Zika Virus Infection Associated with Autism Spectrum Disorder: A Case Report

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Keywords

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did not reveal any pathogenic variant in genes related to ASD. **Conclusion:** Accordingly, this report firstly associates ZIKV exposure to ASD.

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Abstract

Introduction: The aim of this case was to investigate the association of the Zika virus infection in utero with the autism spectrum disorder (ASD) as clinical outcome that presented no congenital anomalies. **Methods:** ASD was diagnosed in the second year of life by different child neurologists and confirmed by DSM-5 and ASQ. After that, an extensive clinical, epidemiological, and genetic evaluations were performed, with main known ASD causes ruled out. **Results:** An extensive laboratorial search was done, with normal findings. SNP array identified no pathogenic variants. Normal neuroimaging and EEG findings were also obtained. ZIKV (Zika virus) IgG was positive, while IgM was negative. Other congenital infections were negative. The exome sequencing

Introduction

Since Zika virus (ZIKV) was associated with microcephaly outbreak in Brazil in 2015, other clinical correlations were described [1–3]. Due to ZIKV neurotropism and molecular footprints left by infection, neurological disorders were reported in in vivo and in vitro experimental studies [3–5]. Infected neural cells produce and release inflammatory mediators that can remain ongoing even after birth [6], which are strongly associated with neuropsychiatric diseases. Interestingly, neuroimmune alterations are common in patients with autism spectrum disorder (ASD) [7, 8]. In addition, molecular markers of

ASD were found differentially expressed in human mesenchymal stem cells infected with ZIKV [9]. Recently, in a Brazilian prospective cohort of ZIKV-exposed children, delayed childhood neurodevelopment and neurosensory alterations were detected [10]. Although in this cohort 3 children (2.1%) were diagnosed with ASD, the study did not present a control group of children analyzed, neither any further clinical evaluation of ASD patients ruling-out other possible causes for this particular pathology. In this case report, we describe the diagnosis of ASD in a 4-year-old child exposed to ZIKV in utero, after deep evaluation.

Materials and Methods

The patient is a 4-year-old boy whose mother developed the whole clinical picture of ZIKV infection during the eighth month of pregnancy. Gestation was normal, with 10 follow-up consultations, until the mother's infection with ZIKV. The infection with the virus was suspected 1 month before delivery, but no molecular diagnostic testing was performed, since it was not commonly available during the peak of outbreak, in 2015, in her hometown. Gestational and prenatal routine laboratorial screening exams were normal, including morphological scan. The mother has no remarkable medical or family history, with no maternal overweight, diabetes, no use of alcohol, tobacco, or medication. The mother used folic acid during pregnancy. Maternal age was 35 at the time of delivery. In this case, mother's age could not be a risk of developing ASD, as previously described [11, 12]. Moreover, during prenatal and first years of child's life, any abnormal occurrence that might be linked to ASD was observed. The only disturbance observed was ZIKV infection during pregnancy, as described.

ASD was diagnosed by 2 different experienced child neurologists (AN and RSR) and confirmed by DSM-5 criteria [13] as well as by 19 points on ASQ (Autism Screening Questionnaire), whose cutoff point is 15 [14]. An informed consent was signed by parents. After that, several clinical, epidemiological, and genetic evaluations were performed. An extensive laboratorial search was done, including screening for congenital infections (CMV, toxoplasmosis, herpes, rubella, and ZIKV).

Results

The patient was born in the first semester of 2015, during the Brazilian outbreak, by elective cesarean section in the 39th week of gestation, with 3,050 g of birth weight, and 51.5 cm of length. There was no microcephaly: head circumference was 35 cm (Zscore Intergrowth 21st = +1). Birth Apgar was 9/10. There were no neonatal problems; the whole routine neonatal screening exams were normal, as well as the visual and hearing neonatal screening tests. No congenital anomaly was identified, and the neonate was discharged from hospital on the 2nd day of life.

During the initial 2 years of life, sleep problems were intensive and prominent. He acquired independent gait at 13 months of age when he was able to say "mom" and "dad." At this time, no eye contact problems were identified. Suddenly, about 20 months of age, he became "different": no more words were pronounced, food selectivity emerged, and the eye contact became progressively poor. At 2 years of age, no creative play occurred: he usually stayed horizontally on the floor, in order to observe toys and spin wheels. No curiosity for other children was present, and eye gaze stereotypies frequently happened. He developed fascination by washing machines, hearing hypersensitivity, and sensorial integration problems. Sphincters were controlled at 3 years-10 months of age. Physical examination at this age: weight, 22.5 kg (>P97); stature, 109 cm (>P97), head circumference: 55 cm (>P97). Vital signs were normal, and no abnormalities were identified in the physical examination. There was no eye contact, some language problems, eye gaze stereotypies, as well as hyperactivity. He had speech delay, but now, at 4 years, he is verbal, with some residual language problems.

The following results were performed after the positive diagnosis for ASD, at the age of 3 years and 8 months old. G band karyotype: 46XYqh+(20); X Fragile PCR: CGG triplets with 26 repetitions. An extensive laboratorial search was done, with normal findings. SNP array identified no pathogenic variants. Normal neuroimaging and EEG findings were also obtained. ZIKV IgG was positive, while IgM was negative according to ELISA test. Other congenital infections were negative. The exome sequencing did not reveal any pathogenic variant in genes related to ASD and was chosen to outperform the chromosomal microarray [15]. One variant in the EP300 gene was classified as of unknown significance (EP300:NM_001429:exon10:c.A2023G:p.M675V) – VUS, with probably no clinical relevance in this case. Pathogenic variants in EP300 were described in Rubinstein-Taybi syndrome, but our patient has no clinical characteristics of this syndrome. Although the VUS identified here could be responsible for ASD, the position of the mutation drives away the causal relationship.

Discussion

Neurodevelopment is a dynamic process that depends on the interaction between neurobiological and environmental factors. Maternal infections have been associated with autism in different studies [16]. Mechanisms proposed include placental inflammation, pro-inflammatory

cytokine production by mother or fetus, and maternal autoantibodies that bind to fetal brain [17]. In addition, the infection in utero by ZIKV was considered a possible risk linked to ASD [10, 18].

This case report presents the diagnostic of ASD in a boy which was exposed to ZIKV during pregnancy. The main known potential causes of ASD were considered, with further extensive clinical exams, and ruled out. It is noteworthy that inflammatory response and molecular alterations after ZIKV exposure in utero can persist after birth. However, this report has 1 main limitation: the molecular confirmation of ZIKV infection was not possible in the mother, although she has presented clinical symptoms, such as cutaneous rash, arthralgia, swelling in the hands, fever, and headache, being clinically diagnosed with ZIKV, at that time. The infection was contracted in their hometown, São Luis, the capital of Maranhão, a northeast Brazilian state with high circulation of ZIKV vector, *Aedes aegypti*, and high prevalence of ZIKV since the beginning of the 2015 Brazilian ZIKV outbreak. In addition, the child's IgG for ZIKV was positive, indicating previous exposure. The case presented here indicates a potential new clinical consequence associated with ZIKV syndrome, raising attention to the need for continuous survey of children exposed to ZIKV in utero during all stages of development. Also, we must take into consideration that children born whose mothers had ZIKV infection symptoms during pregnancy must be clinically monitored, even if the child's early neurodevelopment is considered normal, as previously highlighted [19] and as seen in this report.

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Statement of Ethics

Written informed consent was obtained and signed by parents for publication of this case report. This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki and approved by the Ethical Committee from Clinical Hospital of Porto Alegre and by the Brazilian Platform under CAAE number 56176616.2.1001.5327.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

L.S., R.S.R., R.L.R., E.F.T., A.Q.-S., L.S.-F., M.B., and W.O.B.S. proposed and drafted the manuscript with revisions and inputs from all other authors; R.S.R. and A.C.C.O. performed clinical care and diagnosis; P.C.C.B.-B. made the exome analysis; D.O.S. and J.A.G. supervised the project and got funding source. All the authors read and approved the final version.

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