Zika Virus

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Introduction:

Zika virus, (ZIKV), is an arbovirus of the Flaviviridae family, which is transmitted by the Aedes mosquitoes (Aedes aegypti or Aedes albopictus) [3]. Zika is currently one of the most significant viruses in the world. The infection is mainly caused by mosquito bite. It has been associated with severe clinical manifestations and congenital malformations. Although, we know a little about the pathogenicity of the virus. Yet, there is no specific treatment, vaccines or other prevention strategies of ZIKV infection. [2] In 1947, ZIKV was first isolated from the serum of a monkey from the Zika Forest (Uganda) during a study of the transmission of yellow fever. [2] The first human infection was documented in Nigeria in 1954. Where, there was a very few documented infections after this initial report. However, 73% of the population in Yap Island of the western Pacific Ocean were infected with ZIKV in the outbreak in 2007. [4] [1] This outbreak of Zika virus, in Yap Island, was the first outbreak of the ZIKV disease outside Africa and Asia. At the beginning, this outbreak was suspected to be caused by dengue virus (DENV), because the infection was caused by a mosquito bite, and it had similar clinical presentations to the dengue infection. However, the serological and molecular findings indicated that ZIKV was the causative agent. [2][5] In 2013, the first imported case of ZIKV infection in Europe was reported, which was a German traveler returning from Thailand. The case was confirmed by the presence of anti-ZIKV IgM and IgG and of ZIKV neutralizing antibodies in the patient's blood. [2] Also, in the same year, there was another ZIKV outbreak in the French Polynesian, which estimated to have affected approximately 28,000 persons, or approximately 11% of the population. [6] In October 2015, Pernambuco, a state in Brazil, was subject to an increasing number of newborns with microcephaly, lissencephaly, cerebral atrophy and brain calcifications. [3] On 1st February 2016, the World Health Organization (WHO) declared a global public health emergency due to the ZIKV threat. [2]

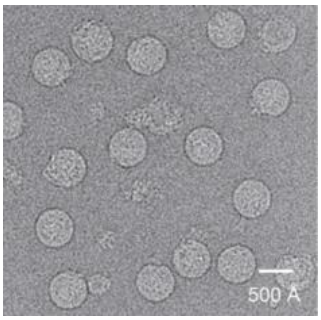

Fig 1. ZIKV [29]

The classification of the Zika virus, and the protein structure:

ZIKV is an arbovirus member of the Spondweni serocomplex within the genus Flavivirus, family Flaviviridae. Other flaviviruses, members of that genus, include Yellow fever virus, Dengue virus (DENV), West Nile virus (WNV) and others. [15] The Complete genome sequence identity and divergence analysis indicated that ZIKV showed the highest identity with Spondweni virus (SPOV). Where, 68.6% identity is in nucleotides and 75% identity is in amino acids. The results showed that ZIKV formed a clade together with SPOV in mosquito-borne flaviviruses. [17] The structure of ZIKV is similar to flavivirus structures, except for the ~10 amino acids that surround the Asparagine 154 (Asn_{154}) glycosylation site in each of the 180 envelope glycoproteins that make up the icosahedral shell. [19] The carbohydrate moiety associated with this residue, it may function as an attachment site of the virus to host cells. The variation of this region is not only among ZIKV strains, but also exist in other flaviviruses, which suggests that the differences in this region may influence the virus transmission and disease. [19] The ZIKV contains a positive single stranded genomic RNA. [15] The length of its genome is 10,794 nucleotides, [18] with 2 flanking non-coding regions (5′ and 3′ NCR). [15]

The 3′ NCR contains about 428 nucleotides, and it includes 27 folding patterns that may be involved in the recognition by cellular or viral factors, translation, genome stabilization, RNA packaging, or cyclization. [16] The 3′ NCR defects in certain critical regions that is called the conserved region, which have a profound impact on the survival and/ or pathogenicity of the virus. Conserved sequences (CSs) in the 3′ NCR of the ZIKV are three: ZIKV ImCS1(Imperfect CS1), ZIKV CS2 and ZIKV ImCS3. The presence of CS2 or CS3 is not considered absolutely essential for viral replication. [18] The 3′ NCR also, contain a single long open reading frame (ORF), [15] encode 3419 amino acids. [18] ZIKV is encoding a polyprotein: 5′C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′,[16] which processed into three structural proteins, the capsid (C), the precursor of membrane (prM) and the envelope (E), and seven nonstructural proteins NS1 to NS5.[15] They are necessary for viral replication and assembly.[14] The NS3 consists of two domains, protease domain and helicase/ NTPase domain, which are involved in the processing of viral polyprotein and the unwinding of structural template regions during viral RNA synthesis, respectively. [17] NS5 protein (≈103 kDa) is the largest viral protein composed of N-terminal methyltransferase domain, which methylates the 5′cap structure of genomic RNA, and C-terminal RNA dependent RNA polymerase (RdRp) domain. [16] [17] The envelope protein is divided into three domains (Domain I, Domain II and Domain III) according to other mosquito-borne flaviviruses. Domain I is involved in the envelope

structure organization, Domain II and Domain III are related to the monomers interaction and receptor binding, respectively.[17]The substitutions in Domain III may result in the alterations of receptor binding efficiency, whereas the stem and transmembrane region had been demonstrated to be involved in virion assembly and membrane fusion, respectively.[17] The E protein is involved in the recognition of the receptor on the surface of the host cell and then the fusion process between the viral envelope and the intracellular membranes. [14] The E protein is, also, involved in various aspects of the viral cycle. [16]

The transmission of the Zika Virus:

ZIKV is transmitted by the bite of several mosquito species, notably, during daytime Aedes aegypti is more active than anthropophilic Aedes albopictus. [13] The transmission of ZIKV typically occurs through the bite of an infected female mosquito during its blood feeding. [2] After 24h of ZIKV infection, all cells were able to produce infectious virions, which proves the critical role of skin compartment in the transmission of ZIKV. [14]

The increasing threat of non-vector-borne transmission includes the ZIKV. [2] There were some reports displayed the range of the non-vector transmission. These reports arranged the probability of the infection of this virus, in order from the highest probability of the infection to the lowest probability of the infection, as follows, the transmission from a mother to a child, the transmission by urine, the sexual transmission that transmits only by semen, because there is no data are available regarding the presence of ZIKV in the female genital tract. [13] [4] Then it is followed by the transmission through saliva, blood transfusion, breast milk, and lastly, the lowest range of the transmission is through animal bites, only one case of this kind of transmission was documented and was caused by a monkey. [13]

Replication:

Once ZIKV particles are in the human body, they must enter individual cells in order to replicate and make more viruses. Cell entry is possible because a ZIKV particle carries specific proteins on its outer envelope that interact with receptor proteins on human cells. When the viral proteins bind to cell receptors, they ‘trick’ the cells into taking up the viral particle. First, the virion attaches to the host cell membrane receptors via the envelope protein which induces virion endocytosis. Next, the virus membrane fuses with the endosomal membrane and the ssRNA genome of the virus is released into the cytoplasm of the host cell. [20] The replication stages of ZIKV have not yet been well defined, but it is thought that they are essentially similar to other members of

flavivirus genus. [14] The virus replication consists of four stages: translation of genomic RNA into viral proteins, replication of viral RNA molecules, assembly of virus particles in the endoplasmic reticulum and the release of virions. [14]

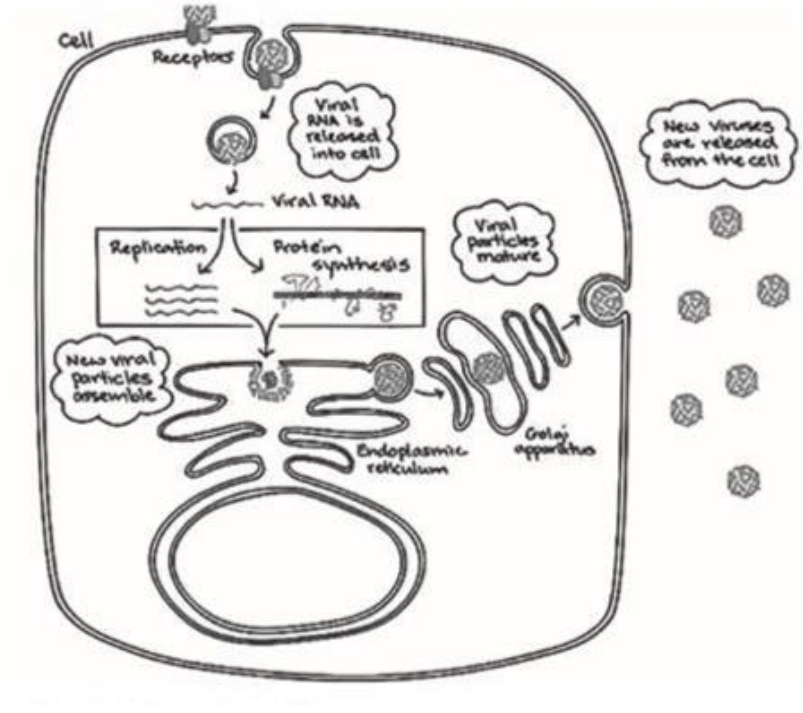

Fig 2. ZIKV life cycle [20]

After the releasing of the ssRNA into the cytoplasm, it's translated into a polyprotein that is subsequently cleaved to form all structural and non-structural proteins. Replication then takes place at intracellular compartments known as cytoplasmic viral factories in the endoplasmic reticulum resulting in a dsRNA genome. The dsRNA genome is then transcribed resulting in additional ssRNA genomes. Viral proteins and copies of the RNA genome assemble on the surface of the endoplasmic reticulum (ER), a membrane compartment that's part of the cell's export system. New viral particles bud off into the interior of the ER, taking a small patch of ER membrane along with them. This 'stolen' membrane will form the viral envelope. [20] The virus assembly in the endoplasmic reticulum is as a noninfectious "spiky" immature particle, [19] The particles then travel through another structure, the Golgi apparatus, where the particles will undergo more processing before release at the cell surface. In the Golgi apparatus the virus assembled into a mature "smooth" virus. [19] then excreted into the intracellular space where the new virions can infect new host cells thus, continuing the infection cycle. [20]

Symptoms:

Only 1 out of 5 patients with Zika infection will show symptoms. [21] When symptoms are shown, the resultant illness is generally mild, comprising low-grade fever, pruritic rash, myalgias, and non-purulent conjunctivitis. The exception to this is a congenital Zika infection, which can result in devastating consequences for the fetus, including death, microcephaly and other brain abnormalities. [23] Microcephaly is a birth defect in which the baby's head is unusually small and under-developed. Infants born with microcephaly may display balance and coordination problems, developmental delays and trouble swallowing and problems feeding. [21] The association between ZIKV infection during pregnancy and microcephaly led the World Health Organization to declare Zika a public health emergency of international concern. [23] ZIKV infection has been attributed in the first two trimesters of pregnancy. [8] Even though the direct effect of ZIKV on infants is not clear, the infection in pregnant woman can be very harmful to her foetus and cause foetal death, placental insufficiency and central nervous system (CNS) injury, because many reports confirm that prenatal transmission of ZIKV to foetus can cause loss of brain development in infants. [24] Also, the Guillain-Barre Syndrome (GBS) is a severe symptom in adults as the microcephaly in infants. The (GBS) is neurological defect that causes acute a reflexic paralysis and damage of peripheral nervous system. A study in 2016 shows that two patients with GBS had ZIKV infection. [24] Physicians considered thrombocytopenia as a possible symptom of ZIKV, since two patients had severe thrombocytopenia after ZIKV infection. The first had 1,000 platelets/mm^3, and died following multiple hemorrhages. The second had 2,000 platelets/mm^3, melena, ecchymoses, and recovered after receiving intravenous immunoglobulin. [25] Symptoms may develop within a few days to a week after infection and last for several days to one week. Severe illness and death are rarely a result of a ZIKV infection. [21]

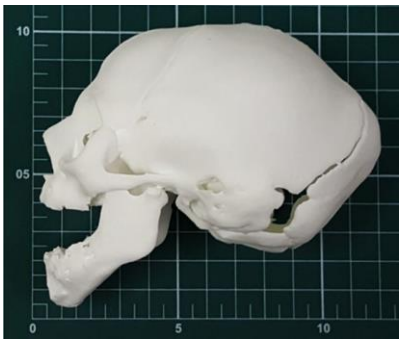

Fig 3. A 3D physical model of Microcephaly brain [3]

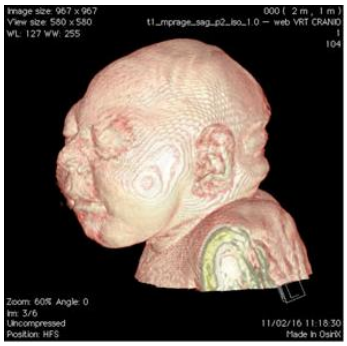

Fig 4. A 3D head reconstruction of Microcephaly brain [3]

Cytopathic effect:

ZIKV RNA has been seen in damaged mononuclear cells (presumably glial cells and neurons) in the brains of newborns with microcephaly, and the virus appears to be neurotropic. Furthermore, ZIKV efficiently infects neural progenitor cells and produces cell death and abnormal growth, thus providing a possible mechanism for microcephaly. [27]

In 1971 there was a study that focused on the ZIKV Infection of the Central Nervous System of Mice, by one ampoule of the MP 1751 strain of ZIKV which was placed in the baby mice brain before lyophilization. The most striking changes were found in Ammon's horn, where there were localized segments of necrosis in the band of pyriform cells (Fig. 5a,5b,6a,6b). Much scattered hyperchromatic nuclear debris was present and the tissue presented a moth-eaten appearance. Astrocyte hypertrophy was prominent (Fig. 7a, b). The relatively plump cells of the newborn animal were enlarged and presented more branched processes. Microglial cells were not prominent. [28]

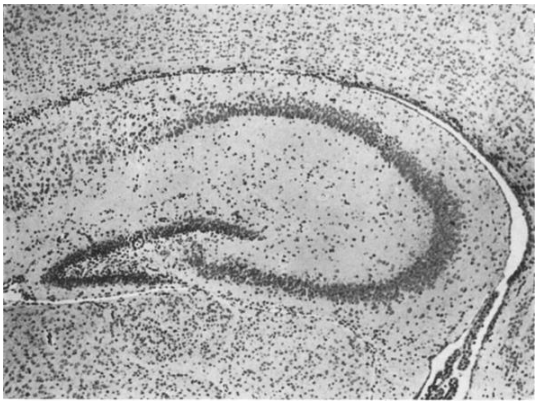

Fig 5a. Normal Ammon's horn of mouse 7 days old. [28]

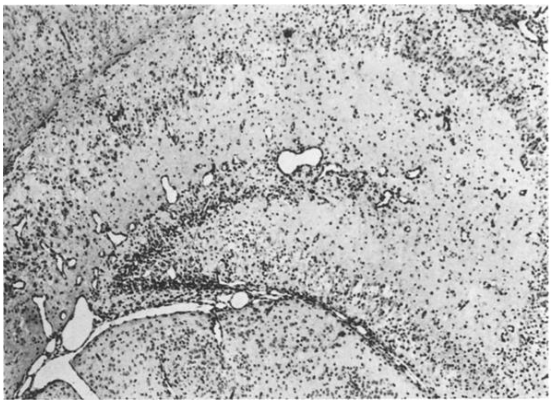

Fig 5b. Ammon’s horn of Zika infected mouse 7 days old. [28]

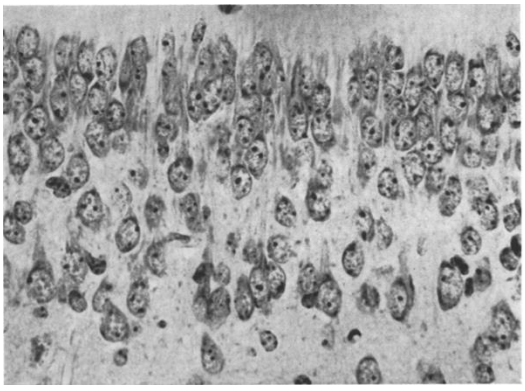

Fig 6a. Normal pyriform cells: 7 days old mouse. [28]

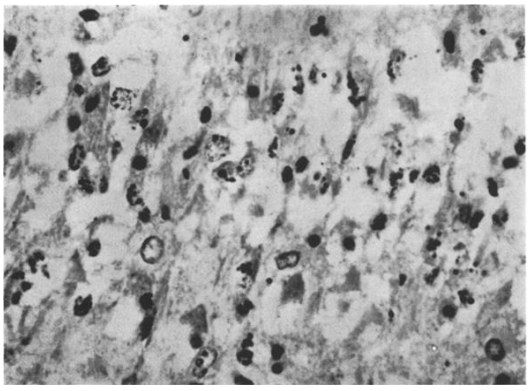

Fig 6b. Same area in 7 days old Zika infected mouse. [28]

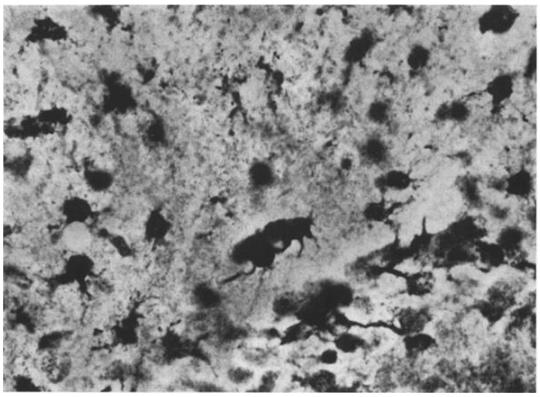

Fig 7a. Astrocytes in Ammon's horn of normal 7 days old mouse. [28]

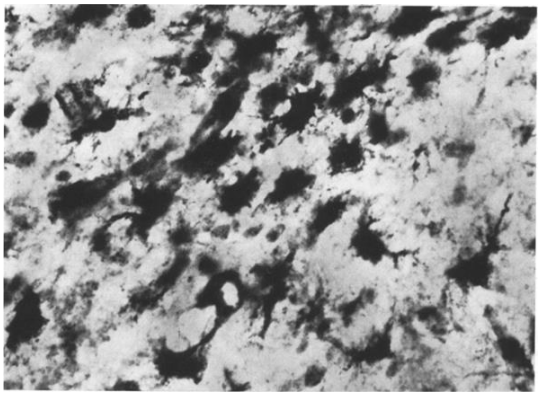

Fig 7b. Astrocytes in Ammon's horn of 7-day old mouse with Zika infection. [28]

In the 5-week-old mouse astroglial changes were 'again remarkable (Fig. 8). [28]

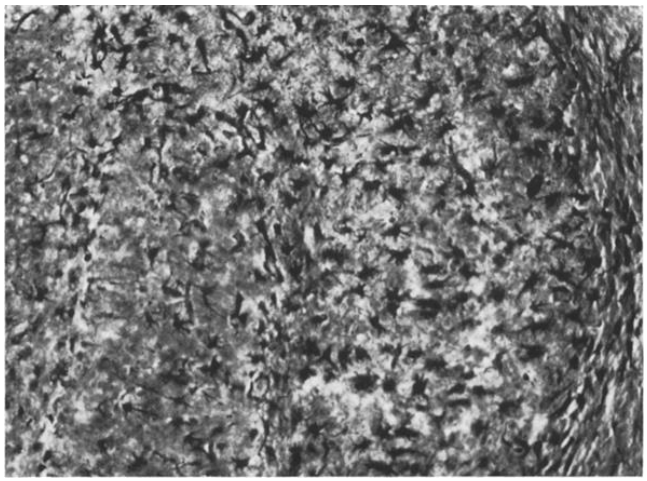

Fig 8. Ammon's horn of 50-day-old mouse with Zika infection. [28]

The normal animal at eight weeks has wispy and poorly stainable astroglial cells in the Cajal preparation. Astroglial changes in the baby mouse were limited to Ammon's horn, but in the 5-week-old animals infected astroglia was prominent throughout the cortex. In the cortex of the baby mouse there were many nerve cells with "empty" vesicular nuclei and some interstitial scattered nuclear chromatin particles (Fig. 9 a, b). Perivascular cuffing was not seen. [28]

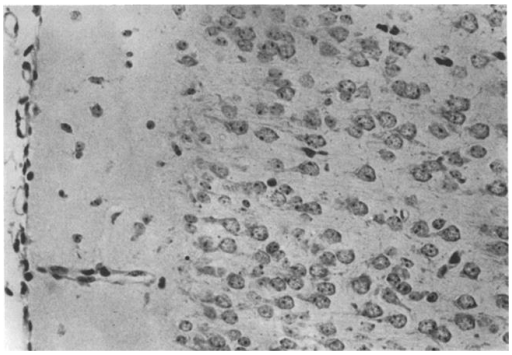

Fig 9a. Cerebral cortex of 7-day-old normal mouse. Note: well stained nuclei. [28]

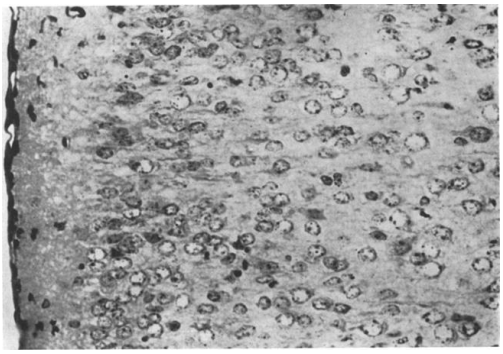

Fig 9b. Same area in 7-day-old Zika infected mouse. Note: margination of chromatin in neurones and nuclear debris. There is no perivasenlar cuffing. [28]

Examination of the cortex and Ammon's horn of 7-day-old mice injected intracerebrally when 1-day-old with the MP 1751 strain of ZIKV revealed numerous cells containing intracytoplasmic inclusions or "virus factories" (Figs. 10,11 and 12). Both glial cells and neurons were affected and more than one "factory" could sometimes be seen in a single neuron process (Fig. 10). In those cell profiles, which included a section of the nucleus, the "virus factories" were usually found in close proximity to it (Fig. 11). [28]

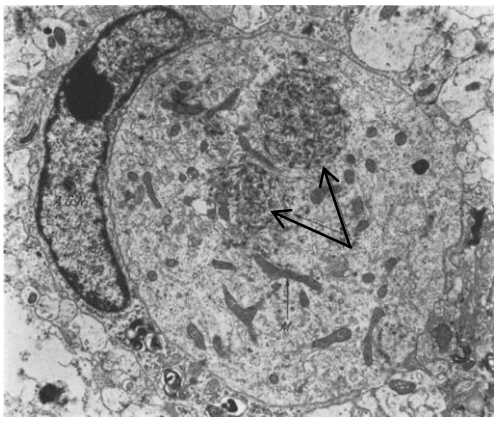

Fig. 10. Ammon's horn of 7-day-old mouse infected with ZIKV. Profile of dendrite embraced by an astroglial cell (AGN). Two virus factories (VF) are visible, of astroglial are dark. [28]

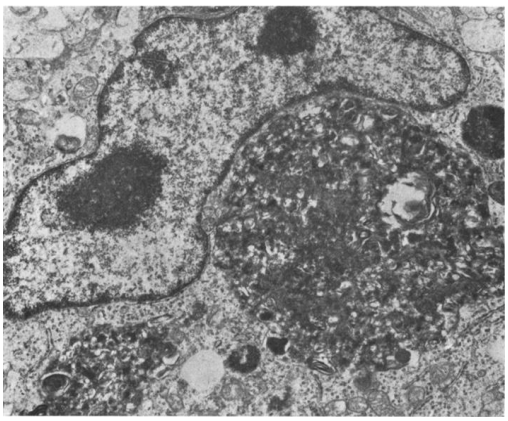

Fig. 11. Virus factory (more advanced stage than fig. 8) in cytoplasm of astroglial cell. [28]

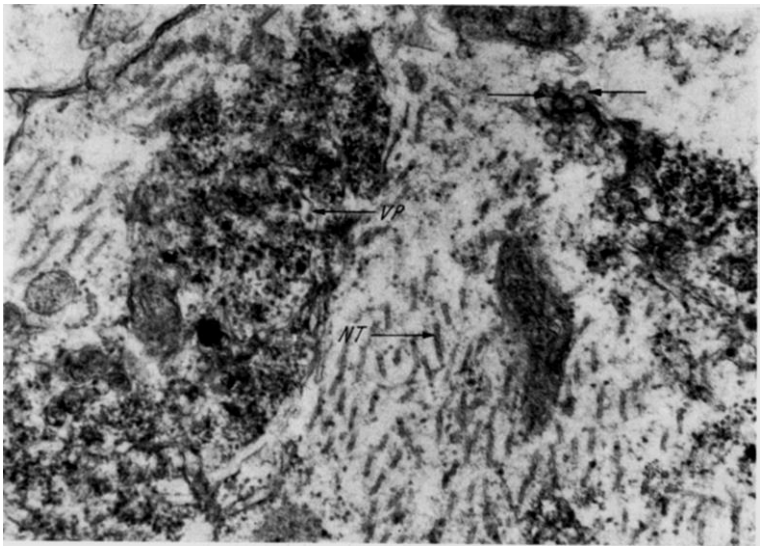

Fig 12. Ammon's horn 7-day-old mouse infected with Zika. Virus factory eontainiug" individual vision (VP) present in cytoplasm of pyriform nerve cell. Neurotubules are prominent (NT). Endoplasmic reticulum contains vesicles (arrows) [28]

The "factories" were composed of a network of endoplasmic reticulum and large numbers of the distended endoplasmic cisternae (Fig. 13). These enclosed numerous vesicular bodies within which there was a fine reticular network. Dense-cored particles, with the typical appearance of group B arboviruses, were found throughout the "factories". The virions were frequently found in short chains within tubular elements of endoplasmic reticulum, which appeared to be in continuity with the distended cisternae (Fig. 13). The cytoplasm of infected cells appeared normal in areas removed from the "virus factories" and large numbers of mitochondria were present in these cells, many adjacent to the "factories". [28]

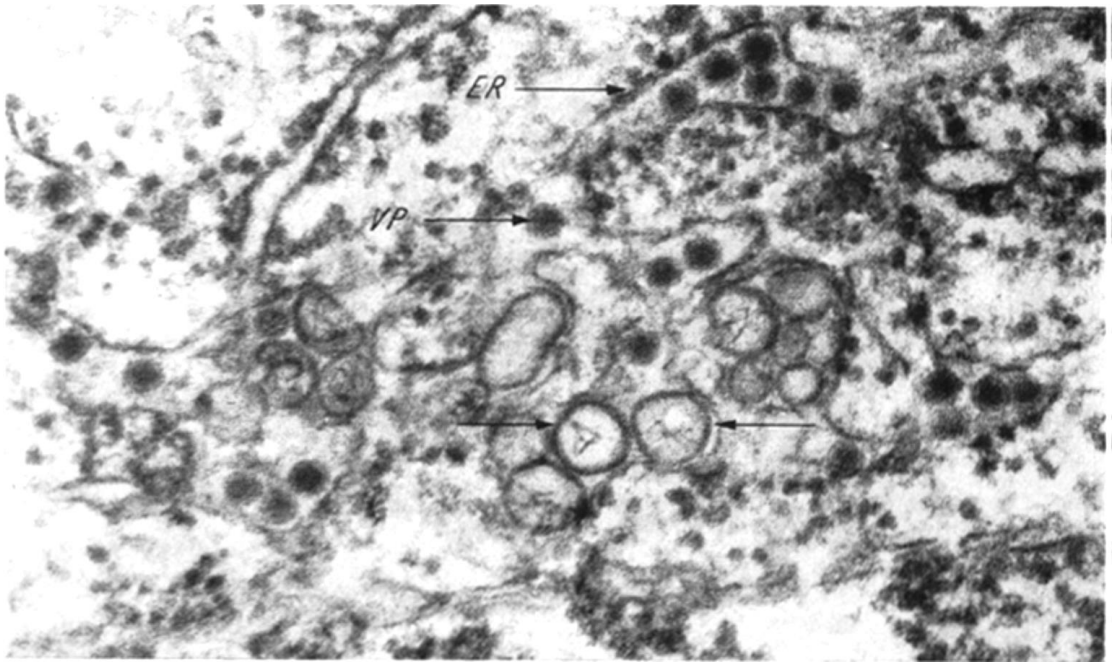

Fig. 13. Thalamus neurone of 7-day-old mouse with Zika infection. Endoplasmic reticulum (ER) contains mature virus particles (VP). Vesicles (arrows) seen in association with endoplasmic reticulum [28]

Diagnosis:

Health care professionals used to diagnose patients by physical exams, revealing any recent travel to areas where Zika is active. [21] Until recently, the test capacity has been limited. Furthermore, Zika cross-reacts serologically with other flaviviruses, such as dengue, West Nile, and yellow fever. Current or past infection, or even a vaccination with another flavivirus, will often cause false positive results. Testing of serum, whole blood and urine is recommended because of the higher viral loads and longer duration of shedding. [22] In addition, laboratory confirmation of diagnosis made with a reverse-transcription polymerase chain reaction (RT-PCR) of viral RNA or serology. However, the RT-PCR has quite limited utility, as it is only positive during a narrow range, 3-7 days after the onset of illness. ZIKV serologic testing has much greater application in the usual clinical setting, but should not be performed until more than 4 days after onset of disease because detectable amount of antibodies immunoglobulin M (IgM) appears only by days 3-5 after an illness. This test measures IgM and neutralizing antibodies. Because they cross react with dengue antibody, specific dengue serology should also be obtained and compared to results for Zika. Acute and convalescent serum determinations are also quite useful in confirming the diagnosis as the level of antibodies increases during acute infection and decreases to during convalescent. [24] Thus, the greatest need and most difficult challenge is the development of accurate antibody tests for the diagnosis of recent Zika infection. Research is urgently needed to identify ZIKV epitopes that do not cross-react with other

flavivirus antigens. [22] The simple ZIKV detection kit that is currently commercially available detects the antibodies to ZIKV (immunoglobulin G and immunoglobulin M) that are produced in the bodies of infected humans, and is therefore not suited for diagnosing Zika fever in the initial stage of infection. In order to make the early identification of ZIKV infection possible, Tanaka Kikinzoku Kogyo applied unique antibody screening technology, which selects antibodies, suited to the target purpose from among a range of antibodies, and the nano-colloidal gold manufacturing technologies, which Colloidally dispersed particles of nano sized gold as a mean to generate visualized color of the assay signal thus helps in rapid diagnosis of ZIKV. [26]

Treatment:

A. Vaccines:

As of now, there is no vaccine available to prevent ZIKV infection. [21] The researchers from The National Institute of Allergy and Infectious Diseases (NIAID) decoded the structure of ZIKV and trying to work on the specific drugs against ZIKV infection. [24]

B. Medication:

Treatment for ZIKV is directed at relieving the symptoms of infection. If diagnosed with Zika, get plenty of rest and drink fluids in order to prevent dehydration. Medications such as acetaminophen (Tylenol) can relieve fever and pain. [21]

Control the virus and Prevention:

We could prevent ZIKV infection by avoiding mosquito bites; the mosquitoes that transmit ZIKV are most active during the daytime. Also by covering as much skin as possible by wearing long sleeves and long pants when indoors. Use a mosquito bed net if your sleeping area is open to the outdoors. Treat gear and clothing with permethrin and follow directions for proper use. You can also purchase permethrin-treated products. Do not use permethrin directly on skin. [21] Recently, two South American metropolises are enlisting bacteria-infected mosquitoes to fight Zika, in the world’s biggest test of an unconventional yet promising approach to quell mosquito-borne diseases. Mosquitoes that carry Wolbachia bacteria which hinder the insects' ability to transmit Zika, dengue and other viruses will be widely released in Rio de Janeiro, Brazil, and Medellin, Colombia, over the next two years. [30] Wolbachia has been used to control the transmission of dengue and other pathogens. [9] Wolbachia pipientis plagues some 60% of insect species worldwide but doesn't naturally infect Aedes aegypti mosquitoes, the species that transmits Zika, dengue and numerous

other viruses. The bacteria can hinder the fertility of their hosts and influence the sex of offspring. They can also block viruses from reproducing in infected fruit flies and mosquitoes. [30]

Host Immune Defense:

In the followed study and many other studies have found that the small membrane-associated interferon-inducible transmembrane proteins (IFITMs) can inhibit the replication of a wide range of pathogenic viruses, including all flaviviruses tested to date, for example; West Nile virus, dengue virus, and reporter viruses carrying the envelope of the Omsk hemorrhagic fever virus. [8]

Murine models of ZIKV pathogenesis require an absence of type I interferon (IFN) signaling, suggesting that IFN-stimulated genes (ISGs) can prevent heightened levels of infection, more evidence of the protective role of ISGs shows in the action of placental cells which can resist ZIKV infection due to the actions of IFN-λ. The IFITMs have been shown to restrict viral replication by blocking fusion-pore formation and the entry of the viral genome and its associated proteins into the cytosol, but the exact mechanism of IFITM-mediated restriction is unknown. However, IFITM1 and IFITM3 inhibit ZIKV infection. IFITM3 plays the predominant role in blocking ZIKV replication, and its effect is dependent on its proper localization within the endosomal pathway. It inhibits the early stages of ZIKV infection that occur after viral-host binding, but before either the viral RNA's cytosolic entry or its early transcription, which suggest that this assay only recognizes viral RNA once it has exited the viral particle and entered the cytosol subsequent to fusion-pore formation. It also prevents the fusion of multiple viruses. [8]

Mutation:

The ZIKV nowadays is not the same as Uganda ZIKV. There are mutations occurring in the Brazil outbreak ZIKV, this mutation is in two amino acid of the sequence of one of its non-structural protein which called NS1. The NS1 is one of the ten Flavivirus multifunction proteins. Some of its functions are unknown, but has had a major role in genome replication and in the modulation of the host immune-system. The mutation in Uganda ZIKV are in the amino acids Glutamate (GLU 146) replaced by lysine (Lys 146) and Tyrosine (Tyr 286) replaced by Histidine (His 286) in Brazil ZIKV. The sequence changes from $ZIKV_{Uganda}$ to $ZIKV_{Brazil}$ show how does the genetic defect has altered the appearance of the NS1 virulence factor, thus its effect on the host immune system. [7] Another study noted that a full-length ZIKV genome traced to the Brazil outbreak and amplified from fetal tissues had five non-synonymous polymorphisms within the ORF when compared with French Polynesian isolate. Remarkably, three amino acid changes were found in NS1. [6] This protein has an

important role in the diagnosis of The ZIKV. By detecting the presence of the secretion of NS1 as hexametric lipoprotein particle of the infected cell. [7] There is a recent study, which illustrated that all the 2015 ZIKV isolates in American and Asian countries, including Brazil, Suriname, Puerto Rico, Guatemala and China clustered closely within the Asian lineage, forming a new American clade. All the circulating strains showed the highest sequence identity to the French Polynesia isolate H/PF/2013, in accordance with the previous analysis. These results suggest the unique imported ZIKA clade from the French Polynesia is spreading through the America. [17]

Zika and Dengue viruses:

Recently there have been several studies evaluating the effect of preexisting DENV antibody on Zika infection. [23] In the case of ZIKV infection, dengue-specific antibodies may do nothing, may lead to enhanced infection of Fcγ receptor-bearing cells, or may protect against infection, depending on the antibody. Broadly crossreactive, highly neutralizing antibodies against dengue also appear to protect against Zika infection. [23] Interestingly, increased numbers of cases of Zika and microcephaly have not been reported from countries in Southeast Asia where dengue has been hyperendemic for generations. In areas such as Thailand, where multiple dengue infections have occurred by early adulthood, which bring out a question, could dengue-induced immunity be broad enough to induce a protective effect against Zika? Discerning the effects of preexisting dengue antibody, both causative and protective, on Zika transmission, transfer across the placenta and clinical outcome is critical to the prevention and control of Zika. [23]

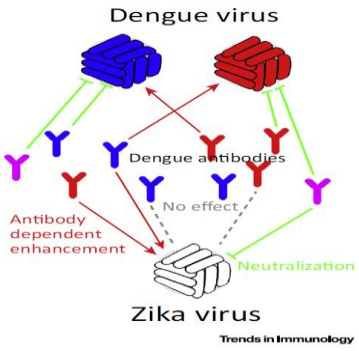

Fig. 14. Antibody-Dependent Enhancement and Neutralization in Flavivirus Infections. [23]

The Insecticides and the Microcephaly:

There are some researchers are denying the linkage between Zika and the microcephaly phenomena. They are relying on the evidences that related the microcephaly to the insecticide as one of its side effects. This insecticide main component is the Glyphosate, which was the major subject

of a study in 2010. This insecticide has been used in the area which documented the outbreak of born babies with brain deficiency. In that study the effects of Glyphosate shown as a gradual loss of rhombomere domains, reduction of the ophthalmic vesicles and microcephaly. It shows also a direct effect of glyphosate on the mechanism of morphogenesis by impairing Retinoic Acid signaling in vertebrate embryos. This increase the apprehension about the clinical findings in the next generations of the population in the regions that have been exposed to this insecticide for a long term. [10] [11]

In 2012, Brazil passed the United States as the largest buyer of pesticides. [10] After the outbreak of the ZIKV in USA the consumed insecticides were enormous that concern the bee keeper after confirming the death of 2.5 million bees by the anti-Zika toxin. [12]

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