Zika virus infection and pregnancy: what we do and do not know

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Recent data strongly suggest an association between the current outbreak of ZIKA virus (ZIKV) in many countries of Central and South America and a sharp increase in the detection of microcephaly and fetal malformations. The link with brain defect, which has been detected mainly in some areas of Brazil, is supported by the following evidence: (1) ZIKV transmission from infected pregnant women to their fetuses; (2) the potential of ZIKV to determine a specific congenital fetal syndrome characterized by abnormalities involving primarily the developing brain and eye. In particular, the risk of transmission and congenital disease appears to be restricted to mother's infection during the first trimester of pregnancy. Among brain defects, microcephaly, brain calcifications, and ventriculomegaly are the most frequent abnormalities of the central nervous system detected so far. However, relevant information on effect of maternal infection with ZIKV on the fetus is still limited. In this review, we focus our attention on current knowledge about ZIKV infection in pregnancy, discussing relevant issues and open problems which merit further investigation.

Keywords: Zika Virus, Pregnancy, Microcephaly, Congenital malformations

Introduction

On 1 February 2016, the World Health Organization declared the cluster of microcephaly and other neurological disorders associated with Zika a 'Public Health Emergency of International Concern.'¹ The statement followed the detection of a possible association between infection by Zika virus (ZIKV) in pregnant women and congenital anomalies – particularly neonatal microcephaly. Without doubt, ZIKV-induced disorders merit serious consideration, taking into account that core information about its biology, mechanisms of action, and pathogenic potential is still largely unknown or rapidly evolving. In fact, until four months ago, Zika was a nearly unknown mosquito-driven infection discovered in central Africa, which was considered capable of causing outbreaks of limited clinical relevance.

The aim of this article is to review currently available knowledge, trying to answer open questions on ZIKV infection, with specific focus on pregnancy and the fetus.

ZIKV: background information

ZIKV is a single-stranded RNA arbovirus. It is a member of the *Flaviviridae* family, that includes dengue, Japanese encephalitis, West Nile, and yellow fever viruses. The ZIKV, which is transmitted by *Aedes spp.*

mosquitoes (Table 1), was isolated for the first time in a monkey, in 1947, in the Zika forest, Uganda.^{2,3} ZIKV remained confined to Central and West Africa, with reports of infection in several countries, including Nigeria, Sierra Leone, Gabon, Senegal,⁴ until 1977–1978, when the first cases of infection out of Africa, namely in Southeast Asia (Indonesia), were described.⁵ Thereafter, ZIKV spread eastward to Thailand, Vietnam, Philippines, Micronesia, French Polynesia,⁶ where the largest outbreak occurred in early February 2014.⁷ Then, ZIKV reached Easter Island, South Pacific, in 2014.⁸ The first documented case of autochthonous transmission of ZIKV from mosquito to human in South America was reported in Brazil in May 2015.^{9,10} A rapid spread of ZIKV infection was observed in this country; by the end of 2015, the Brazilian Ministry of Health estimated a cumulative number of infections ranging from 440,000 to 1,300,000.¹¹ ZIKV is now spreading well beyond its original African niche, causing a high burden of disease in East Asia and South America.

ZIKV and congenital fetal abnormalities

Maternal–fetal transmission of ZIKV has been well described,¹² and the possible routes of fetal infection are presented in Figure 1. While there is no evidence that the clinical course of ZIKV infection in pregnant women is more severe than that observed in non-pregnant individuals,^{12,13} it is now becoming clear that maternal infection with ZIKV is associated with several potentially relevant

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Table 1 Mosquito vectors of ZIKV

Mosquito Spp. with demonstrated capacity to infect humans	Mosquito Spp. in which ZIKV has been infrequently identified	Mosquito Spp. likely to be enzootic vectors of ZIKV in Africa and Asia
<i>Aedes Aegypti</i> <i>Aedes Albopictus</i> <i>Aedes Hensilli</i> <i>Aedes Polynesiensis</i>	<i>Aedes Unilineatus</i> <i>Anopheles Coustani</i> <i>Mansonia Uniformis</i> <i>Culex spp.</i>	<i>Aedes Africanus</i> <i>Aedes Furcifer</i> <i>Aedes Luteocephalus</i> <i>Aedes Taylari</i>

Figure 1 Potential and documented routes of prenatal and perinatal routes of ZIKV transmission.**Table 2 Fetal/neonatal major complications reported to be associated with maternal infection with ZIKV in pregnancy**

Type of complication	Specific complication
General	Fetal demise (early miscarriage), fetal growth restriction, polyhydramnios, severe arthrogryposis, low birth weight
Neurologic	Microcephaly, brain atrophy, agyria, hydrocephalus, severe ventriculomegaly, coarse multifocal intracranial calcifications, white matter atrophy, cerebellar agenesis, corpus callosal and vermian dysgenesis
Ophthalmologic	Microphthalmia, cataracts, asymmetric eyes, macular lesions, intraocular calcifications
Placental	Placental calcifications

fetal and placental abnormalities, the major of which are reported in Table 2. These abnormalities are delineating a congenital ZIKV syndrome,¹⁴ which partially resembles those occurring during infections with other viruses, such as rubella and CMV. However, maternal infection with ZIKV appears to have unique, distinctive characteristics that render it a potential global threat. These characteristics are represented by the strong tropism of the virus for the developing brain and eye – even though at present the full spectrum of fetal and/or neonatal outcomes is still undefined – and the rapid spread potential attributable to the main route of transmission (through mosquito bite). Maternal infections due to other mosquito-borne viruses – dengue, chikungunya, West Nile, and yellow fever – have some potential to cause fetal abnormalities,^{15–18} but their overall impact on the fetus appears to

be limited compared with the potentially high number of birth defects which are attributed to maternal ZIKV infection.

Microcephaly and CNS abnormalities

Concomitantly to the description of a large outbreak of ZIKV infection the Brazilian Health Ministry reported an unusual, impressive 20-fold increase – compared with the preceding 5 years – in the rate of birth defects, particularly microcephaly.¹⁹ In Pernambuco, a state of the northeast of Brazil particularly affected by ZIKV outbreaks, more than 3500 newborns with microcephaly were reported in 2015.²⁰ These observations were followed by reports of cases showing: (a) an association between fetal microcephaly and the detection, by RT-PCR, of ZIKV in the amniotic fluid of pregnant women with recent symptoms consistent with ZIKV infection,¹⁹ and (b) the detection of the viral agent in the blood and tissues of a newborn with multiple anomalies and microcephaly.²¹ Taken together, these findings supported the possibility of maternal–fetal prenatal and/or perinatal ZIKV transmission as well as of an association between prenatal ZIKV and serious congenital abnormalities. Therefore, as reported above, WHO declared the ZIKV epidemic a global health emergency¹ and several other important health organizations and authorities focused their attention on this emerging problem.^{22–24} Guidelines for pregnant women potentially exposed to ZIKV and for their health care providers were produced,^{25,26} together with recommendations to minimize or prevent contamination by ZIKV.^{27,28}

Table 3 Major ultrasonographic, CT, and MRI findings so far described in the CNS of fetuses whose mothers were infected by ZIKV during pregnancy (infection documented and/or presumed)

Reduced head circumference
Brain atrophy
Lissencephaly
Diffuse brain parenchymal calcifications (mainly involving the subcortical–cortical transition and the basal ganglia)
Cerebellar hypoplasia
Corpus callosum hypoplasia
Brain stem hypoplasia
Thin cerebral cortex
Pachygyria
Agyria
Polymicrogyria
Absence of the cavum septum pellucidum
Displacement of the midline
Hydrocephalus ex vacuo
Ventriculomegaly
Enlarged cisterna magna
Enlarged subarachnoid space
Delayed myelination

Note: Modified from references.^{13,42–45}

Even though the real impact of some brain anomalies, particularly microcephaly, was initially questioned,^{29,30} current evidence strongly suggests that ZIKV can seriously damage the developing fetal brain. This concept has been further supported by recent well-documented cases in which ZIKV genome³¹ and viral particles³² were detected in brain tissue from fetuses of mother infected by ZIKV after termination of pregnancy. The conclusion, based on the above-mentioned findings, is that there is a robust, although still not definitive, evidence supporting a causal relationship between the maternal ZIKV infection and the development of microcephaly and other severe brain abnormalities in the fetus.³³

Microcephaly is a clinical finding in which the size of the head of the fetus/neonate is smaller than expected for gestational age, sex, and ethnicity.^{34,35} It is an expression of an underlying disturbance of the growth of the brain and, when severe, can be associated with several, significant neurologic problems, including seizures, cerebral palsy, developmental delay and intellectual disabilities.^{36,37} Microcephaly is the consequence of a process of fetal brain disruption sequence,^{14,38} leading to the destruction of fetal brain tissue with diminished intracranial pressure, followed by the collapse of the fetal skulls.³⁴ Microcephaly can be determined by multiple different conditions,³⁵ including genetic and infectious causes (congenital infection by rubella, cytomegalovirus, toxoplasmosis, herpes virus, HIV) as well as exposure to alcohol, drugs or other environmental toxic substances. Microcephaly is defined as primary when detected at birth, or secondary when it is detected postnatally. The microcephaly associated with maternal ZIKV infection is of the acquired congenital type³⁶ and is likely due to the effects of ZIKV on the developing fetal brain. There is still no full agreement on the definition of microcephaly: in fact, it has been defined as an head circumference ≥ 2 or ≥ 3 SD below the mean¹⁴

or, alternatively, lesser than the 3rd or 5th percentile.³⁷ Several nomograms have been built up in order to aid in the prenatal diagnosis of microcephaly by ultrasound.³⁷ As a consequence of non-univocal definition of microcephaly, at present it is difficult to clearly establish the actual prevalence of this condition in a specific population. It is estimated that in the United States it ranges from 2 to 12/100,000 births.³⁹ Another potentially relevant issue concerning the current diagnostic limitations of microcephaly is represented by a possible overdiagnosis of this condition, particularly of the less severe cases, that can lead to the termination of pregnancies with nearly healthy fetuses.

Microcephaly related to maternal infection with ZIKV may be associated with several neurologic and brain abnormalities—such as hypertonia, spasticity, and seizures,⁴⁰ in roughly 50% of cases, and to neuroimaging abnormalities, such as brain calcifications, ventricular enlargement, and neuronal migration disorders, in virtually all the cases.⁴⁰ Many other brain abnormalities have been described fetuses from mothers infected by ZIKV: agyria, internal hydrocephalus of the lateral ventricles, small cerebellum and brain stem, open sylvian fissures, multiple disseminated calcifications in the cortex and in the subcortical white matter probably resulting from neuronal death, Wallerian degeneration of the brain stem and the spinal cord,³¹ decreased brain parenchymal volume, abnormal development of the corpus callosum,³² cerebellar atrophy.⁴¹ Viral particles consistent with flavivirus, suggesting an active viral replication, have been detected in brain cells from a fetus of a ZIKV-infected pregnant woman,³¹ and a high ZIKV load in fetal brain was detected by RT-PCR.³²

In summary, currently available knowledge strongly suggests that ZIKV: (1) has a strong neurotropism for the fetal brain; (2) is probably able to replicate and persist in the fetal brain, that could represent an immunologically protected environment; (3) exerts its main damage on the intermediately differentiated postmigratory neurons in the neocortex, which appears to undergo an apoptotic loss,³² whereas the well-differentiated neuronal cells, as well as the primitive cells in the germinal matrix, appear to be unaffected or relatively spared.³²

The corresponding ultrasonographic, CT and MRI findings, reflect the variability in the lesions observed in prenatal ZIKV infection (Table 3).^{42–45} Although there is still no evidence of cerebral lesions pathognomonic of congenital ZIKV infection, the lesions most frequently detected are sparse brain calcifications mainly involving the subcortical–cortical transition areas, ventriculomegaly, and lissencephaly.^{45,46} Although ultrasonographic diagnosis of microcephaly in the prenatal period can be very difficult, microcephaly can be detected by ultrasound as early as in the second trimester (18–20 weeks).¹³ In a prospectively investigated case, it has been reported that MRI can be more sensitive than ultrasonography in detecting early neurologic abnormalities.³²

Table 4. Major ocular abnormalities described so far in the offspring of mother infected by ZIKV in pregnancy.

Focal pigment mottling of the retina
Chorioretinal atrophy
Cataract
Iris coloboma
Optic nerve abnormalities (hypoplasia and severe cupping of the optic disk)
Optic nerve atrophy
Lens subluxation
Asymmetrical eye size
Intraocular calcifications
Foveal reflex loss
Optic nerve double-ring sign
Increased cup-to-disk ratio

Note: Modified from references.^{43,47–50}

Ocular abnormalities

In addition to microcephaly and other severe CNS abnormalities, congenital ZIKV infection has been associated with several relevant ocular findings. In a series of 29 infants with microcephaly presumably attributable to congenital ZIKV infection followed in an ophthalmology tertiary center, ocular abnormalities were detected in 10 cases (34.5%), with bilateral findings present in 70% of them.⁴⁷ The most common findings were focal pigment mottling of the retina and chorioretinal atrophy.⁴⁷ The ocular abnormalities described in the offspring of mother infected by ZIKV during pregnancy are reported in detail in Table 4.^{43,47–50} Interestingly, a major risk factor for ocular abnormalities in infants with microcephaly and presumed congenital ZIKV infection was the occurrence of maternal symptomatic infection during the first trimester.⁴⁹ Definitive information on ophthalmologic findings in infants born to ZIKV-infected mothers but without microcephaly is not available; therefore, it is uncertain whether these infants should undergo ophthalmologic screening.

Other abnormalities

In addition to CNS, ZIKV has been detected in other organs and tissues from fetuses of mother infected during pregnancy. Driggers et al. found high ZIKV RNA loads in the placenta, fetal membranes, and umbilical cord, and lower amounts in fetal muscle, liver, lung, and spleen.³² Conversely, Mlakar et al. could not be able to detect neither ZIKV nor macroscopic or microscopic lesions in any of the fetal organs examined apart from the brain.³¹ Therefore, while it is clear that ZIKV can infect the placenta, which could represent a reservoir of the virus, the role as well as the clinical relevance of the presence of the virus in other non-CSN organs or tissues it is still undetermined. It is possible, although still not demonstrated, that placental infection could be responsible for ZIKV-related miscarriages. With respect to the localization of ZIKV in the placenta, it has been demonstrated that ZIKV can infect and replicate in human primary placental macrophages (Hofbauer cells) and, although to a lesser extent, also in syncytiotrophoblasts (differentiated cytotrophoblasts).⁵¹

Therefore, placental macrophages are, at least to some extent, permissive to productive ZIKV infection and could have a key role in the maternal–fetal ZIKV transmission.⁵¹

Rates of maternal–fetal transmission

There are several key questions concerning congenital ZIKV infection that are still unsolved: (a) the overall rate of fetal transmission of the virus from an infected mother; (b) the rate of fetal transmission of the virus from an infected mother by trimester of pregnancy; (c) the rate of microcephaly or other fetal abnormalities by trimester of pregnancy when maternal infection occurs. Information on these issues is still largely incomplete. However, current evidence suggests that maternal infection in the first trimester of pregnancy carries the highest risk of fetal infection.^{50,52} Cauchemez et al. built up a mathematical model to analyze retrospectively serological and surveillance data from the large ZIKV outbreak occurred in French Polynesia in 2013–2014.⁵³ According to their model, they concluded that the first trimester is the period of pregnancy associated with the highest risk for fetal microcephaly, with an estimated rate of 95 cases/10,000 infected women (95% CI: 34–191).⁵³ As the author stated, the risk of about 1% is much lower than that observed for other well-known maternal viral infections, such as CMV and rubella, that are known to cause fetal congenital syndrome at rates of 13% and 38–100%, respectively.^{54,55} However, the population risk may be potentially high during epidemics, when a high number of ZIKV infection occurs among pregnant women. Moreover, the study considered only microcephaly, that could represent only the tip of the iceberg of the congenital ZIKV syndrome, and was based only on eight cases. In contrast, using an integral equation model applied to the outbreak in northeastern Brazil in 2015, Nishiura et al.⁵⁶ estimated that the theoretical risk of fetal microcephaly after maternal infection in the first trimester could be as high as 46.7% (95% CI: 9.1, 84.2), which is comparable to the risk of congenital rubella syndrome. However, there is also some evidence suggesting that maternal infection in the second trimester could also be associated, although probably to a lesser extent – with fetal microcephaly⁵⁶ and other abnormalities and problems, including stillbirth.⁵⁷ Prospective investigation is urgently needed to clarify this important issue.

Finally, in a recent study conducted in Colombia, Pacheco et al. did not observe any case of microcephaly in fetuses/neonates of women infected during the third trimester of pregnancy,⁵⁸ suggesting that fetal damage from ZIKV occurs mainly when the mother became infected in early pregnancy. This hypothesis has been further supported by another recent study carried out in Bahia, Brazil, in which the projection of birth months in relation to the occurrence of microcephaly revealed that the first trimester and the early second trimester of pregnancy are the periods of highest risk for ZIVK transmission.⁵⁹

Mechanisms of cell damage with specific application to the fetus

The molecular mechanisms and the host pathways leading to the pathogenesis of ZIKV-induced damage, with specific regard to fetal abnormalities, are still largely unknown. However, emerging experimental evidence suggests that ZIKV, although being capable to infect several cell types, has a high and selective tropism for nerve progenitor cells (NPCs) in which may create several types of damage. Mouse models have shown that ZIKV may determine fetal demise⁶⁰ and microcephaly,⁶¹ as well as significant histopathological lesions selectively limited to brain tissue.⁶² Tang et al. demonstrated that ZIKV can efficiently infect human NPCs, in which it is able to determine a profound perturbation of the cell cycle, leading to a reduction of cell growth, to activate the caspase-3 mediated apoptosis pathway, and to substantially increase cell death.⁶³ A study carried out using a bioinformatic approach showed that infection of human NPCs results in substantial changes in gene up- and down-regulation.⁶⁴ In this context, prominent features are represented by the hyper-expression of genes involved in the regulation of nucleic acid metabolism (hallmark of virus replication) and the down-regulation of genes involved in chromosome segregation and DNA replication and repair.⁶⁴ In addition, ZIKV infection of the above-mentioned cells results in the activation of several innate immunity pathways, that are different from those observed in other infections known to cause congenital fetal abnormalities, such as CMV.⁶⁴ Experiments carried out in more complex systems, such as human cerebral organoids, are consistent with the above reported observations.^{65,66}

Open questions

Although the knowledge about ZIKV infection in pregnancy has rapidly improved in the last six months, several issues remain unclear and many questions still need to be answered. More detailed information is urgently needed on the overall rate of fetal transmission of ZIKV from an infected pregnant mother, as well as on the specific fetal transmission rate by each trimester of pregnancy. In particular, there is an urgent need to confirm whether the risk of malformations is limited to ZIKV transmission during the first trimester of pregnancy. Additional evidence is needed on the occurrence of fetal damages other than CNS and eye abnormalities. Moreover, it is still undefined whether ZIKV can be associated also with more subtle alterations in the CNS or in other organs or systems, even in absence of microcephaly and/or ocular findings. More information is needed on the possibility that the genetic evolution and changes in the ZIKV are associated with an increased fetal susceptibility to damage. Finally, more information is needed on the molecular, genetic and immunologic mechanisms and pathways through which ZIKV can determine damage in NPCs.

An expanded knowledge on the above relevant points could be particularly important in order to make evidence-based decisions, avoiding unnecessary pregnancy terminations, and to allow physicians to offer appropriate counseling to pregnant women.

Finally, appropriate guidelines on the precautions to be adopted in order to minimize the risk of infection during early pregnancy are of paramount importance, while waiting for the availability of an effective vaccine.

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