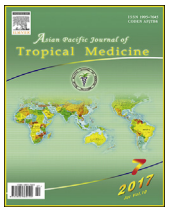
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Review http://dx.doi.org/10.1016/j.apjtm.2017.07.003

Alternate paradigms on Zika virus-related complications: An analytical review

Baburajan Radha[1]✉, Gnanaraj Muniraj[2]

[1]*MVK Hospital-Hitech Diagnostic Laboratory, Tanjavur 02, Tamil Nadu, India*

[2]*Department of Plant Morphology and Algology, School of Biological Sciences, Madurai Kamaraj University, Madurai 21, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 29 Apr 2017
Received in revised form 25 May 2017
Accepted 20 Jul 2017
Available online 28 Jul 2017

Keywords:
Zika virus
Microcephaly
Neurotropism
Koch's postulates
Placenta
Vaccines

ABSTRACT

The proportion of the reported cases of Zika virus (ZIKV) infection reached the status of a pandemic. Numerous studies are being conducted on the isolation of ZIKV strains from various epidemics, diagnosis of the infections, various animal models and cell culture designs to study the pathogenesis of ZIKV in the attempts to find an effective ZIKV vaccine. This review focuses upon the 'Off-Spectrum' body of studies which analyses the epidemiology, pathogenesis and other attributes of ZIKV in the light of various dissident hypotheses.

1. Introduction

Begun from a humble report [1] on its discovery in the Ugandan Zika forests, to its present day threat at a pandemic level, the Zika virus (ZIKV) is really intriguing in various ways. Wide arrays of studies and reviews are being reported regarding its epidemiological, structural and functional aspects. There are no drugs or vaccines officially declared against this virus, even though various strategies are being proposed.

The main stream body of reports [2–4] considers ZIKV as a main causative agent of the fever, rashes, Guiiles-barrion syndromes in adults and, importantly, of microcephaly of the neonates and microcephaly-related intellectual disabilities, and encourages corresponding studies on various animal models and cell lines in this perspective. But it must be noted that there is a dissident group of reports, which maintain that ZIKV is not that 'guilty' and virulent enough to provoke such adverse reactions so as to infiltrate the immune system or to cross the placenta to affect the fetus. Hence, there available are these interesting bodies of proposals and communications, and this review is all about them.

2. On microcephaly

The interest on microcephaly well precedes that on ZIKV. Babies born with small heads invoked interesting speculations across all races and, of course, various theories were constructed and studies reported in this discipline.

The Japanese myth of the curse for having killed pregnant monkeys [5], Virchow's theory of premature synostosis [5], Vogt's Atavastic theory [5], Jelgersma's theory on 'Advanced-type Individuals' [6] and Giacomino's classification on microcephaly [5] are some of the theories worth mentioning in this regard. Prasad *et al.* attributed the mental retardation, supposedly caused by microcephaly, to malnutrition [7]. Later, the measurement standards for normal and microcephalic heads were established by various authors and were either accepted or followed flexibly, in the subsequent studies.

2.1. Measurement of microcephaly

One of the earlier standards on microcephaly was proposed by Böök *et al.* [8], which considered a head microcephalic only if

✉First and corresponding author: Baburajan Radha, MVK Hospital-Hitech Diagnostic Laboratory, Tanjavur 02, Tamil Nadu, India.
E-mail: babumkubct@gmail.com
Peer review under responsibility of Hainan Medical University.

it has the (head) circumference (HC) of less than three standard deviations (SD) from the mean for age and sex. This standard was accepted and followed by Brandon *et al.* [9] in their subsequent study on microcephaly in monozygotic twins. They followed the standards of Westropp and Barber [10] for the normal head size. Interestingly, Westropp and Barber, in their study with children measured in Oxford, London and Aberdeen, admitted the difficulty in the construction of these standards. They repeatedly mentioned the word 'normal' in quotes. Later, Roche *et al.* proposed the HC to be greater than 3SD [11]. Modern studies on microcephaly consider the Fenton growth curves or InterGrowth curves.

Recently, in their comment, Victora *et al.* explicitly stated the distortion of standard parameters by various organizations such as Brazil's Ministry of Health, Pan American Health Organization (PAHO) and World Health Organization (WHO), in calculating the number of microcephalic neonates [12]. This paper also questions the reliability of using the Fenton curves and the diagnostic methods proposed by WHO, and strongly anticipates effective anthropometric measurements on both scientific and moral grounds.

2.2. Causes of microcephaly

The genetic etiology of microcephaly was strongly contemplated in the earlier periods of literature in this field, though the non-genetic factors were not completely ignored. This preponderance over the genetic factors was advocated with strong supporting evidences.

One of these evidences is an unique study by Plummer in 1952 [13], which reported that among the 11 children, whose mothers were exposed to the radiation emitted from the atom bomb dropped in Hiroshima, 7 were microcephalic. The mothers were within 1 200 m from the hypocenter and were in their first 20 weeks of gestation. Following this study, came the report by Komai T *et al.*, which studied microcephaly in 78 Japanese sib-ships [5]. The primary conclusions of this study are as follows: i) An autosomal recessive gene is the cause of microcephaly; ii) Males are genetically more disposed than females, and iii) Contribution of non-genetic factors is not important.

But Brandon *et al.* suggested other factors, which could operate during pregnancy [9]. They claim that factor such as multiple pregnancies could also result in low birth weights and brain defect, as the probands in this study were not exposed to any radiation *in utero*. In this context, they also questioned the measurement of microcephaly among these children, as these low weight children could be falsely considered to be microcephalic.

3. Evidences on the pathogenicity of ZIKV

Certain studies [14–18] report the diagnosis of ZIKV infection utilizing the presence of ZIKV antibodies, ZIKV RNAs and ZIKV particles among the subjects. But mere presence of antibodies, nucleic acids or particles does not suggest the pathogenicity of ZIKV, especially when it violates the Koch postulates. Koch postulates are the golden standard to assess pathogenicity and, in our opinion, are technically far superior to the Bradford Hill criteria [19]. While the latter infer certain statistical causalities, the former gives a Fool-proof standard in analyzing infective potential.

Various interesting and extensive studies are being reported regarding the pathogenicity and neurotropism of ZIKV. Albeit technically sophisticated, analytically they are less helpful. One such report is that of Onorati *et al.* [20]. This group devised and characterized cell lines to simulate neocortical (NCX) and neuroepithelial stem cells (NES), infected them with the 2010 Cambodian ZIKV strain FSS 13025 and Brazilian ZIKV strain PE243, used both strains to infect *ex vivo* human fetal brain slices (1.5 DPI and 15 pcw, respectively) and attempted to elucidate the pathogenic mechanisms. They observed NS1+/VIM+ cells and scaffold disorganization of radial glial cells starting at 3.5 DPI. Infection of radial glial cells could also be accomplished by intraperitoneal injection of ZIKV strain in offspring mice [21].

Another important part of this paper is that the investigators, to their credit, conducted immunohistochemistry with the post-mortem forebrain and spinal cord slices of the first reported ZIKV-infected microcephalic fetus. ZIKV RNA from this fetus was first isolated by Mlakar *et al.* [22]. Significant morphological differences in ventricular and sub-ventricular zones in this ZIKV-infected fetus were observed and compared with age-matched postmortem controls. Yet, the genetic, nutritional and cephalic profile of that child, whose brain samples were analyzed, should have been discussed.

Based on immunoblotting, immunoelectron microscopy and droplet digital PCR (on both the devised NES cell lines and organotypic fetal slices), this group elucidated that ZIKV translocates phosphorylated TANK binding kinase (pTBK), which is already reported to mediate innate immunity and proliferation pathways, from centrosomes to mitrochondria. They studied the pTBK1 translocation with Dengue-2 virus and human cytomegalovirus. Though human cytomegalovirus induced the translocation, Dengue-2 virus did not. In addition, they treated the NES cells with KIN1408, an agonist of RIG 1 like receptor. The treatment resulted in the similar translocation of pTBK1 in mitochondria and CASP3-mediated cell death. This experiment suggests that ZIKV may activate RIG 1 like receptor for its pathogenicity. The group indicated, finally, that the nucleotidyl prodrug, Sofosbuvir, could protect the NES cells from ZIKV related infections.

Incidentally, Victora *et al.* report a case, in their study with 31 Brazilian microcephalic children with no intrauterine exposure to infectious diseases such as syphilis, toxoplasmosis and pathogens such as rubella virus, cytomegalovirus and herpes virus [12]. This report, apparently, does not advocate microbial infections to be the cause of microcephaly. Despite being extensive, the studies performed by Onorati *et al.* does not consider the protection conferred by the maternal immune system within the fetus via the placenta [20,23–26]. Even the normal flora [27], certain drugs ingested during pregnancy [28,29] and other compounds and regulatory molecules [30–34] also could pass via placenta regulating or affecting the fetus. Placenta is an intriguingly unique organ [35–39] and mimicking it using models is highly challenging [36] and, if not properly structured, could yield misleading results. Most importantly, the stability of ZIKV in normal environmental conditions is reported [40,41] to be very less. Hence it is unlikely that ZIKV could potentially infiltrate the mucosal immune system.

Infecting the NES, NCS and other neural cell lines and brain tissue slices with highly virulent neurotropic viruses such as polio virus and rabies virus could plausibly yield valuable results and help us make an extensive and comparative analysis.

4. Discussion

Ironically, no vaccines are officially declared against the major epidemics of the third world countries, caused by Ebola virus, SARS virus and ZIKV, except that by dengue virus, against which a vaccine named Dengvaxia (CYD-TDV) is available with much restriction [42].

Yet, hopes are expressed in finding therapeutic strategies, including vaccines, against ZIKV on the grounds that ZIKV's fetal pathology much resemble that of rubella virus [43,44]. The penetrance [45] and immunogenic potential of ZIKV [46] also are reported to be very low. It should be noted that an effective vaccine presupposes its corresponding pathogen to be reasonably virulent so as to generate a strong immune response in the host.

It is our conviction that the scientific community has a moral responsibility to understand the etiology and pathogenicity of ZIKV and assess the epidemiology with genuine reasoning, given the complicated nature of this worldwide enterprise.

Conflict of interest statement

The authors declare no conflict of interests.

References

[1] Dick GW, Kitchen SF, Haddow AJ, Zika virus I. Isolation and serological specificity. *Trans R Soc Trop Med Hyg* 1952; **46**(5): 509-520.

[2] Fauziah MI. Zika – a pandemic in progress? *Malays J Med Sci* 2016; **23**(2): 70-72.

[3] Radha B, Muniraj G. Trends and perspective of the Zika pandemic: a short review. *Int J Pharm Biol Sci* 2016; **6**(3): 166-183.

[4] Jamil Z, Waheed Y, Durrani TZ. Zika virus, a pathway to new challenges. *Asian Pac J Trop Med* 2016; **9**(7): 626-629.

[5] Komai T, Kenichi K, Yasunosuke O. Genetic study of microcephaly based on Japanese material. *Am J Hum Genet* 1954; **7**(1): 51-65.

[6] Jelgersma HC. Die sogennante entartungszeichen der mikrocephalein und die fetalisationslehre. *Zeit Neur U Psychiat* 1928; **112**: 88-100.

[7] Prasad KMR, Girimaji SR, Manjunatha KR, Khanna Neelam, Rao BSS, Ravi V, et al. The etiologic patterns in microcephaly with mental retardation. *Indian J Psychiatry* 1995; **37**(2): 70-80.

[8] Böök JA, Schut JW, Reed SC. A clinical and genetical study of microcephaly. *Am J Ment Defic* 1953; **57**: 637-660.

[9] Brandon MGW, Kirman BH, Williams CE. Microcephaly in one of monozygous twins. *Arch Dis Child* 1959; **34**(173): 56-69.

[10] Westropp CK, Barber CR. Growth of the skull in young children. Part 1: standard of head circumference. *J Neurol Neurosurg Psychiatry* 1956; **19**: 52.

[11] Roche AF, Guo SS, Wholihan K, Casey PH. Reference data for head circumference-for-length in preterm low-birth-weight infants. *Arch Pediatr Adolesc Med* 1997; **151**(1): 50-57.

[12] Victora CG, Schuler-Faccini L, Matijasevich A, Ribeiro E, Pessoa A, Barros CF. Microcephaly in Brazil: how to interpret reported numbers? *Lancet* 2016; **387**(10019): 621-624.

[13] Plummer G. Anomalies occurring in children exposed in utero to the atomic bomb in Hiroshima. *Pediatrics* 1952; **10**: 687-693.

[14] Gourinat A, Connor OO, Calvez E, Goarant C. Detection of Zika virus in urine. *Emerg Infect Dis* 2015; **21**(1): 84-86.

[15] Musso D, Roche C, Robin E, Nhan T, Teissier A. Potential sexual transmission of Zika virus. *Emerg Infect Dis* 2015; **21**(2): 2013-2015.

[16] Shinohara K, Kutsuna S, Takasaki T, Moi ML, Ikeda M, Kotaki A, et al. Brief communication Zika fever imported from Thailand to Japan, and diagnosed by PCR in the urines. *J Travel Med* 2016; **23**(1). pii: tav011.

[17] Barzon L, Pacenti M, Berto A, Sinigaglia A, Franchin E, Lavezzo E, et al. Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveler returning from the Dominican Republic to Italy. *Euro Surveil* 2016; **21**(10): 30159.

[18] Barr KL, Anderson BD, Prakoso D, Long MT. Working with Zika and Usutu viruses in vitro. *PLoS Negl Trop Dis* 2016; **10**(8): e0004931.

[19] Bradford A. The environment and disease: association or causation? *Proc R Soc Med* 1965; **58**(1): 295-300.

[20] Onorati M, Li Z, Liu F, Sousa AM, Nakagawa N, Li M, et al. Zika virus disrupts phospho-TBK1 localization and mitosis in human neuroepithelial stem cells and radial glia. *Cell Rep* 2016; **16**(10): 2576-2592.

[21] Wu KY, Zuo GL, Li XF, Ye Q, Deng YQ, Huang XY, et al. Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. *Cell Res* 2016; **26**(6): 645-654.

[22] Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, et al. Zika virus associated with microcephaly. *N Engl J Med* 2016; **374**(10): 951-958.

[23] Abu-Raya B, Smolen KK, Willems F, Kollmann TR, Gray CM. Transfer of maternal antimicrobial immunity to HIV-exposed uninfected newborns. *Front Immunol* 2016; **7**: 338.

[24] Stach SC1, Brizot ML, Liao AW, Palmeira P, Francisco RP, Carneiro-Sampaio MM, et al. Placental transfer of IgG antibodies specific to Klebsiella and Pseudomonas LPS and to Group B Streptococcus in twin pregnancies. *Scand J Immunol* 2015; **81**(2): 135-141.

[25] Ishida Y, Zhao D, Ohkuchi A, Kuwata T, Yoshitake H, Yuge K, et al. Maternal peripheral blood natural killer cells incorporate placenta-associated microRNAs during pregnancy. *Int J Mol Med* 2015; **35**(6): 1511-1524.

[26] Le Doare K1, Taylor S, Allen L, Gorringe A, Heath PT, Kampmann B, et al. Placental transfer of anti-group B Streptococcus immunoglobulin G antibody subclasses from HIV-infected and uninfected women to their uninfected infants. *AIDS* 2016; **30**(3): 471-475.

[27] Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci Rep* 2016; **6**: 23129.

[28] Macchiaverni P, Ynoue LH, Arslanian C, Verhasselt V. Early exposure to respiratory allergens by placental transfer and breastfeeding. *PLoS One* 2015; **10**(9): e0139064.

[29] Ewing G, Tatarchuk Y, Appleby D, Kim D. Placental transfer of antidepressant medications: implications for postnatal adaptation syndrome. *Clin Pharmacokinet* 2016; **54**(4): 359-370.

[30] Torres-Mendoza BM, Coronado-Medina DE, Gómez-Meda BC, Vázquez-Valls E, Zamora-Perez AL, Lemus-Varela Mde L, et al. Micronucleated erythrocytes in newborn rats exposed to raltegravir placental transfer. *Biomed Res Int* 2014; **2014**: 851820.

[31] Li J, Zhang Y, Li D, Liu Y, Chu D, Jiang X, et al. Small non-coding RNAs transfer through mammalian placenta and directly regulate fetal gene expression. *Protein Cell* 2015; **6**(6): 391-396.

[32] Culina S, Gupta N, Boisgard R, Afonso G, Gagnerault MC, Dimitrov J, et al. Materno-fetal transfer of preproinsulin through the neonatal Fc receptor prevents autoimmune diabetes. *Diabetes* 2015; **64**(10): 3532-3542.

[33] Cleal JK, Day PE, Simner CL, Barton SJ, Mahon PA, Inskip HM, et al. Placental amino acid transport may be regulated by maternal vitamin D and vitamin D-binding protein: results from the Southampton Women's Survey. *Br J Nutr* 2015; **113**(12): 1903-1910.

[34] Holder B, Jones T, Sancho Shimizu V, Rice TF, Donaldson B, Bouqueau M, et al. Inflammatory cytokines: a novel mode of maternal-placental messaging. *Traffic* 2015; **17**(2): 168-178.

[35] Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. *Philos Trans R Soc Lond B Biol Sci* 2015; **370**(1663): 20140066.

[36] Sferruzzi-Perri AN, Camm EJ. The programming power of the placenta. *Front Physiol* 2016; **7**: 33.
[37] Hayward CE, Lean S, Sibley CP, Jones RL, Wareing M, Greenwood SL, et al. Placental adaptation: what can we learn from birthweight: placental weight ratio? *Front Physiol* 2016; **7**: 28.
[38] Zhang S, Regnault TR, Barker PL, Botting KJ, McMillen IC, McMillan CM, et al. Placental adaptations in growth restriction. *Nutrients* 2015; **7**(1): 360-389.
[39] Shafiee A, Fisk NM, Hutmacher DW, Khosrotehrani K, Patel J. Fetal endothelial and mesenchymal progenitors from the human term placenta: potency and clinical potential. *Stem Cells Transl Med* 2015; **4**(5): 419-423.
[40] Müller JA, Harms M, Schubert A, Jansen S, Michel D, Mertens T, et al. Inactivation and environmental stability of Zika virus. *Emerg Infect Dis* 2016; **22**(9): 1685-1687.
[41] Goo L, Dowd KA, Smith ARY, Pelc RS, Demaso CR, Pierson TC. Zika virus is not uniquely stable at physiological temperatures compared to other Flaviviruses. *MBio* 2016; **7**(5). pii: e01396–16.
[42] World Health Organization. [Online] Available from: www.who.int [Accessed on 29th April, 2017]
[43] Nguyen T Van, Pham VH, Abe K. Pathogenesis of congenital rubella virus infection in human fetuses: viral infection in the ciliary body could play an important role in cataractogenesis. *EBioMedicine* 2014; **2**(1): 59-63.
[44] Geyer H, Bauer M, Neumann J, Lüdde A, Rennert P, Friedrich N, et al. Gene expression profiling of rubella virus infected primary endothelial cells of fetal and adult origin. *Virol J* 2016; **13**: 21.
[45] Katz BZ. Zika Virus is more like rubella than human immunodeficiency virus. *JAMA Pediatr* 2016; **170**(11): 1115-1116.
[46] Hanners NW, Eitson JL, Usui N, Richardson RB, Wexler EM, Konopka G, et al. Western Zika virus in human fetal neural progenitors persists long term with partial cytopathic and limited immunogenic effects. *Cell Rep* 2016; **15**(11): 2315-2322.