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An Update on the Transmission, Pathogenesis, Diagnosis, Treatment and Prevention of Zika Virus Infection

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Abstract

Zika virus is an arthropod-borne flavivirus, related to other flaviviruses such as dengue virus, yellow fever virus, and West Nile virus. Though Zika virus was first isolated in 1947, virus remained in relative obscurity for nearly 70 years. The epidemiology of Zika virus changed since 2007 when an outbreak occurred on Yap Island of the Federated States of Micronesia. Then, Zika virus was introduced into Brazil from the Pacific Islands and spread rapidly throughout the Americas. Zika virus has infected over a million people in the countries of South and Central America. Zika virus infection generally leads to self limiting mild, febrile illness. However, many of the recent outbreaks were linked to upsurge in cases of Guillan Barré syndrome and a rise in infants born with microcephaly. Because of these complications and rapid spread of the Zika virus infections, the world health organization declared Zika fever as a public health emergency of international concern. This review describes the current understanding about the transmission, pathogenesis, clinical features, and diagnosis of Zika virus infection.

Keywords: *Zika virus; pathogenesis; microcephaly; Guillan Barré syndrome*

1. Introduction

Arboviruses are an important group of viruses of medical relevance due to the wide range of illnesses they cause. Zika virus is an arbovirus, related to yellow fever (YF), dengue, West Nile, and Japanese encephalitis viruses, and most closely to Spondweni virus. It is an envelope, icosahedral positive strand RNA virus belongs to the genus flavivirus and replicate mostly in intracellular compartments associated to endoplasmic reticulum and golgi complex. Zika virus is was first isolated from a Rhesus macaque obtained from the Zika forest of Uganda during 1947[1]. After its initial discovery in 1947, it was isolated on several occasions from *Aedes africanus* mosquitoes[2]. For many years, it was not known whether the virus can cause human disease. A serosurvey involving residents of multiple areas of Uganda revealed that the antibodies against Zika virus are present in 6.1% of the tested population, suggesting the human infection with Zika virus[3]. Additional serosurveys indicated a much broader geographic distribution of human infection. The first human case was reported in Nigeria in 1954 and since then sporadic cases have been reported from different regions around the globe[4]. The epidemiology of Zika virus changed since 2007 when an outbreak occurred on Yap Island of the Federated States of Micronesia[5]. After that several Zika virus outbreaks were reported from New Caledonia, French Polynesia, the Cook Islands, Easter Island, Vanuatu, Samoa, Brazil and several countries in the Americas[6-8]. Zika virus infection generally leads to self limiting mild, febrile illness[9]. However, many of the recent outbreaks were linked to upsurge in cases of Guillan Barré syndrome (GBS) and a rise in infants born with microcephaly[10-12]. Because of these complications and rapid spread of the Zika virus infections, the world health organization declared Zika fever as a public health emergency of international concern[7]. Hence, scientific knowledge regarding the transmission, clinical features and diagnosis of Zika virus infection is necessary to implement effective prevention and control measures.

2. Transmission

2.1 Vector-borne transmission

Zika virus is transmitted to humans by mosquito bites. Two mosquito species belonging to stegomyia subgenus of aedes — *A. aegypti* and, to a lesser extent, *A. albopictus* are responsible for nearly all known Zika virus outbreaks[13]. Zika virus is maintained in the community through its sylvatic cycle, where the virus circulates between non-human primates and Aedes mosquitoes, and urban cycle. In Asia, a sylvatic transmission cycle has not yet been identified. Both *A. aegypti* and *A. albopictus* are daytime feeders and are widely distributed throughout the tropical and subtropical world. Very rarely Zika virus has been identified in other mosquito species, such as *A. unilineatus*, *Anopheles coustani*, and *Mansonia uniformis*; however, these species have a low potential for transmission of the virus. So far only one report suggested the presence of Zika virus in culex species, which suggests that mosquitoes in this genus have a low vectorial capacity[14].

2.2 Non-vector-borne transmission

Many studies now indicates that Zika virus can be transmitted from the mother to the fetus during pregnancy. Zika virus RNA has been identified in the amniotic fluid of mothers whose fetuses had cerebral anomalies as detected by ultrasonography[15-17]. Brain tissue and placentas of children who were born with microcephaly and died soon after birth, as well as in tissues from miscarriages were reported to have Zika virus antigen and RNA[17,18]. Zika virus infection is also transmitted by sex[19]. In 2013, during a Zika virus outbreak in French Polynesia, a patient sought treatment for hematospermia, and replicative Zika virus could be detected from semen samples[20]. However, the risk factors and the duration of the risk of sexual transmission have not been determined. Male population infected with Zika virus were reported to have replicating viral particles, as well as viral RNA often in high copy numbers in sperm[20,21]. So far there are no reports on the transmission of Zika virus through a blood transfusion. However, during the Zika virus outbreak in French Polynesia, viral RNA was detected in 3% of donated blood samples by reverse-transcriptase polymerase chain reaction (RT-PCR)[22]. A woman who was infected with Zika virus on the day of delivery contained high titer of infective Zika viral particles in breast milk[23]. However, there are no reports on transmission through breast milk. Serosurvey studies have detected antibodies to Zika virus in bats, goats, and rodents[24]. However, such serological data should be interpreted carefully, since there is cross reaction between flaviviruses. Hence, there is no well-documented reservoir animal for Zika virus.

3. Pathogenesis and clinical features

Probability of Zika virus transmission is related to the volume of fluid held in the insect's proboscis from a prior blood meal, volume of insect salivary glands, and viral replication levels. Once Zika virus enters into host, viral envelope protein binds to specific receptors such as DC-SIGN, AXL, Tyro3, and TIM-1 expressed on the susceptible cells. Zika virus infects different cell types including skin fibroblasts, epidermal keratinocytes, and skin dendritic cells. Immature dendritic cells appear to be an important initial Zika target. This interaction triggers transcriptional activation of Toll-like receptor 3 (TLR3), RIG-I, MDA5, interferon stimulated genes including OAS2, ISG15, and MX1, and beta interferon[25]. Similar to other flaviviruses, Zika virus infection might trigger apoptosis of infected cells, thereby evading innate immune responses and increasing initial release of infectious viral particles[26]. Zika viruses subsequently exploit autophagy to enhance replication[27]. One study reported that treating Zika virus infected cells with 3-Methyladenine (3-MA), an inhibitor of autophagosome formation, strongly reduces viral copy numbers in infected fibroblasts[25]. Many other studies using murine models have suggested that autophagy plays an important role in the pathogenesis of Zika-associated primary microcephaly[28].

Zika virus has an incubation period of 3 to 12 days[29]. Among French Polynesian blood donors who tested positive for Zika virus RNA by RT-PCR, 11 (26%) reported conjunctivitis, rash, arthralgia, or a combination of these symptoms 3 to 10 days after donation[22]. Serosurvey results from Yap island indicated that only 19% of Zika virus infected persons developed sympyoms[5]. Common clinical symptoms were maculopapular rash (90% of patients), fever (65%), arthritis or arthralgia (65%), nonpurulent conjunctivitis (55%), myalgia (48%), headache (45%), retro-orbital pain (39%), edema (19%), and vomiting (10%). No patient was hospitalized during this outbreak in Yap. Similar clinical manifestations were observed in a group of pregnant women with Zika virus infection in Brazil[32]. The rash is generally maculopapular and pruritic. Fever is generally low-grade and persists for short-term[30]. Other rare symptoms that are associated with acute Zika virus infection include hematospermia, transient dull and metallic hearing, swelling of the hands and ankles, and subcutaneous bleeding[31-33]. These clinical symptoms are usually self-limiting and may last for four to seven days[34]. Other arboviruses such as Dengue and Chikungunya also produce similar symptoms, the only difference is Zika virus-induced symptoms are milder than those of others[35]. Hence, it is difficult to diagnose Zika virus infection based on clinical symptoms alone.

4. Neurologic complications

Recently, Many reports suggested the association of Zika virus infection and an increase in cases of fetal abnormalities like microcephaly, hydranencephaly, ventriculomegaly, cerebral calcifications, abnormally formed or absence of brain structures, cataracts of both eyes, calcifications of eye, and hydrops fetalis during pregnancy and yet an unproven association with Guillain Barre syndrome (GBS) in adult people[36-39]. All of the microcephaly cases were reported only from Brazil and outside of Brazil, no other country has reported the association of Zika virus infection and an increase in microcephaly cases. However, several countries in the Americas and Australias also reported that an increase in GBS cases coincided with Zika virus outbreaks. The association of an increase in GBS incidence with Zika virus infection is not as solid as microcephaly. Interpretation of any change in overall GBS incidence in the region attributable to Zika virus is complicated by local fluctuations in the incidence of dengue and chikungunya[40].

An Asian-lineage strain has been associated with the recent increase in microcephaly cases in Brazil. Zika virus RNA has been detected in the placenta and amniotic fluid of women with microcephalic fetuses and in the blood of microcephalic newborns suggesting that the virus can cross the placental membrane. The virus has also been identified in the brains and retinas of microcephalic fetuses. Despite accumulating clinical evidence, direct experimental evidence showing that the Zika virus causes birth defects remains absent. Very recently, Cugola and colleagues demonstrated that Zika virus infects fetuses, causing intrauterine growth restriction, including signs of microcephaly, in mice[41]. They also showed that the Zika virus infects human cortical progenitor cells *in vitro*, leading to an increase in cell death. The exact mechanism by which virus might cause brain malformations is also not known. To study the mechanism, Dang et al. (2016) used human embryonic stem cell-derived cerebral organoids to recapitulate early stage, first trimester fetal brain development and showed that Zika virus efficiently infects organoids and causes a decrease in overall organoid size through activation of the innate immune receptor Toll-like-Receptor 3 (TLR3)[42]. During the earliest stages of foetal development, the nervous system consists of a hollow tube running along the back of the growing embryo. The inner lining of this neural tube is packed with cells radial glia, which have fine processes spanning the thickness of the tube. Microcephaly is thought to result from a depletion of these founder population of radial glia, the neural stem cells in developing brain, either through cell death or premature differentiation. Nowakowski and colleagues reported that the AXL is a candidate viral entry receptor and is highly expressed by human radial glial cells, astrocytes, endothelial cells, and microglia in developing human cortex and by progenitor cells in developing retina[43]. They also proposed that Zika virus reaches the developing brain by hematogenous spread or via the cerebrospinal fluid and

invades radial glia cells with highest AXL expression. By preferentially destroying radial glia cells, the founder cell population that generates all cortical neurons, Zika virus can produce severe microcephaly.

Though Zika virus was discovered in 1947, why a possible correlation between Zika infection, microcephaly and GBS was not detected in outbreaks prior to the 2013–2014. It is possible that in endemic areas girls are infected and become immune well before childbearing age. The recent upsurge in microcephaly cases in Brazil might be due to the availability of large susceptible population, including pregnant women. Flaviviruses can appear significantly more pathogenic when introduced into new niches and populations, but when the virus becomes established, herd immunity develops and the virulence of that particular virus will be gradually reduced. For example when West Nile virus was introduced into North America in 1999, caused very high mortality. This high virulence was associated with specific mutations that increased viral reproductive fitness in avian hosts and the North American environment[44]. The rapid spread of chikungunya into India was the result of a single nucleotide change that promoted the adaptation of Chikungunya virus to a different mosquito vector[45].

An alternative hypothesis is that viral evolutionary changes such as mutations or recombination events might be responsible for the increased virulence and a new spectrum of Zika disease. Recombination events were reported to occur in different Zika viral strains[46]. The same study also identified that a few Zika viral genes such as envelope and NS5 are under strong negative selection pressure. Hence, episodes of negative selections together with no sign of positive selection are indicative of eradication of unwanted polymorphism in genes with functional significance. One can assume that deletions that remove glycosylation sites in the envelope gene which, in turn, increase the infectivity of Zika virus and the outbreak of Zika virus is due to negative selection. The insertion of few amino acids is seen at position 441–442 in the genomic region endcoding E protein[47]. The role of those newly inserted aminoacids in the virulence of Zika virus or host-virus interaction needs to be studied. Very recently, Shrinet and colleagues analysed 50 Zika virus genomes and reported the distinct amino acid variations in the structural and nonstructural proteins of all Zika virus stains responsible for 2015-2016 outbreaks[48]. They also reported unique motifs in the un-translated regions (UTRs) of the new Zika virus strains. However, all these speculations need experimental validation.

Zika virus can be carried by a variety of Aedes mosquitoes, but the principal species responsible for the current outbreaks is thought to be *A. aegypti*. *A. aegypti* mosquitoes also serve as vectors for dengue and chikungunya viruses. In the past two decades, dengue virus has spread through areas of South America, and the seroprevalence of dengue in some Zika virus affected areas exceeds 90%. Many reports demonstrated a degree of antigenic similarity between the dengue and zika viruses. Antibody-dependent enhancement (ADE) of infection is common among Dengue virus serotypes. The recent Zika virus outbreaks are associated with neurological complications and this could be explained by the occurrence of recent outbreaks in hyper-endemic regions of Dengue virus. Very recently, using a panel of monoclonal antibodies to dengue virus, Dejnirattisai et al., 2016 reported that most antibodies that reacted to dengue virus envelope protein also reacted to Zika virus[49]. Using human myeloid cell line U937, they demonstrated that those antibodies were able to bind Zika virus but were unable to neutralize the virus and instead promoted ADE[49]. Hence, immunity to dengue virus might enhance replication of Zika virus and play an important role in pathogenesis and disease outcome in Zika infection.

The exceptional climatic conditions, arising from the strong El Niño event in 2015, in northeastern South America might have contributed to the rapid dispersal of Zika virus. Recently, a study revealed that Zika virus and man have a peptide in common, hence this may be the reason for microcephaly and GBS[50]. Along with the Zika virus, there could be other associated risk factors/agents responsible for microcephaly. Nogueira and colleagues analyzed protein extracts of three Zika positive brains by shotgun mass spectrometry and reported the presence of peptide(s) from the polyprotein of a Bovine-like viral diarrhea virus

(BVDV)[51]. They hypothesized that Zika virus may not be the only etiological agent responsible for microcephaly[51]. However, BVDV is not known to cause disease or even infection in humans. It is also a known contaminant in many cell culture reagents. Of the 25 identifications of BVDV derived peptides, 24 turned out to be identical to human proteins, many from the ubiquitin-family. Hence, their findings are of doubtful significance and needs experimental validation.

5. Diagnosis

Zika virus infection can be diagnosed based upon clinical symptoms, the prevalence of vector in the region, and by serological and molecular detection assays. The Zika virus can be isolated from mosquitoes and different samples of patients using animal inoculation and cell lines[52,53]. However, isolation is less frequently employed for diagnosis because it requires long time, laborious and less sensitive because of low level of viremia. Zika virus infections are routinely diagnosed by the detection of viral nucleic acid using RT-PCR and the detection of IgM antibodies by IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA). The detection of viral RNA in serum provides a definitive diagnosis; however, in most instances viremia is transient, and diagnosis by RT-PCR has been most successful within 1 week after the onset of clinical illness[54]. Zika virus RNA can also be detected in various other samples such as umbilical cord sample of infants, urine, nasopharyngeal swab, saliva, amniotic fluid, CSF, and frozen and fixed placenta [55-58]. Persistance of Zika virus in various samples is inconsistent hence, shedding of virus in different body fluids has to be elucidated to establish a better diagnostic method combined with exact sample of choice[59]. A report suggests that Zika virus is present in urine for more than 15 days after the onset of symptoms: if verified, this would extend the period during which a definitive diagnosis of Zika virus infection can be established by RT-PCR[60]. Another study has compared RT-PCR results in serum and saliva samples. The study results suggested that RT-PCR had higher sensitivity in saliva than in serum. However, samples from some patients were positive in serum but not saliva, and testing of saliva samples did not extend the duration of viral RNA detection after the onset of illness[61]. ELISA based methods can be used to detect Zika virus infection during the first week of illness, if they target virus specific antigens. Very recently, BioFront Technologies Inc. has developed the MonoTrace Zika Virus NS1 ELISA kit for specific detection of Zika virus non-structural 1 (NS1) protein. They also reported that the assay demonstrates strong reactivity to all major Zika virus genotypes yet shows no cross-reactivity with Dengue virus NS1.

IgM antibodies to Zika virus will appear as viremia wanes within the first week after symptoms onset and will persist for several months[62]. Hence, for sera samples RT-PCR is useful within the first week of clinical illness and MAC-ELISA will be useful after first week of clinical illness. Hence, combination of RT-PCR and MAC-ELISA is likely to have the highest diagnostic yield[63]. The considerable cross-reactivity between members of flaviviruses hinders the use of serological techniques for diagnosis of Zika infection. For example, dengue infection may also evoke a positive MAC-ELISA for Zika virus. The plaque reduction neutralization test (PRNT), the most specific test used to differentiate antibodies of closely related viruses, can be used to verify MAC-ELISA results[64]. However, this test is labor-intensive and costly, involves handling of live virus, takes up to a week to perform, requires standardized reagents that often are not available, and is not widely performed. In settings, where PRNT is not available, specimens that are found positive by Zika virus MAC-ELISA and negative by dengue MAC-ELISA may be interpreted as a presumptive recent Zika virus infection. However, the diagnostic accuracy of this approach needs to be validated.

Reliable testing regimens for the diagnosis of prenatal and antenatal Zika virus infection have not been established. Congenital Zika virus infection can be diagnosed by screening the amniotic fluid for Zika virus RNA by RT-PCR. However, the sensitivity of RT-PCR in this context is not established[16, 17]. At the time of delivery, cord blood can be tested by RT-PCR and MAC-ELISA, but the sensitivities of these tests for detecting prenatal Zika virus infection

are unknown. Zika virus infection in tissues of fetal losses and full-term infants who died shortly after birth can be diagnosed by RT-PCR and immunohistochemical testing[18,65]. Microcephaly is detected by measuring occipitofrontal circumference as suggested by standard charts (WHO, 2006). Ultrasonography can be employed for detection of microcephaly in pregnant women[16]. Although microcephaly and other fetal abnormalities may be detected as early as 18 to 20 weeks of gestation, they are often not detected until later in pregnancy. Furthermore, the use of ultrasonography to detect microcephaly is dependent on clinical and technical factors, and ultrasonography is not highly sensitive for detection of microcephaly. Results of ultrasonography can be verified using molecular and serological tests[55]. Molecular diagnosis involves detecting of viral RNA by RT-PCR. Blood picture reveals neutropenia and thrombocytopenia[66].

6. Treatment

Currently, there is no specific antiviral drug available to treat Zika virus infections. Similar to other mosquito-borne flaviviruses, treatment for uncomplicated Zika virus infection focuses on symptoms. Only supportive treatment such as use of fluids, and analgesics to reduce pain and antipyretics to reduce fever is in use to treat Zika infections. Xiyanping is a semi-synthetic component extracted from Andrographis paniculata, a Chinese herb, possesses anti-inflammatory and antiviral activity[67,68]. Very recently, there is a report from China on the use of Xiyanping injection combined with supportive therapy with promising results[69]. As of now, not much attention has been given to explore new therapeutic regimens for treating Zika infection. Hence, there is a dire need to find out the suitable antiviral drug for prevention and control of Zika virus. Considering the public health significance of this virus and global concerns, future studies should concentrate on exploiting valuable therapeutic options of novel and emerging/upcoming regimens such as cytokines, RNA polymerase inhibitors, microRNA (mi-RNAs), small interfering RNA (si-RNA), probiotics, herbs/plant extracts, nutritional immunomodulation.

7. Prevention and control strategies

Currently there is no vaccine exists to counter the Zika virus infection. Though Zika virus is known since 1947, only after the recent outbreaks, the virus has assumed great significance and efforts are being made towards developing a vaccine. As many as 23 groups from different countries are working on the development of vaccine against Zika virus[70]. Since, there is no vaccine, prevention and control of Zika virus are mainly aimed at prevention of vector population (mosquitoes) as they play an important role in the transmission of this virus. These vectors can be controlled either by mechanical, chemical, and biological measures. Mechanical control methods involve removal of any objects that helps in unwanted storage of water in the premises that serve as a breeding point for female mosquitoes. Chemical control of insects and vectors can be used with caution as it can cause toxicity to animals[71]. The major disadvantage in the use of this method is the problem of resistance development and also these compounds are toxic to higher mammals thereby questioning its use for the control of vectors[9].

As part of biological control measures, bacteria such as *Bacillus thuringiensis israelensis* can be used for mosquito control[72]. A report suggests that a fungi named *Beauveria bassiana* can also be used to control Aedes mosquitoes[73]. Another approach is the use of larvivorous fishes (e.g. *Gambusia affinis*) in the water-logging areas and flower pots which can eliminate larvae of mosquitoes[74]. Lately, the use of microbes to control vector population gained interest. For example, a bacteria named Asaia has been explored for its ability to colonize the gut and reproductive tract of mosquitoes and also to get transmitted vertically to its progeny[75]. Another bacterium named Wolbachia has the potential to feminize male vectors thereby preventing the reproduction of vectors hence controlling their population[76]. Other techniques such as irradiation have been used to generate sterile males to control the mosquito population[77].

Apart from vector control, proper surveillance and monitoring has to be carried out at the highest level in countries where there are reports and also countries adjoining them. All international airports, harbors, and places of international tourist attraction is needed to be under the scanner for the screening of this important disease. Spread of Zika infection to pregnant women can be prevented by avoiding unnecessary travel to areas of ongoing Zika virus transmission, avoiding unprotected sexual contact with partners who are at risk for Zika virus infection, and using mosquito repellent, permethrin treatment for clothing, bed nets, and window screens.

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