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Negative RT-PCR in a Newborn Infected with Zika Virus: A Case Report

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***Abstract*—**Congenital Zika Virus Syndrome is an entity composed by a variety of birth defects presented in newborns that have been exposed to the Zika Virus during pregnancy. The syndrome characteristic features are severe microcephaly, cerebral tissue abnormalities, ophthalmological abnormalities such as uveitis and chorioretinitis, arthrogryposis, clubfoot deformity and muscular tone abnormalities. The confirmatory test is the Reverse transcription polymerase chain reaction (RT-PCR) associated to the physical findings. Here we present the case of a newborn with microcephaly whose mother presented a confirmed Zika Virus infection during the third trimester of pregnancy, despite of the evident findings and the history of Zika infection the RT-PCR in amniotic and cerebrospinal fluid of the newborn was negative. RT-PCR has demonstrated a low sensibility in samples with low viral loads, reason why, we propose a clinical diagnosis in patients with clinical history of Zika Virus infection during pregnancy accompanied by evident clinical manifestations of the child.

***Keywords*—**Zika Virus, polymerase chain reaction, microcephaly, amniotic fluid.

I. Introduction

THE Zika virus (VZ) is an Arbovirus belonging to the genus Flavivirus of the Flaviviridae family [1]. Zika virus is an arthropod borne pathogen that targets neural progenitor cells. Viral cerebritis can affect cerebral embryogenesis resulting in neurological abnormalities. The virus is transmited by infected Aedes mosquitoes, sexual contact, saliva, urine, breast milk, blood transfusion and during pregnancy [2], [3]. In 2015 and 2016 there was a global pandemic of VZ infections, evidencing an increase in the number of cases of microcephaly in Brazil in [4] children of mothers infected during pregnancy [1], [5], [6], which obliged the health services to explore this relationship, reporting different neonatal alterations, possibly associated with maternal infection of the VZ and within which were clinical findings of neurological predominance such as: microcephaly, facial disproportionality, hypertonia and / or spasticity, hyperreflexia and irritability, seizures, brainstem dysfunction [1]. It has been discovered that the virus has a special tropism for the AXL surface receptors which are expressed in developing neuronal cells. It is believed that in the infected cells the virus transcription is affected causing an alteration in the neurogenesis and finally leading to the different neurological alterations characteristic of the syndrome [1], [5].

The complete mechanism by which he virus induces microcephaly remains under study, although, literature suggest Zika virus infection acts as an antagonist of the phosphorylation of STAT1 and STAT2 proteins, which leads to a blockage of IFN1, resulting in a greater viral replication and alteration in the regulation of neurogenesis [7]. Other pathways have also been proposed in the pathogenesis of the central nervous system anomaly secondary to prenatal infection with the Zika virus. The first of these is autophagy and cell death through the deterioration of mTOR signaling pathways that alter the neuromorphism of the fetus [8]. The second theory that would explain how congenital anomalies secondary to prenatal infection with the Zika virus would occur corresponds to the regulation of Retinoic Acid Respond Elements (RARE) sequences. These viral RARE sequences can be inserted into regulatory regions of RARE-dependent genes in the DNA of the fetal host which can lead to alteration of gene expression resulting in a congenital fetal defect. All previous routes dysregulate cell cycle, transcription and apoptotic pathways in neural progenitor cells leading to reduced growth and hence microcephaly. Zika virus infection can be diagnosed by serology through IgM ELISA and RNA detection by RT-PCR which is more specific [8].

In Colombia, according to the Ministry of Health (Minsalud), the first case was detected in October 2015 in the department of Bolívar; Subsequently, 26 of the 36 territorial entities confirmed VZ autochthonous circulation, 11944 pregnant women with VZ infection, of which 1484 (12%) were confirmed cases [4], [9], [10]. Regarding congenital diseases and injuries in the Central Nervous System (CNS) (sixth epidemiological week, 2018) a national level of a prenatal infection by VZ, 247 cases were reported per clinic, one confirmed by laboratory, the national incidence is 0, 96 cases per 100,000 people at risk and zero fatalities, with 19.6% of Zika cases in pregnant women who ended the pregnancy (11

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cases) [11]. According to the Pan American Health Organization to date that has been reported 98803 suspected autochthonous cases, 9927 autochthonous confirmed, incidence rate of 22349 per 100,000 people year and zero deaths [10].

II. Case Report

We present the case of a 18 months female patient, daughter of a teenage mother whose pregnancy was not controlled until week 28. The maternal STORCH was negative and there was not other teratogenic risk factor present at the pregnancy. The mother´s patient had Zika Virus (ZV) infection confirmed by positive serology during stay in endemic area (Amazon rain Forest) in her first trimester of pregnancy. She arrived at our hospital at week 28, where she underwent a prenatal ultrasound during the same week that showed microcephaly, ventriculomegaly and dandy Walker variant (Table I), which is compatible with a diagnostic suspicion of Congenital Zika Virus Syndrome (CZVS).

Amniocentesis was requested to perform a serologic study of Zika virus and it was taken until week 31.6 due to a delay in the procedures of her health insurance, finally yielding a negative result for zika virus infection. At 33.5 weeks, the mother enters the emergency room with regular uterine activity, delivery is carried out and a newborn baby is received with a weight of 2.210 g; size of 42.5 cm, cephalic perimeter of 26 cm, APGAR of 8-9-9, AB + hemoclasification, normal vital signs and physical findings of: microcephaly, micrognathia, short neck, hypertelorism, long nasal bridge, hypoplastic maxilla, high arched palate, small anterior fontanelle and closed posterior fontanelle (as shown in Fig. 1). Neurological evaluation showed poor visual monitoring, no fixation of the gaze, reaction to auditory stimuli, adequate suction, hypotonia in the axial axis with hypertonia and hyperreflexia of both extremities with predominance in the upper limbs and presence of bilateral flexor plantar reflex. Cerebral Nuclear Magnetic Resonance was performed to confirm prenatal findings and cerebrospinal fluid was taken, which was sent to the National Health Institute for RT-PCR study of the ZV, yielding a negative result (ZV IgM was negative) (as shown in Table II).

Continuous patient follow-up in our institution presenting at 18 months a head circumference of 36 cm, height of 66.2 cm, micrognathia, hypertelorism, evidence of mongoloid spot, single palmar crease in right hand, digital shortening and tenar hypoplasia (as shown in Fig. 2), in addition to an evident neurological delay for the age (2 months compared to 18 months of chronological age), determined by the absence of complete supine cephalic support, incomplete rolling, inadequate trunk control, inability to sit down, immature language for age and absence of segmentation of upper limb movements.

III. Discussion

The Zika virus has been detected in blood, urine, cerebrospinal fluid, amniotic fluid, semen and saliva. The virus can be detected for a longer period in urine and semen, however, the recommendation of the World Health Organization recommends as a diagnostic test blood RT-PCR.

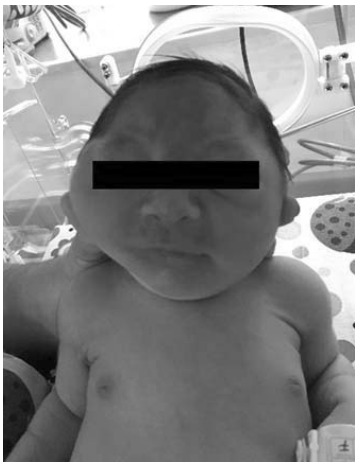

Fig. 1 Newborn patient showing microcephaly, short neck and hypertelorism

TABLE I
Obstetric Ultrasound

Obtetric Ultrasound Date	Gestatioal Age	Adnormal findings
11/08/2016	28.6 weeks	Microcephaly Ventriculomegaly communicating Parenchymal calcifications Megacisterna magna Dandy walker variant Discard alteration of neuronal migration Concurrent fetal grown
25/08/2016	31.6 weeks	Microcephaly Ventriculomegaly communicating Parenchymal calcifications Megacisterna magna Dandy walker variant It is considered to rule out alteration of neuronal migration

TABLE II
Serology in Patient`s Cerebrospinal Fluid

Laboratory findings	**Results**
Proteins	129
Glucose	58
Xanthochromia	moderate
Leukocytes	3X mm3
Red blood cells	18Xmm3
Fresh Red blood cells	95%
Red cells	5%
Chinese ink process cultivation	Negative
Serology	Not reactive
IgM toxoplasms	Negative
CMV IgG	12.4 Negative
iGm CMV	0.12 Negative
Toxoplasm IgG	Negative
Zika RT-PCR	Negative

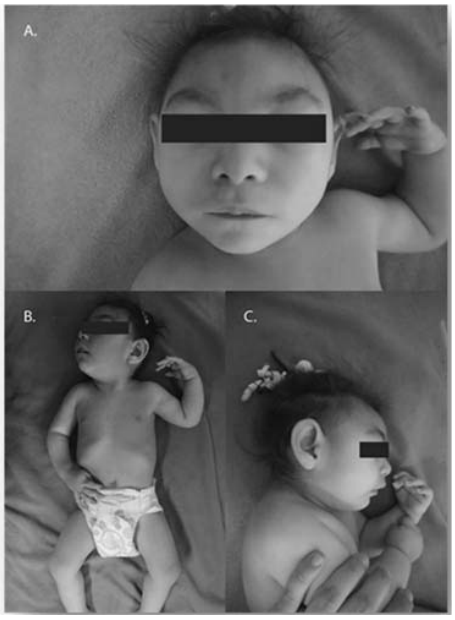

Fig. 2 Pictures of 18 months patient: A. Anterior image of the face showing microcephaly and hypertelorism B. Full body image showing cephalic disproportion compared to body length C. Lateral image showed microcephaly and micrognathia

The test should be taken within the first 7 days after the onset of symptoms, because the viremia drops rapidly 7 days after the onset of symptoms. It is recommended to perform IgM in serum, however, it can also be performed in cerebrospinal fluid to diagnose congenital.

The mother of the patient reports a positive RT-PCR test for ZV during her first trimester of pregnancy, which is why a congenital infection was suspected from the beginning.

In Colombia all live newborns that are classified as suspicious cases, that is, all those whose mothers have presented a picture compatible with Zika during the gestation period should be studied to rule out all diseases belonging to the S-TORCH complex (Syphilis, Toxoplasmosis, Cytomegalovirus and HIV). A part of the serum sample must be sent to the National Reference Laboratory of the National Health Institute where the respective PCR-RT processing for ZIKA will be done. In the previously mentioned case, after ruling out S-TORCH infections in the mother (as shown in Table III) two tests were performed, the first was in amniotic fluid at week 31.6 and the second in cerebrospinal fluid just after birth; despite the typical of the table, both tests were negative, which may be due to a false negative, taking into account previous reports where it was concluded that in samples with viral load the sensitivity of the RT-PCR for the ZV decreases and therefore both a negative result does not rule out the infection when it is present.

The infection by VZ, after its introduction in the Americas has allowed significant advances against knowledge, after this epidemic for the year 2015 [12] several hypotheses have emerged about the types of VZ circulating in the Americas, phylogenetic studies established that the Asian strain predominated during outbreaks in Colombia [13], [14]. Several case series and reviews worldwide have shown the high teratogenic power of the VZ in the prenatal period, producing several malformations of the disruptive type in the fetus [15], [16]. VZ has a special tropism for AXL surface receptors that are expressed in developing neuronal cells [1], [8]. It is believed that in infected cells the virus affects transcription causing alterations in neurogenesis and the different neurological alterations characteristic of the syndrome [8], [17] and as it was evidenced in this cohort, patients with a low brain development coefficient.

TABLE III
SEROLOGY MOTHER

Laboratory findings	Results
VDRL	Not reactive
HIV	Negative
HbsAG	Negative
Dengue IgM	Negative
Rubella IgG	Positive
Rubeola IgM	Negative
CMV IgG	Positive
CMV IgM	Negative
Vaginal smear	Negative
Partial Urine	Suggestive of infection- treated
Treponemic test	Negative
IgM Dengue (Elisa)	3.12 negative
IgM Chicungunya (PT-PCR)	3.28 negative
Zika (RT-PCR)	Positive

One of the hypotheses described for this mechanism is the possible alteration in the pathway of retinoic acid essential for biological processes such as cell growth, reproduction and differentiation in embryonic CNS cells [8], its functions are regulated through the RAR receptors (retinoic acid receptors) and RXR (rexinoid receptors) that regulate the expression of several regulatory genes in the axes of neural tube development in this pathway (NODAL gene, fibroblast growth factor and others) [8]. Modeling in mice has shown that VZ can infect and destroy niches of adult neuronal progenitor cells (18) important for plasticity and learning of the nerves, which implies that the infection could also affect developing brains that have not had prenatal infection [18]. therefore, we believe that it is important to follow the children possibly exposed to VZ (residence in endemic areas) for the first five years of life.

The guidelines of Minsalud indicate that newborns with microcephaly suggestive of VZ infection and negative PCR-RT results, a CSF sample can confirm the infection [19], [20]. However, it is not available in many rural centers that hinder the timely diagnosis for the clinical diagnosis of the syndrome with the findings of this study has an important space. There is evidence of the sensitivity of this test in CSF may be low in samples with reduced viral load [21]. Given the above, it is recommended to optimize the diagnosis, which is based on the taking of molecular tests (polymerase chain reaction, real time reverse transcription [RT-PCR]) and serological tests (immunoglobulin M [IgM] 23 [21], [22] being the RT-PCR the test of choice It is important that the institution responsible for

carrying out the processing of such tests has the number of personnel required to process them and thus avoid the loss of them.

Fig. 3 shows the current phenotype of the patient at 20 months of age with persistence of the typical congenital Zika virus the picture given by microcephaly and peculiar fascies (microstomia, on-screen ears and small forehead). Which leads us to think that it is important to continue studying this patient and thus begin to study the other children of our country to be able to compare with other children from other countries.

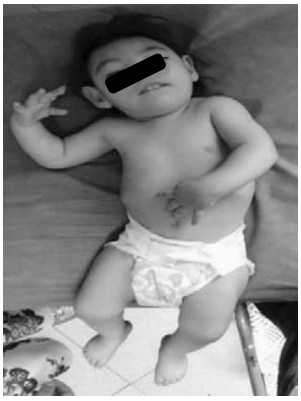

Fig. 3: Current phenotype of the patient at 20 months of age

IV. Conclusions

Taking into account the infant symptoms and the recent outbreak of Zika Virus we diagnosed the case as CZVS and we suggest treating physicians to analyze ZV cases not only with molecular results but with the findings in the history and phenotypes of patients with high suspicion for CZVS.

We propose that a clinical diagnosis be made in patients with a clinical history of Zika virus infection during pregnancy accompanied by evident clinical manifestations of the child because RT-PCR has shown low sensitivity in samples with low viral loads.

Suggestions to deepen the interpretation and analysis of the results of negative RT-PCR tests given that negative results do not exclude the presence of prenatal infection (2), (5), (21), (22), positive results in amniotic fluid can confirm The diagnosis of congenital infection by VZ, but it is not a negative result. 30 days of life in whom other causes of microcephaly have been ruled out, with diagnosis of infection confirmed in the mother.

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References

[1] Group MER. Microcephaly in Infants, Pernambuco State, Brazil, 2015. Emerg Infect Dis. 2016;22(6):1090-3.

[2] Smithburn K. Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa. J Immunol. 1952;69(2):223/34.

[3] Dick G, Kitchen S, Haddow A. Zika Virus Isolations and serological specificity. Trans R Soc Trop Med Hyg 1952;46(5):509/20.

[4] Social MdSyP. Plan de respuesta frente a la fiebre por el virus Zika: Dirección de promoción y prevención subdireccion de enfermedades transmisibles; 2016 [20]. Available from: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/PP/ET/plan-respuesta-fiebre-zika-colombia.pdF.

[5] Faizan M, Abdullah M, Naqvi I, Ahmed A, Parveen S. Zika Virus-Induced Microcephaly and Its Possible Molecular Mechanism. Intervirology 2016;59:152- 8.

[6] Velho Barreto de Araujo T, Cunha Rodrigues L, Arraes de Alencar Ximenes R, de Barros Miranda-Filho D, Ramos Montarroyos U, Paula Lopes de Melo A, et al. Association between Zika virus infection and microcephaly in Brazil, January to May, 2016: preliminary report of a case-control study. Lancet Infect Dis. 2016;16(12):1356-63.

[7] Kublin J, Whitney J. Zika virus research models. Elservier. 2018;254:15-20.

[8] Kumar A, Singh H, Pareek V, Raza K, Dantham S, Kumar P, et al. A Possible Mechanism of Zika Virus Associated Microcephaly: Imperative Role of Retinoic Acid Response Element (RARE) Consensus Sequence Repeats in the Viral Genome. Front Hum Neurosci 2016;10.

[9] Pacheco O, Beltrán M, Nelson CA, Valencia D, Tolosa N, Farr SL, et al. Zika Virus Disease in Colombia - Preliminary Report. N Engl J Med. 2016.

[10] Paho.org. Washington, DC: Organización Panamericana de la Salud y Organización Mundial de la Salud; [updated 1 Dic 2015; cited 2017 4 Abr]. Available from: https://www.paho.org/hq/dmdocuments/2015/2015-dic-1-cha-alerta-epi-zika-sindrome-neuro.pdf.

[11] Ins.gov.co. Bogota, DC: Instituto Nacional de Salud; [updated 18 May 2018; cited 2018 25 May]. Available from: https://www.ins.gov.co/buscador-eventos/Informesdeevento/ZIKA%20PE%20IV%202018.pdf.

[12] Theel E, Hata D. Diagnostic Testing for Zika Virus: a Postouthbreak Update. J Clin Microbiol. 2018;26(56):4.

[13] Costello A, Dua T, Duran P, Gülmezoglu M, Oladapo OT, Perea W, et al. Defining the syndrome associated with congenital Zika virus infection. Bull World Health Organ. 2016;94(6):406-A.

[14] Malkar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj. M, Mraz J, et al. Zika Virus Associated wth Microcephaly. N Eng J Med. 2016;374(10):951-8.

[15] Fontaina A, de Laval F, Belleoud D, Briolant S, Matheus S. Duration of Zika viremia in serum. Clinical Infect. 2018.

[16] Hendrixon DT, Newland JG. Zika virus infection in childrens. Infect Dis Clin N Am. 2018;32:215–24.

[17] Weaver SC, Costa F, Garcia-Blanco MA, Ko AI, Ribeiro GS, Saade G, et al. Zika virus: History, emergence, biology, and prospects for control. Antiviral Res. 2016;130:69-80.

[18] Li H, Saucedo-Cuevas L, Regla Nava JA, Chai G, Sheets N, Tang W, et al. Zika Virus Infects Neural Progenitors in the Adult Mouse Brain and Alters Proliferation. Cell Stem Cell. 2016;19(5):593-8.

[19] Salud. Md. Guía para la vigilancia integrada de la infección por el virus del Zika y recomendaciones para el equipo de salud. Anexo 12016 [cited 2017 24 noviembre]:[72 p.]. Available from: https://dtoepidemiologia.files.wordpress.com/2017/02/2017-337929955-legislacion-leg84401-anx-anexo-resolucion-117-e2017-pdf.pdf.

[20] Ruiz J, Romero R, Buitrago A. Guía de Practica Clínica: Detección de anomalías congénitas del recién nacido Bogota Centro Nacional de Investigación en Evidencia y Tecnologías en Salud CINETS2013 [Available from: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/INEC/IETS/GPC_Ptes_AC.pdf.

[21] Paiva M, Guedes D, Leal W, Ayres C. Sensitivity of RT-PCR method in samples shown to be positive for Zika virus by RT-qPCR in vector competence studies. Genetics and Molecular Biology. 2017;40(3):597-9.

[22] Gueneret M, Jolivet E, Adenet C, Volumenie J, de Gynécologie Obstétrique Schaub S, Monthieux A, et al. Analysis of blood from Zika virus-infected fetuses: a prospective case series. Lancet Infect Dis 2017.