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Zika Virus and Pregnancy: Association between Acute Infection and Microcephaly in Newborns in the State of Rio de Janeiro, Brazil

Zikavirus und Schwangerschaft: Assoziation zwischen akuter Infektion und Mikrozephalie bei Neugeborenen im Bundesstaat Rio de Janeiro, Brasilien

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Key words

congenital infection, Zika virus, microcephaly, low birth weight, first trimester of pregnancy

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ABSTRACT

Introduction Aim of the study was to evaluate the association between microcephaly and acute infection with Zika virus (ZIKV) in pregnant women in the state of Rio de Janeiro, Brazil. Infection was confirmed by laboratory testing.

Materials and Methods A cross-sectional retrospective study of pregnant women with symptoms occurring between 2015 and 2016 suggestive of acute ZIKV infection was carried out, with confirmation of infection done by blood or urine RT-PCR. The relative proportions of categorical variables were calculated for two distinct groups: pregnant women whose newborns had microcephaly and pregnant women who gave birth to infants without microcephaly. Confidence intervals with a 95% level of agreement were estimated for the relative ratios.

Results A total of 1609 pregnant women with a mean age of 26.4 ± 6.5 years were evaluated. As regards the time of acute infection, 19.6% (316) of cases occurred in the first trimester of pregnancy. Nineteen (76%) of the 25 cases with microcephaly (1.5%) were associated with an infection contracted in the first trimester of pregnancy ($p < 0.001$, OR = 13.7, 95% CI: 5.6–37.7). 48% (12/25) of the newborns with microcephaly had a birth weight of < 2500 grams, while only 7% (116/1597) of the group of newborns without microcephaly had a similarly low birth weight ($p < 0.001$, OR = 11.7, 95% CI: 5.2–26.2). Logistic regression showed that a birth weight of < 2500 g (OR = 12.54) and ZIKV infection in the first trimester of pregnancy (OR = 14.05) were associated with microcephaly (area under ROC curve = 0.86).

Conclusion Acute ZIKV infection in the first trimester of pregnancy and low birth weight are associated with microcephaly.

ZUSAMMENFASSUNG

Einleitung Ziel dieser Studie war es, die Assoziation zwischen Mikrozephalie und akuter Infektion mit Zikavirus (ZIKV) bei schwangeren Frauen im Bundesstaat Rio de Janeiro, Brasilien zu untersuchen. Die Infektion wurde durch Laboruntersuchungen bestätigt.

Material und Methoden Es wurde eine retrospektive Querschnittsstudie von schwangeren Frauen mit Symptomen, die zwischen 2015 und 2016 auftraten und die auf eine Infektion mit ZIKV hindeuteten, durchgeführt. Die Infektion wurde mittels RT-PCR von Blut- bzw. Urinproben bestätigt. Die relativen Anteile der kategorischen Variablen wurden für 2 verschiedenen Gruppen kalkuliert: schwangere Frauen, die Neugeborene mit Mikrozephalie zur Welt brachten, und schwangere Frauen, deren Kinder keine Mikrozephalie aufwiesen. Die 95%-Konfidenzintervalle für die relativen Anteile wurden geschätzt.

Ergebnisse Insgesamt wurden 1609 schwangere Frauen mit einem Durchschnittsalter von 26,4 ± 6,5 Jahren evaluiert. Was den Zeitpunkt der akuten Infektion angeht, stellte sich heraus, dass 19,6% (316) der Infektionen im 1. Schwangerschaftstrimenon aufgetreten waren. Bei 25 Fällen mit Mikrozephalie (1,5%) bestand bei 19 (76%) eine Assoziation mit einer Ansteckung im 1. Trimester der Schwangerschaft ($p < 0{,}001$; OR = 13,7; 95%-KI [5,6–37,7]). Von den Neugeborenen mit Mikrozephalie hatten 48% (12/25) ein Geburtsgewicht von < 2500 g, wohingegen nur 7% (116/1597) der Neugeborenen in der Gruppe ohne Mikrozephalie ein ähnlich niedriges Geburtsgewicht aufwiesen ($p < 0{,}001$; OR = 11,7; 95%-KI [5,2–26,2]). Das logistische Regressionsmodell zeigte, dass ein Geburtsgewicht von < 2500 g (OR = 12,54) sowie eine ZIKV-Infektion im 1. Trimenon der Schwangerschaft (OR = 14,05) mit Mikrozephalie assoziiert sind (Fläche unter der ROC-Kurve = 0,86).

Schlussfolgerung Akute Infektion mit ZIKV im 1. Schwangerschaftstrimester und ein niedriges Geburtsgewicht sind mit dem Auftreten von Mikrozephalie assoziiert.

Introduction

The Zika virus (ZIKV) is a single-stranded RNA arbovirus of the virus family *Flaviviridae*, a family which also includes yellow fever, dengue, and Nile fever viruses. ZIKV was first identified in 1947 in Uganda in the Zika forest on a Rhesus monkey [1]. The virus was only detected in humans in 1952 in Nigeria [2]. The first outbreak of the disease occurred on Yap Island in 2007, where 49 confirmed cases and 59 probable cases of ZIKV disease were identified [3]. The virus crossed the Pacific and reached Brazil, Suriname, and Colombia [4, 5]. In Brazil, ZIKV was first identified in Bahia in 2015 after testing the serum of patients who presented with symptoms similar to dengue [6]. In September 2015, research reported a significant increase in cases of microcephaly in newborns in Northeast Brazil which was followed by an increase in cases in the Southeast of Brazil [7].

ZIKV is transmitted by mosquitoes of the Aedes aegypti species, which also transmits yellow fever, dengue, and Chikungunya virus. Mother-to-fetus transmission is the most worrying form of transmission because the virus has a teratogenic effect and can cross the placenta in any trimester of gestation [8]. It was estimated that more than 40% of cases in a Brazilian prospective observational study with pregnant women showed symptoms of ZIKV infection [9].

Neural progenitor cells are the primary target of ZIKV, which explains the extent of fetal central nervous system (CNS) changes found in neuroimaging studies [10]. The most common fetal CNS abnormalities are microcephaly, ventriculomegaly, and intracranial calcifications. Microcephaly is defined as a head circumference (HC) of more than 2 standard deviations (SD) below the mean for age and sex. The most severe microcephaly (> 3 SD) is correlated with maternal disease in the first trimester of gestation [11]. Ultrasonography (US) is the method of choice to monitor pregnant women living in areas at increased risk of congenital ZIKV infection. HC is easy to measure, and microcephaly is the most common finding in cases with congenital ZIKV infection [12]. A study conducted in Brazil to monitor fetuses with microcephaly by US showed that microcephaly was severe in 73.7% of the cases. Only 10.5% of the fetuses had isolated microcephaly, 89.5% had additional CNS malformations, including periventricular or parenchymal calcifications (63.2%), symmetric or asymmetric ventriculomegaly (47.4%), cerebellar abnormalities (42.1%) and cortical atrophy (15.8%) [13]. Neuroimaging patterns obtained with transfontanellar ultrasound are accurate and diagnostic of brain pathology in newborns affected by ZIKV infection [14].

This study aimed to study the association between microcephaly and acute ZIKV infection, with laboratory confirmation of the infection in symptomatic pregnant women living in the state of Rio de Janeiro, Brazil.

Materials and Methods

Patients

A cross-sectional retrospective study was performed of pregnant women with symptoms of infection living in the state of Rio de Janeiro, Brazil in 2015 and 2016, with acute ZIKV infection confirmed by laboratory tests. This study was approved by the Ethics Committee of the State University of Rio de Janeiro (UERJ).

During the ZIKV epidemic in Brazil, all pregnant women with symptoms suggestive of acute ZIKV infection were instructed to collect blood and urine samples to confirm the infection by RT-PCR.

Databases

The study used three databases: the laboratory environment management system (GAL), the live births information system (SINASC), and the public health record of events (RESP). The GAL database contains the records of all pregnant women who showed clinical symptoms suggestive of acute ZIKV infection and whose blood or urine samples were collected to confirm disease. The analysis process was monitored and controlled from the time of registration of the examination request to the issue of a report. The SINASC database was developed by DATASUS (IT Department of the Unified Health System) to gather epidemiological information on reported births throughout Brazil. RESP is an integrated surveillance and health monitoring system for conditions related to infections during pregnancy, childbirth and the puerperium.

Preparation of the database

The GAL database contains the records of all ZIKV-positive tests confirmed by RT-PCR of blood or urine samples obtained from symptomatic pregnant women from November 18, 2015 through to January 3, 2017. A total of 2635 samples were analyzed with RT-PCR and found to be positive for ZIKV, of which 1824 were positive blood samples and 811 were positive urine samples. The number of positive samples does not correspond to the actual number of pregnant women, since the records were compiled based on the type of sample and the date of collection. Several pregnant women had positive samples of both blood and urine and were examined on different dates; the database therefore can have more than one record for the same person. To deal with this, the records were filtered using the name of the pregnant woman.

The SINASC database contains the records of live births in Brazil. A total of 237 541 and 215 974 births were recorded for 2015 and 2016, respectively, in the state of Rio de Janeiro.

By cross-referencing data from the GAL and SINASC databases, we obtained the gestational outcomes of pregnant women selected from the GAL database for the study sample. Cross-referencing the GAL and RESP databases enabled us to identify the confirmed cases of microcephaly diagnosed at birth or during puerperium.

The preparation of the database consisted of successive linkages from the database of pregnant women to append and update variables, identifying the group of pregnant women who had a newborn with microcephaly and a group of women without a neonate with microcephaly.

Statistical analysis

The data were transferred to an Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) and analyzed using the Statistical Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL, USA). Descriptive analysis was done to calculate the frequencies and proportions for the categorical variables collected from the selected bases. The relative ratios of the categorical variables were calculated for two distinct groups: one group consisting of pregnant women whose newborns had microcephaly, and a second group consisting of pregnant women with newborns without microcephaly. The confidence intervals were estimated for the relative ratios with a 95% confidence level. The hypothesis test was used to compare proportions, and the level of significance (p) was set at < 5%. Logistic regression models were used to jointly evaluate the effect of variables on the likelihood of microcephaly. The odds ratio for the adjusted variables in the logistic regression model, the respective ranges of the odds ratio and the 95% confidence intervals were calculated. The performance of the adjusted model was evaluated using the receiver operating characteristics (ROC) curve.

Results

Sample

A total of 2635 RT-PCR positive ZIKV samples were found in the GAL database, of which 1824 were positive blood samples and 811 were positive urine samples. The number of samples did not correspond to the actual number of pregnant women, since some pregnant women had positive blood and urine samples, and some repeated the tests at different dates, i.e., a pregnant woman could have more than one positive sample. The first positive test was recorded on November 27, 2015, and the last one on January 3, 2017, with 355 examinations carried out in 2015, 2279 exams in 2016 and only one positive exam in 2017. It was found that 81.1% of cases occurred in the metropolitan region of the city of Rio de Janeiro. After excluding repeated datasets for the same pregnant women in the GAL database, the databases were cross-referenced, resulting in the data of 1609 pregnant women with laboratory confirmation of ZIKV infection whose gestational outcome was recorded in the SINASC database.

Characteristics of pregnant women

The mean maternal age at pregnancy was 26.4 ± 6.5 years. In terms of ethnicity, 57.4% (913) were non-white. Regarding the number of pregnancies, 606 pregnancies (38%) were first gestation. As regards the timing of acute infection, 19.6% (316) of infections occurred in the first, 44.9% (723) in the second, and 35.5% (570) in the third trimester of pregnancy.

Perinatal outcomes

A total of 1622 live newborns were registered (13 were double pregnancies) to the 1609 pregnant women studied. Of these recorded live newborns, 25 had microcephaly (1.5%), 21 of which were reported at birth and recorded in the SINASC database, with 4 cases reported in the postnatal follow-up period and recorded in the RESP. Of the 25 cases with microcephaly, 19 (76%) were associated with infection in the first trimester of gestation; in 3 cases (12%) infection occurred in the second and in 3 cases (12%) in the third trimester. The likelihood that a pregnant woman infected with ZIKV in the first trimester of pregnancy would have a newborn with microcephaly was 6% (19/316). Of the 25 microcephaly cases studied, 19 (76%) were associated with an infection contracted in the first trimester of gestation ($p < 0.001$; OR = 13.7, 95% CI: 5.6–37.7) (▸ **Table 1**).

Of the 25 newborns with microcephaly, 20% (5/25) were preterm births, four cases were delivered between 32 and 36 weeks of gestation, and one was born between 22 and 27 weeks. 52% (13/25) of newborns with microcephaly and 43% (681/1584) of the non-microcephaly group ($p = 0.49$) were delivered by vaginal birth. Only one newborn with microcephaly (4%) and 16 newborns in the non-microcephaly group (1%) had an Apgar score of < 7 at 5 minutes ($p = 0.60$). Of the infants with microcephaly, 48% (12/25) had a birth weight of < 2500 grams while only 7% (116/1597) of the infants in the non-microcephaly group had a similarly

▶ **Table 1** Analysis of gestational characteristics according to the presence or absence of microcephaly.

Variable		With microcephaly (%)	Without microcephaly (%)	OR (95 % CI)
Number of gestations	1	36	38	0.91 (0.40–2.07)
	≥ 2	64	62	
Gestational age	1st trimester	76	19	13.72 (5.43–34.65)
	2nd and 3rd trimesters	24	81	
Type of pregnancy	Singleton	100	99.2	–
	Multiple	0	0.8	

OR: odds ratio; CI: confidence interval.

▶ **Table 2** Distribution of perinatal outcomes according to the presence of absence of microcephaly.

Variable		With microcephaly (%)	Without microcephaly (%)	OR (95 % CI)
Type of delivery	Vaginal	52	43	0.70 (0.32–1.54)
	Cesarean	48	57	
Gestational age (weeks)	< 37	20	9	0.40 (0.15–1.08)
	≥ 37	80	91	
Birth weight (g)	≤ 2500	48	7	11.68 (5.21–26.18)
	> 2500	52	93	
Apgar score in the 5th min	< 7	4	1	0.23 (0.03–1.78)
	≥ 7	96	99	

OR: odds ratio; CI: confidence interval.

▶ **Table 3** Odds ratio of the adjusted variables in the logistic regression model.

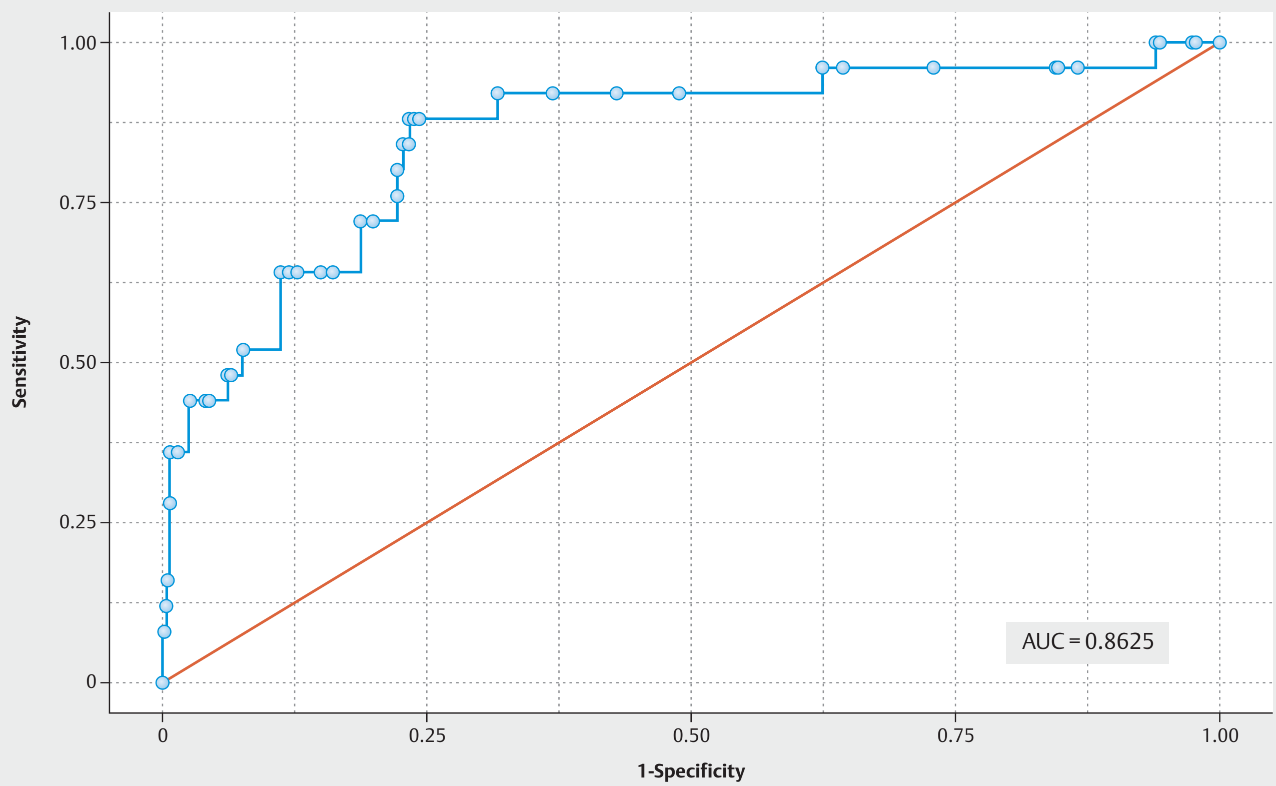
Variable	Adjusted odds ratio	Confidence interval (95 %)	p-value
Ethnicity (white)	0.62	0.26–1.48	0.284
Number of gestations (2 or more)	0.84	0.34–2.08	0.713
Birth weight < 2500 g	12.54	5.27–29.85	< 0.001
Male gender of newborn	0.74	0.31–1.76	0.499
Gestational age (1st trimester)	14.05	5.41–36.47	< 0.001
Maternal age (< 35 years)	0.53	0.11–2.56	0.433

low birth weight ($p < 0.001$, OR = 11.7, 95 % CI: 2–26.2) (▶ **Table 2**).

The logistic regression model showed that a birth weight of < 2500 g (OR = 12.54) and ZIKV infection in the first trimester of pregnancy (OR = 14.05) were associated with microcephaly (▶ **Table 3**). The performance of the adjusted model was evaluated using the ROC curve, and the graph showed an area under the ROC curve of 86 %, confirming the good performance of the model as a classifier between cases with and without microcephaly (▶ **Fig. 1**).

Discussion

The outbreak of ZIKV in Brazil contributed to an increased number of reported cases of microcephaly in newborns. Severe microcephaly and CNS abnormalities are associated with congenital ZIKV syndrome [15]. Infection in early pregnancy may result in long viral shedding and severe brain malformations that only become detectable later in pregnancy [16]. Neuroimaging investigations contribute to the prenatal detection of microcephaly and other brain abnormalities [17, 18]. Because dengue has been endemic in Brazil for more than 30 years, diagnosis of ZIKV infection in Brazil is difficult due to the high rates of cross-reactivity between Flavivirus antibodies. A serological surveillance study found



▶ **Fig. 1** Receiver operating characteristics (ROC) curve of the model to predict the risk of microcephaly in infants born to pregnant women with acute Zika virus infection.

evidence of antibodies against dengue in over 90% of the population of the city of Recife [19]. Another Brazilian study showed that 88% of pregnant women had anti-IgG antibodies [9].

Microcephaly possibly associated with maternal infection with Zika virus was observed in 25 of the newborns in a cohort of 1609 pregnant women (1.5%). Similar numbers were reported in a study of 546 pregnant women in the French territories of the Americas (Martinique, French Guiana, and Guadeloupe), which found microcephaly in 9 of 527 newborns (1.7%) [20].

The highest reported rates of fetal birth defects potentially associated with ZIKV were 6% in the U. S. [21] and 42% in Rio de Janeiro, Brazil in 2016 [9], possibly because not all pregnant women included in the studies had RT-PCR laboratory confirmation and also because the studies evaluated all congenital anomalies and not only microcephaly. When analyzing the likelihood of microcephaly in the Brazilian study alone, two cases of microcephaly were found in 117 newborns exposed to ZIKV (1.7%), that is, a similar rate to that in our study [9].

Microcephaly has been observed as a consequence of ZIKV infection in any trimester of pregnancy, but the risk is reported to be higher when infection occurs in the first trimester of gestation [22]. In this study, it was verified that the likelihood of a pregnant woman with ZIKV infection in the first trimester having a newborn with microcephaly was 6%. This rate decreased in the subsequent trimesters to 0.4% in the second and 0.5% in the third trimester. Comparable results have been reported in a study carried out in the French territories of the Americas, where the rates were, respectively, 5.8% in the first, 1.6% in the second and 2.6% in the third trimester [20].

The percentage of infants with a birth weight of < 2500 g in the group without microcephaly was 7%, but the percentage increased to 48% in the group with microcephaly, evidencing a strong association between low birth weight and microcephaly. A previous study conducted in the state of Bahia showed a 37.2% prevalence of low birth weight in newborns with congenital ZIKV syndrome, including microcephaly [23]. In a study carried out in Guatemala, a birth weight of < −1 standard deviation and small for gestational age were associated with microcephaly [24].

The study has some limitations, the main one being that it was only performed in pregnant women with acute symptoms who sought medical care. Despite the expectation that complications are more significant with symptomatic infections, an observational study involving women in the U. S. showed that that the difference in the rate of fetal malformations born to pregnant women with and without symptoms of ZIKV infection is insignificant, and the association between severity of symptoms or viral load with adverse perinatal outcomes was also insignificant [25]. Other limitations of the study were the impossibility of evaluating gestational outcomes such as abortions or gestational discontinuation, as well as failure to identify fetal infection without microcephaly.

In summary, in this cohort of pregnant women with ZIKV infection in the state of Rio de Janeiro, Brazil, acute infection in the first trimester and low birth weight were associated with microcephaly.

Conflict of Interest

The authors declare that they have no conflict of interest.

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