Entre os investigados, 55 (59,8%) crianças eram do gênero masculino; 46 (50,0%) mães foram infectadas no segundo trimestre da gestação; duas (2,2%) crianças apresentaram microcefalia ao nascimento e uma (1,1%) apresentou características clínicas compatíveis com microcefalia pós-natal. Ademais, no acompanhamento, constatou-se a existência de alterações de comportamento que podem comprometer o neurodesenvolvimento infantil, como a irritabilidade extrema, com incidência significativa ($p < 0,0001$), seguida de agressividade e hiperexcitabilidade, apesar de exames de imagens normais. CONCLUSÃO: Diante desses achados, reforça-se a necessidade de um acompanhamento multiprofissional sistemático das crianças expostas intraútero, para identificar alterações precoces e tardias associadas ao ZIKV e implementar atividades psicomotoras que possam atenuar as sequelas no neurodesenvolvimento infantil.

Palavras-chave: Zika Vírus; Gravidez; Infecção; Desenvolvimento Infantil; Microcefalia.

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INTRODUCTION

The emergence of *zika virus* (ZIKV) in the Americas, which severely affected northeastern Brazil in the first semester of 2015, revealed the occurrence of congenital malformations during pregnancy, showing to the need for integrated monitoring with other pathogens more frequently related to intrauterine infections[1]. Accumulated evidence of clinical studies, particularly those conducted in Brazil, serve as a basis for American researchers from the Centers for Disease Control and Prevention who recognized the causal relationship between the occurrence of microcephalyand other brain damage identified in fetuses with zika virus[2,3].

Currently, changes have been recorded among neonates: microcephaly, craniofacial disproportion, spasticity, convulsions, irritability and brain abnormalities detected by neuroimaging[4]. Such changes referred to ZIKV infection during pregnancy may suggest a congenital syndrome similar to congenital rubella or cytomegalovirus infection[5,6]. The evaluation of these newborns showed that ZIKV, besides being a neurotropic virus, also presents tropism for other organs, such as liver and heart, in addition to important visual and hearing alterations[7].

Current clinical studies have found that other abnormalities can be identified at birth, besides microcephaly, such as craniocaudal disproportion, arthrogriposis (joint contractures) and central nervous system disorders (cerebral calcifications and atrophy, ventricular dilation and cerebellar hypoplasia)[8,9]. It was also observed, in the clinical follow-up of children with in utero Zika virus exposure, the presence of abnormalities in their development and growth, such as hypertonicity, clonus, hyperreflexia, involuntary movements, spasticity, contractures and convulsions, justifying the current recommendation of the Ministry of Health to follow those children up to 3 years of age by a multidisciplinary team[6,8,9].

Based on these findings and the absence of clinical and epidemiological data in the Northern Region, the present study aims to identify early and late clinical and imaging changes, in children exposed to in utero ZIKV infection. From this perspective, a multiprofessional approach will be sought through clinical and laboratory follow-up, with emphasis on the investigation of, vision and hearing neuropsychomotor development in order to obtain more information about maternal and fetal infections.

MATERIALS AND METHODS

This is a prospective, longitudinal research with clinical follow-up of children exposed to intrauterine ZIKV infection, in the Metropolitan Region of Belém, from August 2017 to July 2018. The research activities (clinical evaluation of patients, blood test and specialized evaluations) were conducted at the Instituto Evandro Chagas, Belém *campus*. The present study was developed following the rules from the Declaration of Helsinki and the Nuremberg Code, in addition to respecting the Ethical Standards of Scientific Research Involving Human Beings, according to Resolution No. 466/12 of the National Health Council, after approval by the Research Ethics Committee of the Instituto Evandro Chagas, with opinion no. 2,288,316, on August 19, 2017. All those responsible for the individuals involved in the research presented the signed Free and Informed Consent Form.

Preceding this study, the investigation was performed with 308 pregnant women with exanthem and suspected of ZIKV infection, who were referred to the Evandro Chagas Institute from November 2015 to December 2017, and clinical follow-up was performed after laboratory-confirmed ZIKV infection in 134 pregnant women and, of these, 109 were followed up until the end of pregnancy, however with the results still under analysis and they should contribute to clarify the macro-regional differences. Of the total number of live births from this group, 92 women were selected for the current research, based on the inclusion criteria and because they live in the Metropolitan Region of Belém. Children whose guardians did not accept to participate in the study or who gave up during its performance were excluded from the study, besides those who could not follow up during the study.

For the clinical evaluation of each child, including identification and description of malformations of newborns and/or infants, the definitions of the Integrated Guidelines for Surveillance and Health care in the Scope of the Public Health Emergency of National Importance were adopted[6]. The protocol used in this research followed the investigation questionnaire for microcephaly of the Ministry of Health in the Surveillance and Response Protocol for the Occurrence of Microcephaly Health - Version 1.3 of 2016[10].

In this group, clinical evaluations of children previously scheduled were performed with an interval of two to three months, for children up to 1 year of age, and an interval of six months from 2 years of age. Of the total number of pediatric consultations conducted during the follow-up period, the average was 5.7 consultations for each child. At the first appointment, the clinical evaluation was performed with a physical examination, with recording of anthropometric measurements (weight, height, head circumference, thoracic perimeter), investigation of congenital anomalies, incidence of seizures and other complications of the neonatal period, assessment of neurological functions and neurocognitive development, newborn screening tests, with emphasis on newborn hearing screening – NHS (hearing test) and vision screening test for infant (eye test) and analysis of the vaccination card. Whenever necessary, specific laboratory tests could be requested for ZIKV and differential diagnosis for dengue *viruses (Dengue virus* – DENV) and Chikungunya *(Chikungunya virus* – CHIKV), in addition to TORCH agents (toxoplasmosis, other infections, rubella, cytomegalovirus infection, herpes).

For the total RNA extraction of the 45 (28.9%) bloods/sera and one (1.1%) umbilical cord blood, the Promega Maxwell extraction system was used, following

the manufacturer's protocol. The virus genome detection was carried out according to the method described by Domingo et al.[11th]. This molecular methodology is recommended in the investigation of newborns exposed to *in utero* ZIKV in order to confirm congenital infection.

The enzyme immunoassay (ELISA) for the detection of IgM antibodies anti-ZIKV, DENV and CHIKV was performed in 64 (69.56%) sera, according to Martin et al.[12], and in 92 sera for TORCH agents. For the detection of viral antigens in the 15 placental fragments, the immunohistochemistry (IHC) was used[13]. In addition, a child with postnatal microcephaly characteristics performed reverse transcriptase polymerase chain reaction (RT-PCR) because s/he presented negative ZIKV tests.

After this stage, the neurological evaluation was indicated in case of changes in the physical examination and/or observations of the guardians, for subsequent indication of imaging exams – computed tomography (CT) and magnetic resonance imaging (MRI) of the skull, besides transfontanela ultrasound (US) and electroencephalogram (EEG). These tests were carried out according to neuropediatric indication and availability in public/private services.

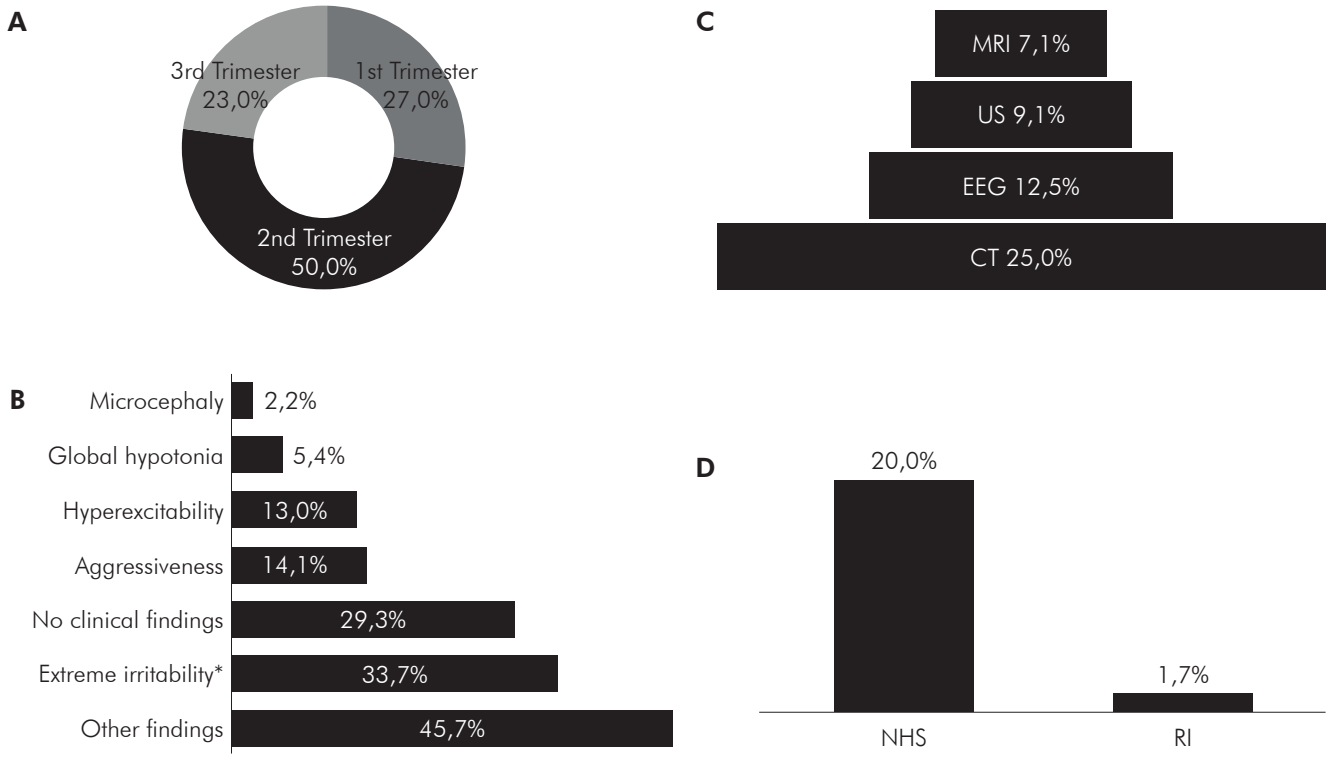
It is noteworthy that, if during pediatric consultations it was found that the child did not perform any newborn screening test (NHS and eye test), the present study created evaluations in groups (task forces), with professional and specialized services to carried out retinal imaging (RI) and otolaryngology evaluation.

Analytical statistics were used in order to evaluate the results of categorical variables of the sample, using the Adherence Chi-Square tests for univariate analysis, and Partitioning Chi-Square for the table of clinical findings per trimester of gestation. Descriptive and analytical statistics were performed using the BioEstat software® v5.4.

RESULTS

Of the total number of children in this study, 55 (59.8%) were boys and 37 (40.2%) girls, there is no significant difference (p = 0.0763) in the gender ratio.

Regarding the gestational age exposed to the virus, it was identified that most mothers were infected in the second trimester of pregnancy (50.0%; n = 46), according to Figure 1A, with a significant incidence of exposure to the virus during this period (p = 0.0028). However, in the relationship between the incidence of clinical changes identified during clinical follow-up and the gestational trimester of infection by this arbovirus, there was no statistically significant difference (p = 0.8727).



A: Distribution of children according to gestational age in which they were exposed to ZIKV. **B**: Clinical findings identified during the clinical follow-up of children. **C**: Distribution of imaging exams with different results in children under clinical follow-up. **D**: Distribution of the altered results found in screening tests of children under clinical follow-up.

NHS: Neonatal hearing screening tests; RI: Retinal imaging; MRI: Magnetic resonance imaging of the skull; US: Transfontanela ultrasound; EEG: Electroencephalogram; CT: Computed tomography of the skull; *: p< 0.0001, AdherenceChi-Square Test.

Figure 1 – Distribution of clinical, laboratory and imaging findings of children exposed to intrauterine ZIKV in the Metropolitan Region of Belém, Pará State, Brazil, 2017–2018

Regarding the prevalence of malformations in the evaluated group, two (2.2%) children presented microcephaly at birth, with laboratory confirmation of ZIKV infection by detecting viral antigens in the maternal placenta by IHC and IgM anti-ZIKV antibodies in cerebrospinal fluid by ELISA. It is emphasized that one (1.1%) ZIKV negative child, by RT-PCR and ELISA, presented clinical characteristics compatible with postnatal microcephaly, due to the deceleration in cephalic growth after the first year of age, with change of the head circumference growth curve adopted by the Ministry of Health[6].

Regarding the changes of sensorineural hearing loss described in the Protocol of the Ministry of Health[6,] irritability, aggressiveness and hyperexcitability were recorded in this study as the most frequent ones, with 33.7% (n = 31), 14.1% (n = 13) and 13.0% (n = 12), respectively. There are also, although less frequent, cases of global hypotonia in five (5.4%) children. Other important findings (45.7%) include tremor, motor and speech disorder, premature thelarche, hyperreflexia, convulsion, hyperactivity, and low-set ear (Figure 1B). Genitourinary alterations were also found, considered rare in association with ZIKV, such as adhesions of the labia minora, cryptorchidism and hypospadias. All these changes were identified during the clinical follow-up of the children.

In the children laboratory analysis, 80.4% (n = 74) were under investigation of vertical transmission of ZIKV infection after birth. Of the samples tested, only three (3.2%) children (two microcephalic children and one without microcephaly) had laboratory confirmation of intrauterine infection by ZIKV. There was one (1.1%) molecular and serological positive result by ELISA technique, and two others resulted from clinical specimens obtained during the neonatal period: one in umbilical cord blood, by RT-PCR, and one in placenta fragment, by IHC. All these samples had increased investigation for other arboviruses, with negative results for DENV, CHIKV and TORCH agents.

Regarding imaging, 53 were carried out, adding 12 CT, 11 US, 14 MRI and 16 EEG, corresponding to 35.9% (33/92) of the children in this study after identifying any change in the clinical and neuropediatric evaluation or from reports of children's guardian that suggest neurological changes.

Of the 12 CT performed, three (25.0%) presented alterations: two in children with microcephaly at birth and one in children under investigation of postnatal microcephaly. All of them had calcifications in common, but in different regions, such as in parietal, parenchyma cells and in the cerebral white matter. Among the other changes found, the following ones stand out: skull reduction, dilation of the ventricles, small cerebellum, corpus callosum dysgenesis, increase of subarachnoid spaces and encephalomalacia areas. In US, a nonmicrocephalic infant showed a reduction in brain volume (frontal area), later confirmed by skull MRI.

It was possible to identify that, among the skull MRI performed, one (7.1%) microcephalic infant showed abnormalities such as calcifications, gyration anomalies, hypoplasia of the corpus callosum, cortical changes and brain disproportion. With the performance of the EEG, irritative activity was recorded with different proportions in the incidence of discharges and in the number of epileptogenic zones in both (12.5%) microcephalic children at birth (Figure 1C).

For the early identification of hearing loss, NHS tests were performed in 60 (65.2%) children in the current study. Of these, 12/60 (20.0%) children had absent response, especially on the left side. In the visual assessment, 58/92 (63.0%) eye test and MR tests were conducted, and no ocular abnormality was identified, such as pigmentary, atrophic or hypoplastic lesions in the macula or optic nerve. However, there was the identification, in MR, of bilateral total congenital cataract in a child and until that present moment, there is no report in the literature of association with ZIKV infection (Figure 1D).

DISCUSSION

Scientific evidence indicates that infants with normal head circumference and without visible congenital malformations may present developmental damage, in a medium to long term periods, associated with ZIKV[14], which justifies the current clinical follow-up recommendations of children exposed to intrauterine ZIKV, with the objective of early identifying signs of restrictions in neuropsychomotor development.

Although gestational age at the time of infection is an important factor[15], data found in the present study do not show that the earliest gestational age of virus exposure induces the most severe condition, and may cause congenital malformations regardless of the gestational age that the mother became infected. Despite this, in 2018, Orofino et al.[7] found children with heart involvement who had intrauterine exposure to ZIKV, most of them (53.8%) in the second trimester of pregnancy.

In addition, one of the infection outcomes in the first trimester of pregnancy, observed in this study, is related to the malformation of the central nervous system in one of the microcephalic children in the study, during the 12th week. On the other hand, in the study by Faria et al.[16], the potential risk peak was reported between the 14th and 17th week of pregnancy, including, therefore, the first period of the second trimester. This period, in the present study, corresponded to the moment of congenital infection of the second child with microcephaly.

Although the congenital syndrome associated with ZIKV brings together well-defined changes, such as microcephaly, specific imaging findings of the central nervous system and visual and auditory deficits[17,18,19], it has been observed that there is a group of other clinical changes that may arise during the clinical follow-up of these children, such as severe irritability, hypotonia, hypertonia, hyperreflexia, spasticity and convulsions[20].

Thus, congenital ZIKV infection may result in atypical neurological manifestations, far beyond isolated

microcephaly, which have been identified during the clinical follow-up of these children with signs and symptoms such as irritability, hyperexcitability and hypotonia, all them considered risk factors for changes in neurodevelopment[21].

In the present study, changes in the genitourinary system were identified, such as hypospadias, cryptorchidism and adhesions of the labia minora, which corroborates the findings of Costello et al.[22], by suggesting that the digestive and genitourinary systems may be included in the congenital syndrome induced by ZIKV, despite the fact that rare clinical changes in the clinical spectrum of children exposed to intrauterine infection are considered.

In the analysis of children submitted to laboratory investigation after birth, three samples were positive, one confirmed by ELISA technique and two by clinical specimens obtained in the neonatal period. The difficulty in performing molecular and serological tests in the present study was mainly due to the absence verification for these tests at the exact time at birth, or specific serological tests to decrease cross-reactions with other flaviviruses, as well as logistical difficulties in transporting and storing the materials to the reference laboratory. This situation is similar to the problems faced by Castro et al.[23] in 2017.

The results of negative laboratory tests in children should not be interpreted as determinants for the interruption of longitudinal clinical follow-up, it is essential screening tests associated with neuroimaging tests in children exposed to intrauterine ZIKV infection[24]. Normocephalic children at birth are also included, such as those found in the present study, but who should receive early attention regarding complementary brain study techniques, since only exposure is a risk factor for possible cognitive delays in this silent and unknown scenario[8].

On skull CT, the presence of coarse heterogeneous calcifications is considered a inclusion criteria for congenital ZIKV infection, as described by Souza et al.[25], and it is associated with microcephaly. As for skull MRI, the main abnormalities already described in the literature were found in a microcephalic child[26,27]. US in a non-microcephalic infant revealed frontal lobe reduction, that is not similar to that described by Aragon et al.[26], in a study conducted in 2016, in which this test had no change in children exposed to ZIKV, but when it is present, may suggest virus-induced damage. Regarding EEG, few data have been found on the importance of this test associated with Zika microcephaly; however, epileptiform discharges were identified in children without structural anomalies, which confirms developmental surveillance in long-term intrauterine exposed children.

It is known that sensory impairment is present with two variables (auditory and visual), among the three main impairments of ZIKV exposure during pregnancy, making observations and periodic monitoring of these organs that are essential targets for the clinical follow-up of children, since only neonatal screening does not exclude the organs in later stages of life[26].

Hearing loss associated with other congenital viral infections is well described in the literature; however, this disorder still needs to be elucidated in children with intrauterine exposure to ZIKV infection[28]. In a study conducted with 70 microcephalic children with evidence of congenital ZIKV infection, it was recorded that 7.1% had hearing loss[29]. That finding corroborates what was found in this study, in which there were changes to the evoked otoacoustic emission testing (EOAE) of 20.0% of the children with absent response in the test. It shows the importance of elucidating the exact mechanism underlying hearing loss.

Ocular alterations have been reported in children with confirmed or presumed congenital ZIKV infection, which are chorioretinal atrophy or scars, pigmentation disorders, optic nerve hypoplasia, optic disc pallor, optic disc augmentation, retinal hemorrhages and retinal vascular abnormalities[19,20]. It is also known that the association of ZIKV with ocular changes is due to the tropism of the virus by the organ and is documented in patients with microcephaly, as reported in the study conducted by Verçosa et al.[30] in 2017, in which he identified that 36% of his microcephalic patients had some abnormalities, which were not found in the present study, but that can be justified by the sample of this cohort involving only two (2.2%) microcephalic children.

CONCLUSION

From the results obtained in this research, it was possible to observe that, in addition to the already well-defined situation of congenital syndrome associated with ZIKV, there is a challenging scenario of clinical follow-up of these children, since clinical findings, such as extreme irritability, aggressiveness and hyperexcitability, observed during child development, showing the need for a biopsychosocial and multiprofessional view, thus allowing the identification of early changes and promote intervention measures aimed at mitigating neuropsychomotor complications.

On neonatal screening and additional tests, such as retinal mapping, periodic screening is essential, and it is clear that changes may arise in later stages of childhood. This highlights the importance of determining whether children who have been exposure to this arbovirus during pregnancy will have fluctuations or progressions to abnormalities.

Therefore, is necessary to have further clinical follow-up studies on children exposed to intrauterine ZIKV infection in order to evaluate the long-term consequences on child development and, thus, to promote knowledge and early interventions in the clinical follow-up of these children.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTION OF THE AUTHORS

Dr. Consuelo Oliveira performed specialized medical consultation, she coordinated the study group, participated in the study logistics and gave assistance during the manuscript writing. Dr. Haroldo Matos assisted in the coordination of study group and during the writing of the manuscript. Luna Gomes, Emilene Serra, Marcella Fraiha and Deborah Nunes worked to support the medical team, during the attendance in task forces and medical consultations; they participated in the study logistics (database and recruitment of children for clinical evaluation); and contributed to the manuscript writing.

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