ISSN: 2415-038X (Print)

Journal of Preventive and Rehabilitative Medicine

2016; 1(1): 35-40
Published Online: 05/09/2016 (http://medicine.unza.zm/research/journal)
doi: 10.21617/jprm.2016.0102.8

Review Paper

Zika Virus and Congenital Microcephaly in Zambia, What are the Chances?

Kunda Ndashe[1], Nova Nalondwa[2], Oswell Khondowe[2], Samuel Munjita[3]

[1]Department of Environmental Health, Faculty of Health Science, Lusaka Apex Medical University, Kasama Road, Lusaka, Zambia
[2]Department of Physiotherapy, School of Medicine, University of Zambia, Lusaka, Zambia
[3]Department of Biomedical Sciences, School of Medicine, University of Zambia, Lusaka, Zambia

Email: ndashe.kunda@gmail.com

To cite this article:
Kunda Ndashe, Nova Nalondwa, Oswell Khondowe, Samuel Munjita. Zika Virus and Congenital Microcephaly in Zambia, What are the Chances? *Journal of Preventive and Rehabilitative Medicine,* Vol. 1, No. 2, 2016, pp. 35-40. doi: 10.21617/jprm.2016.0102.8

ABSTRACT

Background: In 2015, Brazil experienced an increase in the incidence of microcephaly cases, 20 times higher than preceding years. Epidemiological and experimental data suggest that microcephaly cases in Brazil might be associated with the introduction of Zika virus. We reviewed literature correlating Zika virus to microcephaly, epidemiology of the virus and its genetic, occurrence and possible transmission in Zambia.
Methodology: We searched online databases such as PubMed, Scopus, Google Scholar, National Center for Biotechnology Information (NCBI) database and ISI Web of Science and critically reviewed appropriate publications to extract consistent findings, identify knowledge gaps, and suggest future studies.
Results: After the screening process, 44 articles and bulletins were critically reviewed. In outbreak studies, pregnant women were either living in areas of ongoing transmission, had resided in, or travelled to ZIKV-affected areas during pregnancy. In the case of the Zambian Study, serology tests were conducted on selected residents in the Western and North-western Provinces to detect Zika virus antibodies. *Aedes* spp. which are vectors of the Zika virus were reported in Zambia as well. There lies a strong correlation between Zika virus infection and microcephaly as proven epidemiologically and experimentally through mice experiments. Genetically the Asian lineage of Zika virus has undergone adaptive changes in the genome resulting in improvement of the NS1 translation in humans.
Conclusions: With globalisation and modern transportation, the presence of *Aedes* spp. in Zambia and strong correlation of Zika virus to microcephaly, the risk of having an outbreak with microcephaly manifestation is very high. Routine Zika virus antigen surveillance in *Aedes* spp. and infants born with microcephaly is highly recommended.

Key Words: Zika virus, congenital microcephaly, *Aedes* spp., Zambia

1. Introduction

The scientific world has been drawn to the recent Zika virus (ZIKV) outbreak in Brazil and its possible link with congenital microcephaly. Since the 2015 ZIKV outbreak in Brazil, the virus has spread to 31 countries in the Americas [1]and just recently cases were reported in Cape Verde, Africa, [2]. There are increasing public health concerns on the possible association between the ZIKV and the approximately 5000 microcephaly cases in new born reported by the Brazilian Ministry of Health (MoH) [3]. Microcephaly is a neurological abnormality that is present at birth and defined as head circumference of at least 2 standard deviations (SD) smaller than the mean for sex, age, and ethnicity, [4] with head circumference at least 3 SD smaller being deemed severe [5]. Although studies have not

yet been conducted in Zambia, Health Grades Inc. Colorado, USA, estimates the prevalence of microcephaly in Zambia at1.0 per 10,000 births [6].In Brazil the prevalence of microcephaly was 0.5 per 10,000 before the sharp change in 2015 thereafter it increased to20 infants per 10,000 which was above the normal reported limits [7].The increase in microcephaly cases is worrying because several authors have suggested that the condition carries a grave prognosis for normal intellectual functioning of the child [8,9]. Children with microcephaly are prone to epilepsy [10,11], cerebral palsy [12,13], mental retardation [14], Ophthalmologic and audiologic disorders [11]. The World Health Organisation (WHO) declared a public health emergency of international concern on February 1, 2016 due to the global threat posed by the possible link of ZIKV to microcephaly [2,15].

The mosquitoes (*Aedes aegypti, Aedes albopictus and Aedes africanus*) responsible for the transmission of ZIKV [16] are currently abundant in Zambia [17] and therefore an introduction of the Brazilian ZIKV into the country would result in an outbreak with possible increase in congenital microcephaly cases. This article reviews the ZIKV and its genetics, association with microcephaly and the possibility of an outbreak in Zambia.

2. Methodology

We searched PubMed, Scopus, Google Scholar, National Center for Biotechnology Information (NCBI) database and ISI Web of Science (up to May 24, 2016) using the following search terms:("Epidemiology of Zika Virus", "Genetics of Zika Virus", "Zika Virus Infection", "Zika Virus and Microcephaly", "Microcephaly", "Zika Virus in Africa" "*Aedes* spp. in Zambia" and "Flaviviruses in Zambia"). We supplemented database searches by screening bibliographies of the articles. We also included epidemiology bulletins from World Health Organization (WHO), Pan American Health Organization (PAHO), and European Centre for Disease Prevention and Control (ECDC). All the articles were published in English. Eligibility criteria were original studies that reported cases of Zika infections, Zika Virus and microcephaly, disease vectors and epidemiology bulletins from the WHO, PAHO, and ECDC.

Two independent reviewers (KN, NN) screened article titles and abstracts to select articles for full-text screening. The reviewers of the current paper assessed full texts independently; in case of disagreement, they consulted a third author (OK), and agreed upon a decision by consensus.

3. Results

The primary search identified 357 papers. We removed 124 duplicates. We screened 233 articles to assess eligibility, and excluded 170 that did not meet the inclusion criteria. We included 13 articles in the synthesis (7 case reports or case series and 6 surveillance or cross-sectional studies). We also included 11 epidemiological bulletins and alerts from WHO, PAHO, and ECDC.

Characteristics of included studies

The studies were conducted largely in South America: Brazil, Colombia, Puerto Rico, and Venezuela. Other studies were conducted in France, the USA and Zambia. In outbreak studies, pregnant women were either living in areas of ongoing transmission, had resided in, or travelled to ZIKV-affected areas during pregnancy. In the case of the Zambian Study serology tests were conducted on selected residents in the Western and North-western Provinces. Diagnostic tests to confirm the presence of ZIKV infection in pregnant women included reverse transcription polymerase chain reaction (RT-PCR) for ZIKV nucleic material and tests on serum, breast milk, amniotic fluid and urine samples. IgG and IgM antibody tests for viral ZIKV exposure were also conducted. For foetal imaging, ultrasound, computed tomography scanning for brain calcifications and magnetic resonance imaging were employed.

4. Discussion

We reviewed 44 studies and epidemiology bulletins reporting genetics of ZIKVs, correlation of ZIKV to microcephaly and epidemiology of ZIKV worldwide.

Genetic Analysis of Zika Viruses

Zika virus is a single-stranded, positive sense RNA virus with a 10.7-kb genome encoding a single polyprotein that is cleaved into three structural proteins (a) Capsid (C), (b) Pre-membrane/Membrane (prM/M), (c) Envelope (E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [18]. It is a member of the family Flaviviridae, genus Flavivirus, and classified by sequence analysis into three genotypes East and West African and Asian [19]. The genus Flavivirus includes West Nile virus, dengue virus and yellow fever virus [18]. Zika transmission can be categorized into two routes (a) mosquito-borne and [20] (b) non-mosquito transmission [21-23]. Mosquitoes known to transmit ZIKV among humans are *Aedes aegypti, Aedes albopictus* and *Aedes africanus* [20]. There is increasing evidence to support non-mosquito transmission of ZIKV in humans, the virus RNA has been detected in amniotic fluid of mothers whose foetus had cerebral abnormalities detected by ultrasonography [24]. In another case, viral antigen and RNA have been identified in the brain tissue and placenta of children who were born with microcephaly and died after birth [25] thus supporting vertical transmission of the ZIKV. Sexual transmission of the ZIKV has been reported to a partner in Paris of returning male traveller who captured ZIKV infection in Rio de Janeiro, Brazil [26].

Changes in nucleotide composition have long been noticed as an important evolutionary mechanism and a tell-tale of

viral adaptation to host [18]. The link of congenital microcephaly to ZIKV has led to scientists extensively studying the genome of the virus for any mutational changes. Until 2012, there were eight genomes available; however, post 2012, 42 genomes have since been reported in the public domain (till 20th March 2016) of which 25 genomes reported post January 2016 [27-29]. Based on serologic and genetic properties, three lineages of ZIKV have been established, namely, East African, West African and Asian [29]. The differences between the African and Asian lineages could explain the emergence of ZIKV in humans and raises concerns about the consequences of the adaptive genetic changes observed in NS1 and the recent increase in viral fitness [28,29]. Moreover, the limited number of human ZIKV cases in Africa could be associated to low viremia in humans, [29]. Researchers suggest that fitness gain is associated with improvement of the NS1 translation in humans by synonymous mutations. Synonymous mutations are a common source of variation, given the constrained nonsynonymous substitutions rate imposed to RNA viruses that have to negotiate successful infections, alternating between humans and mosquitoes [29]. It remains to be evaluated how the NS1 structural and immunological similarities associate to the aggravated symptoms observed when ZIKV and DENV co-circulate [27,28].

Shrinet and colleagues performed several genetic analyses to 50 ZIKV genomes currently available in the public domain (NCBI database). Year 2015 and 2016 outbreak samples (n=25) were compared against the year 1966 sequence from Malaysia ((HQ234499.1) [29]. The study revealed that the viral capsid (C) protein showed variations at five aa positions, namely, N25S, L27F, R101K, I110V and I113V in all the sequences. Envelope (E) protein of 2015-2016 isolates of ZIKV when compared to the reference Malaysian strain revealed changes at three positions, D393E, V473M and T487M in all sequences. Sequence comparison of pre-membrane (pr) protein showed three aa variations, namely, V1A, S17N, V31M in all the 2015 and 2016 sequences. The researchers further observed the non-structural protein sequences comparison of the isolates of 2016 and 2015 with the reference sequence from Malaysia isolate indicated that the non-structural proteins of ZIKV is more conserved than the structural proteins. They reported that non-structural proteins namely, NS1, NS2A, NS2B and NS3 showed very few conserved changes as compared to NS4B and NS5 which showed 7 and 15 aa variations respectively. Malaysian strain did not have 5' and 3' UTR sequence available for analysis and also UTR information were absent for two sequences each from 2015 and 2016 isolates respectively. The analysis revealed that both UTR sequences (5' UTR and 3' UTR) were mostly conserved. Untranslated regions (5' and 3') are known to play important roles in flavivirus replication and virulence. The study revealed a balancing selection of the identified amino acid variations thereby favouring fitness to the strains [29].

Possible Associations of Zika Virus Infection with Microcephaly

Just after the reported increase of congenital microcephaly cases in Brazil, scientists have undertaken extensive research work to ascertain the association of ZIKV to microcephaly. In a review by Teixeira and colleagues (2016), they proposed two main points as evidence to support the association of microcephaly to ZIKV infection. Firstly, the geographical distribution of the cases of congenital microcephaly is consistent with areas of ZIKV outbreak. Re-examination of the Brazil MoH registry data revealed that the cases of congenital microcephaly were experienced by women that lived or visited ZIKV outbreak areas during the first or early second trimester of their pregnancy [24]. The second evidence was the identification through Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) of ZIKV in amniotic fluid of two pregnant women whose foetuses had microcephaly [25], in tissue samples of deceased case and placenta of a woman who was reported to have had rash and later miscarriage within 8 weeks of pregnancy [28].

The second attempt to associate microcephaly to ZIKV outbreak was done using the Shepad's criteria for identifying teratogens [28]. The first Shepad's criterion states that a proven exposure to the agent must occur at a critical time during prenatal development. Data from Brazil MoH registry has shown that women with foetuses or infants with congenital microcephaly were either residence or at some point in the first trimester or early second trimester of the pregnancy had travelled to ZIKV outbreak areas [24]. The second criterion requires that two epidemiological studies of high quality support the association. The first epidemiological consideration is a study which was conducted during the Brazil outbreak, 88 pregnant women presented with rash that had appeared in preceding 5 days were tested for ZIKV RNA. Among all the pregnant women that were tested 72 were ZIKV RNA positive, further prenatal ultrasonography was performed on 42 women and only fetal abnormalities were only observed in 12 women [31]. The second epidemiological consideration is retrospective analysis of the ZIKV outbreak that took place in French Polynesia 2013- 2014 [32]. During that outbreak eight cases of microcephaly were recorded; the researchers used serologic and statistical data and mathematical modelling to estimate that 1% of the foetuses and neonates that were born to mothers who had been infected with ZIKV in the first trimester had microcephaly [32] a prevalence that was approximately 50 times as high as the estimated baseline prevalence. However, this estimate was based on small numbers, confidence intervals were wide, and the risk of other adverse outcomes (e.g., other brain anomalies) was not assessed [32]. The third Shepad's criterion states that there

should exist careful delineation of clinical cases with the findings of a specific defect or syndrome and in the case of the Brazil outbreak many foetuses or infants with presumed "congenital ZIKV infection" had a consistent pattern of microcephaly, intracranial calcification and other brain anomalies [24,31]. The fourth criterion states that with rare exposure comes rare defects. The reports of foetal or infant microcephaly among pregnant women that travelled to ZIKV outbreak areas fits in this criterion [24,26,33]. The fifth criterion requires the need for an animal model that shows teratogenicity. Recent studies have further linked the Brazilian ZIKV to congenital microcephaly through mice model experiments [34-36]. Cugola and colleagues reported that that Brazilian ZIKV crosses the placenta and causes microcephaly by targeting cortical progenitor cells, inducing cell death by apoptosis and autophagy, and impairing neurodevelopment [35]. This study further reinforces the growing body of evidence linking the Brazilian ZIKV outbreak to the alarming number of cases of congenital brain malformations [35]. The historical African ZIKV (MR766) was recently shown to infect cultured human neural precursor cells (NPCs), but unlike the contemporary ZIKV strains, it is not believed to cause microcephaly [37]. LI and colleagues investigated whether the Asian ZIKV strain (SZ01) could infect NPCs in vivo and affect brain development and the result was that it does replicate efficiently in embryonic mouse brain by directly targeting different neuronal linages. The research concluded that ZIKV infection leads to cell-cycle arrest, apoptosis, and inhibition of NPC differentiation, resulting in cortical thinning and microcephaly. The two studies on mice strongly link the Brazilian ZIKV to microcephaly [37]. The sixth Shepad's criterion states that the association should make biological sense. ZIKV appears to be neurotropic and can be seen in damaged presumably glial cells and neuron in the brain of new born with microcephaly [35,36].

Zika Virus and its vector in Zambia and a possibility of a microcephaly-related Zika Virus outbreak.

In 2013, Babaniyi and colleagues conducted a sero-survey to determine the prevalence of arbovirus Zika, Dengue fever, Yellow fever and Rift Valley fever infections in the Western and North-western provinces of Zambia and they recorded a ZIKV antibody prevalence rate of 6.1% [38]. In the study the researchers tested for IgG and IgM antibodies against ZIKV. The mainstays of the routine diagnosis of ZIKV infection are the detection of viral nucleic acid (RNA) by RT-PCR and the detection of IgM antibodies by IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) [39]. The considerable cross-reactivity of flavivirus antibodies presents major challenges for the interpretation of serologic test results. For example, a recent ZIKV infection may also evoke a positive MAC-ELISA result for dengue [39]. In the case of the study by Mazaba-Liwewe and colleagues, 4.1% of the participants tested positive for Dengue IgG in the same study area (North-western and Western provinces) where the Zika IgG and IgM was found [40]. In order to rule out cross reactivity with other flavi viruses including dengue, Mazaba-Liwewe and colleagues conducted differential antibody tests by ELISA [40]. Antigen detection by RT-PCR offers a more definitive result and the test further provides information on the molecular characteristics of the circulating ZIKV [19]. Since antibodies (IgG and IgM) against ZIKV were detected in Zambia there is a possibility that the virus could be in circulation in the Western and North-western provinces [38].

The *Aedes* spp. (*Aedes aegypti, Aedes albopictus and Aedes africanus*) are abundant in tropical climates and Zambia provides suitable ecological and climatic conditions for mosquito vector survival [18,41]. Masaninga and colleagues conducted a study on the distribution of Yellow Fever (YF) Virus vectors in North-western and Western provinces of Zambia and it was observed that the presence of *Aedes aegypti* was mainly in peri-urban areas and *Aedes africanus* in forested areas of North-western Province [41]. The two species (*Aedes aegypti* and *Aedes africanus*) formed 0.68% of the overall mosquito collection in the North-western province, where low densities and sparse distribution were observed for larvae and adult [41]. This study therefore reported the presence of the *Aedes* spp. in Zambia, mostly in North-western province.

Increased human interaction through globalized trade and travel and the presence of *Aedes*spp in North-western province of Zambia possess very high risk of an Asian ZIKV outbreak in Zambia [42]. In May 2016, it was reported that in Cape Verde a ZIKV of Asian lineage and not African was isolated [43]. The country lies on the western coast of Africa and serves a tourist destination. It is therefore important that routine surveillance of the ZIKV is conducted by detection of viral antigens in areas (North-western and Western provinces) previously reported with seropositive populations. Deliberate steps should be taken to record all congenital anomalies such as microcephaly in all regions reported with ZIKV seropositive communities in Zambia. Routine ZIKV screening by detection of the virus antigen should be conducted in all cases of infants born with congenital microcephaly to rule out any possibility of ZIKV infection. Vector strategies including destroying mosquito breeding sites indoor residual spraying should be prioritised [42]. All Zambians preparing to attend the Olympic Games in Rio de Janeiro, Brazil, this year are advised to avoid mosquito bites, seek medical attention should they develop symptoms compatible with ZIKV infection (rash, fever, joint pains or conjunctivitis), and use condoms to prevent potential sexual transmission during or after visiting Brazil.

Acknowledgements

We would like to thanks Dr. Margaret Mweshi for the constructive criticism and guidance during the preparation of the manuscript.

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