Introduction

Since October 2015, Brazil has had an alarming number of suspected cases of newborns with microcephaly. During that period, the concomitance with zika virus infection in the country aroused the suspicion of an association between these conditions. Therefore, the World Health Organization (WHO) declared a public health emergency in February 2016 [1, 2].

Microcephaly is defined as a clinical sign of congenital malformation in children presenting occipitofrontal circumference two standard deviations lower than the mean compared to children of the same age, sex, and ethnicity [3]. It is a rare condition, with an incidence of 5.9 cases per 10,000 live births in the United States of America [4], and its diagnosis can be confirmed as delayed brain development identified by imaging tests, such as intrauterine ultrasonography (US) or computer tomography (CT) after childbirth.

This congenital malformation has many causes, such as genetic factors and congenital infections, and it is also associated with social factors. Among the congenital infections, cytomegalovirus [5], rubella [6–8], toxoplasmosis [9], and syphilis [10] stand out as the leading agents. Low education, alcohol abuse, and inadequate prenatal care are also associated with microcephaly [4]. However, a study revealed that 41% of microcephaly cases do not have a definitive cause and are categorized as idiopathic cases [11].

Current studies have confirmed the biological plausibility of a link between zika virus and microcephaly: case reports have confirmed stillbirths infected by zika virus [12]; other studies have shown brain tissue tropism caused by zika virus [13], the vertical transmission of zika through...
the placenta [12, 14], an ecological association [15], and a higher incidence of malformations in pregnancies with confirmed Zika infections [16].

According to many authors [17, 18], studies must be developed to obtain more information about this malformation outbreak, including the temporal patterns of the complications after the epidemic waves [19] and its repercussion in the present and future generations.

This present case-control study was performed in the state of Ceará, Northeastern Brazil, to contribute to the understanding of the association between Zika virus infection and microcephaly and to draft new political-administrative measures for its management.

**Methods**

**Study design**

This exploratory case-control study intended to identify protective factors and risk factors for microcephaly in the state of Ceará, Northeastern Brazil – the Brazilian region with the highest incidence of microcephaly.

The cases were defined as children with a clinical diagnosis of microcephaly with an imaging diagnosis of microcephaly performed by computed tomography (CT) or intrauterine ultrasonography (US). The children were born after October 2015, correlating the first trimester of pregnancy with the period of the Zika virus epidemic. These newborns were identified not only in public and private maternity records but also in reference services for tracking and infant development until June 2017.

The controls were children without microcephaly, showing a cephalic perimeter larger than the 95% percentile for age according to WHO curves, as well as no signs or symptoms of congenital malformations. They were identified near the residence of each child in the group of cases, with the same age or within a two-month range, ensuring the homogeneity of temporal and environmental exposure in both groups.

**Study setting and population**

The study population included children of both sexes born from October 2015 until June 2017 who live in the State of Ceará.

Ceará is one of the poorest States in Brazil, ranking 17th in the Human Development Index (0.682) out of the 27 States in the country [20]. Ceará is in the northeastern region of Brazil, and approximately 93% of its land area of 148,000 km² presents a semiarid climate [21]. With a population of 8 million inhabitants [18], and an annual average of 128,000 live births [21], the state is the fourth in total confirmed microcephaly cases (137 cases) and the first in total of fetal and neonatal deaths resulting from microcephaly (24 cases, 16.9%) [22]. The territory of Ceará comprises 184 municipalities, and there have been confirmed cases of microcephaly in 53 (28.8%) of them [22].

**Study sample**

This study of prevalent cases aimed to enroll a sample of children with a confirmed diagnosis of microcephaly born in Ceará through June 2017. The control group of children was recruited using a ratio of two controls for each case to increase the sample power [23]. The data were collected from 58 randomly selected cases, and from this sample size, it is possible to detect an odds ratio (OR) of 2.3, with a 5% significance level and 80% sample power for exposure with 28% prevalence in the control group.

**Data collection**

Children diagnosed with microcephaly were identified by the infant care network, collecting mothers’ names and their respective addresses. We obtained the data from these units: the Assis Chateaubriand Maternity School – MEAC, the main public maternity of Ceará; the Albert Sabin Children’s Hospital – HIAS, reference for infant diseases of greater complexity in the Northeastern region of Brazil; and the Core of Treatment and Precocious Stimulation – NUTEP, reference service for children’s precocious stimulation in Ceará.

The data collection was conducted in the children’s households through a questionnaire containing data from the mothers, the children, and their families. The field research team composition was one supervisor and six interviewers, all of them with nursing or physiotherapy degrees and with proper training for the interview and anthropometric measurements. The team made five attempts to find the mother and her child at home to collect the data.

The anthropometric measurements of the children (cases and controls) were carried out just after the mother’s interview. The occipitofrontal perimeter was measured using a retractable inelastic measuring tape with a security lock (1.5 m in size and 1 mm precision). A portable digital scale measured the weight with a capacity of 180 kg and an accuracy of 100 g, and the weight was obtained with the child on the mother’s lap followed by subtracting the mother’s weight. The measurement of the children’s length was performed with the child lying down on an infant anthropometer, with a size of 1.5 m and precision of 1 mm.

**Quality control**

To reduce memory bias, we checked the data from the pregnancy and the prenatal care in the Pregnancy Booklet (official medical record of pregnant women in Brazil), which is provided by the maternity ward presenting the gestation registers and the prenatal consultations. Children’s data, such as cephalic perimeter and weight at birth, were confirmed by the Child’s Health Booklet (official medical record for children in Brazil), which is provided by the Ministry of Health to all children at birth.

The scales were calibrated according to the producer’s indication. A random sample of 10% of the questionnaires was repeated by the field supervisor to identify possible errors and fill the gaps to validate the quality of the data collection. The data was double entered in the software program to solve typing errors or data loss.

**Variables**

The dependent variable, the outcome, refers to the presence or absence of microcephaly, with a confirmed diagnosis by clinical or imaging criteria (CT scan or intrau-
The independent variables were grouped and categorized into the blocks described below. Diseases during pregnancy included the following – self-reported and confirmed by the pregnancy booklet: zika virus infection diagnosed by a medical doctor, joint pain, clinical syndromes with a skin rash, eye pain, cytomegalovirus, syphilis, rubella, toxoplasmosis, AIDS, flu or other viruses.

The exposures during pregnancy included the following – self-reported: if one had contact with solvents, inks, varnishes, poisons, hair dye or smoothing agents with ammonia; if the mother underwent tests with radiation during pregnancy (X-ray or CT), magnetic resonance imaging, tobacco use, and alcohol consumption.

**Data analysis**

Bivariate analysis was performed, calculating the proportions of categorical variables and measures of central tendency and dispersion for numeric variables. The differences found between the cases and controls were evaluated for statistical significance using the chi-square test for categorical variables, students’ t-test was used for numeric variables with normal distribution, and the Mann-Whitney test was used for numeric variables with nonnormal distribution. The Kolmogorov-Smirnoff test evaluated the normality of variables. Additionally, if one cell registered fewer than five cases, we used Fisher’s exact test.

Analyses were performed using SPSS software, version 17, SPSS Inc., considering a p-value ≤ 0.05.

**Ethical aspects**

The study was submitted to the Ethics Committee through the Brazil Platform, following all the norms of the 466/2012 Resolution of the National Council of Health of the Ministry of Health [25], and it was approved under protocol number 1.449.427. All the mothers who agreed to participate in the research signed informed consent forms.

**Results**

**Children’s baseline characteristics**

The median age and sex of cases and controls were not different (nine months and 57% male). However, there was a difference between cephalic perimeters (35 and 31 centimeters), birth weights (2.67 and 3.22 kg), and gestational ages, as shown in Table 1.

**Diseases during pregnancy**

The mothers of the patients had two-times more fevers than the mothers of the controls (p-value = 0.022), and these cases of illness were earlier in the first trimester in cases (p = 0.032). Among the women who received a diagnosis during pregnancy, there was a predominance of zika virus syndrome in the pregnant women from the cases, while the majority of the mothers of the controls had urinary tract infections or conditions other than zika (p = 0.040). The signs and symptoms were muscular pain, joint pain, weakness, and skin changes, also showing a difference between the groups (0.028, 0.002, 0.041, <0.001, respectively). Mothers of the cases showed almost twice as many reports of insect bites during pregnancy. The odds ratio of having zika virus of the cases compared to the controls was 10.35 (p-value < 0.001) (Table 2).

**Exposures during pregnancy**

From all the evaluated exposures, only the exposure to radiation before and during pregnancy had different odds between the groups (0.010 and 0.036 for radiography and tomography, respectively). Contact with chemical agents, repellents, and other substances did not present significant p values (Table 3).

**Multivariate analysis**

After the multivariate analysis, the variable infection by zika virus remained independently associated, with a p-value of 0.018 and odds ratio of 14.68 (1.59–134.83) (Table 4).

| Condition                        | Case    | Control | Total     |
|----------------------------------|---------|---------|-----------|
|                                  | n or median | % or interquartile | n or median | % or interquartile | n or median | % or interquartile |
| Sex                              |          |         |           |                 |             |                  |
| Male                             | 29       | 50.9%   | 69        | 60.0%           | 98          | 57.0%            |
| Female                           | 28       | 49.1%   | 46        | 40.0%           | 74          | 43.0%            |
| Age in months                    | 6.50     | 4.0–21.0| 11.00     | 4.0–20.0        | 9           | 4.0–20.0         |
| Birth weight                     | 2.67     | 2.3–3.0 | 3.22      | 2.8–3.7         | 3.010       | 2.6–3.4          |
| Cephalic perimeter               | 31.00    | 29.5–32.0| 35.00    | 34.0–35.5       | 33.0        | 31.0–35.0        |
| Timing of birth                  |          |         |           |                 |             |                  |
| Normal                           | 45       | 77.6%   | 94        | 81.7%           | 139         | 80.3%            |
| Preterm                          | 12       | 20.7%   | 9         | 7.8%            | 21          | 12.1%            |
| Post term                        | 1        | 1.7%    | 9         | 7.8%            | 10          | 5.8%             |
| Not specified                    | 0        | 0.0%    | 3         | 2.6%            | 3           | 1.7%             |

Table 1: Baseline and birth characteristics of the sample.
Table 2: Ratios of diseases presented by the mother during pregnancy in cases and controls.

| Condition                                | Case                        | p value |
|-------------------------------------------|-----------------------------|---------|
| **Fever**                                 | n or median | % or interquartile | n or median | % or interquartile |
| Yes, once                                 | 16 | 28.1% | 16 | 13.9% | **0.022** |
| Yes, more than once                       | 9  | 15.8% | 11 | 9.6%  |
| No fever                                  | 32 | 56.1% | 88 | 76.5% |
| **Gestation month of fever**              | n or median | % or interquartile | n or median | % or interquartile |
| **Days of fever**                         | 2  | 1.0–3.0 | 3  | 3.0–5.0 | **0.057** |
| **Fever intensity**                       | n or median | % or interquartile | n or median | % or interquartile |
| Low                                       | 11 | 50.0% | 6  | 26.1% | 0.151 |
| Moderate                                  | 8  | 36.4% | 9  | 39.1% |
| High                                      | 3  | 13.6% | 8  | 34.8% |
| **Any appointment with a physician**      | n or median | % or interquartile | n or median | % or interquartile |
| Yes                                       | 18 | 58.1% | 26 | 66.7% | **0.449** |
| No                                        | 12 | 38.7% | 13 | 33.3% |
| Do not remember                           | 1  | 3.2% | 0  | 0.0% |
| **Diseases during pregnancy**             | n or median | % or interquartile | n or median | % or interquartile |
| Other                                     | 8  | 47.0% | 12 | 46.1% | **0.040** |
| Urinary infection                         | 1  | 5.2% | 12 | 46.1% |
| Zika                                      | 8  | 47.0% | 2  | 7.6% |
| **Headache intensity**                    | n or median | % or interquartile | n or median | % or interquartile |
| Low                                       | 8  | 15.1% | 16 | 17.4% | 0.865 |
| Moderate                                  | 10 | 18.9% | 20 | 21.7% |
| High                                      | 14 | 26.4% | 19 | 20.7% |
| None                                      | 21 | 39.6% | 37 | 40.2% |
| **Stiff neck**                            | n or median | % or interquartile | n or median | % or interquartile |
| Yes                                       | 5  | 9.6% | 14 | 14.4% | 0.599 |
| No                                        | 47 | 90.4% | 81 | 83.5% |
| Do not remember                           | 0  | 0.0% | 1  | 1.0% |
| **Eye pain**                              | n or median | % or interquartile | n or median | % or interquartile |
| Yes                                       | 12 | 22.2% | 14 | 15.2% | **0.227** |
| No                                        | 41 | 75.9% | 78 | 84.8% |
| Do not remember                           | 1  | 1.9% | 0  | 0.0% |
| **Photophobia**                           | n or median | % or interquartile | n or median | % or interquartile |
| Yes                                       | 10 | 18.5% | 13 | 13.5% | **0.417** |
| No                                        | 44 | 81.5% | 83 | 86.5% |
| **Seizures**                              | n or median | % or interquartile | n or median | % or interquartile |
| Generalized                               | 2  | 3.7% | 1  | 1.0% | **0.527** |
| Focal                                     | 2  | 3.7% | 4  | 4.1% |
| **Disorientations**                       | n or median | % or interquartile | n or median | % or interquartile |
| Yes                                       | 0  | 0.0% | 5  | 5.2% | **0.172** |
| No                                        | 54 | 100.0% | 90 | 93.8% |
| Do not remember                           | 0  | 0.0% | 1  | 1.0% |
| **Amnesia**                               | n or median | % or interquartile | n or median | % or interquartile |
| Yes                                       | 2  | 3.8% | 5  | 5.4% | **0.678** |
| No                                        | 51 | 96.2% | 87 | 93.5% |
| Do not remember                           | 0  | 0.0% | 1  | 1.1% |
| **Behavioral changes**                    | n or median | % or interquartile | n or median | % or interquartile |
| Yes                                       | 13 | 24.1% | 29 | 30.2% | **0.422** |
| No                                        | 41 | 75.9% | 67 | 69.8% |
| **Dyspnea**                               | n or median | % or interquartile | n or median | % or interquartile |
| Yes                                       | 16 | 30.2% | 27 | 28.7% | **0.851** |
| No                                        | 37 | 69.8% | 67 | 71.3% |

(Contd.)
| Condition       | Case | Control | p value |
|-----------------|------|---------|---------|
|                 | n or median | % or interquartile | n or median | % or interquartile |
| Precordialgia   | Yes  | 4 7.4% 14 14.4% | 0.202 |
|                 | No   | 50 92.6% 83 85.6% |
| Dysarthria      | Yes  | 0 0.0% 6 6.3% | 0.062 |
|                 | No   | 53 100.0% 89 93.7% |
| Epigastralgia   | Yes  | 10 18.5% 17 17.5% | 0.879 |
|                 | No   | 44 81.5% 80 82.5% |
| Diarrhea        | Yes  | 5 9.4% 10 10.3% | 0.864 |
|                 | No   | 48 90.6% 87 89.7% |
| Nausea          | Yes  | 33 61.1% 58 60.4% | 0.933 |
|                 | No   | 21 38.9% 38 39.6% |
| Sore throat     | Yes  | 6 11.1% 21 21.6% | 0.105 |
|                 | No   | 48 88.9% 76 78.4% |
| Cough           | Yes  | 13 24.1% 23 24.0% | 0.987 |
|                 | No   | 41 75.9% 73 76.0% |
| Back pain       | Yes  | 27 50.0% 58 59.8% | 0.245 |
|                 | No   | 27 50.0% 39 40.2% |
| Muscle pain     | Yes  | 14 26.9% 12 12.5% | **0.028** |
|                 | No   | 38 73.1% 84 87.5% |
| Joint pain      | Yes  | 23 42.6% 19 19.6% | **0.002** |
|                 | No   | 31 57.4% 78 80.4% |
| Difficulty moving | Yes | 12 22.6% 14 14.9% | 0.237 |
|                 | No   | 41 77.4% 80 85.1% |
| Joint edema     | Yes  | 17 31.5% 25 26.0% | 0.600 |
|                 | No   | 37 68.5% 70 72.9% |
|                 | Do not remember | 1 0.0% 3 1.0% |
| Paralysis       | Ascending | 1 1.9% 3 3.2% | 0.629 |
|                 | No   | 53 98.1% 91 96.8% |
| Weakness        | Generalized | 13 24.1% 9 9.6% | **0.041** |
|                 | Focal | 4 7.4% 13 13.8% |
|                 | No   | 37 68.5% 72 76.6% |
| Conjunctivitis  | Yes  | 0 0.0% 2 2.1% | 0.290 |
|                 | No   | 52 100.0% 92 97.9% |
| Bleeding        | Yes  | 10 19.6% 16 17.2% | 0.720 |
|                 | No   | 41 80.4% 77 82.8% |
| Skin changes    | Yes  | 25 48.1% 14 14.7% | **<0.001** |
|                 | No   | 27 51.9% 81 85.3% |
| Thick skin      | Yes  | 11 42.3% 5 27.8% | 0.325 |
|                 | No   | 15 57.7% 13 72.2% |
| Spot with a border | Yes | 3 12.0% 0 0.0% | 0.128 |
|                 | No   | 22 88.0% 18 100.0% |

(Contd.)
| Condition                        | Case | Control | p value |
|---------------------------------|------|---------|---------|
|                                 | n    | median  | %       | n    | median  | %       |
| Erythema                        | 17   | 68.0%   | 7       | 38.9% |
| No                              | 8    | 32.0%   | 11      | 61.1% |
| Vesicles                        | 1    | 4.0%    | 0       | 0.0%  |
| No                              | 24   | 96.0%   | 18      | 100.0% |
| Itchy spots                     | 11   | 44.0%   | 6       | 33.3% |
| No                              | 14   | 56.0%   | 12      | 66.7% |
| Petechiae                       | 16   | 64.0%   | 7       | 38.9% |
| No                              | 9    | 36.0%   | 11      | 61.1% |
| Bruises                         | 3    | 12.0%   | 0       | 0.0%  |
| No                              | 22   | 88.0%   | 18      | 100.0% |
| Stings during pregnancy         | 17   | 30.9%   | 18      | 16.4% |
| No                              | 38   | 69.1%   | 92      | 83.6% |
| Zika infection                  | 27   | 46.6%   | 9       | 7.8%  |
| No                              | 31   | 53.4%   | 107     | 92.2% |
| Diagnosis of zika by a health professional |  |  |  |  |  |  |
| Yes                             | 18   | 81.8%   | 6       | 66.7% |
| No                              | 4    | 18.2%   | 3       | 33.3% |
| Gestation month of zika diagnosis | 3  | 2.0–4.0 | 5     | 3.0–6.0  |
| What was the severity of zika?  | Low  | 63.6%   | 7       | 87.5% |
| Moderate                        | 4    | 18.2%   | 1       | 12.5% |
| High                            | 4    | 18.2%   | 0       | 0.0%  |
| Internment due to zika          | Yes  | 0.0%    | 1       | 11.1% |
| No                              | 21   | 100.0%  | 8       | 88.9% |
| Had dengue fever                | Yes  | 1.8%    | 1       | 0.9%  |
| No                              | 56   | 98.2%   | 115     | 99.1% |
| Had chikungunya                 | Yes  | 1.8%    | 0       | 0.0%  |
| No                              | 56   | 98.2%   | 116     | 100.0% |
| Had rubella                     | No   | 57.0%   | 116     | 100.0% |
| Had toxoplasmosis infection      | Yes  | 3.5%    | 3       | 2.6%  |
| No                              | 55   | 96.5%   | 113     | 97.4% |
| Had cytomegalovirus infection   | Yes  | 3.5%    | 0       | 0.0%  |
| No                              | 55   | 96.5%   | 116     | 100.0% |
| Had herpes                      | Yes  | 3.5%    | 1       | 0.9%  |
| No                              | 55   | 96.5%   | 115     | 99.1% |
| Had syphilis                    | Yes  | 1.8%    | 1       | 0.9%  |
| No                              | 56   | 98.2%   | 115     | 99.1% |
| Had AIDS                        | No   | 56.0%   | 116     | 100.0% |
| Had hypertension                | Yes  | 5.5%    | 18      | 15.8% |
| No                              | 52   | 94.5%   | 96      | 84.2% |

(Contd.)
Discussion

The results of this case-control study indicate that infection by zika virus diagnosed by a physician in the first trimester was, independently, the risk factor most strongly associated with microcephaly, with an adjusted OR of 14.68 (95% CI 1.59–134.83) and high statistical significance (p < 0.001) despite the small sample size. The prevalence of zika virus infection in mothers of children born with microcephaly, confirmed by diagnostic methods suggested by the WHO, was 46.6%, against only 7.8% of mothers who had healthy children during the same period, as suggested by case series [16] and ecological [15] studies and also from another case-control study, in a state near Ceará [26].

Symptoms of the infectious arboviruses syndrome, mainly occurring during the first trimester of pregnancy, associated with skin rash or joint pain, have shown an association with the development of microcephaly in the bivariate analysis. Additionally, there was a higher risk of mosquito bites during pregnancy reported by the mothers of children with microcephaly.

A preliminary laboratory case-control study conducted in another state in the northeastern region of Brazil identified a high prevalence of zika virus infection in mothers of infants with microcephaly (80%) and the mothers of controls (64%) using RT-PCR and new serological methods. However, zika virus infection did not occur in any children in the control group [27].

This study contributed to the investigation of epidemiological factors, not only the maternal infections and exposures already studied, but also identified factors such as radiation exposure. Additionally, it found an association of microcephaly with maternal exposure to radiological examinations during pregnancy, although this association did not remain after controlling for confounders. Other authors [28, 29] have reported this association with radiation and congenital disabilities; however, this is not fully established. We have not yet found mention in the literature of studies that have specifically evaluated this association.

This study was validated by the difference in the means of cephalic perimeter (CP) among the case and control groups. The average CP of the children with microcephaly was below that found by Rocha et al. (2016), who performed an evaluation study to check the normality parameters of CP in children born at term in the Brazilian Northeast, before the context of the epidemic of microcephaly [30]. Other biological criteria evaluated ensured uniformity between the groups in addition to CP, including gestational age and sex.

Despite alcohol abuse and smoking during pregnancy being causes of congenital malformations [4, 31], no association with microcephaly was found in this study.

As Von der Hagen et al. (2014) discussed, 41% of microcephaly cases are idiopathic [11]. The absence of a significant correlation in this study between the classic factors of teratogenesis, such as alcohol or tobacco [32], led to an investigation of other causes, such as the epidemic of the zika virus.

| Condition     | Case       | Control    | p value |
|---------------|------------|------------|---------|
|               | n or median| % or interquartile | n or median | % or interquartile |     |
| Had eclampsia | Yes        | 1          | 3.3%    | 2          | 3.3%    | 1.000 |
|               | No         | 29         | 96.7%   | 58         | 96.7%   | 0.078 |
| Had preeclampsia | Yes     | 0          | 0.0%    | 6          | 10.0%   | 0.292 |
|               | No         | 29         | 100.0%  | 54         | 90.0%   | 0.370 |
| Had diabetes  | Yes        | 1          | 1.8%    | 6          | 5.3%    | 0.947 |
|               | No         | 54         | 98.2%   | 108        | 94.7%   | 0.390 |
| Had kidney disease | Yes  | 1          | 3.3%    | 5          | 8.3%    | 0.390 |
|               | No         | 29         | 96.7%   | 55         | 91.7%   | 0.390 |
| Had anemia    | Yes        | 12         | 21.1%   | 23         | 20.2%   | 0.534 |
|               | No         | 45         | 78.9%   | 91         | 79.8%   | 0.390 |
| Had the flu   | Yes        | 18         | 31.6%   | 44         | 38.3%   | 0.390 |
|               | No         | 39         | 68.4%   | 71         | 61.7%   | 0.390 |
| Had diarrhea  | Yes        | 8          | 14.0%   | 7          | 6.1%    | 0.082 |
|               | No         | 49         | 86.0%   | 108        | 93.9%   | 0.082 |
| Had an allergy| Yes        | 5          | 8.9%    | 5          | 4.3%    | 0.225 |
|               | No         | 51         | 91.1%   | 111        | 95.7%   | 0.225 |
| Had asthma    | Yes        | 1          | 3.3%    | 1          | 1.7%    | 0.613 |
|               | No         | 29         | 96.7%   | 59         | 98.3%   | 0.613 |
Table 3: Ratios of exposures of the mother during pregnancy in cases and controls.

| Condition | Case | Control |
|-----------|------|---------|
|           | n or median | % or interquartile | n or median | % or interquartile |
| Worked during pregnancy | | | | |
| Yes, outside home | 20 | 35.1% | 31 | 27.2% | 0.235 |
| Yes, at home | 8 | 14.0% | 10 | 8.8% |
| No | 29 | 50.9% | 73 | 64.0% |
| Contact with ink | | | | |
| Yes, during pregnancy | 16 | 27.6% | 35 | 30.7% | 0.822 |
| Yes, before | 1 | 1.7% | 1 | 0.9% |
| No | 41 | 70.7% | 78 | 68.4% |
| Contact with varnishes | | | | |
| Yes, during pregnancy | 8 | 13.8% | 9 | 7.9% | 0.168 |
| Yes, before | 1 | 1.7% | 0 | 0.0% |
| No | 49 | 84.5% | 105 | 92.1% |
| Contact with solvents | | | | |
| Yes, during pregnancy | 8 | 13.8% | 12 | 10.4% | 0.291 |
| Yes, before | 1 | 1.7% | 0 | 0.0% |
| No | 49 | 84.5% | 103 | 89.6% |
| Contact with tails | | | | |
| Yes, during pregnancy | 9 | 15.8% | 14 | 12.2% | 0.637 |
| Yes, before | 0 | 0.0% | 1 | 0.9% |
| No | 48 | 84.2% | 100 | 87.0% |
| Contact with repellents | | | | |
| Yes, during pregnancy | 20 | 34.5% | 55 | 47.8% | 0.095 |
| No | 38 | 65.5% | 60 | 52.2% |
| Contact with pesticides | | | | |
| Yes, during pregnancy | 4 | 7.0% | 7 | 6.1% | 0.814 |
| No | 53 | 93.0% | 108 | 93.9% |
| Contact with poisons | | | | |
| Yes, during pregnancy | 7 | 12.3% | 19 | 16.8% | 0.438 |
| No | 50 | 87.7% | 94 | 83.2% |
| Contact with pesticides | | | | |
| Yes, during pregnancy | 0 | 0.0% | 2 | 1.8% | 0.314 |
| No | 57 | 100.0% | 112 | 98.2% |
| Contact with hair dye | | | | |
| Yes, during pregnancy | 8 | 13.8% | 21 | 18.4% | 0.443 |
| No | 50 | 86.2% | 93 | 81.6% |
| Contact with enamels | | | | |
| Yes, during pregnancy | 33 | 56.9% | 67 | 59.3% | 0.953 |
| Yes, before | 1 | 1.7% | 2 | 1.8% |
| No | 24 | 41.4% | 44 | 38.9% |
| Contact with capillary smoothing with ammonia | | | | |
| Yes, during pregnancy | 2 | 3.4% | 6 | 5.3% | 0.667 |
| Yes, before | 0 | 0.0% | 1 | 0.9% |
| No | 56 | 96.6% | 107 | 93.9% |
| Contact with capillary straighteners without ammonia | | | | |
| Yes, during pregnancy | 2 | 3.4% | 6 | 5.2% | 0.672 |
| Yes, before | 0 | 0.0% | 1 | 0.9% |
| No | 56 | 96.6% | 108 | 93.9% |
| Performed radiographs | | | | |
| Yes, during pregnancy | 6 | 10.5% | 2 | 1.8% | 0.010 |
| No | 51 | 89.5% | 112 | 98.2% |
| Performed CT scans | | | | |
| Yes, during pregnancy | 0 | 0.0% | 1 | 0.9% | 0.036 |
| Yes, before | 3 | 5.4% | 0 | 0.0% |
| No | 53 | 94.6% | 113 | 99.1% |

(Contd.)
| Condition                                    | Case | Control | p value |
|----------------------------------------------|------|---------|---------|
| Performed Magnetic Imaging                   | Yes, during pregnancy | 2 | 4.8% | 0 | 0.0% | 0.074 |
| Resonance                                    | Yes, before | 1 | 2.4% | 1 | 1.0% |
| Imaging                                      | No | 39 | 92.9% | 98 | 99.0% |
| Smoked during pregnancy                      | Yes, all the days | 1 | 17% | 5 | 4.3% | 0.272 |
|                                             | Yes, some days | 2 | 3.4% | 1 | 0.9% |
|                                             | No, stopped smoking | 3 | 5.2% | 2 | 1.7% |
|                                             | No, never smoked | 52 | 89.7% | 108 | 93.1% |
| Number of cigarettes per day                 | 15 | 10.0–20.0 | 7 | 4.0–10.0 | 0.333 |
| Alcohol consumption during pregnancy         | Yes, a little | 5 | 8.8% | 11 | 9.5% | 0.999 |
|                                             | Yes, moderate | 1 | 1.8% | 2 | 1.7% |
|                                             | Yes, a lot | 1 | 1.8% | 2 | 1.7% |
|                                             | No | 50 | 87.7% | 101 | 87.1% |

Table 4: Multivariate analysis of the determinants of microcephaly.

| Condition                                    | B    | S.E.  | Adjusted OR | Adjusted OR CI 95% | p-value |
|----------------------------------------------|------|-------|--------------|---------------------|---------|
| Zika virus                                   | 2.686| 1.131 | 14.680       | 1.598               | 134.833 | 0.018 |
| Muscle pain                                  | 0.480| 0.711 | 1.617        | 0.401               | 6.517   | 0.499 |
| Joint pain                                   | 0.731| 0.602 | 2.078        | 0.638               | 6.768   | 0.225 |
| Skin changes                                 | –0.321| 0.905| 0.725        | 0.123               | 4.277   | 0.723 |
| Sting during pregnancy                       | 0.115| 0.796 | 1.122        | 0.236               | 5.345   | 0.885 |
| Radiography                                  | 0.261| 0.908 | 1.298        | 0.219               | 7.692   | 0.774 |
| CT Scan                                      | –0.434| 0.899| 0.648        | 0.111               | 3.777   | 0.630 |

Block 1: Zika virus, muscle pain, joint pain, skin changes, stings during pregnancy.
Block 2: Radiography, CT scan.

Microcephaly may not be the only outcome in children infected with zika virus, and other neurological disorders can develop after birth [33, 34]. This study was developed with a community design that is well-suited for other risk factors beyond zika infections but with their environmental and socioeconomically correlated factors. Additionally, the emergency situation requires an exploratory study of a broad spectrum. In this sense, we evaluated more than 200 variables, including various epidemiological factors.

Limitations
The memory bias was considered during the data collection using health records whenever possible. Confirmation bias is possible, but there was great diffusion in Brazil of the possible association between zika virus and microcephaly, which has generated a widespread awareness further than the mothers of infants with microcephaly.

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Competing Interests
The authors have no competing interests to declare.

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