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Short Communication

Co-infection with Zika and Chikungunya viruses associated with fetal death—A case report

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ABSTRACT

We describe a case of fetal death associated with a recent infection by Chikungunya virus (CHIKV) in a Brazilian pregnant woman (positive RT-PCR in blood and placenta). Zika virus (ZIKV) infection during pregnancy was also identified, based on a positive RT-PCR in a fetal kidney specimen. The maternal infection caused by the ZIKV was asymptomatic and the CHIKV infection had a classical clinical presentation. The fetus had no apparent anomalies, but her weight was between the 3rd and 10th percentile for the gestational age. This is the second case report of congenital arboviral co-infection and the first followed by antepartum fetal death.

Introduction

The first reports of congenital Zika virus (ZIKV) infection occurred in 2015 in Brazil (Pan American Health Association, 2015). Although many studies have been published on this topic, there is little information about the consequences to the fetus of maternal co-infection by ZIKV and other arboviruses. A case of co-infection by ZIKV, Chikungunya virus (CHIKV), and Dengue virus (DENV) in a Colombian pregnant woman followed up to the 29th week of gestation was recently reported, with no harm to the fetus (Villamil-Gómez et al., 2016). Other articles have described the potential consequences to the fetus of arboviral infections during pregnancy (Marinho et al., 2017; Brasil et al., 2016; Honein et al., 2017; Hoen et al., 2018). In cases of ZIKV infection, up to 25% may result in abortion when occurred in the first trimester and 6-42% may present some abnormal findings, including microcephaly and intrauterine growth restriction (Brasil et al., 2016; Hoen et al., 2018). CHIKV infection has been associated with a range of symptoms in the newborn, since mild manifestations, as fever, exanthema, and irritability, to meningoencephalitis, microcephaly, and delay in global neurodevelopment (Torres et al., 2016; Gérardin et al., 2014). DENV infection during pregnancy also is associated to a higher risk of abortion, fetal death, preterm birth and low birth weight (Paixão et al., 2016). Recently, in a mouse model, a study compared the ability of four arboviruses to infect the placenta and the fetus. The authors found that only the infection with the neurotropic flavivirus was associated with fetal demise (Platt et al., 2018).

In this article, we report the first case of fetal death associated with a co-infection by ZIKV and CHIKV during pregnancy.

Methods

For serology testing, enzyme-linked immunosorbent assay (ELISA) for anti-arboviruses IgM and IgG detection were used (Euroimmun AG, Luebeck, Germany). Reported sensitivity and specificity of the serologic tests used are 83-92% and 65-81%,

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respectively, for ZIKV (Huillier et al., 2017), 98% and 97.5%, respectively, for CHIKV (Johnson et al., 2016), and 100% and 100%, respectively, for DENV (Euroimmun Medizinische Labordiagnostika AG, 2018).

To detect RNA of ZIKV, CHIV, and DENV in clinical samples, viral RNA was first extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany). Then, real-time quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was performed using the following primers: CHIKV 6856, CHIKV 6981 and CHIKV 6919-FAM; ZIKV 1086, ZIKV 1162c and ZIKV 1107-FAM; DENV-F (10546)-generic, DENV-R (10711)-generic and DENV generic-FAM. RNA was amplified by qRT-PCR in an ABI Prism 7500 Real-Time cycler (Applied Biosystems, USA). The reaction was performed with 10 μL of RNA by using the TaqMan One-Step Real-Time PCR Master Mixes reagents (Applied Biosystems, USA). A cut-off cycle threshold (Ct) value corresponding to 38.5 was defined for a positive result.

Case description

In March 2016, a 20-year-old, pregnant woman, in the 21st week of gestation, reported a maculopapular diffuse rash and high fever (39–40 °C) that lasted for 24 hours, with no other complaints. Four days after, the routine ultrasonography revealed no fetal heartbeats. Labor induction was attempted, without success. A curettage was then performed. The fetus was female, had no apparent identifiable anomalies, but her weight (220 g) was between the 3rd and 10th percentile for the gestational age. After written informed consent provided by the family, an autopsy was performed on the fetus, and maternal serum specimens were also collected. The patient also gave informed consent for research and eventual scientific publication.

This young woman, 72 Kg before pregnancy, 75.4 Kg in the date of admission, 1.67 m height, was in her first pregnancy, with no history of miscarriage or abortion, had two prenatal consultations (the first on 19/jan/2016), had no previous related symptoms suggestive of another arboviral infection during this pregnancy, was not in use of any medication, did not report smoking or alcohol consumption, had no other risk factors for fetal death, such as maternal pre-eclampsia, diabetes, drug addiction, or other congenital infections, and had no history of consanguinity or familiar genetic abnormalities. A fetal ultrasound was performed on 13/jan/2016, that was compatible with a 12 weeks and 6 days gestational age, with no other reported abnormality.

Tests of the maternal serum were negative for herpes simplex I and II and *toxoplasma gondii* (IgM and IgG Enzyme Immune Assay, EIA), for HIV (Enzyme Linked Immunosorbent Assay, ELISA), and for *treponema pallidum* (VDRL, Venereal Disease Research Laboratory). IgG were detected by EIA for *parvovirus B19*, *rubella virus*, and *human cytomegalovirus*, but with negative IgM (EIA), and no signs or symptoms suggesting that these serology findings reflected an infection during this pregnancy. DENV IgM and IgG ELISA, and CHIKV IgM and IgG ELISA were positive. ZIKV IgG ELISA was positive, but IgM ELISA was negative. The test to detect DENV NS1 antigen was not available. RT-PCR analysis of the blood sample was negative for ZIKV and DENV, but positive for CHIKV (Table 1). Unfortunately, we could not perform other tests to rule out chorioamnionitis by bacterial infections.

The autopsy of the fetus was performed with extensive sampling of lung, heart, liver, kidney, brain and placental tissues. Placental and renal calcifications were observed. There were signs of moderate to advanced stage of maceration. The lung structures were compatible with the presumed gestational age.

ZIKV, CHIKV, and DENV real-time qRT-PCR tests were performed on RNA-later preserved fresh fetal tissues (one block per tissue). DENV was not detected in any sample, but CHIKV was detected in the placenta, and ZIKV was detected in the kidney (Table 1).

Discussion

DENV, ZIKV, and CHIKV are transmitted by the same vector, and co-infection by two or three of these viruses is possible (Pessoa et al., 2016). In this case, maternal IgG serology was positive for DENV, ZIKV and CHIKV, and IgM positive for CHIKV and DENV. The only positive RT-PCR result in the mother's blood sample was for CHIKV, which suggests that this infection was the most recent in the infection sequence. We could not confirm recent DENV infection despite the positive IgM reaction, as the Plaque Reduction Neutralization Test (PRNT), used to differentiate ZIKV and DENV infection, was unavailable (Rabe et al., 2016). Anti-DENV IgM cross-reactivity with anti-ZIKV IgM may have occurred, however a DENV infection in the last three months is also plausible considering the high DENV seroprevalence in Rio de Janeiro (61%) (Honorio et al., 2009).

We hypothesize that the ZIKV infection occurred early in this gestation, since a blood sample of the mother resulted positive for anti-ZIKV IgG but not IgM, and the RT-PCR was negative. The finding of a positive RT-PCR for ZIKV in the fetal kidney confirms the vertical transmission of this virus. How ZIKV reached the fetal kidneys and why it persisted there remains to be elucidated. It has previously been shown that ZIKV RNA can be detected in urine for a longer time and with a higher viral load compared to blood (Gourinat et al., 2015). A recent study described that ZIKV can infect renal proximal tubular epithelial cells in immunodeficient mice in vivo and human renal proximal tubular epithelial cells in vitro, with cytopathic effects and for extended duration (Chen et al., 2017). The positivity of CHIKV IgM, IgG and blood RT-PCR suggests a long-lasting viremia, especially as it is known that IgG usually appears in the second week. However, the patient had fever, exanthema, and other symptoms compatible with a flavivirus infections just four days before the final outcome. Nevertheless, because the fetus had signs of maceration, the recent CHIKV infection may be incidental and not the cause of fetal death.

There are some limitations we must address in this case report. First, we could not confirm a recent DENV infection, because the RT-PCR was negative for this virus and IgM serology may cross-react with ZIKV. We could not perform a NS1 or PRNT test to help to

Table 1
Laboratory tests results.

	Pregnant woman				Fetus (viscera) RT-PCR						
	RT-PCR (blood)[a]	RT-PCR (urine)[a]	IgM[b] (ratio)	IgG[b] (ratio)	Placenta	Fetal Membranes	Brain	Heart	Lungs	Liver	Kidney
CHIKV	+ (**Ct 36.5**)	N/A	+ (**2,8**)	+ (**1,2**)	+ (**Ct 29.2**)	NR	NR	NR	NR	NR	NR
ZIKV	NR	NR	– (0,1)	+ (**3,2**)	NR	NR	NR	NR	NR	NR	+ (**Ct 37.2**)
DENV	NR	N/A	+ (**5,13**)	+ (**5,31**)	NR	NR	NR	NR	NR	NR	NR

Bold values signifies abnormal elevated results.
[a] Blood (serum) and urine collected perinatally.
[b] ELISA (Euroimmun AG, Lübeck, Germany), reference range: reactive >1.1; NR: non-reactive; N/A: not available; Ct: RT-PCR cycle threshold value.

clarify this point. Second, other causes of chorioamnionitis, especially bacterial infections, could not be ruled out. Third, the moderate/advanced stage of maceration of the fetus may suggest that the fetal demise have occurred before the last clinical symptoms reported by the patient. However, one of the strengths of this report is that we could describe for the second time in the medical literature a confirmed case of ZIKV and CHIKV co-infection during pregnancy.

Though we cannot conclude which congenital infection caused fetal death, the detection of CHIV in the placenta and ZIKV in the fetal kidneys suggests that both infections may have contributed to it.

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Conflict of interest

No competing interest declared.

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Ethical approval

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