Chapter

Clinical Manifestations in Pregnant Women and Congenital Abnormalities in Fetus and Newborns during a Zika Transmission Period in South Mexico

Norma Pavía-Ruz, Silvina Noemí Contreras-Capetillo, Yamila Romer, Nina Valadez Gonzalez, Hector Gómez-Dantés, Gonzalo Vázquez-Prokopec and Pablo Manrique-Saide

Abstract

Dengue, Chikungunya and Zika are arboviruses transmitted by *Ae. aegypti* with significant public health impact. In the first trimester of 2015, autochthonous Zika transmission was reported in Mexico. The state of Yucatan is an endemic region where pregnant women with acute infection and related congenital abnormalities in fetus and newborns were observed. We describe results from a cohort of pregnant women and their babies followed up in Yucatan during the first Zika transmission outbreak (2016–2018). Clinical manifestations of acute ZIKV infection, persistence of viral RNA in pregnant women, as well as congenital abnormalities were observed. In addition, we describe the phenotype of newborns from confirmed or suspected ZIKV prenatal infection.

Keywords: Zika infection, pregnant women birth defects, pregnancy, congenital Zika syndrome

1. Introduction

Dengue (DEN), Chikungunya (CHIK) and Zika (ZIKV) are arboviruses transmitted by the mosquito *Aedes aegypti* and known as *Aedes*-borne-diseases. These diseases are associated with high morbidity and low mortality and considered a public health problem [1]. In the 2015, the ZIKV outbreak was considered an international emergency because infection in pregnant women was related to the increase of congenital abnormalities in the fetuses [2–4]. Vertical transmission of ZIKV was demonstrated by the RNA viral detection in placenta, amniotic fluid, serum and fetal brain in products with microcephaly, abortions or in autopsies of affected newborns and offspring of symptomatic or asymptomatic mothers [5, 6].

The clinical manifestations of Zika in general population and pregnant women were mild rash, conjunctivitis and low fever, although up to 80% remain asymptomatic, higher than DEN (19%) and CHIK (45%) [1, 2, 7, 8].

ZIKV is a Flavivirus with an Asian and African lineages [9, 10]. Its RNA genome (10.8 kb) encodes for a 3419-amino acid polyprotein which form a capsid (C), a membrane precursor (prM), a wrap (E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [11]. ZIKV interferes with the neural development through decreased neural progenitor cells, arrest in neuronal migration and/or disruption of the maturation process of the fetus central nervous system (CNS) [12, 13]. The congenital Zika virus syndrome (CZVS) is a pattern of congenital defects associated with ZIKV infection during the pregnancy so ZIKV pathogenicity and virulence is currently studied [14–18].

The surveillance of ZIKV infection during pregnancy in endemic regions requires screening and detection of fetal morphological abnormalities [19]. An integrated intervention model for the prevention of Zika and *Aedes*-borne diseases, that includes primary health care services, gynecologists, obstetricians, pediatricians, geneticists and neurologists should be mandatory. Strategies to prevent and control the vectors and reduce the risk for diseases transmission should be strengthened, particularly for protection of women in reproductive ages [20, 21].

Here we report final the clinical manifestations observed in a cohort of pregnant women and the congenital abnormalities in fetus and newborns during a Zika transmission period (2016–2018) in South Mexico.

2. Methods

We developed a prospective study to quantify the incidence of disease and infection in a cohort of pregnant women and newborns during an epidemic period of Zika (2016–2018). One of the main objectives of the study was to know the effect of prenatal exposure to ZIKV. The cohort included pregnant women, preferably in the first trimester of pregnancy. The follow up included clinical and molecular detection of ZIKV, DEN and CHIK. Obstetric ultrasound was performed to recognize morphological abnormalities in the fetuses.

During the development of this study, information on health care was provided to pregnant women and their partners, highlighting the importance of family planning and the use of condoms as a method to prevent the transmission of ZIKV, in addition to the implementation of measures to prevent the breeding of the *Aedes aegypti* and mosquito bites at home. We also provided information about general healthy habits, family planning and prevention of sexually transmitted diseases.

2.1 Study population

After the informed consent was signed, women were interviewed, and their medical records including periconceptional and pregnancy history, were collected. The recruitment included 884 families (3993 people) from the cities of Merida, Ticul, and Progreso de Castro in the Yucatan State, South-east Mexico [16]. Merida and its metropolitan area (≈1 million inhabitants), comprises ≈50% of the Yucatan population. Progreso de Castro (37,400) and Ticul (32,000) are smaller urban areas. We enrolled consenting pregnant women from these areas from July 1, 2016 to June 2018 including pregnant women referred by physicians in primary care facilities or hospitals within the areas of our cohort study.

2.2 Clinical follow-up of pregnant women

Patient monitoring included a monthly visit for clinical assessment and sample collection (blood and urine for RTC DEN/CHIK/ZIKV), weekly follow-up by text messages, and complete access to a telephone to report any clinical signs in pregnant women, their newborns, or any family contact. Tissues as umbilical cord blood, placental, amniotic fluid and breastmilk were collected, when possible for assessment of RT-PCR RNA ZIKV (TRIOPLEX) [22]. Other biochemical tests were performed to rule out Toxoplasma, Rubella, Cytomegalovirus, Herpes, Syphilis and HIV [23]. Depending of the mother or/and fetus risks, other test such as biochemical serum test and karyotype in amniotic fluid were taken. Ultrasound scanning was performed at the first contact and every 2 months. At the first visit, a questionnaire was fill up to establish the clinical-epidemiologic profile. The follow-up ended when the pregnancy was completed by delivery or fetal loss, or the participant withdrew from the study.

2.3 Clinical follow-up of newborns

Newborn follow up included: clinical evaluation (anthropometric measurements, APGAR score and physical exam) and sample collection for RT-PCR for DEN/CHIK/ZIKV [20–22, 24]. Patients were followed up for 24 months of life for early recognition of morphological anomalies and recorded the neurological development. These evaluations included genetic, neurologic, ophthalmologic, and audiologic evaluation. Microcephaly was defined as a cranial circumference ≥2 SDs below the mean for the age and sex of the baby, following the recommendation of the World Health Organization [25–28].

2.4 Patients not initially integrated into the cohort

At the time when the cohort study was ongoing, in the Genetic Service in the center of Investigations Dr. Hideyo Noguchi/UADY received pregnant women with morphological abnormalities detected in their fetuses, or newborn patients in whom ZIKV prenatal infection was suspected. These patients were not integrated to the cohort, but a clinical follow-up was granted. Sample collections for RT-PCR for DEN/CHIK/ZIKV were offered.

3. Results

3.1 Pregnant women cohort

The study integrated 130 pregnant women with average age of 25 years. No major differences in age distribution and socioeconomic status between ZIKV-positive and ZIKV-negative mothers were observed. Of all women, 40 (30%) were in the first trimester of pregnancy upon admission to the study, 62 (48%) in the second trimester and 28 (22%) in the third trimester (**Table 1**).

Positive results in *blood/urine* for ZIKV were found in 39 pregnant women, 31% (n = 13/39) at the first trimester, 52% (n = 20/39) in the second trimester and 15% (n = 6/39) in the third trimester.

Of 130 pregnant women, 39 (30%) were RNA-ZIKV positive at the time of the recruitment and 91 were negative (70%). From these, 11 (12% of initially RNA-ZIKV negative) became positive during the surveillance.

	Total pregnant women	Positive for ZIKV in blood/urine	Negative for ZIKV in blood/urine	Trimester of pregnancy when enrolled pregnant women (130)		
	N:130	**N:39**	**N:91**	**1st N:40**	**2nd N:62**	**3rd N:28**
Mérida	75 (58%)	27 (69%)	47 (52%)	16 (40%)	37 (60%)	22 (79%)
Progreso	4 (3%)	0	4 (4)	0	2 (3%)	2 (7%)
Ticul	51 (39%)	12 (31%)	41 (44%)	24 (60%)	23 (37%)	4 (14%)
15–19 years	29 (22%)	8 (28%)	21 (72%)	6 (15%)	16 (26%)	7 (25%)
20–29 years	70 (54%)	22 (31%)	48 (69)	22 (55%)	34 (55%)	15 (54%)
30–39 years	30 (23%)	9 (23%)	21 (%)	12 (30%)	12 (19%)	6 (21%)
40–49 years	1 (1%)	0	1 (%)	0	0	1
Signs and symptoms		21/39 (%)				
Exanthema		21/21 (100%)				
Pruritus		11/21 (52%)				
Joint edema		7/21 (33%)				
Conjunctivitis		16/21 (76%)				
Retro-orbital pain		10/21 (55%)				
Hyperemia		5/21 (22%)				
Clinical profile in persistence in serum samples		**Total N:15**	**Symptomatic N:9**	**Asymptomatic**		**p**
Age (years)		24.7 ± 4.4	24.2 ± 3.3	23.8 ± 6		0.874
Persistence (days) (interval)		45.93 ± 24.4 (17–19)	50.11 ± 30.58 (17–97)	39.67 ± 9.89 (29–52)		0.363
Pregnancy trimester						
First		6 (40%)	3	3		
Second		8 (53%)	6	2		
Third		1 (7%)		1		

Table 1.
Summary of health, medical and laboratory data collected from pregnant women in the cohort.

Of the 28 symptomatic patients (negative or positive ZIKV), the most common symptoms were exanthema (75%), pruritus (39%) and conjunctivitis (57%). Of the 21 patients with RT-PCR ZIKV-positive, 75% were symptomatic and 25% were asymptomatic. Even so, more than half (64%) of the women had at least, more than one sign or symptom compatible with an *Aedes*-borne acute infection. Most prevalent symptoms were exanthema (100%), conjunctivitis (76%), pruritus (52%) headache (50%), retro-orbital pain (55%), arthralgia (33%), hyperemia (22%) and joint edema (6%). No hemorrhagic or systemic complications were observed in any patient (**Table 1**). Differences in the distribution of ZIKV-positive vs. ZIKV-negative women between the studied cities were not founded.

3.2 Persistence of ZIKV viral RNA in serum and urine in pregnant women

Of the 39 ZIKV-positive pregnant women, persistent RNA-ZIKV was detected in 38.5% (15/39) of the patients during 14 days after the initial symptoms or the last

PCR detection. Of these, six women were in the first trimester of gestation, eight in the second and one in the third. Within the group of symptomatic women (n = 28), nine (9/28) presented persistence of RNA-ZIKV, three in the first trimester and six

Newborn residence	**Merida N:67**		**Progreso N:2**		**Ticul N:46**		**Total births N:115**
Percentile	**Percentile <3 N (%)**		**Percentile 3–97 N (%)**		**Percentile >97 N (%)**		**Total**
	At birth	**End of the study**	**At birth**	**End of the study**	**At birth**	**End of the study**	
Birth weight percentiles	5 (4)	1 (1)	110 (96)	114 (99)	0 (0)	0 (0)	115 (100)
Birth height percentiles	3 (3)	0 (0)	112 (97)	115 (100)	0 (0)	0 (0)	115 (100)
Birth head circumference percentiles	2 (2)	1 (1)	112 (97)	114 (99)	0 (0)	0 (0)	115 (100)
Newborn evaluations							
	From mothers ZIKV negative during pregnancy N:80				**From mothers ZIKV positive during pregnancy N:35**		
	N (%)		**Alterations N (%)**		**N (%)**		**Alterations N (%)**
Newborn blood	29 (36)				27 (77)		
Newborn urine	32 (40)				14 (40)		
Pediatrics	39 (49)				30 (86)		
Ophthalmology	19 (24)				31 (89)		3 (10)
Auditory screening	51 (64)				30 (86)		2 (7)
Placenta	NA		NA		17 (49)		
Umbilical cord	NA		NA		17 (49)		
Genetics	NA		NA		35 (100)		
Metabolic screening	NA		NA		34 (97)		
Transfontanelar and abdominal ultrasound	NA		NA		22 (63)		
Age of newborns who completed the study							
Age	**Mother ZIKV –**		**Mother ZIKV +**		**Total**		
1–6 months	16 (22%)		0		16 (16%)		
7–12 months	25 (34%)		3 (10%)		28 (27%)		
13–18 months	20 (27%)		17 (59%)		37 (36%)		
19–23 months	12 (17%)		9 (31%)		21 (21%)		
Total	73		29		102		

Table 2.
Newborn cohort follow-up.

Patient/ sex	Mother Zika symptoms (MoG[1])	WoG[2]	Neonatal head circumference (z score)	Birth weight for gestational age	Phenotype	Intracranial calcification	Zika IgM InBios newborn/ mother[6]	Final diagnosis
1/Male	No	38	**Microcephaly (−3)**	Small	**Microcephaly MMC[3]**	**Yes**	−/−	CZS/neural tube defect
2/Male	**Yes (2)**	37	Normocephaly (10)	Small	IUGR[4]	NR[5]	−/−	IUGR
3/Male	No	38	**Microcephaly (−3)**	Appropriated	**Microcephaly**	**Yes**	−/−	CZS
4/Male	**Yes (2)**	37	Normocephaly (3)	Small	Asymptomatic	NR	−/−	IgM positive for toxoplasma
5/Male	No	33	Normocephaly (10)	Appropriated	**Microcephaly Arthrogryposis**	**Yes**	Positive in serum/−	CZS
6/Male	No	39	**Microcephaly (−3)**	Appropriated	**Microcephaly**	**Yes**	−/−	CZS
7/Female	**Yes (2)**	40	Normocephaly (3)	Small	**Postnatal microcephaly**	NR	−/−	CZS
8/Female	No	38	Macrocephaly (97)	Appropriated	**Macrocephaly Arthrogryposis**	Normal	−/−	Amyoplasia congenital

[1]*Month of gestation.*
[2]*Weeks of gestation.*
[3]*Myelomeningocele.*
[4]*Intrauterine growth retardation.*
[5]*NR: not reported.*
[6]*Serum and urine were taken at born in newborns and theirs mothers.*

Table 3.
Main findings of eight patients evaluated for suspected ZIKV prenatal infection.

in the second. Of these, 100% (9/9) presented rash, 55% (5/9) conjunctivitis and 33.3% (2/9) fever. Only 22.2% (2/28) reported having three symptoms, fever, rash and conjunctivitis (**Table 1**).

3.3 Newborn evaluations

All pregnancies of women in the cohort have ended the pregnancy. Two fetal losses (one in the first trimester and one in the third trimester) occurred in ZIKV-negative mothers. Of all newborns alive, 3% were preterm (two in ZIKV-negative mothers and one in a ZIKV-positive moth group). No newborns or products of conception were positive for RNA-ZIKV. Clinical evaluation of Apgar scores in the newborns did not show significant differences between positive/negative ZIKV mothers. Also, microcephaly was not founded in any newborn. One newborn of Zika-positive mother died the first days of life due to gastroschisis (**Table 2**).

During the ZIKV epidemiological period in Yucatan, 10 patients attended the medical genetics clinic in CIR Hideyo Noguchi with a reference diagnosis of microcephaly, arthrogryposis and/or ventriculomegaly. Two of them were excluded from the follow up because ZIKV prenatal symptoms were absent in the mothers and microcephaly and intracranial calcifications were discarded in the patients. Preconceptional, prenatal and perinatal backgrounds were investigated in all patients. Only one woman took folic acid 6 months before the conception and none used mosquito repellent during pregnancy, even they were living in an endemic region.

Of the newborns evaluated, clinical symptoms of Zika were reported only in two mothers in the first trimester of gestation, but only one was tested positive for RT-PCR Zika. One more woman reported symptoms of Zika 1 month before conception. During the pregnancy, intrauterine growth restriction in 4/8 (50%) and oligohydramnios in 2/8 (25%) were reported. Only one patient was reported prenatally with microcephaly, intracranial calcifications and ventriculomegaly (patient 5) (**Table 3**). Five pregnancies were ended by caesarean section and three by vaginal delivery between second semester of 2016 and the second semester of 2017.

All newborns were at term, except one. Of them, six were males and two were females. Three males had microcephaly with less than three standard deviations and one male with microcephaly less than two standard deviations. One female had macrocephaly. All babies from the three mothers with positive ZIKV symptoms during o before pregnancy were born with normocephaly, but were small for gestational age. One of these babies, a female, developed microcephaly within the first 6 months of life. Of all babies follow up, two had arthrogryposis, one with microcephaly and one with macrocephaly. Both of them were child from non-symptomatic mothers. Only five patients were evaluated with axial computerized tomography with positive intracranial calcifications founded in four. All of them were from asymptomatic mothers.

All patients and their mothers were tested for RT-PCR for ZIKV/DEN/CHIK in serum and urine. TORCH was also performed. RNA-ZIKV was obtained only in serum of one male with microcephaly, arthrogryposis and intracranial calcifications. This male was from an asymptomatic mother. Antibodies IgM of toxoplasma were detected in one asymptomatic male whom has clinical symptoms of Zika during first trimester of pregnancy.

4. Discussion

The WHO declared the ZIKV outbreak in South America and the associated increase in neurological disorders and neonatal malformations a "*public health*

emergency of international concern" [2], and the CDC issued the ZIKV epidemiological alert, recommendations of high-risk Mexican territories was recognized by the National Health Service. Even so, the recognition of the ZIKV infection symptoms by patients and health workers, were underestimated [29]. In Mexico, first patients with ZIKV infection were documented early in 2016 even so, captured mosquitoes in early 2015 were recognized with ZIKV infection [30].

After the observed relationship between prenatal Zika infection and the risk of congenital defects in Brazil and Colombia, we realized a cohort with detailed evaluation of pregnant women searching ZIKV infection and congenital abnormalities [1, 31, 32].

The women in this study with the highest proportion of symptoms with ZIKV were those from 20 to 29 years, different from other studies that have reported the highest symptomatic disease ratio among women >30 years of age [33, 34]. In our studies this can be explained because the highest incidence of pregnancies in women was aged 20–29 years [1]. In clinically affected women, univariate analyzes showed that the most sensitive clinical sign was the exanthema, but it was also the least specific. The triad, conjunctival hyperemia, joint edema and exanthema had the highest level of specificity [35].

In pregnant women with exanthema without fever or other symptoms should to suspect ZIKV infection. In this study, ZIKV/DEN/CHIK co-infection was not identified neither [36].

The persistence of RNA viral in pregnant women has been described previously, reported a patient positive for RNA-ZIKV until 10 weeks after the onset of symptoms. In this study, the maximum viremia persistence was 97 days, but a quarter of positive mothers had viremia for more than 8 weeks [37]. From all RNA ZIKV-positive mothers, only 50% of the symptomatic group had persistence of ZIKV for more than 8 weeks. Other series reported longer RNA ZIKV viremia in symptomatic women than asymptomatic and other study obtained similar results in serum, in a range of 14–63 days; although in this study, we detected two pregnant women who had viremia older than 90 days [33, 34]. A study reported viral persistence in five pregnant women, of which 80% were symptomatic and only one case was asymptomatic. In this study, a higher percentage of asymptomatic viral persistence cases were found with 40%. In the symptomatic group, the average number of days of persistence after the onset of symptoms was greater than that reported, no relationship was observed between viral persistence and the presence of abnormalities in pregnancy products [38, 39].

The confirmatory tests which provide evidence for prenatal infection by ZIKV were conducted based on tests in the mother and the newborn were made with viral RNA isolated from biological fluids and placental tissues performed with the Trioplex kit of the CDC. The ZIKV genome was detected in cerebrospinal fluid, cardiac fluid, chorionic villi, fetal face of the placenta, serum and urine [26]. Vertical transmission studying the placental and fetus tissue also failed been demonstrated [40, 41].

Clinical variability in patients in whom ZIKV infection were prenatally suspected was described previously, in this cohort, congenital abnormalities associated to ZIKV were not observed in fetus, stillborn or newborns [1, 17]. Only one patient with gastroschisis was detected but the prenatal ZIKV infection in her mother was after detection of this abnormality.

Congenital Zika syndrome (CSZ) was observed in the patients evaluated from the Genetics Service out of the initial cohort. In them, Zika symptoms in mothers, microcephaly, arthrogryposis and intracranial calcifications were observed as in other reports [1, 17, 18]. Establishing the final diagnosis of ZIKV prenatal infection was difficult especially in asymptomatic mothers or in mild affected babies [31].

In two patients, the final diagnosis was toxoplasma and congenital amyoplasia. Asymptomatic or mild symptomatic women would be a seriously limitation from early ZIKV infection. To define diagnostic of prenatal ZIKV is important: (1) to establish the follow up of the affected patient, (2) to limit the tests related to others genetic diseases that share symptoms with CSZ and (3) to establish the specific risk of recurrences of congenital abnormalities in subsequent pregnancies in the mother.

5. Conclusions

In this study, symptomatic or asymptomatic pregnant women with a high prevalence for ZIKV are reported, however there was no positive newborn or with malformations associated with ZIKV, despite the genetic consultation, the presence of CSZ, laboratory-confirmed if present so doctors should maintain a realistic perspective of the impact of ZIKV on pregnancy. And despite the uncertainties, future mothers should receive adequate, systematic advice and the best planned obstetric surveillance, always considering the gestational moment of maternal ZIKV infection and accept that there is gestational risk for different elements of the CZS phenotype in risk areas ZIKV transmission.

Acknowledgements

This research and publication was made possible through support provided by and the Canadian Institutes of Health Research (CIHR) and IDRC (preventing Zika disease with novel vector control approaches Project 108412) and the Office of Infectious Disease, Bureau for Global Health, US Agency for International Development, under the terms of an Interagency Agreement with CDC. The opinions expressed herein are those of the author(s) and do not necessarily reflect the views of the US Agency for International Development. The recipient of this support was the Yucatan Autonomous University and its affiliated Regional Research Center "Hideyo Noguchi."

Author details

Norma Pavía-Ruz[1], Silvina Noemí Contreras-Capetillo[1], Yamila Romer[2], Nina Valadez Gonzalez[1], Hector Gómez-Dantés[3], Gonzalo Vázquez-Prokopec[2] and Pablo Manrique-Saide[1*]

1 Autonomous University de Yucatan, Merida, Yucatan, Mexico

2 Emory University, Atlanta, GA, USA

3 Public Health Institute of Mexico, Cuernavaca, Morelos, México

*Address all correspondence to: msaide@correo.uady.mx; pablo_manrique2000@hotmail.com

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