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Clinical and epidemiological profile of the mother–neonate binomy with microcephaly and suspected infection by zika viruses in the gestation: a sectional study

Abstract

Introduction: The association between the increased incidence of microcephaly and the epidemic outbreak of Zika virus infection (ZIKV) in Brazil between 2015 and 2016 was observed by many authors. World Health Organization declared it as a public health emergency of international concern. Maternal infection with the Zika virus can be transmitted vertically and lead to Congenital Zika Syndrome (CZS) in infants. So that it is important to investigate the clinical-epidemiological profile of the mothers and their newborns.

Aims: To characterize the clinical- epidemiological profile of pregnant women associated with presumed ZIKV in pregnancy and their newborns diagnosed with microcephaly at birth and associated with CZS.

Methods: A sectional study, using medical record data, between September 2015 and June 2016.

Results: 82 cases of microcephaly were reported in maternity during this period. Of these, 32 cases were excluded because they did not fit the new microcephaly criteria according to the World Health Organization (WHO) and the Ministry of Health of Brazil. The mean maternal age was 25 years, varying from 13 and 43 years old; exanthema was the only symptom related to Zika virus infection and it was observed in 60% (27/45) of the pregnancies, which occurred predominantly during the first or the second trimester of pregnancy. Other mothers were asymptomatic. Related to the newborns, 62% (31/50) were female; 32% (16/50) were low weight; 2% (1/50) were premature. Neonatal brain ultrasound showed 70,4% (31/44) of the neonates with abnormal findings, mainly calcifications that occurred in 87% (27/31). 29 newborns were submitted to fundoscopy examinations and 38% (11/29) were abnormal.

Conclusion: Despite the majority of pregnant women did not have classic symptoms of arboviruses and because of this the suspicion and confirmation of Zika viruses infection through serologies are difficult in clinical practice, it is important to understand clinical and epidemiological characteristics related to Zika viruses infection and the CZS to allow mapping where preventive measures should be directed and better investigated as well as to offer an adequate follow-up to the infected neonates according to their outcomes.

Keywords: zika virus, microcephaly, arboviruses, congenital infection, Congenital Zika Syndrome

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Introduction

Zika virus (ZIKV) is an arbovirus of the Flavivirus genus, originally isolated in 1947 from a rhesus monkey utilized as bait for a study of Yellow Fever Virus (YFV) in the Zika forest, near Kampala, Uganda.[1–3] It has been isolated in several African countries (Uganda, Tanzania, Egypt, Central African Republic, Sierra Leone, and Gabon), Asian countries (India, Malaysia, the Philippines, Thailand, Vietnam, and Indonesia), and in Micronesia.[1,2]

Zika virus was probably introduced in Brazil during the World Soccer Cup in 2014 when many tourists visited the city of Natal and other Brazilian capitals, possibly contributing to the infection of mosquitoes of genus *Aedes* (*Stegomyia*).[3–5] ZIKV was identified for the first time in Brazil by reverse transcriptase-polymerase chain reaction (RT- PCR) in blood samples from patients in the Northeast region of the country[6] and autochthonous transmission was confirmed in Brazil months later.[3,7,8] Since then ZIKV transmission has been confirmed in at least 35 countries.[3]

Microcephaly associated with Zika virus infection has been described in the last decade in Latin America, but in 2015/2016, there was an epidemic outbreak in northeastern Brazil (South America). In 2016, the World Health Organization declared that the cluster of neurological disorders, mainly microcephaly cases, reported in Brazil in 2015/2016 had constituted a Public Health Emergency of International Concern.[8]

Also in 2016, the virus was identified by RT-PCR in tissue samples from two neonates with severe microcephaly[9] and by the detection of ZIKV in the amniotic fluid from two fetuses of women with a history of exanthema during pregnancy.[10–12] Although the pregnant women may present symptoms as rash, fever, arthralgia and conjunctivitis, most of them are asymptomatic.[3] Seroprevalence studies showed that

80% of ZIKV infections were asymptomatic.[3] Thus, diagnosis of vertical exposure to ZIKV is based on positive RT-PCR in mothers with suspected symptoms during pregnancy.[10] ZIKV is able to cross the placental[3] and can cause Congenital Zika syndrome (CZS). The CZS is resulting from vertical transmission of the virus and includes microcephaly, specific findings from imaging of the central nervous system, ophthalmic and auditory deficits, arthrogryposis and cardiac defects.[13–16]

Considering public health problems caused by Zika virus, its infection is a compulsory notification disease since 2017 in Brazil. According to the Information System of Compulsory Notification Diseases, up to epidemiological week 11 in 2019 (December, 2018 to March, 2019), there were 2.344 probable cases of Zika virus infection in the country (incidence of 1,1 case/100.000 inhabitants) and 393 probable cases in pregnant women, with 59 confirmed cases.[17]

Regarding on deaths and cases suspected of abnormalities in growth and development related to Zika virus infection and other infectious etiologies from August, 2015 (epidemiological week 45/2015) to December 2018 (epidemiological week 52/2018) in Brazil, 17.041 suspected cases were reported. Most of them in the Northeast region of the country (58.5%), which Pernambuco (16.4%) and Bahia (15,6%) were the states with the highest number of reported cases. Of these, 3.332 (19.6%) were confirmed; 643 (3.8%) were classified as likely to be related to congenital infection during pregnancy.[18]

The states of Pernambuco and Bahia were mainly affected causing a huge impact on family planning and the health of the general population, especially for the households of children affected.[5,6,7,18] Most neonates had nervous system severely affected, with the commitment of growth and development[11]. The eye lesions have been described, and severe involvement of the vision constituted another major aggression of the virus to the newborn.[15] In addition to the clinical repercussions, ZIKV infection brought socio-economic and hygienic-sanitary concerns to the country.[19–22] According to the Brazilian Ministry of Health, there were 4.180 reported cases of microcephaly or malformation of the central nervous system suggestive of congenital infection in Brazil in 2015-2016. Of these, 270 cases were confirmed after investigation with imaging exams and/or serologies.[22]

Although the recent epidemics in the Americas and other countries have confirmed vertical transplacental transmission of Zika virus and its association with congenital anomalies, particularly severe central nervous system lesions, the exact burden of disease remains unclear.[23] The objective of the present study was to characterize the clinical-epidemiological profile of pregnant women that are associated with presumed ZIKV in pregnancy and their newborns diagnosed with microcephaly at birth and associated with the CZS in a Maternity of Salvador – Bahia.

Methods

Study design

We performed a sectional study at Climerio de Oliveira Maternity (MCO), a public Federal University Maternity located in Salvador city from September 2015 to June 2016. The data were collected between April and June 2016 from medical records of neonates diagnosed with microcephaly at birth at MCO and their mothers.

Inclusion criteria

All newborns with microcephaly born at MCO in the period from September 2015 to June 2016 were included in the study. Considering criteria established by the World Health Organization (WHO) and the Ministry of Health of Brazil, microcephaly was considered when the head circumference was ≤31.5cm (girl) and ≤31.9cm (boy) at birth or when premature or low-weight newborns whose cephalic perimeter was below the third centil (<P3) according to Fenton Preterm Growth Chart[24] and Intergrowth Chart.[25]

Clinical and epidemiological data of newborn`s mothers were also included in the study. The suspicion of congenital ZIKV infection of the newborn was done according to clinical criteria: one or more manifestations caused by pathogenic arbovirus in the mothers including cutaneous rash, fever, arthralgia, headache, and malaise. There was not possible to use laboratory criteria in order to confirm ZIKV infection because the RT- PCR technique and antibody dosage were not universally available at that time during prenatal care and they were not used routinely during birth of children with microcephaly. Viral RNA is detected up to seven days after the onset of symptoms.[20] Immunoglobulin IgM and immunoglobulin IgG are detected after seven and fourteen days, respectively.[20]

Exclusion criteria

Term newborns were excluded with head circumference measuring ≥31,6 in girls and ≥32 centimeters in boys and in preterm babies with the head circumference above the third centile (≥P3) according to Fenton Preterm Growth Chart[24] and Intergrowth Chart[25] after confirmation by neurological examination. The WHO´s criteria of microcephaly had been changed during epidemic and we considered the most recently one. Were excluded stillborn babies and abortion.

Data collection

Data were collected from the medical records and from the notification form of microcephaly´s cases developed by the Ministry of Health of Brazil[22] and available on the website www.resp.saude.gov.br.

Epidemiological data of their mothers and clinical data related to the pregnancy were collected from the medical records. The maternal variables were age; race; educational level; civil status; occupation; origin; parity; signs and symptoms of arboviruses. Gestational timing of probably arboviruses infection was reported according to symptom onset date.

Epidemiological and clinical data of the newborn with microcephaly were also collected from the medical records. The newborn variables were gender; gestational age at birth; twinning; birth weight; length at the birth; head circumference; classification of microcephaly according to Fenton Preterm Growth Chart[24] and Intergrowth Chart;[25] neonatal examination report (brain ultrasound, abdominal ultrasound, echocardiogram, hearing screening and fundoscopy).

Laboratory analysis

The laboratory analysis of Zika virus was not used routinely at the beginning of the epidemic. So, not all newborns and mothers were tested laboratory. Some blood samples (serum) were sent to the Central Public Health Laboratory of Bahia (LACEN-BA) and some investigative tests for arboviruses (Zika viruses, Chikungunya viruses and Dengue viruses) and congenital infection (Cytomegaloviruses, Rubella, Toxoplasmosis, Parvoviruses and Herpes viruses) have been performed.

The methods used were Reverse Transcription-PCR (RT-qPCR) for Chikungunya Virus and Zika Virus identification; Chemiluminescence for anti-Cytomegalovirus IgM antibodies; anti-Rubella IgM antibodies and anti-Toxoplasmosis IgM antibodies; Enzyme-Linked Immunosorbent Assay (ELISA) for Anti-Human Parvovirus B19 IgM and IgG antibodies; Anti-Chikungunya IgM antibodies; Anti-Herpes 1 e 2 IgM antibodies and Anti-Dengue IgM antibodies and immunochromatographic method for qualitative determination of the Dengue virus`NS1 antigen.

The definition of laboratory evidence of possible recent Zika virus infection was: 1) recent Zika virus infection detected by RT-qPCR for Zika Virus identification on maternal or infant blood sample or 2) Detection of recent unspecified flavivirus infection by serologic tests (Positive Dengue immunoglobulin virus [IgM]) on maternal or infant blood sample.[24] Serologic tests Zika virus immunoglobulin M [IgM] were not available.

Statistics

The sampling plan was not probabilistic, due the convenience sample. The statistical analysis was descriptive and non-inferential, due to the impossibility of estimating the standard error appropriately.[27,28]

Data were entered into a database and analyzed statistically using SPSS Statistics software version 21.0. The statistical analysis of the quantitative variables was performed from frequencies, measures of central tendency (mean) and dispersion (standard deviation). Quantitative variables were: maternal age, length of newborn at birth, circumference of the newborn's head. The statistical analysis of the qualitative variables was performed from frequencies and proportions. Maternal qualitative variables were categorical: race, age range, education level, marital status, occupation, origin city, parity, cutaneous manifestation, prenatal care. Qualitative variables of newborns were also categorical: sex, gestational age, twinning, birth weight, findings of cranial ultrasound, abdominal ultrasound and fundoscopy.

Ethics

The study was submitted to the Research Ethics Committee of MCO, in accordance with Resolution 466/12 of the National Health Council and the 2009 Code of Medical Ethics of the Federal Medical Council. Approved by the Ethics Committee of the MCO.

Results

82 cases of microcephaly were reported at MCO during the period of September 2015 to June of 2016. Of these, 32 cases were excluded because they did not meet microcephaly criteria. Therefore, 50 newborns with microcephaly and their mothers were studied. Although 92% of the mothers were from Salvador (capital of Bahia), there was no concentration of housing in any specific neighborhood. 17,5% were mothers from the metropolitan area.

The mean maternal age was 25 years old; the youngest mother was 13 years old and the oldest was 43 years old. 48% (24/50) were 20 to 29 years; 24% (12/50) were teenagers (13 to 19years). Single mothers corresponded to 55% (24/44) of the total group. About 57,5% (23/40) of all mothers had a middle educational level and only 2,5% (1/40) had higher education, 35,9% (14/39) were freelance. Regarding the mother's signs and symptoms, 60% (27/45) reported exanthema (maculopapular rash) which occurred predominantly (81,5%) in the first and second trimesters of pregnancy (22/27). 40% (18/45) of the mothers did not report exanthema. Most mothers (93,5%) (43/46) held prenatal care. Other information about the mother's population is reported on Table 1.

Table 1 Clinical-epidemiological profile of mothers

Variables	n	%
Age range (N=50)		
13–19	12	24
20–29	24	48
30–35	7	14
>35	7	14
Race (N=45)		
White	1	2
Black	22	49
Mixed	22	49
Educational Level (N=40)		
Fundamental	16	40
Middle / Technical	23	57,5
University	1	2,5
Civil Status (N=44)		
Single	24	55
Married	7	16
Separated / Divorced	1	2
Stable union	12	27
Occupation (N=39)		
Primary activity	15	38,5
Secondary activity	4	10,3
Self employed	14	35,9
Unemployed	2	5
Student	4	10,3
Origin (N=50)		
Salvador-Bahia-Brazil	42	84
Metropolitan region of Salvador	4	8
Other cities in Bahia	4	8
Primigravida (N=48)		
Yes	21	44
No	27	56
Rash report (N=45)		
Yes. Before gestation	2	4,4
Yes. 1st trimester	17	38,1*
Yes. 2nd trimester	5	11
Yes. 3rd trimester	1	2
Yes. Unknown period	2	4,4
Did not present	18	40,1*
Prenatal care (N=46)		
Yes	43	93,5
No	3	6,5

*The values were approximated in 0.1 due to the presence of periodic tithe

The newborn's results showed that 98% (49/50) were born at term and only 2% were premature (1/50), 62% (31/50) were girls, 32% (16/50) were born with low birth weight (1500 to 2499g) and 68% (34/50) were born with weight ≥2500g (2500 to 3566 g). There was a pair of twin and just one of the twins was born with microcephaly (Table 2).

Table 2 Clinical-epidemiological characteristics of the newborns

Variables	n	%
Gender (N=50)		
Male	19	38
Female	31	62
Gestational age (N=50)		
Preterm	1	2
Term	49	98
Twin pregnancy (N=50)		
Yes	1	2
No	49	98
Birthweight (g) (N=50)		
Low weight	16	32
≥2500	34	68
Length at birth (cm) (N=49)		
Minimum	39,5	
Maximum	50	
Head circumference (cm) (N=50)		
Mean (SD) (Boys) (n = 19)	28,9 (2,1)	
Mean (SD) (Girls) (n = 31)	29,4 (1,8)	
Z score [Intergrowth] (N=50)		
0 e -2 (no microcephaly)	7	14
≤ -2 to -3 (microcephaly)	13	26
≤ -3 (severe microcephaly)	30	60
Media (SD)	-3,3032 (1,2294)	
Minimum	-60,691	
Maximum	-0,6367	

Regarding the cranial ultrasound findings, 88% of the neonates had the ultrasound scan performed: only 29.6% (13/44) were normal and 70,4% (31/44) were abnormal. Of these, 87% (27/31) had calcifications; 43,2% (19/44) had ventriculomegaly; 11,4% (5/44) had cerebral atrophy; 4,5% (2/44) had hemorrhage subependymal. In addition to the alterations evidenced in cranial ultrasound, the following findings were described: signs of hypoplasia and agenesis of the corpus callosum, malformation of posterior fossa, coarse parenchymal and periventricular calcifications.

Abdominal ultrasound scanning was performed in 68% (34/50): only 14,7% (5/34) were abnormal with incidental findings, such as hepatic nodule suggestive of hemangioma and biliary cholestasis, apparently not related with the CZS. Echocardiogram was performed in 30% (15/50) and 93,3% (14/15) were abnormal: mild mitral valve insufficiency, patent oval foramen, persistence of the ductus arteriosus, ostium secundum inter-atrial communication, minimal interventricular and perimembranous communication and moderate dilation of atria and slight ventricles. Hearing screening was performed in 76% (38/50): 18,4% (7/38) were abnormal, that is, evoked otoacoustic emissions were not captured and further investigation was indicated (Table 3) (Table 4).

Table 3 Newborn´s exams

Variables	n	%
Brain ultrasound (N=44)		
Normal	13	29,6*
Calcifications	9	20,5*
Calcifications AND Ventriculomegaly	15	34,1
Calcifications AND Cerebral atrophy	1	2,3
Calcifications AND Ventriculmegaly AND Cerebral atrophy	2	4,5*
Ventriculomegaly AND Cerebral atrophy	2	4,5*
Hemorrhage I (subependymal)	2	4,5*
Fundoscopy (N=29)		
Normal	18	62
Abnormal findings	11	38
Abdominal ultrasonography scan (N=34)		
Normal	29	85,3
Abnormal findings	5	14,7
Echocardiogram (N=15)		
Normal	1	6,7
Abnormal findings	14	93,3
Hearing screening (N=38)		
Normal	31	81,6
Abnormal findings	7	18,4

*The values were approximated in 0.1 due to the presence of periodic tithe

Table 4 Characteristics of the mothers according to fundoscopy (N=29)

Variables	Fundoscopy	
	Normal	**Abnormal**
	18 (36%)	**11 (22 %)**
Clinical-epidemiological profile of mothers		
Exantema in the pregnant (n=27)		
Yes. Before gestation	2 (12,5%)	0
Yes. 1st trimester	3 (18,75%)	6 (54,5%)
Yes. 2nd trimester	3 (18,75%)	0
Yes. 3rd trimester	1 (6,25%)	0
Yes. Unknown period	0	1 (9,1%)
No	7 (43,75%)	4 (36,4%)

Table Continued...

Variables	Fundoscopy	
	Normal	Abnormal
	18 (36%)	11 (22 %)
Mother´s race		
White	1 (5,6%)	0
Black	10 (55,6%)	4 (36%)
Mixed	6 (33,3%)	6 (54%)
Missing data	1 (5,6%)	1 (9%)
Mother´s age range		
13-19	5 (27,8%)	0
20-29	5 (27,8%)	8 (73%)
30-35	5 (27,8%)	0
>35	3 (16,7%)	3 (27%)
Mother´s age (years)		
Mean (SD)	26 (8,5)	27 (8)
Range	13 - 38	20 - 43

Fundoscopy was performed in 58% (29/50) of the neonates: 38% (11/29) of them presented ophthalmological abnormalities. Among the fundoscopy's abnormalities, we found refraction of the retinal pigment epithelium, unilateral atrophy, important hypoplastic papule, optic nerve hypoplasia, reduced retinal pigment epithelium in macula, atypical macula lesion, small areas of bleeding, retinopathy of prematurity, papillary pallor, macular refractions and extensive coloboma in macula. There was no detailed information about the abnormal findings in the medical records (Table 5).

Among the newborns with abnormal fundoscopy, 73% (8/11) were female and 27% (3/11) male; 18.2% (2/11) had normal cranial sonography. Among the newborns that were diagnosed with abnormal fundoscopy and brain ultrasound, 45.5% (5/11) had calcifications and ventriculomegaly; 18.2% (2/11) had calcifications, ventriculomegaly and cerebral atrophy; 9.1% (1/11) had ventriculomegaly and cerebral atrophy and 9.1% (1/11) had only calcifications. The mother´s age range varied predominantly from 20 to 29 years old, 36.4% (4/11) had no rashes, 54.5% (6/11) had rashes in the 1st trimester and 9.1% (1/11) had rash but did not know the gestational period. Mothers who had reported rash maculopapular before gestation, had newborns with normal fundoscopy.

Table 5 Characteristics of newborns according to fundoscopy (N=29)

Variables	Fundoscopy	
	Normal 18 (36%)	Abnormal 11 (22 %)
Clinical-epidemiological profile of newborns		
Gender		
Male	6 (33,3%)	3 (27%)
Female	12 (66,7%)	8 (73%)
Gestational age (weeks, days)		
Media (SD)	38w5d (1w3d)	39w (1w2d)
Range	37w1d–41w2d	37w2d–41w1d
Birth weight (g)		
Media (SD)	2746 (401,5)	2518,5 (627)
Range	2008-3566	1626–3466
Length at birth (cm)		
Media (SD)	45,9 (1,8)	45,2 (3,2)
Range	43-49	39,5–48,5
Cephalic perimeter (cm)		
Media (SD)	30 (1,4)	28,5 (2,2)
Range	27–31,5	24–31,5
Cranial Ultrasound (N=29)		
Normal	8 (44,4%)	2 (18,2%)
Calcifications	4 (22,2%)	1 (9,1%)
Calcifications AND Ventriculomegaly	3 (16,7%)	5 (45,4%)
Calcifications AND Ventriculomegaly AND Cerebral atrophy	0	2 (18,2%)
Ventriculomegaly AND Cerebral atrophy	0	1 (9,1%)
Hemorrhage I (subependymal)	2 (11,1%)	0
Unrealized	1 (5,6%)	0

In relation to the neonate IgM and IgG serologies for the TORCHS group and arboviruses (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes, Sifilis, Parvovirus, Dengue and Chikungunya): one neonate was reagent for Dengue virus (IgM) and another for Chikungunya virus (IgM). 20 children were tested for Zika virus by RT-qPCR after birth: 85% (17/20) of them were negative and 15% (3/20) were inconclusive. Other information about serologies is on the Table 6.

Table 6 Newborns´ serologies

VARIABLES	n	%
Toxoplasmosis IgM (n=28)		
Negative	19	68
Inconclusive	9	32
Toxoplasmosis IgG (n=01)		
Inconclusive	1	100
Rubella IgM (n=27)		
Negative	20	74
Inconclusive	7	26
Cytomegalovirus IgM (n=28)		
Negative	20	71,4
Inconclusive	8	28,6
Cytomegalovirus IgG (n=02)		
Negative	1	50
Inconclusive	1	50
Herpes 1 and 2 IgM (n=24)		
Negative	23	95,8
Inconclusive	1	4,2
Herpes 1 and 2 IgG (n=02)		
Negative	1	50
Inconclusive	1	50
Chikungunya IgM (n=21)		
Positive	1	4,8
Negative	19	90,4
Inconclusive	1	4,8
Chikungunya rt-qPCR (n=14)		
Negative	13	92,9
Inconclusive	1	7,1
Zika virus RT-qPCR (n=20)		
Negative	17	85
Inconclusive	3	15
Dengue IgM (n=21)		
Positive	1	4,8
Negative	19	90,4
Inconclusive	1	4,8
Dengue ns1 (n=04)		
Negative	4	100
Parvovirus B19 IgM (n=27)		
Negative	25	92,6
Inconclusive	2	7,4
Parvovirus B19 IgG (n=01)		
Positive	1	100

Discussion

Congenital ZIKV transmission can happen during any time during gestation, but the risk of severe malformations to the fetus is greater when the infection occurs in the first and second trimesters. Transmission is much more common and can cause more problems in the newborn of mothers who present with a cutaneous rash but asymptomatic mothers can also transmit ZIKV to the fetus.[2,3,29] The first trimester is probably the period of pregnancy that there is the greatest risk of microcephaly[3].This study corroborates the fact that the majority of mothers with presumed Zika virus infection were oligosymptomatic, presenting mostly rash maculopapular during the first or the second trimesters.

In clinical practice, it is difficult to differentiate ZIKV infection and other viral illness such as other arboviruses also transmitted by *Aedes* mosquitoes.[3] This fact confirms the importance of implementing preventive measures against arboviruses before and during pregnancy, in addition to the implementation of routine diagnostic tests in prenatal care as well as specialized care for affected children. 40% of mothers were asymptomatic during pregnancy for arboviruses, reinforces the necessity of early investigation.

Pregnant women with presumed or confirmed ZIKV infection should be referred to a fetal medicine service for evaluation and follow up.[3] In pregnancy, ZIKV RNA was found in some mother´s samples until 3 months after the beginning of the disease. Perinatal ZIKV transmission was reported for the first time during the outbreak in French – Polynesia in 2013/2014 and also occurred during the Brazilian outbreak[8]. The viral RNA was detected in the amniotic fluid of pregnant women with symptoms of ZIKV infection, also in fetal brain and abortion products, those data support mother-fetal viral transmission.[2,30–32]

Most cases of ZIKV in Brazil were concentrated in the northeast region (60,7%) followed by southeast (23,8%) and middle-west regions (7,3%). The states of Pernambuco (16,9%) and Bahia (16,2%) were the most affected, followed by São Paulo (9,0%), Paraiba (7,4%) and Rio de Janeiro (7,4%).[17,18,19,20,33] In our study, the results showed that most of the mothers were under 29 years. About 40% (16/40) had fundamental education and 57,5% (23/40) had medium or technical educational level and only 2,5% (1/40) had higher education. Regarding race, 98% (44/45) of them were afro-Brazilian descendants. That profile shows that ZIKV infection, in our study, correlated with lower social-economic groups. This fact corroborates with new associations of protein malnutrition with the emergence of CZS.[34] Pregnant women with low purchasing power generally have a low protein diet and high content of carbohydrates. Regarding origin, 84% (42/50) were from Salvador and metropolitan area (Table 1) but there was not a specific distribution of these pregnant women in the city of Salvador.

The correlation of ZIKV and microcephaly in Brazil was first described in northeast in October of 2015.[35,36] In 2016 authors showed the profile of newborns with microcephaly and their mothers: 71% were from the northeast region, 77% were afro Brazilian descendants, 51% of the mothers were under 24 years old and 27% had less than 8 years of schooling.[37] The neurological findings of the newborns with microcephaly call attention to the important irritability, hypertonia, convulsions, progressive impairment of the coordination of the swallowing suction, evolving with cerebral palsy; severe mental retardation. More recently there have been reports of central nervous system disorders such as ventriculomegaly and reduced cerebral parenchyma in children who were exposed to the Zika virus during pregnancy and did not present microcephaly at birth.[3,6,16,35,38,39] Studies show that comorbities were highly prevalent such as pneumonia, urinary tract infections, epilepsy and dystonia.[40]

The newborns enrolled in our work had severe microcephaly in 60% (30/50) confirming how aggressive the virus can be to the brain. 70,4% of newborn with microcephaly were detected with one or more brain abnormalities such calcifications, ventriculomegaly; and cerebral atrophy. Brain ultrasonography in fetus can be a sensitive screening test for microcephaly and these other abnormalities[3]. Although microcephaly was the first signal associated to Congenital Zika virus infection, other brain abnormalities can occur without microcephaly.[26,40]

Besides, neuroimaging findings in infants with other congenital infections have been correlated with neurodevelopmental outcomes.[26] In this study, other infections of the TORCHS group that could cause these manifestations were excluded. So, neuroimaging and serologies for other congenital infections such as rubella, varicella and parvovirus,[3] and for arboviruses such as dengue and chikungunya should be implemented in prenatal and postnatal care, mainly if the pregnant presents symptoms. Sofisticated techniques such as amniocentesis can be also used to test for ZIKV and other causes of congenital infection in the amniotic fluid.[3]

Regarding ocular findings, the literature shows that some patients had involvement of the retina and low vision. These alterations have their characteristics such as chorioretinal atrophy, especially of the macular area and abnormalities of the optic disc, differing from other perinatal infections. No uveitis or vasculitis has been described.[15,41–43] Birth defects potentially associated with Zika virus infection during pregnancy include brain abnormalities and/or microcephaly, eye abnormalities.[26] Retinopathy of prematurity was detectable in a newborn in this study which can cause impairment of irreversible visual acuity by retinal detachment.

In order to prevent and mitigate the severity of Congenital Zika virus infection, scientific knowledge must be applied in clinical practice. In addition to continuous health monitoring and surveillance, scientific knowledge should be applied in the clinical practice of childcare services, early stimulation and specialized care for affected children. In family planning care, it is important to advise women who have had a suspected or confirmed Zika virus infection to wait at least three months to become pregnant[44]. In prenatal care, it should be advised to practice safer sex using condom or abstain from sexual activity for at least the whole if their sexual partners returning from areas where transmission of Zika virus occurs.[3,44,45]

Besides, due the broad spectrum of clinical manifestations and a lack of sensitive and specific diagnostic assays, vaccines, drugs for primary and post-exposure prophylaxis and treatment should also be developed in order to prevent the disease and reduce its incidence and impact on public health.[8,46,47] At the present moment, the control of mosquito populations and the prevention of mosquito bites are the most important preventive measures.[8] All Brazilian pregnant women should be advised to us insect repellents during all gestation. If foreign pregnant women come to Brazil, they should also be advised to take care to protect themselves against mosquito bites.[3]

There were some limitations of this study such as:

a. The use of a convenience sample bias because only newborns with microcephaly were included in this study (selection bias). There are most likely other consequences of central nervous system dysfunction beyond microcephaly.
b. Missing data related to epidemiological profile of mothers because some medical records were incomplete (information bias).
c. Other maternal risk factors for microcephaly were not reported and considered in this study.
d. Incapacity of testing fetus and mother in the beginning of gestation when the infection probably occurred, when malformations occur and when Zika virus RNA could be detectable.
e. Serologic testing cannot readily discriminate between flaviviruses because of the possibility of cross reactivity and similarity of clinical manifestations.
f. Current recommendations for neuroimaging or testing have not been implemented.

This study will contribute to mapping where preventive measures should be directed and better worked, as well as enable an adequate follow-up of infected neonates. Most of the patients of our study are being followed at the Congenital Infections Outpatient Clinic of the Federal University of Bahia. It is necessary to ratify the recommendation of physical examination, including neurologic examination, and postnatal neuroimaging[25] for all newborns whose mothers reported signs and symptoms of arboviruses disease before or during pregnancy or mothers with laboratory evidence of Zika virus infection. Detecting brain abnormalities can ensure appropriate follow up and intervention in order to understand the full clinical spectrum of congenital Zika Syndrome.

Conclusion

Despite the majority of the pregnant women did not have classic symptoms of an arboviruses infection and because of this the suspicion and confirmation of infection of Zika virus through serologies are difficult in clinical practice, it is important to understand clinical and epidemiological characteristics related to Zika viruses' infection and the CZS. It is also important to highlight the necessity of women avoiding Zika virus exposure before and during pregnancy and the necessity of screening pregnant women for possible Zika virus exposure at every prenatal visit. Besides, postnatal neuroimaging and testing should be routine for all infants born to women with laboratory evidence of Zika virus infection during pregnancy.

The knowledge of the epidemiological clinical profile of the mothers and newborns involved allows mapping where preventive measures should be directed and better worked, as well as enable an adequate follow-up of infected neonates. Appropriate clinical services should be available to follow up infants who were born with microcephaly.

Such data will allow a possible causal relation of predisposing factors, to create better quality policies for women who are planning the pregnancy, pregnant women and newborns as well as understand the scope of clinical repercussion, and to promote theoretical-practical basement for future investigations which can carry implications for a better Public Health Practice.

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Conflicts of interest

The authors confirm the absence of any other conflict of interest that needs to be reported.

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