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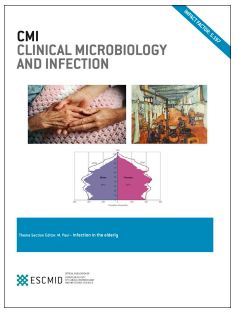

In utero negativization of Zika virus in a case with serious Central Nervous System abnormalities

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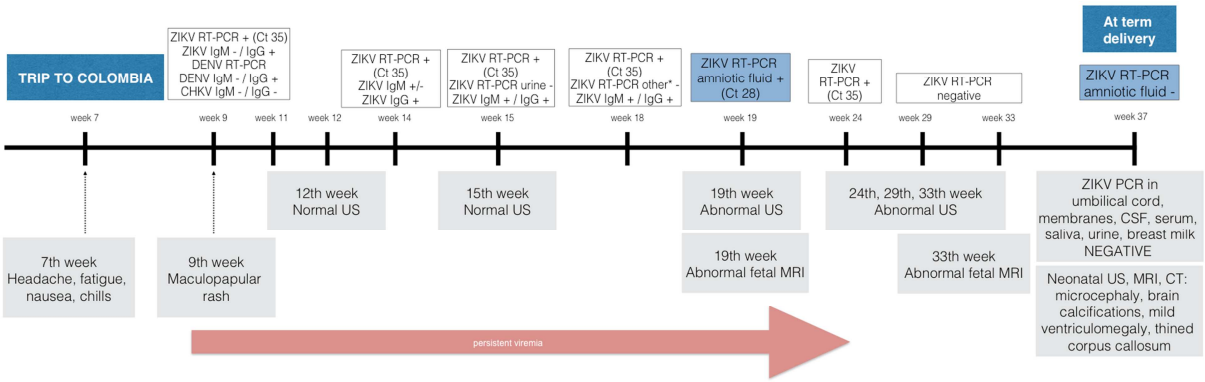

Category: Research note

Running title: *In utero* resolution of ZIKV

Title*: In utero* negativization of Zika virus in a case with serious Central Nervous System abnormalities

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Abstract

Objectives: To describe a case of a pregnant woman with Zika virus infection and severe fetal brain malformations.

Methods: Serial ultrasound measurements, fetal magnetic resonance imaging results, laboratory and amniocentesis results, and perinatal outcomes of the pregnant woman and her neonate are reported.

Results: Zika virus tested positive in amniotic fluid at 19 weeks while being negative at delivery. The newborn did not meet the case definition of congenital Zika virus syndrome because neither the Zika virus RNA nor immunoglobulin M antibodies were detected; however, prenatal brain lesions were confirmed after birth.

Conclusions: The presence of Zika virus in amniotic fluid plays a role in the diagnosis of Zika virus congenital syndrome.

Graphical abstract.

Introduction

Zika virus has been demonstrated to be a new infectious disease potentially harmful for the foetus if vertical transmission occurs. There are some reports suggesting that persistent maternal viremia can be the consequence of viral replication on the foetus or the placenta [1]. However, some concerns remain regarding the difficulties in laboratory confirmation of Zika virus infection in neonates due to the lack of accurate diagnostic tests.

The purpose of this report is to analyze the role of amniotic fluid testing in prenatal diagnosis of Zika virus congenital syndrome.

Methods

We report the case of a woman with Zika virus infection during pregnancy. Her foetus

had prenatal ultrasonographic findings consistent with Zika virus congenital syndrome. Reverse-transcriptase-polymerase-chain-reaction analysis was performed with a *RealStar® Zika Virus RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany*) and serologic screening was performed with Indirect Immunofluorescence Tests (IIFT, *Arboviral fever Mosaic, Euroimmun, Germany*). We used a pan-flavivirus endpoint one-step RT-PCR for the amniotic fluid testing [2]. The amplification cycle thresholds (Ct) of *Altona RT-PCR* were used as an indirect marker of viral load. Serial ultrasound measurements, fetal magnetic resonance imaging results, laboratory and amniocentesis results, and perinatal outcomes are reported.

Results:

A 41-year-old Colombian woman was referred to the Maternal Fetal Medicine Unit of Vall d'Hebron University Hospital (Barcelona, Spain) due to a confirmed maternal Zika virus (ZIKV) infection. She was infected in her first trimester of pregnancy in Colombia and presented with symptoms consistent with ZIKV infection (a maculopapular rash affecting trunk and limbs, with no fever or other concurrent symptoms) at eight weeks' gestation, after returning to Spain.

Reverse-transcriptase-polymerase-chain-reaction in maternal serum was positive at first determination (9th week) and remained positive until 24 weeks of gestation (107 days after the onset of symptoms). Serologic testing was negative for IgM-ZIKV antibodies and positive for IgG-ZIKV by Indirect Immunofluorescence Tests. She was also screened for Dengue (DENV) and Chikungunya (CHKV) viruses, and serologic testing showed DENV IgG positive and IgM negative, and CHKV IgM and IgG both negative. Serological screening for other infections was negative, namely Rubella, Toxoplasmosis, Syphilis, Hepatitis B and C and Human immunodeficiency virus.

At 19 weeks' gestation we suspected a congenital ZIKV infection due to abnormal fetal ultrasound findings, which showed bilateral mild ventriculomegaly, and a shortened corpus callosum. The posterior fossa showed no abnormalities. We did not observe brain calcifications until the week after. The fetal magnetic resonance (MRI) confirmed ultrasound findings. Amniocentesis was performed after obtaining maternal informed consent. ZIKV RT-PCR in amniotic fluid was positive, and screening for DENV, CHKV, Cytomegalovirus, Varicella Zoster virus, Parvovirus B19, *Toxoplasma gondii* and sexually transmitted infectious agents (*Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma hominis, Ureaplasma parvum, Mycoplasma genitalium, Ureaplasma urealiticum* and *Trichomonas vaginalis*) was negative. We also discarded other flaviviruses in serum and amniotic fluid by means of a pan-flavivirus endpoint one-step RT-PCR. The cycle thresholds suggested a higher viral load in amniotic fluid than in the maternal serum samples (serum Ct 35 (median 33-36) vs amniotic fluid Ct 28). Phylogenetic analysis showed that the partial NS5 sequence from ZIKV detected in amniotic fluid belonged to Asian ZIKV lineage (figure 1). Genetic test (*Quantitative Genomic Hybridization Array, 8x60K Agilent G4827A CGH ISCA v2 array*) was normal.

The mother delivered at 37 weeks. She consented for a second amniocentesis before delivery (18 weeks after the first amniocentesis) and amniotic fluid ZIKV RT-PCR tested negative. Maternal serum and urine, placenta, umbilical cord and amniotic membranes tested negative for ZIKV RT-PCR. Moreover, all the analyzed biological samples in the neonate (urine, serum and cerebrospinal fluid (CSF)) were negative for ZIKV RT-PCR. Serologic response in the newborn was negative for IgM-ZIKV and positive for IgG-ZIKV antibodies by means of IIFT, both in serum and CSF, and it remains positive in serum at 12 months of age. Postnatal ultrasounds and MRI studies were consistent with microcephaly with brain atrophy and polymicrogyria,

mild ventriculomegaly with thinned corpus callosum, and cortical and subcortical calcifications, confirming the spectrum of images compatible with congenital Zika virus syndrome (CZS) in the neonate (graphical abstract). CSF showed a high rate of proteins (214 mg/dl, range 15 – 45 mg/dl), with cells account within the normal range (leukocytes 3.00 cel/µl, rec cells 40.00 cel/µl).

Discussion

We report a case of congenital Zika virus syndrome with complete prenatal and postnatal follow-up. In this case, the newborn did not meet the laboratorial case definition of CZS [3] because neither ZIKV RNA nor IgM antibodies were detected in the neonate. However, since June 2016 CDC allows the confirmation of a suspected case of Zika virus disease when the neonate meets the clinical criteria for congenital ZIKV (central nervous system malformations not explained by another cause) and ZIKV-RNA is detected in fetal tissues, cord blood or amniotic fluid [4].

There is no available data regarding sensitivity, specificity, positive and negative predictive values of ZIKV RT-PCR in amniotic fluid [5], hence we currently decide the optimal time to perform amniocentesis on the basis of the presence of fetal malformations; or 5 weeks after the presumed infection (in cases of symptomatic women) or 5 weeks after the first positive screening test (in asymptomatic women) when there are no fetal abnormalities [6].

In our case, Zika virus RNA was not detected in the neonate, or amniotic fluid, placenta, or membranes at birth. However, the positivity of ZIKV RT-PCR in amniotic fluid at mid gestation and the positivity of serum ZIKV-IgG antibodies in the newborn beyond 9 months of age should be criteria to classify this baby as a CZS case. All the children born to ZIKV-infected mothers without CZS that are being followed-up in our Unit have seroreverted ZIKV-IgG antibodies by 9-months of age (unpublished data).

Our group previously published the persistent maternal viremia as a marker of the viral replication in the foetus [1]. The negativization of the maternal viral loads during pregnancy (between 24 and 29 weeks of gestation) raises the hypothesis of the resolution of the infection during the fetal life being only apparent the consequences of the infection (fetal central nervous system (CNS) malformations). Schaub et al [7] also observed the negativization of ZIKV RT-PCR in amniotic fluid. They coincide with our hypothesis of the fetal immune system itself being able to resolve the infection during intrauterine life; despite they attribute this finding to false-negative results of the technique. They also comment on the virus' persistence in foetal CNS. However, despite IgG antibodies in cerebrospinal fluid were positive at birth and there was no serum contamination in the CSF sample, we were not able to demonstrate the presence of the virus in the CNS of the neonate, and the unique laboratory evidence for congenital ZIKV infection was the persistence of ZIKV-IgG antibodies beyond 9-months of age.

Further studies would be necessary to confirm our findings and to evaluate the usefulness of amniotic fluid testing in the diagnostic algorithm for congenital ZIKV infection.

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References

[1] Suy A, Sulleiro E, Rodó C, Vázquez É, Bocanegra C, Molina I, et al. Prolonged Zika Virus Viremia during Pregnancy. N Engl J Med 2016;375(26):2611-3.

[2] Balm MN, Lee CK, Lee HK, Chiu L, Koay ES, Tang JW. J Med Virol 2012;84(9):1501-5.

[3] Van Kerkhove M, Broutet N, Reveiz L. Standardized Protocol: Prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus during pregnancy. 2017 [cited 2017 Aug 27]; Available from: https://pdfs.semanticscholar.org/34f2/6796897fe1285053013142db8d39f761c35f.pdf

[4] Zika Virus Disease and Zika Virus Infection 2016 Case Definition, Approved June 2016. https://wwwn.cdc.gov/nndss/conditions/zika/case-definition/2016/06/

[5] Eppes C, Rac M, Dunn J, Versalovic J, Murray KO, Suter MA, et al. Testing for Zika virus infection in pregnancy: key concepts to deal with an emerging epidemic. American Journal of Obstetrics and Gynecology. 2017 Mar;216(3):209–25.

[6] Bocanegra C, Sulleiro E, Soriano-Arandes A, Pou D, Suy A, Llurba E, et al. Zika virus infection in pregnant women in Barcelona, Spain. Clinical Microbiology and Infection. 2016 Jul;22(7):648–50.

[7] Schaub B, Vouga M, Najioullah F, Gueneret M, Monthieux A, Harte C, et al. Analysis of blood from Zika virus-infected fetuses: a prospective case series. The Lancet Infectious Diseases. 2017 May;17(5):520–7.

Figure 1. Phylogenetic reconstruction of partial ZIKV NS5 coding sequences (from nucleotide position 10088 to 10606 of homologous sequence from MR 766 strain with accession number NC_012532) constructed using the Neighbor-Joining method with the Kimura 2-parameter method. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.21). The evolutionary distances are in the units of the number of base substitutions per site. The sequence of the present study is marked with a black point.

Graphical abstract. Timeline of significant events.

(*) Serum, urine, vaginal swab, cervico-vaginal lavage and endocervical swab.

Abbreviations: ZIKV: Zika virus. DENV: Dengue virus. CHKV: Chikungunya virus. IgM: Immunoglobulin M, IgG: Immunoglobulin G, RT-PCR: Real time-polimerase chain reaction, Ct: amplification cycle thresholds, US: ultrasound, MRI: magnetic resonance, CT: computerized tomography, CSF: cerebrospinal fluid