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A REVIEW ON ZIKA VIRUS ASSOCIATED MICROCEPHALY

Reza Rajesh [1*], Sharon Liza Koshy[2], Renuka R[3], Dr. Elessy Abraham[4]

[1]Third Year Pharm D Student, Nazareth College of Pharmacy, Othera P.O, Thiruvalla, Kerala, India

[2]Third Year Pharm D Student, Nazareth College of Pharmacy, Othera P.O, Thiruvalla, Kerala, India

[3]Assistant Professor, Nazareth College of Pharmacy, Othera P.O, Thiruvalla, Kerala, India

[4]Principal, Nazareth College of Pharmacy, Othera P.O, Thiruvalla, Kerala, India

Abstract:

Microcephaly is a condition where a baby's head is much smaller than expected. During pregnancy, a baby's head grows as brain is enlarging. Microcephaly can occur because a baby's brain has not developed properly during pregnancy or has stopped growing after birth, which results in a smaller head size. Zika virus is a mosquito-borne flavivirus transmitted primarily by Aedesaegypti mosquitoes. These vectors also transmit dengue and chikungunya virus and are found throughout much of the Americas, including parts of the United States. Zika virus infections have been confirmed in infants with microcephaly. In the current outbreak in Brazil, a marked increase in the number of infants born with microcephaly has been reported. Studies are under way to investigate the association of Zika virus infection and microcephaly.There are currently no drugs approved for the treatment of ZIKV-infection.Clinical trials are going on for the development of Zika vaccine. The aim of drug development is primarily to reduce viral load, reduce symptoms, and protect the unborn fetus from neurological sequelae. In order to develop effective anti-ZIKV vaccines and therapeutics, improved animal models and a better understanding of immunological correlates of protection against ZIKV are required. This review will summarize what is currently known about ZIKV, the clinical manifestations and epidemiology of Zika as well as, host immune responses against ZIKV, and the current state of development of vaccines and therapeutics against ZIKV.

Key Words: *zikavirus (ZIKV), microcephaly, zikavaccine, flavivirus, Aedesaegypti*

***Correspondence to Author :**

Ms. Reza Rajesh,

Third Year Pharm D Student

Nazareth College of Pharmacy, Othera P.O,

Thiruvalla, Kerala, India

Ph No-9847065025

E-mail: rezarajesh2015@gmail.com

Osmanabad-413501

Ph. No.: +917620610555

Email: vikrant.nawale71@gmail.com

QR code

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INTRODUCTION:

Zika is a rapidly emerging public health threat. The Zika virus has spread rapidly in the Americas since its first identification in Brazil in early 2015. Adverse effects in pregnancy such as microcephaly and other serious brain anomalies and birth defects were found to be linked withprenatal Zika virus infection.Zika virus is a mosquito-borne flavivirus transmitted primarily by *Aedesaegypti* mosquitoes. [1,24]

Almost 80% of persons infected with Zika virus are asymptomatic. Most commonly found symptoms are acute onset of fever, maculopapular rash, arthralgia, or non-purulent conjunctivitis.The primary mode of ZIKV transmission is through the bite of infected mosquitoes, Aedesaegypti and Aedesalbopictus.They are considered as the predominant vectors.Other than vector-borne transmission, sexual transmission also contributes to ZikaVirusspread. Other modes of transmission include transmission from mother to child, blood transfusion, laboratory transmission, and transmission by physical contact [9,16,18,19,21,22,28,42].

EPIDEMIOLOGY

From the reports of human cases of ZIKV infection from 1947 until the 2007 it was found that ZIKV has now spread dramatically to over 80 countries and territories with vector-borne transmission. ZIKV was first reported in Brazil in 2015 with large numbers of suspected cases and the initial observation that the number of newborn infants with microcephaly was increased in ZIKV-affected areas later that year.The studies conducted in French Polynesia in 2013-2014 revealed that the risk of microcephaly due to ZIKV infection in the first trimester of pregnancy was 0.95%.According toZika virus outbreak which began in October, 2013, and ended in April, 2014,it was estimated that 66% of the general population were infected.Among a cohort of 35 infants with microcephaly born during August–October 2015 in eight of Brazil's 26 states and reported to the registry, the mothers of all 35 had lived in or visited Zika virus-affected areas during pregnancy, 25 infants had severe microcephaly, 17 had at least one neurologic abnormality, and among 27 infants who had neuroimaging studies, all had abnormalities[11,24,49].

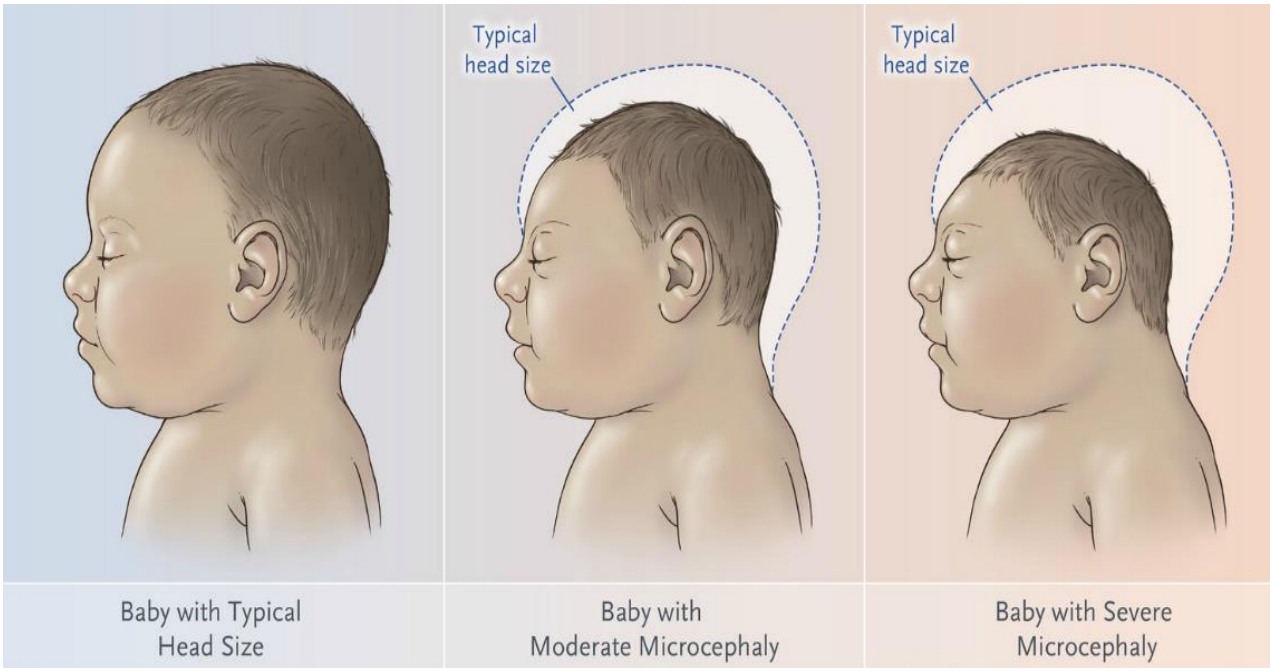

Fig. 1: Microcephaly in babies

ETIOPATHOGENESIS

ZIKV belongs to the family Flaviviridae, genus Flavivirus and is a mosquito-borne virus in the Spondweni group. Flaviviruses are arboviruses and include pathogens such as yellow fever virus (YFV), Japanese encephalitis virus (JEV), West Nile Virus (WNV), tick-borne encephalitis virus (TBEV), and the four dengue viruses (DENV1–4) in addition to ZIKV.[29]

Usually Flaviviruses are enveloped RNA viruses.They have viral genome complexed with multiple copies of the capsid protein (C) and is surrounded by an icosahedral shell composed of 180 copies of the envelope (E) glycoprotein and precursor membrane (prM) or membrane (M) proteins which are anchored in a lipid membrane.Capsid protein(C), Envelope glycoprotein (E) and Membrane protein (M) are structural proteins. When we analyze the full-length genome of ZIKV it consists of 10,794 nucleotides and encodes 3419 amino acids, in addition to the structural proteins. They constitute seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) which are involved in replication and assembly of the virus and antagonizing the host innate immune response [29,44].

NS3 and NS5 are large, multifunctional proteins.They perform several enzymatic activities involved in polyprotein processing (NS3) and RNA replication (NS3 and NS5). NS5 also have the ability to antagonize the interferon (IFN) response [39].

Mature ZIKV structure is similar to mature West Nile Virus(WNV) and Dengue virus(DENV) structures. Eventhough there exist certain differences in the E protein structure which are thought to be responsible for cellular tropism which contribute to various disease outcomes [44].

Phylogenetic analyses of ZIKV strains isolated have shown two distinct classes (lineages), African and Asian.The causative agent of the current ZIKV infection is the Asian lineage. It is not closely related to the African lineage but shares a common ancestor.On comparing the E protein sequences from the two lineages it has been found that the Asian lineage contains insertions in the E protein glycosylation motif which are not present in the African lineage. When the amino acid sequences of the E protein of ZIKV isolates from human,monkey and mosquito were compared, sixteen amino acid substitutions were identified, which results in subtle structural changes. These changes, may impact ZIKV virulence and host tropism [25].

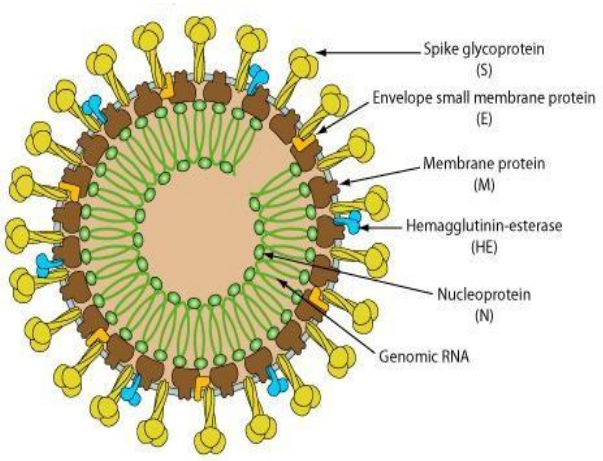

Fig. 2: Structure of Zika Virus

CLINICAL MANIFESTATION

About 80%, of individuals infected with ZIKV are asymptomatic. Pruritic maculopapular rash, low grade fever, arthritis, arthralgia, non-purulent conjunctivitis, and edema of the extremities are the most commonly found symptoms.Headache, myalgia, retro-orbital pain, low-back pain, lymphadenopathy, and vomiting are also seen in some patients.Symptoms last from several days to 1 week. Severe disease requiring hospitalization and fatalities are rare. Guillain-Barré syndrome has also been reported in patients.The disease is frequently mild but fatal cases have been reported in those with underlying medical conditions.Neurological complications associated with ZIKV infection are encephalitis, meningoencephalitis and acute myelitis.There are multiple reports of microcephaly and other birth defects following ZIKV infection during pregnancy. A causal link was suspected following the recognition of increase in the number of infants with microcephaly in Brazil and French Polynesia which were associated with outbreaks of ZIKV infection. Rassmussen et al. suggested sufficient evidences that had been accumulated to establish a causal relationship between ZIKV infection and microcephaly and other brain anomalies [7,11,22,39].

Evidence to support the role of ZIKV infection in causing microcephaly and other neurological abnormalities during pregnancy includes:

(a) Increase in microcephaly with increased ZIKV transmission

(b) The first trimester is the primary risk period of pregnancy according to data modelling

(c) ZIKV is identified in the brains of foetuses and infants (who died) with microcephaly

(d) Human neural progenitor cell growth following in vitro ZIKV infection
(e) in vitro infection of cytotrophoblasts and placental macrophages
(f) Microcephaly associated with ZIKV infection in mouse and non-human primate models [34].

IMMUNITY

IFN production is a major component of the innateresponse. Studies show that mice deficient in various components of the IFN response have increased susceptibility to ZIKV infection. Human primary trophoblast cells, isolated from full-term placentas were found to release antiviral typeIII IFNλ1 which helps to protect cells from ZIKV infection. Flaviviruses antagonize IFN signaling through multiple mechanisms. In-vitro studies show that, for proteosomal degradation, ZIKV NS5 binds to and targets the human IFN-regulated transcriptional activator STAT2.Type I and Type II IFN use different cell-surface receptors which signal through the Jak-STAT pathway, including STAT2. Thus there is possibilityfor ZIKV to evadeType III IFN signaling by degrading STAT2 mediated by NS5 even though they are produced by villous trophoblasts[1,27,41,48].

In humoral immunity, neutralizing antibodies have a major role in the protection against ZIKV infection. Multiple groups have identified antibodies from Dengue (DENV) patients, which cross-react, and in some cases, neutralize ZIKV.There is also concern for antibody dependent enhancement (ADE), an immunological phenomenon in which non-neutralizing or weakly neutralizing antibodies facilitate viral entrance into Fc-receptor bearing cells such as monocytes and macrophages[7,49].

Coming towards cell mediated immunity T cell responses contribute to protection and/or disease enhancement.ZIKV-specific cytokine production by CD4+ and CD8+ T cells was identified in ZIKV-infected people[33].

TRANSMISSION

- Zika virus is transmitted to people primarily through the bite of an infected *Aedes* species mosquito (*Ae. aegypti* and *Ae. albopictus*).
- Zika virus can be passed through sex from a person who has Zika to his or her sex partners.
- A pregnant woman can pass Zika virus to her fetus during pregnancy. Zika infection during pregnancy can cause serious birth defects. Zika is a cause of microcephaly and other severe fetal brain defects.
- Zika virus may be spread through blood transfusion.
- Anyone who lives in or travels to an area with risk of Zika and has not already been infected with Zika virus can get it from mosquito bites [15].

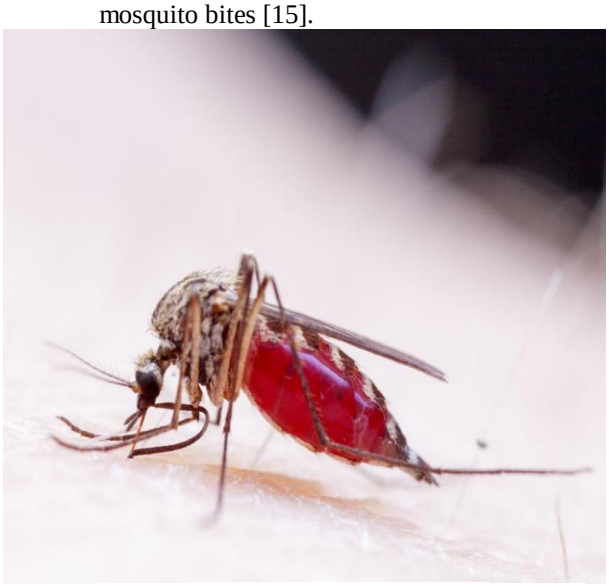

Fig.3*: Ae.aegypti* mosquito

DIAGNOSIS

Zika virus IgM antibodies can persist beyond 12 weeks after infection.We cannot reliably differentiate Zika infection occurring before pregnancy from that occurring during pregnancy from positive test results for ZikaIgM.

Molecular Test for Zika Virus

Zika virus RNA can sometimes be detected early in the course of illness,for symptomatic persons with Zika virus infection. On paired serum and urine specimens RNA NAT (nucleic acid testing) is performed. NAT should be performed concurrently with IgM serology for symptomatic pregnant women with possible exposure to Zika virus. NAT is recommended three times during pregnancy for asymptomatic pregnant women with ongoing possible exposure to Zika virus.

Trioplex Real-time RT-PCR Assay

For the differentiation of RNA from Zika virus and for the qualitative detection of Zika virus RNA in urine and amniotic fluid the Trioplex Real-time RT-PCR Assay (TrioplexrRT-PCR) is used. The assay is is usually performed in specimens collected from individuals meeting CDC Zika virus clinical criteria and/or CDC Zika virus epidemiological criteria.

Serologic Test for Zika Virus

In most cases Zika virus-specific IgM and neutralizing antibodies develop toward the end of the first week of illness. IgM levels are variable, but generally are IgM is found positive from almost the day 4 after onset of symptoms and it continues up to 12 weeks after the onset of symptoms sometimes may persist longer.Thus the test for IgM is performed concurrently with NAT.

Zika MAC-ELISA

For the qualitative detection of Zika virus IgM antibodies in serum or cerebrospinal fluid, the Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) is used. But sometimes the results may be difficult to interpret due to the cross-reaction with other flaviviruses and possible nonspecific reactivity [15].

Nucleic acid amplification test (NAAT)

ZIKV RNA detection in the serum, whole blood, urine, and cerebrospinal fluid (CSF) are possible through NAAT. It is more specific than serological tests but have limited by the short duration of RNAemia and RNAuria. In general, ZIKV RNA can be detected from serum for approximately seven days and in urine for approximately 15–20 days of post-infection. Different NAAT have different sensitivities. Corman et al.,in a comparison of seven published and two new RT-PCR assays, found that due to lack of sensitivity or difficulty in obtaining necessary reagents some published RT-PCR assays may be of limited value for diagnostics in the current outbreak.Currently, 12 molecular assays are available through EUA [47].

TREATMENT

There are no specific drugs for the treatment of Zika virus.

Vaccines against Zika virus are under clinical trials.They include:

- **Inactivated Whole Organism (with or without Adjuvant)**

Inactivated vaccines against flaviviruses including JEV (Japanese Encephalitis virus) and TBEV(tick-borne encephalitis virus) have been used successfully providing support for this method. Benefits of an inactivated whole organism vaccine include multiple antigenic targets and non-replicating virus, which may improve safety.The Phase I trials of anti-ZIKV PIV vaccine candidates are under studies [32].

- **DNA vaccines**

The plasmid is injected and DNA is allowed to be taken up by antigen presenting cells, which then express it as plasmid-encoded genes and generate the target antigen. DNA vaccine against WNV (West Nile Virus) was previously tested in humans and demonstrated excellent safety and immunogenicity[28,17].

- **RNA vaccines**

RNA vaccines contain an open reading frame encoding the antigen of interest which is then translated by the host cellular machinery.A single immunization of a different ZIKV prM/E encoding mRNA lipid nanoparticle vaccine was done in rhesus macaque model and it resulted in neutralizing antibody titres that were fifty times greater than those induced by a single immunization of a DNA vaccine and more than twice as high as those induced by two immunizations of a DNA vaccine.Since there is no potential for genomeintegration, RNA-based vaccines may be safe than DNA vaccines[3,40].

- **Recombinant Viral Vector**

In rhesus monkeys, a rhesus adenovirus serotype 52 (RhAd52) vector-based vaccine elicitedZIKV-specific neutralizing antibodies following a single immunization which demonstrated asubstantial breadth of antibody responses against linear ZIKV E protein epitopes (peptide microarrayassays)[23].

- Additional vaccine candidate platforms in pre-clinical studies include live-attenuated vaccines, recombinant subunit vaccines, peptide vaccines, and ZIKV exosome vaccines [32].

Table 1: Summary of anti-ZIKA virus (ZIKV) vaccine candidates currently in clinical trials

Type of vaccine	Developers/collborators	Candidate vaccine name (if available)	Stage of development	Clinical trial registration number
Inactivated whole organism	WRAIR/BIDMC/Harvard/ NIAID/Sanofi Pasteur		Clinical (Phase I)	NCT02963909NCT 02952833NCT0293 7233
DNA	GenOne Life Science,Inc/Inovio Pharmaceuticals	GLS-5700	Clinical (Phase I)	NCT02809443NCT 02887482
DNA	VRC/NIAID	VRCZIKV DNA	Clinical (Phase I)	NCT02840487NCT 02996461
Synthetic peptide	NIAID	AGS-v	Clinical (Phase I)	NCT03055000
Measles-vectored	Themis Bioscience	MV-ZIKA	Clinical (Phase I)	NCT02996890
mRNA	Valera(Moderna)	Mrna-1325	Clinical (Phase I)	NCT03014089
WRAIR:Walter Reed Army Institute of Research , BIDMC:Beth Israel Deaconess Medical Center, NIAID:National Institute of Allergy and Infectious Disease , VRC:Vaccine Research Center				

No drugs are currently approved for the treatment of ZIKV-infection. The primary aim of drug development is to reduce viral load, symptoms, andto protect the unborn fetus from neurological disorders. “Re-purposing” existing compounds for the treatment of ZIKV are under studies. Multiple compounds, including ribavirin and polymerase inhibitor 7-deaza-20-C-methyladenosine (7DMA) are tested by Zmurko et al., to determine potential anti-ZIKV drugs. Of the compounds tested, 7DMA inhibited ZIKV replication in vitro, and, also reduced viremia when administered for 10 consecutive days (beginning 1 h prior to infection).It also had shown to delay time taken for disease progression in ZIKV-infected AG129 mice. But one of the main challenges is that the initiation of treatment prior to infection is impractical in non-research settings. Various other techniques such as rapid, high-throughput screening of drug/compound libraries were also utilized inorder to identify compounds with in vitro anti-ZIKV activity. On analysing 727 compounds for anti-ZIKV activity using this technique, ZIKV had shown sensitivity to pyrimidine synthesis inhibitors (e.g., brequinar). Moreover, Barrows et al. screened 774 FDA-approved drugs for anti-ZIKV activity.He found that over 20 compounds, including mycophenolate mofetil, daptomycin and sertraline, are able to reduce viral infection in vitro. Then ~6000 compounds, which include approved drugs, clinical trial drug candidates, and pharmacologically active compounds, were tested to determine whether they are able to inhibit ZIKV infection or suppress infection-induced caspase-3 activity in neuronal cells .Thus it was found that emricasan, a pan-caspase inhibitor, was the most potent anti-cell-death compound .Furthermore it has shown neuroprotective activity for human neuronal progenitor cells but they were not able to suppress ZIKV replication. Several anti-malarial compounds were alsostudied using a cell-based cytotoxicity assay .It had been demonstrated that they are having anti-DENV2 and anti-ZIKV properties. An FDA-approved hepatitis C virus (HCV) anti-viral, sofosbuvir was studied by Bullard-Feibelman et al. ,and he found that the drug was able to inhibit ZIKV replication and infection in tissue culture as well as protected mice from ZIKV-induced death [15,51].

All at a glance there are multiple challenges to be faced for the development of anti ZIKV agents. Though the clinical infection with ZIKV is typically mild, pregnant women and those at increased risk for neurological complications are the primary populations for whom treatment would be indicated.For the development of therapeutics to be used during pregnancy,there are multiple ethical considerations. In general, from clinical trials of new investigational compounds, pregnant women are excluded. But the agent should be of low risk to the mother, foetus, effective in preventing adverse effects on foetus, and practical for use in resource-limited settings [5,10].

Currently used strategies of treatment include:-

- Treat the symptoms.
- Get plenty of rest.
- Drink fluids to prevent dehydration.

- In order to reduce fever and pain take medicine such as acetaminophen.
- Do not take aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) until dengue can be ruled out to reduce the risk of bleeding.
- Talk to your healthcare provider before taking additional medication for some other medical conditions [15].

CONCLUSION:

Zika virus is a mosquito-borne flavivirus transmitted primarily by *Aedesaegypti* mosquitoes. It is a rapidly emerging virus with a complex clinical picture. Despite mild clinical symptoms in the mother, ZIKV infection during pregnancy produceharmful effects in fetus and is associated with fetal death, growth restriction, and a spectrum of central nervous system abnormalities most commonly microcephaly. Microcephaly is a condition in which baby's brain has not developed properly during pregnancy or has stopped growing after birth, which results in a smaller head size. Today, much progress has been made in understanding the epidemiology and pathogenesis of this infection. The vaccines to arrest the spread of ZIKV are under clinical trials.

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