

Available Evidence of Association between Zika Virus and Microcephaly

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Abstract

Objective: To clarify the possible association between the Zika virus (ZIKV) and microcephaly and understand where we are in terms of research and the debate on the causation between mild maternal clinical features and severe fetal microcephaly.

Data Sources: We did a comprehensive literature review with the keywords “zika” and/or “microcephaly” from inception to May 27, 2016, with PubMed.

Study Selection: Studies were included and analyzed if they met all of the following criteria: “probable or confirmed infant microcephaly” and “probable or confirmed ZIKV infection among mothers or infants”.

Results: We emphasize the diagnosis of ZIKV infection, including maternal clinical manifestations, maternal and fetal laboratory confirmation, and possible autopsy if need. Other confounders that may lead to microcephaly should be excluded from the study. We presented the results from clinical manifestations of ZIKV infection, testing methods evolving but the mechanism of microcephaly uncertain, flexible definition challenging the diagnosis of microcephaly, and limited causal reference on pregnant women. We made analog comparison of severe acute respiratory syndrome and chikungunya virus in terms of DNA mutation and global movement to provide further research recommendation. The chance of catch-up growth may decrease the number of previous “diagnosed” microcephaly.

Conclusions: There are some evidence available through mice models and direct isolation of ZIKV in affected pregnancies on kindly causal relationship but not convincing enough. We analyzed and presented the weakness or limitation of published reports with the desire to shed light to further study directions.

Key words: Microcephaly; Pregnant Woman; Zika Virus

INTRODUCTION

The Zika virus (ZIKV) is a mosquito-borne flavivirus with single-stranded positive RNA,^[1] which is related to dengue, yellow fever, and Japanese encephalitis virus. ZIKV was first isolated from rhesus monkeys in the Zika forest of Uganda in 1947,^[2] and later, the virus was named after that place. It is potentially transmitted through sex (heterosexual or homosexual transmission), blood, direct contact, or mother-to-fetus.^[3-7] Detected ZIKV in blood,^[4] semen,^[3] amniotic fluid,^[8] saliva,^[5] breast milk,^[5] and cerebrospinal fluid^[9] provided further evidence of its transmission route and sent an alert to public health. It was

first detected in humans in 1954,^[10] with classic and mild clinical manifestations such as rash, conjunctivitis, fever, arthralgia, and arthritis.^[11,12] The disease was sporadically found in Asia and Africa, and then there were outbreaks in Micronesia (Yap Island), French Polynesia, and Brazil^[12-14]

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in 2007, 2013, 2015, respectively. In early 2015, physicians in Northeast Brazil first found patients with a “dengue-like syndrome”, and ZIKV was later identified as the cause.^[15] High transmissibility was found for ZIKV, with a range of 2–6.6 among the basic reproduction number,^[16] especially among those in a high migration flow.^[17] This virus can adapt to harsh conditions, even can keep stable structure in the temperature of 40°C.^[18]

Microcephaly is a rare condition defined as a smaller head size, compared to other fetuses/babies of the same age/sex/ethnicity,^[19] often with prognosis of intellectual disabilities. There is still no uniform and universally accepted diagnostic standard of microcephaly^[20] because of its complex and diverse etiology. The possible etiology of microcephaly includes genetic cause, perinatal brain injury, postnatal brain injury, or craniosynostosis. No cause for microcephaly was found in 40% of the child victims.^[20]

In November 2015, physicians in Brazil began to report that there was a surge of the number of microcephaly among newborns, which was possibly linked to ZIKV infection during the mothers’ pregnancy.^[21] A Public Health Emergency of International Concern was dispatched to explore the suspected association between ZIKV and microcephaly.^[22] We reviewed the published literature to search for hints of further research to clarify or verify the association. The potential association between ZIKV and microcephaly was reported in Brazil,^[8,9,23-36] French Polynesia,^[23,37-40] Columbia,^[41] America,^[42,43] and Slovenia^[44] [Table 1]. The studies were mainly conducted from 2013 to 2016. The cases in the above studies included both resident and nonresident patients.

We did a comprehensive literature review with the keywords “zika” and/or “microcephaly” from inception to May 27, 2016, with PubMed. No publication date, language, location, or age restrictions were employed. In this review-based viewpoint, studies were included if they met all of the following criteria: “probable or confirmed infant microcephaly” and “probable or confirmed ZIKV infection among mothers or infants”. The information from selected articles was extracted and entered into Microsoft Excel 2010™ (Microsoft Corporation, Redmond, Washington, USA) by one reviewer and separately reviewed by another independent reviewer according to the selection criteria. A third independent reviewer reconciled any discrepancy. We extracted the following variables: title of the study, study type, reporting place, probable infection place, study period, study description, maternal ZIKV identification, other maternal infections exception, fetal ZIKV identification, other fetus infections exception, microcephaly identification, and sample size [Table 1].

The characteristics of the included studies are 4 ecological studies, 19 case reports, 2 cohort studies (1 in Brazil and 1 in Columbia), and 1 modeling study. In total, 25 articles

covered 26 studies: 16 in Brazil, 5 in France, 2 in Columbia, 2 in USA, and 1 in Slovenia.

Zika virus infection and its clinical manifestations

At present, what we know about ZIKV is limited. A few studies in process are trying to uncover the mystery and gain a better understanding. It is estimated that about 80% of ZIKV infections are asymptomatic. The disease often presents as mild and nonlethal among symptomatic infections and the self-limiting disease usually lasts about 1 week, while the incubation period varies from several days to 2 weeks.^[12] The characteristics of ZIKV make the infection process unnoticeable due to no obvious symptoms/signs. People do not care about the self-limiting disease with mild symptoms/signs. Such characteristics hindered the diagnosis and ecological studies in Brazil made the diagnosis imprecise by mainly relying on self-reported symptoms/signs from the patients.^[29] The clinical manifestations of ZIKV infections are similar to dengue or chikungunya fever^[46] and are even called a “dengue-like” illness.^[29] Nonisolated patients without symptoms or with mild symptoms may be a potential source of infection while mosquitos with ZIKV are another infection source. ZIKV confirmation is very challenging according to the published studies. Maternal ZIKV identification mainly relies on clinical manifestations during pregnancy.

Specific and clear clinical manifestations of ZIKV are keys to identify probable patients although not unique. Later then, we confirm the disease with other diagnosis techniques. Further monitoring should be conducted among the fetus if the patients are pregnant. However, unapparent clinical manifestations hinder the detection of ZIKV patients, not to mention monitoring among pregnant women.

Testing techniques evolving while the mechanism of microcephaly uncertain

The virus was often misdiagnosed as dengue virus because of a high cross-reactivity rate between them. During the early stage of the ZIKV outbreak of Yap in Micronesia, the laboratory test results from the dengue IgM kit mistakenly identified the dengue virus.^[12] Now, the frequently used laboratory testing methods to confirm ZIKV include both antibody and RNA detection in serum, plasma, urine, saliva, amniotic fluid specimens, cerebrospinal fluid specimens, tissues of autopsy and placenta, and conception products.^[3,9,43,44,47] A recent testing technique in the final validation may differentiate the three viruses: zika, dengue, and chikungunya in early of infection,^[48] when a physician cannot confirm the infection from symptoms/signs only. Although laboratory ZIKV testing methods are advanced, at moment, no treatment is pharmaceutically effective to reduce viral load. Clinical trials are absent due to no specific treatment of the ZIKV infection. However, mice model for ZIKV has provided directions of therapy and vaccine products.^[49] Interferon may be an option to treat and control acute ZIKV infections and prevent ZIKV pass the placenta.^[50] Until, microcephaly is diagnosed by ultrasonography, and women often had an inaccurate recall

Table 1: Published literature on ZIKV and microcephaly

Study	Study type	Reporting place	Probable infection place	Study period	Study description	Maternal ZIKV identification	Other maternal infections exception	Fetus ZIKV identification	Other fetal infections exception	Microcephaly identification	Sample size
WHO, 2016 ⁽²³⁾	Etiological study	Brazil	Brazil	October 22, 2015, to March 5, 2016	Of 6158 reported microcephaly cases, 745 cases were confirmed for ZIKV infection and 1927 were excluded. About 163 microcephaly cases per year were reported from 2001 to 2014 in Brazil	A ZIKV outbreak in 2015–2016	NR	A ZIKV outbreak in 2015–2016	NR	Microcephaly diagnosis confirmation on the way	745
WHO, 2016 ⁽²³⁾	Etiological study	French Polynesia	French Polynesia	March 2014 to May 2015	8 microcephaly newborns reported versus 0–2 cases/year	A ZIKV outbreak in 2014–2015	NR	A ZIKV outbreak in 2014–2015	NR	NR	19
Besnard <i>et al.</i> , 2016 ⁽²⁹⁾	Case study	French Polynesia	French Polynesia	March 2014 to May 2015	Mean age of the mothers in Group 1 was 29.7 years (range 22.8–38.9), no genetic history and no alcohol and cocaine use during pregnancy; cerebral malformations and dysfunction were reported by imaging examination	Clinical features	Negative for hepatitis B, HIV, rubella, toxoplasmosis, and syphilis	4 ZIKV-positive in amniotic fluids	Negative for dengue virus, enterovirus, herpes simplex virus, lymphocytic choriomeningitis virus, rubella, and varicella zoster virus, but only for 5 samples	A head circumference below the third percentile for gestational age and sex, by ultrasonography and magnetic resonance imaging	8
Brasil <i>et al.</i> , 2016 ⁽²⁴⁾	Cohort study	Rio de Janeiro, Brazil	Rio de Janeiro, Brazil	September 2015 to February 2016	Exposure group versus nonexposure group: 4/42 versus 0/16	Clinical features, tested by RT-PCR in blood/urine	Rubella, cytomegalovirus, and venereal disease outcomes from medical records, but no report; dengue history	NR	NR	By ultrasonography, newborns outcomes still under investigations	88
Butler <i>et al.</i> , 2016 ⁽⁴¹⁾	Case study	Colombia	Colombia	NR	The first newborn with microcephaly	NR	NR	Yes, no detail	NR	Yes, no detail	1
Calvet <i>et al.</i> , 2016 ⁽⁸⁾	Case study	Paraiba, Brazil	Paraiba, Brazil	NR	A description of cases, including clinical features and microcephaly diagnosis	Clinical features, tested negative by RT-PCR in blood/urine at 28 weeks of gestation	Negative for toxoplasmosis, HIV, syphilis, measles, rubella, cytomegalovirus, and herpes simplex	Tested positive in amniotic fluids at 28 weeks of gestation; sequence analysis	NR	By ultrasonography	2
Cauchemez <i>et al.</i> , 2016 ⁽³⁸⁾	Mathematical and statistical model	French Polynesia	French Polynesia	2013–2015	Trimester 1: $RR = 53$ (95% $CI: 7-1061$) Trimester 1 and 2: $RR = 26$ (95% $CI: 3-352$) Trimester 1, 2, and 3: $RR = 21$ (95% $CI: 2-424$) Trimester 2: $RR = 23$ (95% $CI: 1-408$) Trimester 2 and 3: $RR = 12$ (95% $CI: 0-178$) Trimester 3: $RR = 0$ (95% $CI: 0-49$)	Estimation according to the local sentinel surveillance data	NR	A ZIKV outbreak in 2015–2016	NR	Head circumference \leq (mean -2 SD) for age and sex, medical records review	8
Cavalheiro <i>et al.</i> , 2016 ⁽³⁶⁾	Case study	Pernambuco, Maranhão, and Rio Grande do Norte, Brazil	Brazil	NR	Microcephaly and decreased brain parenchymal volume were found	All with rash symptoms	NR	NR	Negative for toxoplasmosis, rubella, cytomegalovirus, herpes virus, and syphilis	A head circumference <32 cm; by computed tomography and/or magnetic resonance imaging	13

Contd...

Table 1: Contd...

Study	Study type	Reporting place	Probable infection place	Study period	Study description	Maternal ZIKV identification	Other maternal infections exception	Fetus ZIKV identification	Other fetal infections exception	Microcephaly identification	Sample size
Cordeiro <i>et al.</i> , 2016 ^[9]	Case study	Pernambuco, Brazil	Brazil	October 21–30, 2015	30 Zika-specific IgM in cerebrospinal fluid samples and in 28 in blood samples	NR	NR	RT-PCR in blood/cerebrospinal fluid samples	Tested for dengue and chikungunya	NR	31
de Fátima Vasco Aragao <i>et al.</i> , 2016 ^[35]	Case study	Pernambuco, Brazil	Brazil	July to December 2015	Severe cerebral damage was found among most of the children, such as brain calcifications, enlarged cisterna magna, abnormalities of corpus callosum, ventriculomegaly, delayed myelination	Clinical features	Negative for cytomegalovirus, toxoplasmosis, rubella, and syphilis	6 ZIKV-positive in the cerebrospinal fluid	Negative for toxoplasmosis, cytomegalovirus, rubella, syphilis, and HIV	A head circumference ≤ 2 cm at gestational age ≥ 37 weeks, or \leq (mean -2 SD) for age and sex for preterm infants By computed tomography and/or magnetic resonance imaging	23
de Paula Freitas <i>et al.</i> , 2016 ^[25]	Case study	Salvador, Bahia, Brazil	Bahia, Brazil	December 1–21, 2015	Of 29 children aged 1–6 months, 18 were female, 23 mothers had ZIKV clinical presentations; anterior segment and retinal, chorioidal, and optic nerve abnormalities were found	Clinical features	Negative for toxoplasmosis, HIV, syphilis, rubella, cytomegalovirus, and herpes simplex virus	NR	NR	A head circumference ≤ 2 cm at birth	29
Faria <i>et al.</i> , 2016 ^[33]	Etiological study	Brazil	Brazil	2015–2016	Significant relationship between total per capita ZIKV incidence and per capita suspected microcephaly cases in each state; and also significant relationship between total per capita ZIKV incidence and per pregnancy suspected microcephaly cases in each state	A ZIKV outbreak in 2015–2016	NR	A ZIKV outbreak in 2015–2016	NR	WHO standard	1118
Guillemette-Artur <i>et al.</i> , 2016 ^[40]	Case study	French Polynesia	French Polynesia	October 2013 to April 2014	Severe cerebral damage was found by prenatal magnetic resonance imaging	Clinical features	NR	ZIKV-positive in amniotic fluids	Negative for cytomegalovirus and lymphocytic choriomeningitis virus	By ultrasonography and magnetic resonance imaging	3
Hazin <i>et al.</i> , 2016 ^[34]	Case study	Pernambuco, Brazil	Brazil	September and December, 2015	13 female infants, head CT images performed after birth (mean age: 36 days; range: 3 days to 5 months), CT outcomes: Severe brain anomalies, including calcifications, cortical hypoglycation, ventriculomegaly, and white-matter abnormalities	Clinical features	NR	Tested ZIKV IgM antibody positive by cerebrospinal fluids of 7 infants	Negative for toxoplasmosis, syphilis, varicella, parvovirus, HIV, rubella, cytomegalovirus, and herpes simplex	NR	23
Jouannic <i>et al.</i> , 2016 ^[37]	Case study	French Polynesia	French Polynesia	2014	A description of cases, including diagnosis of ZIKV and microcephaly	NR	NR	Tested by PCR in amniotic fluids	Karyotype and cytomegalovirus detection by PCR in amniotic fluids, but outcomes not reported	Head circumference less than the third percentile	4

Contd...

Table 1: Contd...

Study	Study type	Reporting place	Probable infection place	Study period	Study description	Maternal ZIKV identification	Other maternal infections exception	Fetus ZIKV identification	Other fetal infections exception	Microcephaly identification	Sample size
Kleber de Oliveira <i>et al.</i> , 2016 ⁽²⁶⁾	Etiological study	Brazil	Brazil	NR	The microcephaly birth prevalence in national level versus the prevalence in the four states without laboratory-confirmed ZIKV infections = 2.80 ($CI = 1.86-4.05$) per 10,000 live births versus 0.60 ($CI = 0.22-1.31$) in 2015; pernambuco: 14.62; $CI = 12.33-17.17$, Paraíba: 10.82; $CI = 8.86-13.04$. The areas where women are living were reported laboratory-confirmed zika infections while women were in their first trimester	A ZIKV outbreak in 2015-2016	NR	NR	NR	A head circumference \leq (mean -3 SDs) for age and sex (≤ 30.3 cm for full-term females at gestational age = approximately 37-42 weeks) and 30.7 cm for full-term males	574
Lucey and Gostin 2016 ⁽⁴²⁾	Case study	Hawaii, United States	Brazil	January 15, 2016	The first case of microcephaly potentially associated with ZIKV	Ever lived in Brazil	NR	NR	NR	Yes, no detail	1
Martines <i>et al.</i> , 2016 ⁽³²⁾	Case study	Rio Grande do Norte, Brazil	Rio Grande do Norte, Brazil	December 2015	Clinical features: Including fever and rash during the first trimester of pregnancy; tested ZIKV-positive by RT-PCR in brain tissues, negative for other tissues	Clinical features, no laboratory confirmation	NR	Tested by RT-PCR in the tissues of autopsy and placenta	Negative for dengue	Yes, no detail	2
Meaney-Delman <i>et al.</i> , 2016 ⁽⁴³⁾	Case study	America	American Samoa, Brazil, El Salvador, Guatemala, Haiti, Honduras, Mexico, Puerto Rico, and Samoa	Up to February 17, 2016	A description of cases, including clinical features, probable infection history, and delivery outcomes	Clinical features; laboratory confirmation	NR	Tested by RT-PCR in the tissues of autopsy, placenta and conception products	NR	WHO standard (a head circumference ≤ 30.3 cm at birth)	9
Mlakar <i>et al.</i> , 2016 ⁽⁴⁴⁾	Case study	Ljubljana, Slovenia	Rio Grande do Norte, Brazil	NR	A case description, including clinical features, microcephaly, and ZIKV diagnosis	Clinical features	NR	Tested by RT-PCR and electron microscopy in the brain sample; sequence analysis in the brain tissues	Negative for dengue virus, yellow fever virus, West Nile virus, tick-borne encephalitis virus, chikungunya virus, lymphocytic choriomeningitis, cytomegalovirus, rubella virus, varicella-zoster virus, herpes simplex virus, parvovirus B19, enteroviruses, and Toxoplasma gondii in the brain tissue	By ultrasonography and autopsy	1

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Table 1: Contd...

Study	Study type	Reporting place	Probable infection place	Study period	Study description	Maternal ZIKV identification	Other maternal infections exception	Fetus ZIKV identification	Other fetal infections exception	Microcephaly identification	Sample size
Oliveira Melo <i>et al.</i> , 2016 ^[27]	Case study	Paraíba, Brazil	Paraíba, Brazil	NR	A description of cases, including clinical features, ZIKV diagnosis	Clinical features; negative in blood at 29/30 weeks of gestation laboratory confirmation	NR	Positive in amniotic fluid	NR	By ultrasonography	2
Sarno <i>et al.</i> , 2016 ^[28]	Case study	Salvador, Brazil	Salvador, Brazil	NR	A description of cases, including clinical features, microcephaly, and ZIKV diagnosis	No ZIKV clinical features and family ZIKV history	Negative for HIV, HTLV, and HCV and infections for toxoplasmosis, rubella virus, and cytomegalovirus	Positive in the fetal brain and amniotic fluids, negative in other tissues	NR	By ultrasonography and delivery measurement	1
Schuler-Faccini <i>et al.</i> , 2016 ^[29]	Case study	Brazil	Brazil	NR	26 rash symptoms, travel history confirmation, 31 full-term delivery	Clinical features	NR	NR	Negative for syphilis, toxoplasmosis, rubella, cytomegalovirus, and herpes simplex virus among fetuses	A head circumference \leq (mean -2 SDs) for age and sex, by CT scan and ultrasonography, delivery measurement	35
Ventura <i>et al.</i> , 2016 ^[30]	Case study	Brazil	Brazil	NR	1 rash, arthralgia in the first trimester	Clinical features	Negative for toxoplasmosis, rubella, cytomegalovirus, herpes simplex, syphilis, and HIV	NR	Negative for toxoplasmosis, rubella, cytomegalovirus, herpes simplex, syphilis, and HIV	By CT scans and delivery measurement	3
Ventura <i>et al.</i> , 2016 ^[31]	Case study	Brazil	Brazil	December 14, 2015	7 mothers with "dengue-like" symptoms in pregnancy; mean age: 29 years (17–42 years), mean gestational weeks: 38 weeks (38–39 weeks); 10 female fetus; mean head circumference at birth: 29 cm (26–32 cm)	Clinical features	NR	NR	NR	A head circumference \leq (mean -2 SDs) for age and sex, by CT scan and ultrasonography, delivery measurement	10
Villamil-Gómez <i>et al.</i> , 2016 ^[45]	Cohort study	Sucre, Colombia	Sucre, Colombia	January 2016	21 mothers with ZIKV symptoms, 28 positive for ZIKV by RT-PCR, the study is still being following up	Clinical features; tested by RT-PCR in blood	28 negative for dengue, chikungunya, HIV, HBV, cytomegalovirus, herpes simplex type, Epstein-Barr virus, syphilis; 3 positive for IgG anti-toxoplasma gondii; 1 for IgG anti-rubella	Tested in amino acid	NR	By ultrasonography	28

ZIKV: Zika virus; RT-PCR: Reverse transcription-polymerase chain reaction; NR: No report; CI: Confidence interval; HBV: Hepatitis B virus; SDs: Standard deviations; RR: Relative risk; CT: Computed tomography.

of their mild symptoms and signs dating back several weeks, even months. Zika-confirmation through the viral load in the acute phase and antibody testing in the convalescence period was absent.^[8,27] There are so many “ZIKV-like” symptoms which might occur in diverse diseases including Zika,^[24] that even latently infected women are still potentially relevant to fetal microcephaly.^[25,28] Scientists have realized that many other infections should be ruled out to explore the association between ZIKV and microcephaly.^[8,25,28,30] In the future, we should study and determine how often patients should be tested to timely identify infection status and have a better response to the virus. It is interesting to explore why the virus was recently reported to be related to microcephaly.

There are two kinds of mechanism of birth defects ascribed to viruses. Some viruses directly pass through the placenta to infect the fetus and some viruses cause a placental inflammatory reaction and cause further damage.^[51,52] However, the detailed mechanism of ZIKV to embryo or fetus is still unclear. Previous case studies reported that ZIKV was detected in amniotic fluids and fetal brain tissues though blood/urine was negative for the ZIKV.^[8,27,28,37,43,44] ZIKV identified in amniotic fluids and the fetal brain showed that the viruses can get through the placental barrier. Microscopic placental examinations showed calcific and focal chorionic villi^[32,44] or ultrasonography showed an abnormal placenta.^[24] An inflammatory response in the placenta has not been observed.^[44,53] Animal experimental models in mice showed that ZIKV had the capability to destroy the central nervous system and had severe pathological changes in mice.^[49] ZIKV may impact the survival and growth of human brain cells by restricting the growth of neurospheres and brain organoids.^[54] More appropriate animal models may be needed to solve the mystery between maternal ZIKV infection and microcephaly. It is necessary to find what happened to the fetus and how ZIKV generated a smaller head circumference after maternal infection.^[27]

A flexible definition challenging an accurate diagnosis of microcephaly

The operational definition of microcephaly is a head circumference below the value of the mean -2 standard deviations or $<2\%$ of the population of the same race, age, and sex.^[55] In Brazil, the value was changed from 33 to 32 cm in all full-term newborns to estimate the number of microcephalics at birth.^[56] Microcephaly diagnosis standard in Brazil after December 8, 2015, showed a higher specificity and an equal sensitivity than before.^[57] A questioned surge in microcephaly prevalence occurred in 2015 (about 20 cases per 10,000 births), compared to that in previous years (about 1 case per 10,000 births).^[29] Considering the uniform diagnostic standard, the number of reported cases was overestimated. Many false diagnosis cases were excluded in the follow-up.^[21,23] Brazil now suffering a severe economic recession is facing a challenge to solve the rising unemployment among young people.^[58] With no or little income, the youth of child-bearing age are in an economic-disadvantaged situation and this directly

affects their nutritional status. Malnutrition has a negative impact on fetal growth, including head circumference.^[59,60] The reproductive process study of the Dutch famine in 1944–1945 showed that head circumference declined 2.7% during the famine, and rose 2.4% afterward.^[61] Randomized controlled studies showed that adequate maternal and fetal nutrition improved prenatal head growth,^[62,63] the difference still existed 7 weeks after birth (corrected value), but catch-up growth at 36 weeks narrowed the gap to borderline significance ($P = 0.046$).^[62,63] The economic condition may contribute to a false diagnosis of microcephaly, and postdiagnosis follow-up is necessary to make a definite diagnosis or complete correct diagnosis, considering the limitation of the operational definition in the size of head circumference.^[64] While microcephaly is often identified after 20 weeks of gestation by ultrasonography, it's much earlier than the possible catch-up opportunity. Nutrition assessment is necessary and cautious for microcephaly diagnosis when ascribe to ZIKV. False diagnosis of microcephaly may report inexact relationship between ZIKV and microcephaly: exaggerated, shrink, or even reverse the links.

Challenges to available evidence of association between Zika virus and microcephaly

Ecological studies in Brazil reported a higher prevalence of microcephaly in 2015 than previous years. A higher prevalence was also found in 15 states among the 19 states having ZIKV infections as reported by laboratory confirmation.^[23,26] Nevertheless, the study cannot generate causal reference because it does not rule out unknown and uncontrolled confounders. Brasil *et al.*'s cohort study showed a positive association between ZIKV and microcephaly, 4/42 versus 0/16 among the exposure group and the nonexposure group, respectively.^[24] The limited-sample size study had a control group with a rash during pregnancy, but the cause of the rash was not clearly diagnosed or analyzed. An American case report showed ZIKV infections happened to pregnant women in the second and third trimester, who still delivered healthy babies.^[43] Based on the nature of retrospective surveys, ZIKV exposure seemed to occur earlier than microcephaly diagnosis and a temporal relation was established in published articles. However, it should be taken with caution because there was either no laboratory ZIKV confirmation or laboratory exclusion for other infections relevant to microcephaly in the studies. In addition, there were some gaps between exposure and disease confirmation reported in the studies to verify the potential association between ZIKV and microcephaly [Table 2]. The interval between the time when the fetus was first diagnosed as microcephaly and the occurrence of maternal clinical manifestations was on average 13 weeks, with a broad range of 3–27 weeks. The ZIKV outbreak may predict a remarkable increase in the number of microcephaly cases 5–10 months later in Brazil.^[65] The long time gap often missed the best time for maternal laboratory ZIKV confirmation. As a nonspecific cause for microcephaly, we need to exclude

Table 2: The interval between ZIKV infection and diagnosis of microcephaly, literature review

Study	Infection, weeks of gestation	Microcephaly diagnosis, weeks of gestation	Interval (weeks)
Brasil <i>et al.</i> , 2016 ^[24]	22	31	9
	8	35	27
	12	29	17
	26	35	9
Calvet <i>et al.</i> , 2016 ^[8]	18	21	3
	10	25	15
Jouannic <i>et al.</i> , 2016 ^[37]	8	22	14
	12	21	9
Mlakar <i>et al.</i> , 2016 ^[44]	13	29	16

ZIKV: Zika virus.

confounders other than ZIKV exposure. An accurate microcephaly diagnosis will benefit the causal reference between ZIKV exposure and outcome of microcephaly. The cerebrospinal fluid test for ZIKV should be performed to verify the effectiveness of the description by the possibly infected fetus.

An ongoing cohort study in Colombia will attempt to confirm ZIKV infection and rule out many confounders or other infections.^[45] The available etiological studies and case studies provide incomplete evidence for the confirmation of a potential association between ZIKV and microcephaly. However, ongoing cohort studies in Brazil and Columbia are expected to generate robust evidence for hypothesis verification.^[24,45] A dose-response relationship was observed in three trimesters, with a higher risk in the first trimester in a model based on data from French Polynesia.^[38] In future, if possible, multicenter study should be performed to explore the dose-response relationship in different trimesters of pregnancy.

Future research recommendations: Enlightenment from global movement tracking by molecular epidemiology

Phylogenetic analysis showed that ZIKV strains of Brazil emerged in a mixed way.^[66] The estimated RNA mutation rate was 0.001–0.002 nt⁻¹ year⁻¹,^[66] lower than the severe acute respiratory syndrome (SARS) mutation rate (0.003 nt⁻¹ year⁻¹),^[67] and far higher than the human DNA mutation rate.^[68] ZIKV has a global movement distribution similar to the chikungunya virus.^[69,70] Perhaps this disease can shed light on the virological study of ZIKV strains isolated in Brazil. A study showed that the isolated strains presented a 99% identification with a strain in French Polynesia in 2013 (KJ776791),^[8,44] but were much closer to the Cambodian strain in 2010.^[13] ZIKV strains in Brazil coming from an Asian lineage had 6–15 amino acids changes, compared to a strain in French Polynesia^[44] with a 1947 preepidemic prototype.^[71] A new clade emergence of chikungunya with mutations caused outbreaks in China and India.^[72,73] Can ZIKV outbreaks in Yap of Micronesia, French Polynesia, and countries of the Americas with an Asian lineage be attributed to micro-mutations of the

viruses?^[13,44,74] What kind of changes happened to the virus from sporadic to outbreak? What about an African lineage? Can the changes of the virus affect the toxicity, replication, and hosting environment? What is the survival time in blood, amniotic fluids, brain, and other tissues? Some studies reported that the viruses had a prolonged life in amniotic fluids and fetal brain. Which genes cause the neurotropism? Can a developing brain structure provide a more hospitable micro-environment for the virus? What is the full spectrum of defects caused by congenital ZIKV infection?

SARS research may shed light on ZIKV evolution at different stages in terms of nucleotide and amino acid mutation.^[67] This may uncover the possible change in virus structure of the epidemic strain. Hu *et al.*^[75] found that China Rhinolophus was the natural host of the SARS coronavirus-like virus, rather than palm civets until 2013, and this was 10 years after the first outbreak of SARS. This result was consistent with the fact that few coronavirus-like viruses were separated from palm civets.^[76] The present review-based viewpoint claims that it is too early to come up with any explanation or conclusion of the ZIKV infection.

CONCLUSION

It is a prerequisite to explore the association of ZIKV and microcephaly from the perspective of the global health. Maternal “ZIKV-like” clinical manifestations can corroborate the diagnosis, but maternal and fetal laboratory confirmation, especially autopsy or detection of miscarriage products, could provide more objective evidence. Microcephaly diagnosis relies on ultrasonography during pregnancy, physical birth examination, and/or autopsy of still-birth or miscarriage products, and postdelivery follow-up. We should exclude potential confounders which might cause microcephaly. Other infections related to microcephaly should be detected. Observational studies, especially in economic-advantaged social environment, should follow the above to ensure an accurate outcome. In addition, animal experiments can disclose the possible mechanism, but robust evidence on gestational human being is overwhelming. In addition, comparable studies with other similar viruses will provide experience and lessons. Bioinformatics techniques and findings on similar damages of central nervous system diseases might shed light on the choice of an appropriate animal model, which will be better than mice.

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Conflicts of interest

There are no conflicts of interest.

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