ZIKA VIRUS INFECTION: CURRENT CONCERNS AND PERSPECTIVES

Mari Kannan Maharajan*, Aruna A/P Ranjan, Chu Jian Feng, Foo Wei Lim,

Chai Zhi Xin, Eileen Lau Yin Yien, Ye Heuy Mien, Theam Xi Jin, Lok Yen Ling

Department of Pharmacy Practice,

School of Pharmacy, International Medical University,

Kuala Lumpur, 57000, Malaysia.

*Corresponding Author:

Mari Kannan Maharajan,

Department of Pharmacy Practice, School of Pharmacy, International Medical University,

Kuala Lumpur, 57000, Malaysia.

Email: kanpharm2002@gmail.com; Marikannan@imu.edu.my

Contact: +60 1127634171

CLINICAL DIAGNOSIS OF ZIKA VIRUS INFECTION AND ITS POTENTIAL MOLECULAR TARGETS

Abstract

The Zika virus outbreaks highlight the growing importance need for a reliable, specific and rapid diagnostic device to detect Zika virus, as it is often recognized as a mild disease without being identified. Many Zika virus infection cases have been misdiagnosed or underreported because of its non-specific clinical presentation. The aim of this review was to provide a critical and comprehensive overview of the published peer-reviewed evidence related to clinical presentations, various diagnostic methods, modes of transmission of Zika virus infection and potential therapeutic targets to combat microcephaly. Zika virus is mainly transmitted through bites from *Aedes Aegypti* mosquito. It can also be transmitted through blood, perinatally and sexually. Pregnant women are advised to postpone or avoid travelling to areas where Zika virus transmission is ongoing, as this infection is directly linked to fetal microcephaly. Due to the high prevalence of Guillain-Barre syndrome and microcephaly in the endemic area, it is vital to confirm the diagnosis of Zika virus and start the treatment as soon as possible to prevent further complication. Zika virus infection should be considered as a public health emergency and of international concern. Governments and agencies should play an important role in terms of investing time and resources to fundamentally understand this infection, so that a vaccine can be developed besides raising awareness.

CLINICAL DIAGNOSIS OF ZIKA VIRUS INFECTION AND ITS POTENTIAL MOLECULAR TARGETS

Introduction

Recent international media attention has focused on microcephaly, which is a potential neurological and immunological complication. Clinical and epidemiological reports showed that there is a link between microcephaly and of Zika virus (ZIKV). ZIKV, a surfacing mosquito-borne disease transmissible from human to human through biting of Aedes species. The genome of the ZIKV, of Flaviveridae family and the Flavivirus genus [1] is a single positive-strand RNA of 10794 bases that it is related closely with Spondweni virus [2, 3]. ZIKV was initially distinguished in rhesus monkeys in the Zika forest of Uganda throughout the period of a yellow fever study [3].

Despite the first isolation, six decades ago, little attention has been given to ZIKV compared with the other mosquito-borne flaviviruses. Only 14 cases of human ZIKV infection had been recognized globally and no outbreak had been reported until 2007, when Zika spread further to the Pacific island of Yap [4, 5]. This was the first outbreak reported beyond the confines of Africa and Asia [4, 6]. The second and largest outbreak occurred in French Polynesia [7, 8]. With 383 confirmed cases, it is estimated that ZIKV disease may been the reason why approximately 32000 patients sought medical care between October 2013 and April 2014 [9]. It has only come to international attention recently after the recent outbreak on Yap Island in 2007 and the current epidemic on Brazil in 2015 [10]. Indigenous dissemination of ZIKV has been detected as ZIKV disease has been confirmed in 18 states of Brazil [9]. The geographical distribution of ZIKV has steadily broadened given the wide distribution of the mosquito vector.

This has negatively impacted the safety of other regions, putting them at greater risk, as there is a greater connectivity between the continents as a result of millions of international travellers across the globe. Between 2007 and 2016, 48 countries and territories reported ZIKV transmission. Upon scrutinizing the cases reported worldwide, the Central and South American countries could be the most burdened by ZIKV outbreak, because the estimated number of cases reported was around 440000 to 1300000, which is much higher than that in other countries [11, 12]. The prospect of ZIKV spreading across regions and continents along with can be anticipated from the recent outbreaks [13]. Furthermore, Aedes mosquitoes are abundant in tropical and subtropical areas throughout the world.

Clinical Presentation

The manifestation of human ZIKV infection ranges from asymptomatic to influenza-like symptoms. Clinical manifestations of infection with ZIKV included fever, headache, retro-orbital pain, arthralgia, asthenia,

malaise, myalgia, anorexia, rash, edema, lymphadenopathy, and diarrhoea; in most cases, the infection appeared mild and self-limiting, with a mean symptomatic duration of 3-6 days [14, 15]. Conjunctivitis is commonly present, whereas arthralgia, headache and malaise are pronounced to a lesser degree. Shock complications and haemorrhagic signs have not been reported. However Guillain-Barre syndrome, a neurologic complication was reported in French Polynesia. Laboratory tests revealed transient leukopenia and thrombocytopenia. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations may or may not be raised [10]. In 2008, Foy *et al*., reported two unusual cases of ZIKV infection presenting with aphthous ulcers, prostatitis and hematospermia which are not common [16]. ZIKV infection has been regarded as benign in most cases until the Brazil Ministry of Health considered a link between the ZIKV infection and microcephaly, with the discovery of the presence of virus in the amniotic fluid of two pregnant women in 2015 whose foetuses had microcephaly. In the Paraiba state of Brazil, six ZIKV-infected children, who were born to ZIKV-infected mothers during pregnancy were found to have neonatal head circumferences (HC) below the 10th percentile [17].

Diagnostic Methods

Serology testing

A serological testing (ELISA/Immunofluorescent) has been used to test the specific anti-Zika immunoglobulin, lgM. However, this method is limited and unspecific as cross-reactions could occur with other arbovirus within the Flavivirus genus [18]. Earlier studies using this technique did not find many differences of antigenic properties to differentiate ZIKV and other flaviviruses (e.g. DENV, Uganda S, Banzi and Potiskum). Recent studies showed there are differences comparing the antigenic properties of ZIKV, (Uganda and Banzi) with yellow fever (Potiskum and Wesselsbron [4]. This could be caused by restricted immune response [19]. Hence, a false positive result could be obtained in this serological test.

Furthermore, the concentrations of lgM and lgG are very low during the early phase of infection [4, 19]. The virus-specific lgM antibodies normally can be detected within 3 days of the onset of infection [19, 20] but sometimes can only be detected after 8 days. Hence, it is vital to collect serum from both acute and convalescent phases to confirm the diagnosis of ZIKV infection [19]. A Plaque Reduction Neutralization Test (PRNT) helps

to differentiate the cross-reacting antibodies in infection with the primary flavivirus [21]. Lanciotti *et al.,* showed that PRNT can improve specificity over immunoassays, although cross-reactive results are still found in secondary flavivirus infections [21]. Drawbacks to serological testing are that it is time-consuming, shows cross-reactivity and is non-specific.

Reverse transcriptase polymerase chain reaction (RT-PCR)

One-step reverse transcriptase polymerase chain reaction (RT-PCR) allows qualitative detection of ZV RNA. RT-PCR is a molecular technology that targets envelope protein-coding region in the early stage of infection [14, 22]. During the first 7 days of the illness, where the acute phase begins with the onset of symptoms, viral RNA is frequently identified in serum hence RT-PCR is the favoured test at this stage to detect ZIKV [20].

Saliva and urine can also be used instead of serum as a sample for routine ZIKV RNA detection through RT-PCR [22, 23]. Using saliva increased the ability of molecular detection in the early stages of disease [22] while urine can be used at later stages (>10 days after onset of disease) because there was a higher virus load with an extended period in urine samples relative to serum samples [23]. However, saliva and urine samples cannot replace blood samples and should be considered only when blood collection cannot be performed, especially in children or neonates [22]. Saliva and urine samples are best used together with serum sample to increase the sensitivity of the molecular detection of ZIKV through RT-PCR.

The detection limit to identify ZIKV is low, varying from 103 pfu/ml to 106 pfu/ml in natural human infection [14], demonstrating the high sensitivity of RT-PCR in detecting ZIKV. This detection limit also rules out the need of modification of PCR procedure to reduce non-specific binding due to the amplification of unexpected primer binding sites [14]. In addition, the assay's analytical specificity is assessed utilizing RNA ZIKV isolates and isolates from other related flavivirus. The amplicons of anticipated sequences and sizes are observed only in ZIKV sample, indicating no cross-sensitivity hence verifying the high specificity of this assay. High sensitivity of RT-PCR makes it clinically helpful by allowing its use for differential diagnosis to distinguish ZIKV from additional clinically related arbovirus infections, including Chikungunya and dengue viruses in endemic areas and areas where they are co-circulating, since the clinical presentation of ZIKV is not

very definite [4]. Furthermore, the assay has great repeatability (100%) in human serum also demonstrated the robustness of RT-PCR [14]. Due to the several advantages of RT-PCR, this assay is now widely used for ZIKV detection.

Cytokine profiling

Cytokines profiling is also another important tool to diagnose Zika Fever (ZF). During the acute phase of ZF, a polyfunctional immune response is activated, as reflected by the increased cytokine levels associated with Th1, Th2, Th9 and Th17 [10].

Table 1 shows the types of cytokine increase during acute and reconvalescent phases of ZF, as well as cytokines with no significant changes in both phases. The elevation of chemokines such as RANTES, MIP-1α, and VEGF was more pronounced than the elevation of inflammatory cytokines in ZF patients [10]. Moreover, most cytokines (other than IL-1β, IL-8, IL-10, IP-10, MIP-1β, and GM-CSF) will generally decrease in the reconvalescent phase compared to acute phase. The level of cytokines are important to distinguish dengue fever (DF) from ZF during the acute and reconvalescent phases. The differences between ZF and DF in term of symptoms associated with level of certain kinds of cytokines are listed in **Table 2** [10, 24-26].

Misdiagnosis with Dengue/ Chikungunya

Many ZIKV infection cases have been misdiagnosed or underreported because its non-specific clinical presentation is easily confused with that of other arboviruses, particularly chikungunya and dengue infection [27, 28]. In addition, serologic cross-reactivity of ZIKV antibodies with dengue virus (DENV) also contributes to the misdiagnosis [21]. According to Lanciotti *et al.,* IgM assay for DENVs showed a great extent of cross-reactivity in patients with ZIKV infections, which could lead to the wrong conclusion of dengue being the cause of the infection [29]. A case report by Kwong *et al*., showed that a ZIKV-infected patient was provisionally given a diagnosis of dengue fever due to the dengue serologic analysis showing a positive result for IgM as well as IgG [28]. Commercial tests for ZIKV infection have not yet been developed, and ZIKV-specific tests are not yet widely available, making it a challenge to properly diagnose ZIKV infection [30]. Therefore, expansion of

ZIKV emphasizes the need to develop a reliable, sensitive and specific tool to detect the virus accurately [27]. Plans to provide treatment in affected patients can be accelerated with the rapid detection of the virus in specimens. However, preventing the transmission among human populations will require appropriate control measures.

Modes of Transmission of ZIKV

ZIKV is mainly transmitted through bites from *Aedes Aegypti* [31, 32] mosquito. Additionally, transmission can be perinatal, as well as by close physical contact between the newborn babies and their mothers [33, 34]. Recent reports suggested that ZIKV can be transmitted sexually by males. The possibility of infection with ZIKV through sexual transmission even after recovery was suggested by the high RNA load of ZIKV and its replicable virus in both semen and urine of a man presenting with hematospermia [32]. However, transmission of ZIKV from an infected woman to her sexual partner has not been reported [32, 33, 35]. In particular, vertical transmission of ZIKV needs to be concretely demonstrated as well as any direct or indirect effects of infection of ZIKV.

Target Cells for ZIKV Tropism

Transmission of ZIKV is majorly by the infected vector, *Aedes aegypti mosquito*. The vector inoculates ZIKV into human by allowing the deposition of ZIKV in the epidermis and dermis layers of the skin [36, 37]. Epidermal keratinocytes and skin fibroblasts are highly susceptible to ZIKV [36]. The infected skin fibroblasts show rapid and active viral replication. ZIKV induces autophagy, an intracellular process by which cytoplasmic constituents are recruited in double-membrane vesicles and afterwards fuse with lysosomes for protein degradation in experimentally-infected skin fibroblasts [36, 38]. This suggests that ZIKV could hijack the autophagy pathway and utilize the components of this biological pathway for replication; this is true for several flavivirus [36, 38, 39]. In addition, ZIKV-infected epidermal keratinocytes were observed to undergo apoptosis, which is also similarly found in dengue-infected human skin explants [36]. It is postulated that ZIKV deters anti-viral immune responses by triggering apoptotic cell death of infected cells, resembling DENV's mechanism, thereby enhancing viral dissemination from dying cells. Another promising target of ZIKV is

dendritic cells [36]. This may be due to flavivirus' replication involving skin antigen-presenting cells, of which dendritic cells are an example [36, 40].

ZIKV was shown to be highly neurotropic in intraperitoneally inoculated mice [41]. Brains of infected mice showed neuronal degeneration, cellular infiltration, and softening in certain areas. This suggested that ZIKV has the potential to cross the blood brain barrier, albeit through unknown mechanism(s). Bell *et al.,* reported that neural cells are involved in the infection of ZIKV [42]. Coinciding with the ZIKV outbreak, an increased number of new-borns with microcephaly were observed in several regions of Brazil [43, 44]. This is also coupled with the fact that mothers and amniotic fluid samples from foetuses revealed positive results for Zika viral RNA. Thus, the virus could have the potential to trigger neurodevelopmental dysfunction, as well as microcephaly [31]. More studies are needed to help provide a platform to determine whether the virus is involved (directly or indirectly) in microcephaly.

Potential targets for therapeutic antiviral strategies

In order to enter the host cell, ZIKV's viral envelope protein needs to interact with various receptors on the cell surface and attachment factors, of all which will determine the viral cellular tropism. Among the abundant surface receptors available, C-type lectin receptors (CLR), transmembrane immunoglobulin and mucin domain proteins (TIM proteins), and TAM proteins (standing for Tyro3, AXL and Mer) are important to facilitate the entry [36]

Dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN, CD209), categorized under CLRs, offers a significant link between viral replication and host infection. DC-SIGN is constitutively expressed in dendritic cells that may reside in the skin, the site of initial infection [37]. Being an essential host factor, it could be exploited by ZIKV to penetrate and infect the immature dendritic cells [36, 37].

Hamel *et al*., reported that TAM proteins (namely AXL and Tyro3) mediate ZIKV entry into the host cell [36]. As cutaneous fibroblasts and epidermal keratinocytes express AXL, this further supports the susceptibility of these cells to ZIKV infection. Astrocytes and microglial cells are known to have AXL and Tyro3 expression [45]. Therefore, astrocytes and microglial cell could be targets for ZIKV infection. These cells can be found within the spinal cord and most importantly, the brain. With the possible capability of ZIKV

crossing the blood-brain barrier, it could target astrocytes and microglia within the brain and trigger the infection there. Hence, this could be a possible pathway necessary for microcephaly.

TIM-1 (a TIM protein) contributes the least to ZIKV infection when compared to DC-SIGN and TAM proteins [36]. Its expression, nonetheless, increases the efficacy of AXL-mediated viral entry. With the potential function as an attachment factor, TIM-1 could bind to viral particles and concentrate ZIKV on the cell surface [36]. TIM-1 could then transfer the viral particles to AXL, facilitating ZIKV interaction with AXL. Since ZIKV's entry into target cells is the main key factor of viral pathogenesis and cellular tropism, it becomes the potential target in preventive measures and therapeutic antiviral therapy.

Management and Prevention

The mainstays of management include supportive care and bed rest [30], along with advice to prevent dehydration. Treatments providing symptomatic relief include combining paracetamol and antihistamines for pruritic rash [7, 11]. ZIKV infection shares similar clinical features with dengue. Before dengue is ruled out, non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided, as they are contraindicated in dengue fever, where they may produce an increased risk of haemorrhagic syndrome [7]. In addition, specific viral diagnosis is essential in managing and preventing complications, especially when there is co-circulating of multiple arboviruses [30].

Vaccines availability

To date, there are no vaccines or antiviral therapy available for ZIKV [30, 46]. However, through recent computational approach, there is a good candidate to be considered for vaccine development, namely Zika viral envelope glycoproteins which are the most immunogenic [47]. Nonetheless, it is predicted that Zika vaccines, if available, would face problems similar to chikungunya vaccines. For instance, the vaccine would be prohibitively expensive, and deployment of vaccines following any rapid outbreak may be too slow to counter the explosive epidemic [30]. This is mostly because epidemics usually appear unexpectedly and sporadically [30].

Destruction of mosquito-bite breeding sites

Air-conditioning, house screens and minimizing mosquitoes-breeding sites are considered as the best available preventive measures against ZIKV [30, 48] Aquatic environments, for instance open water storage containers and small puddles are known as their common breeding sites [5]. In order to destroy the breeding sites, larvicides can be used in natural and peridomestic breeding sites. If larvicides fail, the density of adult mosquitoes can be reduced through space spraying with pyrethroids [49]. Deltamethrin is currently the only satisfactorily effective pyrethroids insecticides [11, 14].

Reduction of urban mosquito-human transmission

In order to reduce disease burden, reductions in urban mosquito-human transmission seem to be the only option [50]. Daytime avoidance of mosquito bites is crucial as Aedes mosquitoes, being the most active near dawn and dusk, are adapted for daytime biting in urban areas [5, 48]. Since 80% of infected individuals are asymptomatic, mosquito-bites avoidance such as personal protection helps to disrupt human-to-mosquito-to-human transmission cycles [46]. The personal protection methods recommended for mosquito-bites avoidance include using insects repellent, mosquito bed nets and wearing light-coloured clothes with long sleeves [11, 30, 48]. In addition, insecticide such as permethrin can be sprayed on clothing to kill mosquitoes on contact [51]. There are also alternative strategies available such as reduction of human-to-vector contact with insect repellents, biologic control (e.g., bio-insecticides use), and genetic control [49, 52]. For genetic control, the genetically modified mosquitoes are either sterile or have lost the ability to transmit diseases to humans [49, 53]. However, the control methods mentioned have their weaknesses such as funding and difficulties their application over a sufficiently wide area [53]. Community-level vector surveillance and disease surveillance enhanced earlier case detection and thus appropriate control measures can be implemented [46].

Travel precautions

Travellers are advised to take extra precautions when travelling to the areas with active ZIKV transmission. The precautions suggested include using insect repellent and sleeping under a mosquito net. The most recommended insect repellents are those containing N,N-diethyl meta toluamide (DEET) as it is safe to be used, even in pregnant and breastfeeding women [51]. As described earlier, there is an association between ZIKV and microcephaly, some countries such as United Kingdom and the United States have issued advice that pregnant women should postpone or avoid traveling to areas where ZIKV transmission is ongoing.[46, 51] If they must travel to the endemic area, they should be assessed and monitored appropriately during antenatal visits [9]. After coming back from the endemic areas, women should avoid getting pregnant for a further 28 days which allows a maximum two weeks of ZIKV incubation period. Moreover, there it is possible that ZIKV can be transmitted sexually. Thus, effective contraception for at least 28 days should be taken if a woman's partner has recently travelled to the endemic areas [51].

Avoidance of transfusion-transmitted ZIKV

Prevention of transfusion-transmitted ZIKV includes nucleic acid testing (NAT) of blood donors (albeit not routinely available) and pathogen inactivation, of which the latter abolishes pathogens infectivity in blood products [53]. NAT reduces risk of transfusion-transmitted infections by providing an additional layer of blood safety through screening blood donations. It detects the viral nucleic acids earlier and specifically by amplifying the targeted region of viral ribonucleic acid or deoxynucleic acid (DNA) [54].

Ultimately, more research is urgently needed on ZIKV including vaccination to control arbovirus spread. In managing this arbovirus pandemics, it is suggested that broad-spectrum antiviral drug which is effective in covering whole class of virus is also required with respect to treatment [30].

Roles of healthcare professionals

Healthcare professionals (especially clinicians) have a significant role in the early diagnosis of ZIKV infection and they are encouraged in notifying the suspected cases cases to public health authorities [18]. If done in a timely fashion, this will facilitate the mitigation of local transmission in regions where *Aedes* mosquitoes

are found [55]. Rapid dissemination of key information is crucial in terms of raising awareness to the public on ZIKV prevention. In light of this, healthcare professionals should get involved in public education, emphasizing the importance of preventing mosquito bites in at-risk individuals, especially pregnant women [18]. It is of utmost importance for healthcare professionals to be alert about the evolution of ZIKV outbreaks, especially those in popular tourist destinations, so that clinicians would include ZIKV in their differential diagnosis [9, 18]. Moreover, healthcare professionals would be able to consult travellers who are planning to visit. endemic countries on effective preventive measures [18, 56]. During treatment, it is important rule out other infectious diseases such as dengue.

Conclusion

The peer reviewed literature demonstrates that the growing importance of developing a reliable, specific and rapid diagnostic device to detect ZIKV, as it is often recognized as a mild disease without being identified. In the absence of ZIKV vaccine, other control measures are especially important to be implemented, predominantly by combating vector mosquito and preventing mosquito bites. For a vector control program to be effective, it must be based on extensively-analysed entomological and ecologic surveillance data to understand the time and viral emergence location. Zika's danger seems confined to pregnant women, as it shows a close association with the startling rise in microcephaly, a severe birth defect. This critical fact of ZIKV infection as a public health emergency emphasizes the urgent need of global attention and concern. Lessons learned from the Ebola epidemic concerning the importance of rapid key information dissemination serve as a reminder to stress the need for an urgent global effort to galvanize resources in understanding more about the disease.

References

[1] Zanluca C, de Melo VC, Mosimann AL, Dos Santos GI, Dos Santos CN, Luz K (2015). First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz 110:569-572.

[2] Kuno G, Chang GJ (2007). Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. Arch Virol 152:687-696.

[3] Campos GS, Bandeira AC, Sardi SI (2015). Zika Virus Outbreak, Bahia, Brazil. Emerg Infect Dis 21:1885-1886.

[4] Shapshak P, Sinnott JT, Somboonwit C, Kuhn J (2015). Global virology I -Identifying and Investigating Viral Disease. Springer New York.

[5] Rodriguez-Morales AJ (2015). Zika: the new arbovirus threat for Latin America. J Infect Dev Ctries 9:684-685.

[6] Hayer EB (2009). Zika virus outside Africa 15:1347-1350.

[7] Musso D, Nhan TX (2015) Emergence of Zika Virus. Clin Microbiol 4:222. doi:10.4172/2327-5073.1000222

[8] Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fasce R, et al (2014). A report on the outbreak of Zika virus on Easter Island, South Pacific. Arch Virol 161:665-668.

[9] European Centre for Disease Prevention and Control (2016). Rapid Risk Assessment. Zika virus disease epidemic: potential association with microcephaly and Guillain–Barré syndrome. Second update. Stockholm.http://ecdc.europa.eu/en/publications/ Publications/zika-virus-americas-association-with-microcephaly-rapid-risk-assessment.pdf 12/10/15 Accessed February 25, 2016.

[10] Tappe D, Perez-Giron JV, Zammarchi L, Rissland J, Ferreira DF, Jaenisch T, et al (2015). Cytokine kinetics of Zika virus-infected patients from acute to reconvalescent phase. Med Microbiol Immunol doi:10.1007/s00430-015-0445-7

[11] Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M (2014). Current Zika virus epidemiology and recent epidemics. Med Mal Infect 44:302-307.

[12] The Lancet, Zika virus: a new global threat for 2016. http://dx.doi.org/10.1016/S0140-6736(16)00014-3

[13] Kindhauser MK, Allen T, Frank V, Santhana RS, Dye C (2016). Zika: the origin and spread of a mosquito-borne virus. Bull. World Health Organ. http://www.who.int/bulletin/online_first/16-171082.pdf.

[14] Faye O, Faye O, Dupressoir A, Weidmann M, Ndiaye M, Alpha Sall A (2008). One-step RT-PCR for detection of Zika virus. J Clin Virol 43:96-101.

[15] Kutsuna S, Kato Y, Takasaki T, Moi M, Kotaki A, Uemura H, et al (2014). Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014. Euro Surveill 19:20683.

[16] Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al. (2011) Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis 17:880-882.

[17] Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM (2016). Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?. Ultrasound Obstet Gynecol 47:6-7.

[18] Zammarchi L, Stella G, Mantella A, Bartolozzi D, Tappe D, Gunther S, et al (2015). Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications. J Clin Virol 63:32-35.

[19] Hayes EB (2009). Zika virus outside Africa. Emerg Infect Dis 15:1347-1350.

[20] Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories. 2016. http://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf.

[21] Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al (2008). Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 14:1232-1239.

[22] Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM (2015). Detection of Zika virus in saliva. J Clin Virol 68:53-55.

[23] Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M (2015). Detection of Zika virus in urine. Emerg Infect Dis 21:84-86.

[24] Guabiraba R, Ryffel B (2014). Dengue virus infection: current concepts in immune mechanisms and lessons from murine models. Immunology 141:143-156.

[25] Mathew A, Rothman AL. Understanding the contribution of cellular immunity to dengue disease pathogenesis (2008). Immunol Rev 225:300-313.

[26] Rathakrishnan A, Wang SM, Hu Y, Khan AM, Ponnampalavanar S, Lum LC, et al (2012). Cytokine expression profile of dengue patients at different phases of illness. PLoS One 7:e52215.

[27] Faye O, Faye O, Diallo D, Diallo M, Weidmann M, Sall AA (2013). Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. Virol J 10:311 doi: 10.1186/1743-422X-10-311.

[28] Kwong JC, Druce JD, Leder K (2013). Zika virus infection acquired during brief travel to Indonesia. Am J Trop Med Hyg 89:516-517.

[29] Johnson AJ, Noga AJ, Kosoy O, Lanciotti RS, Johnson AA, Biggerstaff BJ (2005). Duplex microsphere-based immunoassay for detection of anti-West Nile virus and anti-St. Louis encephalitis virus immunoglobulin m antibodies. Clin Diagn Lab Immunol 12:566-574.

[30] Fauci AS, Morens DM (2016). Zika Virus in the Americas--Yet Another Arbovirus Threat. N Engl J Med 374:601-604.

[31] Tetro JA (2016). Zika and microcephaly: causation, correlation, or coincidence? Microbes Infect 18(3):167-168.

[32] Oster AM, Brooks JT, Stryker JE, Kachur RE, Mead P, Pesik NT, et al (2016). Interim Guidelines for Prevention of Sexual Transmission of Zika Virus - United States 2016. MMWR Morb Mortal Wkly Rep 65:120-121.

[33] Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D (2014). Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill 19:20751.

[34] Triunfol M (2016). A new mosquito-borne threat to pregnant women in Brazil. Lancet Infect Dis 16:156-157.

[35] Goorhuis A, von Eije KJ, Douma RA, Rijnberg N, van Vugt M, Stijnis C, et al (2016). Zika virus and the risk of imported infection in returned travelers: Implications for clinical care. Travel Med Infect Dis 14:13-15.

[36] Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, Luplertlop N, et al (2015). Biology of Zika Virus Infection in Human Skin Cells. J Virol 89:8880-8896.

[37] Fernandez-Garcia MD, Mazzon M, Jacobs M, Amara A (2009). Pathogenesis of flavivirus infections: using and abusing the host cell. Cell Host Microbe 5:318-328.

[38] Blazquez AB, Escribano-Romero E, Merino-Ramos T, Saiz JC, Martin-Acebes MA (2014). Stress responses in flavivirus-infected cells: activation of unfolded protein response and autophagy. Front Microbiol 5:266.

[39] McLean JE, Wudzinska A, Datan E, Quaglino D, Zakeri Z (2011). Flavivirus NS4A-induced autophagy protects cells against death and enhances virus replication. J Biol Chem 286:22147-22159.

[40] Schmid MA, Diamond MS, Harris E (2014). Dendritic cells in dengue virus infection: targets of virus replication and mediators of immunity. Front Immunol 5:647.

[41] DICK GW (1952). Zika virus. II. Pathogenicity and physical properties. Trans R Soc Trop Med Hyg 46:521-534.

[42] Bell TM, Field EJ, Narang HK (1971). Zika virus infection of the central nervous system of mice. Arch Gesamte Virusforsch 35:183-193.

[43] Zika Virus. 2016; http://www.who.int/mediacentre/factsheets/zika/en/. Accessed February 25, 2016.

[44] Martines RB, Bhatnagar J, Keating MK, et al (2015). Notes from the Field: Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses- Brazil, 2015 65:159-160.

[45] Ji R, Tian S, Lu HJ, Lu Q, Zheng Y, Wang X, et al (2013). TAM receptors affect adult brain neurogenesis by negative regulation of microglial cell activation. J Immunol 191:6165-6177.

[46] Bogoch II, Brady OJ, Kraemer MU, German M, Creatore MI, Kulkarni MA, et al (2016). Anticipating the international spread of Zika virus from Brazil. Lancet 387:335-336.

[47] Shawan, MMAK, Hossain MM, Hasan MA, Hasan MM, et al (2015). Design and Prediction of Potential RNAi (siRNA) Molecules for 3' UTR PTGS of Different Strains of Zika Virus: A Computational Approach 13:37-50.

[48] Kelser EA C (2015). Meet dengue's cousin, Zika. Microbes Infect 18:163-166.

[49] Ngoagouni C, Kamgang B, Nakoune E, Paupy C, Kazanji M (2015). Invasion of Aedes albopictus (Diptera: Culicidae) into central Africa: what consequences for emerging diseases? Parasit Vectors 8:191.

[50] Weaver SC (2013). Urbanization and geographic expansion of zoonotic arboviral diseases: mechanisms and potential strategies for prevention 21:360-363.

[51] Zika Virus Infection and Pregnancy Information for Healthcare Professionals. Interim RCOG/RCM/PHE/HPS clinical guidelines. 2016; Available at: https://www.rcog.org.uk/globalassets/documents/news/zika-virus-interim-guidelines.pdf. Accessed March 4, 2016.

[52] Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D (2009). Aedes albopictus, an arbovirus vector: from the darkness to the light. Microbes Infect 11:1177-1185.

[53] Aubry M, Richard V, Green J, Broult J, Musso D (2016). Inactivation of Zika virus in plasma with amotosalen and ultraviolet A illumination. Transfusion 56:33-40.

[54] Hans R, Marwaha N (2014). Nucleic acid testing-benefits and constraints. Asian J Transfus Sci 8:2-3.

[55] Malone RW, Homan J, Callahan MV, Glasspool-Malone J, Damodaran L, et al. (2016). Zika Virus: Medical Countermeasure Development Challenges. PLoS Negl Trop Dis 10: e0004530.

[56] Derraik JG, Slaney D (2015). Notes on Zika virus--an emerging pathogen now present in the South Pacific. Aust N Z J Public Health 39:5-7.