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Association between Zika virus infection and microcephaly in Brazil, January to May, 2016: preliminary report of a case-control study

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Summary

Background The microcephaly epidemic, which started in Brazil in 2015, was declared a Public Health Emergency of International Concern by WHO in 2016. We report the preliminary results of a case-control study investigating the association between microcephaly and Zika virus infection during pregnancy.

Methods We did this case-control study in eight public hospitals in Recife, Brazil. Cases were neonates with microcephaly. Two controls (neonates without microcephaly), matched by expected date of delivery and area of residence, were selected for each case. Serum samples of cases and controls and cerebrospinal fluid samples of cases were tested for Zika virus-specific IgM and by quantitative RT-PCR. Laboratory-confirmed Zika virus infection during pregnancy was defined as detection of Zika virus-specific IgM or a positive RT-PCR result in neonates. Maternal serum samples were tested by plaque reduction neutralisation assay for Zika virus and dengue virus. We estimated crude odds ratios (ORs) and 95% CIs using a median unbiased estimator for binary data in an unconditional logistic regression model. We estimated ORs separately for cases with and without radiological evidence of brain abnormalities.

Findings Between Jan 15, 2016, and May 2, 2016, we prospectively recruited 32 cases and 62 controls. 24 (80%) of 30 mothers of cases had Zika virus infection compared with 39 (64%) of 61 mothers of controls (p=0·12). 13 (41%) of 32 cases and none of 62 controls had laboratory-confirmed Zika virus infection; crude overall OR 55·5 (95% CI 8·6–∞); OR 113·3 (95% CI 14·5–∞) for seven cases with brain abnormalities; and OR 24·7 (95% CI 2·9–∞) for four cases without brain abnormalities.

Interpretation Our data suggest that the microcephaly epidemic is a result of congenital Zika virus infection. We await further data from this ongoing study to assess other potential risk factors and to confirm the strength of association in a larger sample size.

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Introduction

In August, 2015, an increase in the number of neonates with microcephaly was detected in Brazil, which by the end of the year had become a major epidemic. Up to June 25, 2016, 8165 cases had been notified, of which 1638 were confirmed, 3466 excluded, and 3061 remained under investigation.[1] Microcephaly results from any insult that disturbs early brain growth, and can be caused by genetic variations, teratogenic agents, or other congenital infections.[2] Because of the temporal and geographical overlap with an ongoing outbreak of Zika virus, the hypothesis was soon formulated that the microcephaly epidemic was caused by Zika virus infection during pregnancy. In November, 2015, the Brazilian Ministry of Health declared the situation a national public health emergency.[3]

Human infection with Zika virus was initially limited to sporadic cases in a small number of countries and perceived not to cause outbreaks or severe disease. Outbreaks were first detected in the Pacific Islands in 2007 and 2013. Since 2007, transmission has been detected in 61 countries and territories, most of them located in the Americas.[4] In February, 2016, WHO

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Research in context

Evidence before this study

The epidemic of microcephaly, which started in Brazil, was declared a Public Health Emergency of International Concern by WHO in February, 2016. The hypothesis that the disorder is caused by congenital Zika virus infection was proposed early on, and the (mostly mechanistic) evidence for this association has since been accumulating. We searched PubMed and LILACS using the search term "Zika". We searched these sources up until May 30, 2016, including publications in English, Portuguese, and Spanish. We did not identify any case-control study of Zika virus infection and microcephaly. In a review of the evidence, published in May, 2016, Rasmussen and colleagues used different frameworks to assess whether the available evidence supports this hypothesis. One of these frameworks, Shepard's criteria for teratogenicity, requires at least two epidemiological studies of high quality confirming the association. The investigators concluded that there was sufficient evidence to accept causation, despite the paucity of epidemiological studies. Here, we report the first case-control study of microcephaly and congenital Zika virus infection, with the aim of adding the missing piece to the process of defining causality.

Added value of this study

To our knowledge, this is the first case-control study to examine the association between microcephaly and in-utero Zika virus infection, investigated by molecular and serological methods in cases of microcephaly and their controls at time of birth. We did this prospective study in the metropolitan region of Recife in Pernambuco State, the hotspot of the microcephaly epidemic in Brazil, between January and May, 2016. We are reporting the preliminary findings of our study because of the striking magnitude of the association between microcephaly and Zika virus infection.

Implications of all the available evidence

We conclude that the microcephaly epidemic is a result of congenital Zika virus infection. We recommend that the list of congenital infections normally referred to as TORCH (toxoplasmosis, others [syphilis, varicella-zoster, parvovirus B19], rubella, cytomegalovirus, and herpes) is renamed as TORCHZ, and that we prepare for a global epidemic of microcephaly and other manifestations of congenital Zika syndrome.

declared, in relation to the epidemic that started in Brazil, that "the cluster of microcephaly cases and other neurological disorders constitutes…a Public Health Emergency of International Concern".[5]

Since the hypothesis that the microcephaly epidemic in Brazil is caused by congenital Zika virus infection was first proposed,[6] there has been an accumulation of evidence supporting the association.[7–17] The relation between Zika virus and birth defects is strong enough to be deemed causal, but the argument would be stronger if confirmed by at least one case-control study and a cohort study.[18] Indeed, the evidence so far comes from case reports,[16] case series,[19,20] modelling studies,[17] and the preliminary report of a cohort study.[7] None of these studies included appropriate population control groups. We report the preliminary analysis of a case-control study, requested by the Brazilian Ministry of Health, to investigate the causes of the microcephaly epidemic in Brazil; the main hypothesis was that it is caused by congenital Zika virus infection.

Methods

Study design and participants

This case-control study, with prospective recruitment of newborn cases and concurrent controls, was done in the metropolitan region of Recife, Pernambuco State, Brazil. We enrolled neonates born to women resident in Pernambuco and delivered in eight public maternity units. Neonates with anencephaly or encephalocele were excluded. This preliminary analysis includes neonates born between Jan 15, 2016, and May 2, 2016.

Cases were neonates with microcephaly, defined as head circumference at least 2 SD smaller than the mean for sex and gestational age in the Fenton growth chart.[21] Controls were live neonates without microcephaly, with no brain abnormalities identified by transfontanellar ultrasonography and no major birth defects detected by physical examination by a neonatologist. For each case, two controls were selected from the first neonates born from the following morning in one of the study hospitals, matched by health region of residence and expected date of delivery (to ensure cases and controls were conceived at the same stage of the epidemic). The criteria for matching for expected date of delivery were specific for gestational age of cases. For cases born at term and post-term (37 weeks or more), controls were the next eligible neonates born at 37 weeks' gestation or more. For early preterm cases (born at <34 weeks), controls were the next eligible neonates who were born at less than 34 weeks' gestation. For preterm cases born between 34 and 36 weeks' gestation, controls were the next eligible neonates born at 34–36 weeks' gestation.

The protocol was approved by the research ethics committees of the Pan American Health Organization and Fiocruz Pernambuco. All mothers provided written informed consent to participate in the study.

Procedures

Gestational age was estimated antenatally by fetal ultrasonography. If ultrasound measurements were not available, gestational age was estimated from the date of the last menstrual period recorded on the antenatal care card or reported by the mother. In cases in which antenatal ultrasound had not been done and the mother

did not know the date of last mentruation, the Capurro method was used to estimate gestational age.[22] Head circumference was measured in the delivery room with a non-stretch Teflon tape. If microcephaly was detected, cord blood was collected. If microcephaly was confirmed by a second head circumference measurement 12–24 h after birth, the neonate was deemed eligible for the study.

After the mothers signed the informed consent form, samples were obtained from mothers and neonates (cases and controls), mothers were interviewed, and children referred for brain imaging. Interviews were done in the hospital by a trained female nurse, using a structured standardised questionnaire. Radiological brain imaging was done by CT scan without contrast in cases and by transfontanellar ultrasonography by radiologist in controls.

Cerebrospinal fluid samples were collected from cases by the study neonatologists. Umbilical cord blood was collected in the delivery room from cases and controls; when necessary, peripheral blood was collected before the neonate left hospital. Blood specimens were sent to the Virology and Experimental Therapy Department, Fiocruz Pernambuco (Recife, Brazil), where they were divided into samples and stored. For neonatal deaths and stillbirths, macerated tissue material was tested by RT-PCR.

RNA was extracted from serum of mothers and neonates (cases and controls) and cerebrospinal fluid samples (of cases) and analysed by RT-PCR for detection of worldwide African and Asian Zika virus genomes using primers designed by Lanciotti and colleagues.[23] Additionally, serum of mothers and neonates and cerebrospinal fluid samples were tested for Zika virus-specific IgM antibodies using a capture ELISA based on the US Centers for Disease Control and Prevention (CDC) Emergency Use Authorization protocol, with reagents from the CDC (Fort Collins, CO, USA).[23] For the ELISA assay, both Zika virus and dengue virus were tested in parallel to check for cross-reactivity.

The presence of neutralising antibodies to Zika virus and dengue virus (serotypes DENV-1–4) was assessed in mothers and neonates by plaque reduction neutralisation test (PRNT) in Vero cells, following a protocol described in detail elsewhere.[24] The cutoff value for PRNT positivity was defined as 50% ($PRNT_{50}$). The $PRNT_{50}$ assay was done using the virus strain ZIKV PE/243 isolated in Pernambuco, Brazil. Serum samples of mothers and neonates were tested for IgM and IgG antibodies specific for toxoplasmosis, rubella, and cytomegalovirus (the main infectious causes of congenital microcephaly[25]).

Laboratory confirmation of Zika virus infection was defined as a positive RT-PCR result or detection of IgM antibody against Zika virus. For the purpose of this analysis, results of brain imaging (CT scan in cases and ultrasonography in controls) were classified as normal or abnormal (including calcification, ventriculomegaly, lissencephaly, and other abnormalities). A neonate was deemed small for gestational age when birthweight was lower than the 10th percentile for gestational age and sex in the Fenton growth chart.

Statistical analysis

The original study aimed to include 200 cases and 400 controls to have 90% power, 95% precision to detect an association with an odds ratio of 2 or greater, assuming that 67% of cases were exposed.

We estimated the crude OR and 95% CI for the association between microcephaly and laboratory confirmation of Zika virus infection, considering the results in serum or cerebrospinal fluid and the results in serum alone, overall and separately for cases with and without radiological evidence of brain abnormalities. To deal with the fact that all controls tested negative for Zika virus, the OR was calculated using a median unbiased estimator for binary data in an unconditional logistic regression model.[26,27] This statistical approach is appropriate for zero cells, and was applicable to our situation in which the sample size for this preliminary analysis is small and data structure sparse. Another consequence of all controls being Zika virus negative was that although the design was matched, a conditional, matched analysis was not needed because matched and unmatched analyses will give the same result. We calculated OR adjusted for maternal age and maternal education (as a proxy of socioeconomic status) for the overall association. We investigated the agreement between the Zika virus-specific IgM for serum and that for cerebrospinal fluid. We also compared the ELISA value (optical density) in serum and cerebrospinal fluid. We used Stata version 14.1 software for the statistical analyses.

Role of the funding source

The funders of the study were involved in the data interpretation and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Figure: **Newborn baby with microcephaly with laboratory-confirmed Zika virus and abnormalities detected on CT scan**

The neonate shows phenotypic features previously described during the microcephaly epidemic, including craniofacial disproportion, prominent externa occipital protuberance, and excessive scalp skin (photo, left). Radiological features found on brain imaging (CT images, centre and right) include reduced volume of cortical brain parenchyma, cortical and subcortical calcifications, simplified gyral pattern, and ventriculomegaly.

	Cases (n=32)	Controls (n=62)	p value
Mothers			
Age, years			
15–24	20 (63%)	34 (55%)	0·76
25–34	9 (28%)	20 (32%)	..
≥35	3 (9%)	8 (13%)	..
Number of years in education			
<4	3 (9%)	4 (6%)	0·52
5–9	9 (28%)	18 (29%)	..
10–12	16 (50%)	37 (60%)	..
≥13, higher education	4 (13%)	3 (5%)	..
Reported rash during pregnancy			
No rash	19 (59%)	46 (74%)	0·09
First trimester	6 (19%)	2 (3%)	..
Second trimester	2 (6%)	4 (6%)	..
Third trimester	3 (9%)	8 (13%)	..
Do not remember	2 (6%)	2 (3%)	..
Specific antibodies, $PRNT_{50}$			
Zika virus positive	24/30 (80%)	39/61 (64%)	0·12
Zika virus negative	6/30 (20%)	22/61 (36%)	..
Not done	2	1	..
Neonates			
Sex			
Girls	22 (69%)	32 (52%)	0·11
Boys	10 (31%)	30 (48%)	..
Head circumference, for gestational age and sex*			
Normal	0	62 (100%)	<0·0001
Between 2 and 3 SD smaller than the mean	21 (66%)	0	..
>3 SD smaller than the mean	11 (34%)	0	..
Birthweight, g			
≥2500	10 (31%)	58 (94%)	<0·0001
1500–2499	17 (53%)	4 (6%)	..
<1500	5 (16%)	0	..
Weight for gestational age			
Normal	5 (16%)	58 (94%)	<0·0001
Small for gestational age†	27 (84%)	4 (6%)	..
Gestational age			
Term, ≥37 weeks	24 (75%)	53 (85%)	0·21
Premature, 33–36 weeks	8 (25%)	9 (15%)	..
Method of estimation of gestational age			
First trimester fetal ultrasonography	14 (44%)	27 (44%)	..
Second trimester fetal ultrasonography	11 (34%)	22 (35%)	..
Third trimester fetal ultrasonography	3 (9%)	5 (8%)	..
Report of last menstrual period	1 (3%)	5 (8%)	..
Capurro method[22]	3 (9%)	3 (5%)	..
Brain imaging findings‡			
Abnormal	11/27 (41%)	0	..
Normal	16/27 (59%)	62 (100%)	..
Not done	5§	0	..

Data are n (%). PRNT=plaque reduction neutralisation test. *For gestational age and sex in the Fenton preterm growth chart.[29] †Defined as birthweight lower than the 10th percentile for gestational age and sex in the Fenton growth chart. ‡Cases assessed by CT scan and controls by ultrasonography. §One stillbirth, three neonatal deaths, and one case in intensive care at the time of analysis.

Table 1: **Characteristics of mothers and neonates**

See Online for appendix

Results

This preliminary analysis included 32 neonates with microcephaly (cases) and 62 neonates without microcephaly (controls). A photograph and cerebral CT image of one of the cases shows features previously described during the current microcephaly epidemic (figure).[28]

Two cases had only one matched control. The participation rate was 100% for cases and 76% for controls. No controls were excluded because of birth defects. Five cases did not have brain imaging: three died in intensive care before CT scan was done, one was stillborn, and another was in intensive care at the time of this analysis. These five cases were included in the analysis for all cases and excluded from the analysis stratified by brain imaging findings.

A higher proportion of mothers of controls reported that they had no rash during pregnancy than did mothers of cases, although this difference was not significant (table 1; see appendix pp 2–5 for characteristics of individual cases and controls). No statistical difference was detected in years of education between mothers of cases and controls. Mothers of cases had a slightly higher, although non-significant, frequency of Zika virus infection detected by $PRNT_{50}$ than did mothers of controls (80% *vs* 64%, respectively). Zika virus was the primary flavivirus infection in nine (10%) of 91 mothers, including the mothers of five cases. Overall, 54 (59%) of 91 mothers had more than one flavivirus infection, with DENV-3 and DENV-4 the predominant dengue serotypes (appendix p 6). Results of $PRNT_{50}$ for Zika virus and dengue virus were similar in mother–neonate pairs (data not shown).

11 (34%) of 32 cases had severe microcephaly (head circumference at least 3 SD smaller than the mean for sex and gestational age at birth). Significantly higher proportions of cases than controls were born with low birthweight and were small for gestational age (table 1). Of the 27 cases investigated by brain imaging, 11 had one or more abnormalities: seven had calcifications, five had ventriculomegaly, one had lissencephaly, and six had other abnormalities (table 1).

None of the controls had a positive result by either RT-PCR with serum samples (none of 62 controls tested) or detection of Zika virus-specific IgM in serum (none of 59 tested). 13 (41%) of 32 cases tested positive for Zika virus (RT-PCR or Zika virus-specific IgM) in cerebrospinal fluid or serum, and nine (28%) cases tested positive in serum only (table 2). No cross-reactivity with dengue virus-specific IgM was seen in samples positive for Zika virus-specific IgM.

The overall crude OR for microcephaly and laboratory-confirmed Zika virus infection was 55·5 (95% CI 8·6–∞), and was similar when adjusted for maternal education or maternal age (table 3). The magnitude of the association in cases with brain abnormalities detected by CT scan was very strong: OR 113·3 (95% CI 14·5–∞). In cases with normal findings on brain imaging the association was still strong and significant: OR 24·7 (95% CI 2·9–∞). When results in serum alone were considered, the crude OR was 31·7 (95% CI 4·7–∞), and similar after adjustment. The magnitude of the association in cases with brain abnormalities on CT scan was very strong, OR 80·9 (95% CI 10·2–∞), but the association in cases with normal findings on brain imaging was not significant (OR 3·9, 95% CI 0·1–∞).

Four of 13 cases with laboratory-confirmed Zika virus infection had normal findings on brain imaging. Of the six cases whose mothers were seronegative to Zika virus ($PRNT_{50}$), five also tested negative (to Zika virus-specific IgM or by RT-PCR in serum or cerebrospinal fluid samples), and one had a positive RT-PCR result.

There was good agreement between serum and cerebrospinal fluid test results for detection of Zika virus-specific IgM (κ 0·91, 95% CI 0·74–1·00). Zika virus-specific IgM was detected in cerebrospinal fluid of nine (36%) of 25 cases (seven cases were not tested) and in serum of eight (27%) of 30 cases (two were not tested); the geometric mean titre was 23·62 (95% CI 18·8–29·6) in cerebrospinal fluid and 16·8 (95% CI 12·3–23·0) in serum. No neonates or mothers had IgM antibodies specific for toxoplasma, cytomegalovirus, or rubella in serum. A high proportion of mothers had IgG in serum (toxoplasma-specific IgG: 17 [53%] of 32 mothers of cases *vs* 27 [44%] of 62 mothers of controls; cytomegalovirus-specific IgG: 28 [88%] *vs* 47 [76%]; rubella-specific IgG: 20 [63%] *vs* 46 [74%]). These proportions did not differ significantly between mothers of cases and mothers of controls (data not shown).

	Cases (n=32)	Controls (n=62)	p value
RT-PCR or Zika virus-specific IgM (cerebrospinal fluid or serum)			
Positive	13 (41%)	0	<0·0001
Negative*	19 (59%)	62 (100%)	
RT-PCR or Zika virus-specific IgM (serum)			
Positive	9 (28%)	0	<0·0001
Negative	23 (72%)	62 (100%)	

Data are n (%). *For one stillbirth and one neonatal death RT-PCR was tested in macerated tissues.

Table 2: **Results based on RT-PCR or specific IgM for Zika virus in cerebrospinal fluid or serum samples for cases and in serum samples for controls**

	Cases	Controls	Odds ratio (95% CI)
Serum or cerebrospinal fluid samples			
All cases			
Crude	13/32 (41%)	0/62	55·5 (8·6–∞)
Adjusted for maternal education	..	..	59·2 (9·0–∞)
Adjusted for maternal age	..	..	55·6 (8·5–∞)
Cases categorised by brain imaging findings*			
Abnormal	7/11 (64%)	0/62	113·3 (14·5–∞)
Normal	4/16 (25%)	0/62	24·7 (2·9–∞)
Serum samples only			
All cases			
Crude	9/32 (28%)	0/62	31·7 (4·7–∞)
Adjusted for maternal education	..	..	38·5 (5·5–∞)
Adjusted for maternal age	..	..	30·2 (4·5–∞)
Cases categorised by brain imaging findings*			
Abnormal	6/11 (55%)	0/62	80·9 (10·2–∞)
Normal	1/16 (6%)	0/62	3·9 (0·10–∞)

Data are number of cases positive for Zika virus by RT-PCR or Zika virus-specific IgM/total number of patients (%), unless otherwise indicated. *Odds ratios in these subgroup analyses are crude because of small numbers; brain imaging was not done in five cases (one stillbirth, three neonatal deaths, and one case in intensive care at the time of analysis).

Table 3: **Association between microcephaly and laboratory confirmation of Zika virus infection**

Discussion

This preliminary analysis shows a strong association between microcephaly and laboratory confirmation of Zika virus infection by RT-PCR or Zika virus-specific IgM in cerebrospinal fluid or serum of neonates. The risk was high in cases with brain abnormalities detected by imaging, but was also present in cases without brain abnormalities. Results of RT-PCR or Zika virus-specific IgM were positive only in neonates with microcephaly and were negative in serum of all neonates in the control group. More than half of neonates with microcephaly had normal findings on brain imaging. There was very good agreement of Zika virus-specific IgM-positive results in serum and cerebrospinal fluid of neonates.

To our knowledge, our study is the first to estimate the seroprevalence of Zika virus infection in pregnant women in an epidemic area in Brazil. The high Zika virus $PRNT_{50}$ seropositivity (64%) in mothers of controls indicates high frequency of Zika virus infection in this population in Recife. Similar frequencies of Zika virus infection were reported in the general population in Yap island[30] and in French Polynesia after the outbreaks in these regions.[31] We cannot determine with any degree of

certainty the timing of the Zika virus infection before or during pregnancy in a case-control study. A cohort study of pregnant women will be able to assess the timing of the onset of Zika virus infection and relate it to the full spectrum of the adverse outcome of pregnancy.

We recorded a high frequency of multitypic flavivirus infections, including Zika virus, and DENV-3 and DENV-4 serotypes, the predominant dengue serotype profile in the study area.[24] We used the best available test authorised by the US Food and Drug Administration (CDC Zika IgM antibody capture ELISA) for antibody testing.[31] All positive results for Zika virus-specific IgM were confirmed by testing for neutralising antibodies. This approach is recommended by CDC guidelines[32] to rule out false-positive results.

Our study has limitations inherent to a preliminary analysis. The management team decided to perform the analysis mainly because the microcephaly epidemic is deemed a Public Health Emergency of International Concern. There was a sense of urgency in finding the answer to the main study question—ie, the association between Zika virus infection and microcephaly. Although our sample size had 82% power to demonstrate an association, we are aware that interim analysis can overestimate the strength of an association, so the magnitude needs to be treated with some caution. The case-control study will continue to investigate the current and alternative hypotheses as well as the role of cofactors, and provide final estimates.

There was a clinical indication to collect cerebrospinal fluid samples from cases, but for ethical reasons (absence of a clinical indication) no cerebrospinal fluid samples were collected from controls; therefore, the association in which laboratory confirmation includes cerebrospinal fluid test results is not strictly a fair comparison. However, presence of Zika virus-specific IgM in cerebrospinal fluid indicates an infection in the neural system of the neonate (because IgM does not cross the placenta or the blood–brain barrier and therefore is produced by the neonate and not by the mother), and given the good concordance between serum and cerebrospinal fluid results, we consider it highly unlikely that neonates of the control group had Zika virus-specific IgM in cerebrospinal fluid. The prospective recruitment of neonates with samples collected at birth ensures that the positive Zika virus-specific IgM or RT-PCR results arise from intrauterine, rather than postnatal, Zika virus infection.

The striking association between microcephaly and laboratory-confirmed Zika virus infection seen in our study adds the necessary epidemiological evidence (in the presence of a rigorously selected control group) to the process of confirming causality.[12,33] We expect one component of the congenital Zika syndrome to be intrauterine growth restriction; we recorded a high proportion of neonates with microcephaly who were small for gestational age. Brasil and colleagues[7] reported fetal growth restriction in fetuses of mothers who had Zika virus infection during pregnancy. In June, 2016, WHO recommended the use of Intergrowth-21 Size at Birth Standards for identifying neonates with microcephaly.[34] In our study, if we had classified cases using the Intergrowth-21 standards instead of the Fenton curve, seven cases would be misclassified, six of them with borderline measurements (two had brain abnormalities on CT scan, another one was Zika virus-specific IgM positive in cerebrospinal fluid). Therefore, the use of the Fenton growth chart in our study does not seem to introduce bias. Even if seven neonates were misclassified, the strength of the association would be underestimated.

Surprisingly, in our study, only seven of 27 cases who had CT scan investigation had brain abnormalities. This finding contrasts with results of the few published series of children with microcephaly, in which neonates with the disorder had brain abnormalities detected by imaging.[19,20,33,35] Whether neonates with microcephaly and normal brain imaging were excluded is not clear.[20,33] Our results showed that the association between laboratory-confirmed Zika virus infection and microcephaly was present in cases who had normal findings on brain imaging, suggesting that congenital Zika virus syndrome can be present in neonates with microcephaly and no radiological brain abnormalities. In view of these findings, patients with microcephaly and normal brain imaging findings should not be excluded from surveillance and diagnosis of congenital Zika virus infection.

Our results suggest that the detection of Zika virus-specific IgM (CDC protocol) in neonates with microcephaly is an adequate method for the diagnosis of congenital Zika virus infection (although not for its exclusion). The question of flavivirus cross-reactivity, particularly for dengue,[24,33] might not be relevant in neonates, because intrauterine infection with dengue is unlikely, and maternal IgM does not cross the placenta. A recent report of Zika virus and dengue virus-specific IgM in neonates with microcephaly showed the usefulness of testing for Zika virus-specific IgM.[36] We suggest that detection of Zika virus-specific IgM in serum is a useful alternative when cerebrospinal fluid collection is a challenge.

The limitations in laboratory confirmation of congenital Zika infection—eg, that no validated diagnostic laboratory test has been developed to confirm congenital Zika virus infection[37]—might partly explain why 19 (59%) microcephaly cases were not confirmed as Zika positive. These cases are unlikely to have been caused by non-Zika virus-related factors, such as toxoplasmosis, rubella, and cytomegalovirus, because no mothers or neonates had IgM antibodies specific for these infections. Although these negative IgM results cannot rule out these other causes of microcephaly, these cases were clustered in space and time during a Zika virus outbreak. Additionally, a neonatologist examined the neonate, and we asked the mother about possible risk factors for microcephaly. Zika virus RNA can be detected by molecular testing during the first stage of

infection, and the timeframe for the detection of Zika virus-specific IgM is uncertain. Sensitivity and specificity of these tests in congenital infection are not known, especially when infection occurs in early pregnancy.

If the causal link between Zika virus infection during pregnancy and microcephaly is true, Zika virus is the cause of the Public Health Emergency of International Concern, and we should prepare for the epidemic of microcephaly to expand to all countries with current autochthonous Zika virus transmission and to those countries where transmission of the virus is likely to spread.[38,39]

We conclude that the microcephaly epidemic is a result of congenital Zika virus infection. We recommend that the list of congenital infections normally referred to as TORCH (toxoplasmosis, others [syphilis, varicella-zoster, parvovirus B1], rubella, cytomegalovirus, and herpes) is renamed as TORCHZ, and that we prepare for a global epidemic of microcephaly and other manifestations of congenital Zika syndrome.

Contributors

TVBA, CMTM, LCR, RAAX, and DBM-F participated in all phases of the study. All other authors participated in data interpretation and critical revision of the manuscript. All authors approved the final version and agree to be accountable for all aspects of the work.

Declaration of interests

We declare no competing interests.

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For further details please see the appendix.

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More pieces to the microcephaly–Zika virus puzzle in Brazil

By October, 2015, the Zika virus epidemic had grown substantially in Brazil with 14 states reporting autochthonous Zika virus transmission. Concurrently, concerns were raised regarding the discovery of a substantial increase in the number of microcephaly cases, particularly in the state of Pernambuco. The following month, a national public health emergency was declared in Brazil in response to growing concerns about the potential association between Zika virus and newborn microcephaly, with 1248 reported cases—20 times greater than the expected number.[1] Following this announcement, additional progress was made in establishing more definitive associations between Zika virus and congenital anomalies, including microcephaly.[2,3]

Studies in mouse models have addressed the causal relation between Zika virus infection in pregnancy and pathological changes in fetuses.[4,5] Although a growing body of evidence suggests that Zika virus causes brain anomalies and microcephaly, describing what has been identified as congenital Zika virus infection syndrome, there is a paucity of published prospective epidemiological studies.[3] A study by Thalia Araújo and colleagues[6] in *The Lancet Infectious Diseases* might be a missing piece to the puzzle, providing necessary epidemiological data to further advance our understanding of the association.

The investigators report preliminary findings from the first case-control study to examine the association between microcephaly and Zika virus infection, done prospectively in the metropolitan region of Recife in Pernambuco state, the hotspot of the microcephaly epidemic in Brazil. Their results highlight the striking magnitude of the association between microcephaly and laboratory-confirmed Zika virus infection: the risk is 50 times higher in all microcephaly cases and more than 100 times higher in cases with brain abnormalities detected by imaging.

However, as acknowledged by Araújo and colleagues, microcephaly remains a poorly defined disorder, and a uniform diagnostic approach is urgently needed. There is much debate in Brazil and worldwide about ascertainment of microcephaly, and the issue of disproportionate and proportionate microcephaly needs further clarification. Infants might be diagnosed with microcephaly when in fact they are globally small—ie, small for gestational age, without true isolated microcephaly.[7] This issue deserves attention, especially because in-utero growth restriction leading to the birth of small-for-gestational age infants is also a feature of congenital Zika virus syndrome.[2] Although disproportionate microcephaly has been the most publicised feature of congenital Zika virus infection, proportionate microcephaly is also identified in the setting of in-utero growth restriction caused by maternal Zika virus infection during pregnancy, not unlike other congenital infections such as cytomegalovirus. The distinction, however, is important because there might be distinct prognostic implications. Although microcephaly has been associated with poor outcome in children with congenital cytomegalovirus disease, other researchers have not found such an association. A possible source of discrepancy is failure to adjust the head size to the weight of the infant when defining microcephaly.[8]

Therefore, proportionality or lack thereof is becoming a very important parameter in ascertainment of microcephaly in Brazil. Likewise, categorising patients according to the presence of microcephaly and other CNS abnormalities as detected by brain imaging can enable the stratification of patients into varying levels of disability risk.

As our knowledge of the clinical repercussions of congenital Zika virus infection advances, it becomes apparent that microcephaly is only one possible adverse outcome among a range of disorders that might be part of congenital Zika virus syndrome. A population-level increase in CNS anomalies was observed in French Polynesia and in Brazil. More data are needed to refine gestational age-specific risk estimates for microcephaly and other adverse outcomes related to Zika virus infection.[9] Therefore, even though the modified Fenton curve[10] or the Intergrowth score[11] provide useful prognostic information, a full clinical assessment of the infant with clinical follow-up should provide more accurate information over time.

As definitions shift and more information is gathered about the pathogenesis and clinical manifestations of Zika virus congenital disease, it is important that surveillance efforts monitoring the current epidemic continue to critically evaluate their data. Newly


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identified clinical and phenotypic criteria should be further analysed, also taking into account findings from imaging studies. This approach will help establish a more definitive gold standard case definition and improve our understanding of the clinical manifestations of congenital Zika virus infection.

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