An immunological outlook of ZIKV as DENV enhanced secondary infection.

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Abstract

Dengue and Zika are tropical diseases with serological similarity of about 50% and recent major concern is the increased pathogenicity and scorching spread of Zika virus. Viral mutation theories are surpassed by cross reactivity theories because of the high dengue seroprevalence in recent Zika infected areas. In vitro studies on serological cross reactivity of DENV and ZIKV points towards antigenic similarity between different serotypes and uniqueness of DENV NS1 protein and explains antibody depended enhancement mechanism exhibited by ZIKV similar to heterologous secondary DENV infection. These verdicts further prove DENV cross reactivity enhanced epidemic nature of ZIKV and inverse case doesn't result increased pathogenicity. Thus, enabling Zika to be considered as the fifth dengue serotype in vaccine development and treatment strategies.

Key words: ZIKV, DENV, Cross reactivity, Antibody Depended Enhancement.

Introduction

Zika virus and Dengue virus found in humans and arthropods are transmitted across an area by mosquito species *Aedes aegypti* [1]. Both these diseases are caused by virus of family *Flaviviridae* and primary symptoms are similar as rashes in body, fever, headache, muscle and joint pain, conjunctivitis [3].

Even though Zika and Dengue are tropical area viral disease, ZIKV was not considered as a serious pathogen until 2007 Yap state outbreak because most of the infected patients were not be showing symptoms. In Yap state case Zika infection confirmed for 75% of susceptible within in a period of 4 months [2][4]. Zika and dengue has equal risk of developing post infection neurological complications like neuropathy, Guillain-Barré syndrome, myelitis in normal patients and apart from dengue severity of Zika infection will be more in pregnancy with congenital Zika syndrome, where the newborn will be suffering from microcephaly, neural development disorders and congenital malformations [3].

Recent Zika cases from brazil in 2015 point towards increased severity of infection and commenced many researches on increased pathogenetic nature of zika virus. Zika fever is milder than dengue but microencephaly associated with Zika infection was totally unexpected since

Flavivirus infection was never before reported with congenital syndrome. There were many theories came up with the epidemic pathogenetic nature of ZIKV like chances of mutations happened to virus, presence of other flavivirus in affected areas, etc. Later in-vitro studies on serological cross reactivity of DENV and ZIKV came up with conclusion that antibody depended enhancement mechanism is exhibited by ZIKV similar to that of DENV infection because dengue immune monoclonal antibodies were found to enhance heterologous secondary DENV infection.

Structural homogeneity of ZIKV with other flaviviruses, specially DENV results in its immunological cross reactivity [5]. Difference among the 4 serotypes of DENV is 30-35% and ZIKV differs from DENV by 41-46% and this indicate the antigenic similarity of ZIKV to DENV and point towards hindrances in identifying the viruses and similarity in post infectious symptoms [6][7].

Antibody-dependent enhancement (ADE), an immunological phenomenon resulting the severity of secondary infection has been reported in dengue cases as the underlying mechanism behind Dengue Shock Syndrome (DSS) or Dengue Hemorrhagic Fever (DHF) [8] and similarly cross-reactive antibodies of DENV will lead to ADE resulting an increased ZIKV viral replication in infected person with preexisting immunity to any of the DENV serotypes [9]. This proves to the argument that increased severity of ZIKV infection in prior dengue infected areas is due to the ADE mechanism resulted from immunogenic cross reactivity and ZIKV can thus be considered as the fifth serotype of DENV in vaccine development.

Viral transmission

Arboviruses ZIKV and DENV are transmitted to a susceptible vertebrate host in tropical and sub-tropical areas by *Flaviviridae* transmission vector *Aedes albopictus* and *Aedes aegypti* with horizontal transmission by infected saliva injection during mosquito-human-mosquito transmission cycle. Cases of non-vector transmission, i.e. human-human transmission has also been reported via organ transplantation, mother to child and sexual transmission [12].

2015 WHO studies on initial outbreak of ZIKV in 44 countries revealed that epidemic ZIKV infected area has seroprevalence of other arboviral presence like DENV, CHIKV etc. with overlapping or prior infections in endemic area. Cross reaction between ZIKV and other arbovirus is a major dilemma in confirming the infection in its initial outbreak.

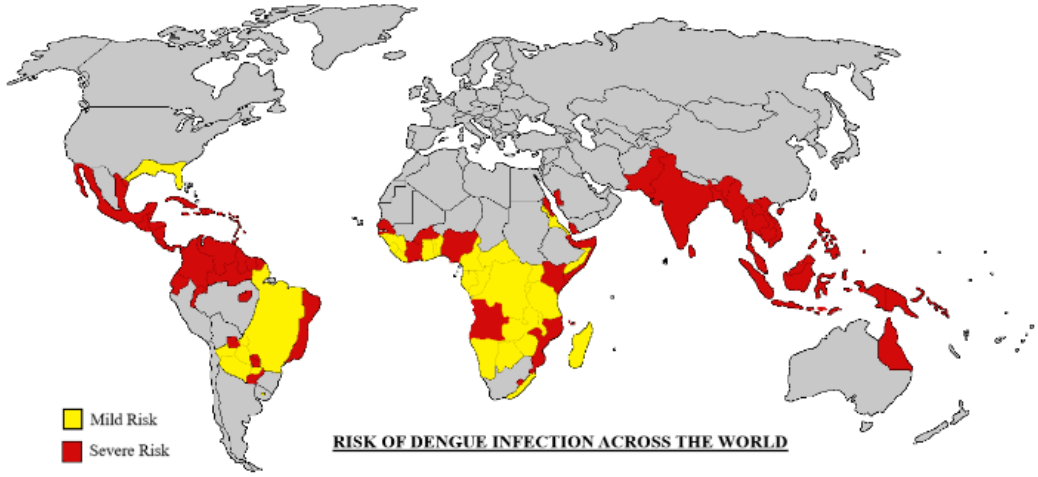

Fig 1. Dengue infected area across the world

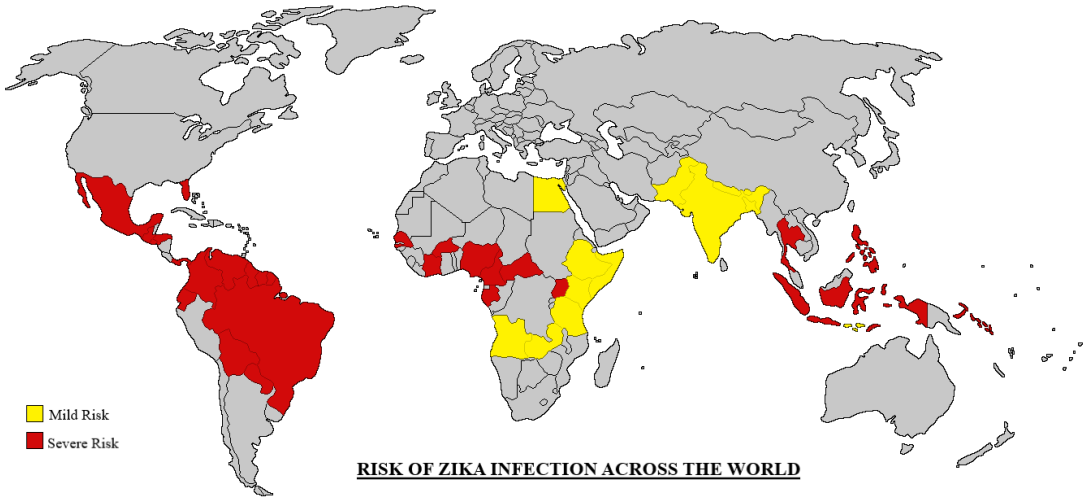

Fig 2. Zika infected area across the world

Topographical similarity to secondary dengue infection

Topographical studies show higher rate of Pre-Zika dengue infection on areas where ZIKV infection became epidemic with congenital Zika syndrome or Guillain-Barré syndrome. Recent ZIKV epidemic areas, viz. Brazil, Yap state, Cameroon, French Polynesia has a prior history of DENV infection which might have resulted in the epidemic status of ZIKV infection.

DENV Antibody Depended ZIKV Enhancement

Dengue virus has a characteristic feature of pathogenicity that infection from one serotype will give immunity against same serotype but if a successive infection from other heterologous serotype occurs existing antibody from primary dengue infection will exacerbate the secondary

infection and this mechanism is called Antibody Depended Enhancement (ADE) of infection. ZIKV also shows the same mechanism with dengue seroprevalence because of high antigenic similarity and this in turn altered evolution of ZIKV pathogenesis.

DENV affected patients were chosen to produce human monoclonal antibodies (mAbs), which interacts with DENV envelope (E) protein in order to examine the cross reactivity of plasma containing antibodies with ZIKV. The results show that these plasma containing antibodies interact with the fusion loop epitope (FLE) of E protein of ZIKV and have very poor neutralizing activity and lead to Antibody dependent enhancement (ADE) in ZIKV [9].

In-Vitro studies shows most of the antibodies target the FLE region of E protein but there are some antibodies that react with an alternative region present on the superficial side of E protein of the virion called E dimer epitope (EDE) [10]. EDE1 and EDE2 are the two subdivisions of EDE which are distinguished on the basis of responsiveness to eviction of N-linked glycan at Asn153 in the envelope protein (the interaction of mAbs to EDE2 results in eviction of Asn 153, on the other hand, no removal of Asn153 takes place while interaction of mAbs to EDE1 region). The mAbs generated against EDE region of DENV was found to be cross reactive to ZIKV. The results have shown, mAbs generated against EDE2 region was found to have lower avidity for ZIKV than EDE1 region, on the other hand, EDE1 has shown neutralizing activity and inhibited ADE of infection with ZIKV [9].

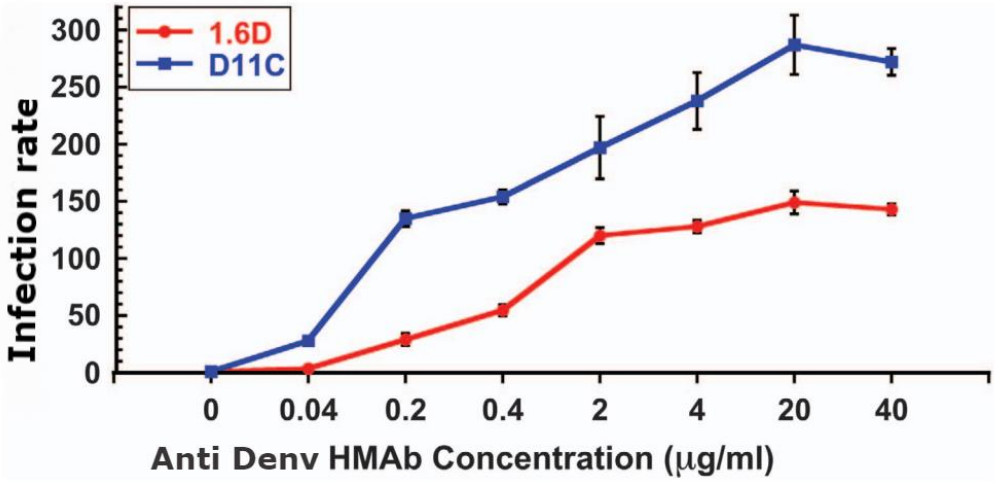

Fig 3. Dengue enhanced Zika infection rate

Anti-dengue human monoclonal antibody (1.6D and D11C) generated against ZIKV indicate strong amplifying infection with ZIKV MR766. From the studies it is shown that a K562 human cell line bearing human Fc receptor is affected by ZIKV ADE from human anti- DENV monoclonal antibodies (HMAbs). These results suggest that an earlier infection from DENV

results in production of a pre-existing antibody which in turn gives rise to ZIKV infection in vivo and also anti-DENV HMAbs, upsurges ZIKV infection as well as the properties of cross-reactivity and non-neutralization are shown. From the investigations, it is clear that human sera play an important role in case of secondary DENV infections where they exhibit both neutralizing and non-neutralizing activity and increase ZIKV infection. From the studies, it is clear that ZIKV ADE and DENV ADE are basically similar and thus increases the concentration of ZIKV in blood of humans [11].

Available studies infer that ZIKV could be regarded as a fifth serotype of DENV, because of the resemblance of ZIKV and DENV, this antigenic similarity results in cross reaction of antibodies and further drives to ADE of infection, which can be regarded as an important parameter for vaccine production of these two viruses.

Inference of prior DENV infection in ZIKV infected areas

Here, ZIKV infection and prior DENV infection of respective area is evaluated from 2000 onwards since this is a cross-reactive infection enhancement study. These data give the interpretation that severity of Zika in a locality was almost similar to the prior dengue infection in same area and all the Zika infected areas after 2000 had a prior history of almost equal severe dengue infection and people were infected with one or multiple strains of DENV in these localities.

Table 1. Epidemic Zika infected area and prior Dengue infection.

Area	**Zika year**	**Zika cases**	**Prior DENV year**
Yap State (Federal States of Micronesia)	2007	414	2004 (DENV 1) 1995 (DENV-4)
Cameroon (West Africa)	2010	12	2006-2009 (DENV-3)
French Polynesia	2014	413	2013 (DENV-1,3)
Brazil	2015	2,952	2007-2014 (DENV-1,2,3,4)

2007 Yap State case- In 2004 May-December there was an outbreak of DENV1 virus in Yap state with 658 clinically reported cases and 12 of them were reported with DHF/DSS. In the April of 2007, Yap State, Federated States of Micronesia was attacked by a sudden epidemic dengue like fever and laboratory testing suggested that the probable causative agent of the outbreak was a dengue virus. flavivirus infection in several patients were confirmed with DENV (IgM)–capture ELISA. RT- PCR with consensus primers for flavivirus generated DNA fragments, which were then when subjected to nucleotide sequencing. Nucleotide sequence was found to be 90% identical to ZIKV. These confirmatory tests indicated that ZIKV and not DENV was the causative agent of the Yap epidemic [13][1].

2010 Cameroon- In western Africa, DENV-3 serotype of Dengue virus was first detected in a traveler from Cameroon returning to Spain in 2006. 19 cases of imported dengue virus infection were reported in travelers from West Africa returning to their countries [14].

2014 French Polynesia- In the January of 2013, Solomon Islands experienced an outbreak of dengue infection. After two months, infections by serotypes DENV-1&3 were identified in patients in French Polynesia of the Pacific Islands. The DENV-3 serotype isolated from French Polynesia was of genotype III. In March of the same year, two patients within the same family were affected by DENV- 3 in French Polynesia. There were also patients affected with DENV-1 that year. By July, 1326 suspected cases of dengue infection were reported. 258 cases were confirmed by laboratory testing with IgM ELISA, NS1 ELISA or RT-PCR. Among the confirmed cases 70 was DENV-1, 73 DENV-3 and 1 cross reactive with both DEN1 and DENV3 [15].

2015 Brazil- There were 440,000 - 1,300,000 cases of Zika infection in Brazil in with 4783 cases of microcephaly, most of which were in the north-eastern region of Brazil and reported 76 ZIKV associated deaths in the region [16]. Brazil had incidence of dengue cases from 2004-2010 with almost 1100000 cases of dengue infection in 2010 and all the four strains of DENV (DENV1, DENV2, DENV3, DENV4) were reported in the area from 2010 onwards [17].

Serotype similarity

In case dengue, T-cell response is in counter to NS3, NS4B and NS5 whereas in Zika T-cell response is towards NS1, NS3 and NS5 proteins. Zika specific T-cell response is towards NS1 protein where as people with seroprevalence of one or more DENV serotype are having higher IFN-γ response against NS5 protein. This implies that cross reactive T-cell identifies more peptides from this region [6].

Table 2. ZIKV similarity to various DENV serotypes.

ZIKV Protein (Strain MR766)	**DENV1 (% Similarity) (Strain Nauru)**	**DENV2 (% Similarity) (strain 16681)**	**DENV3 (% Similarity) (strain Sri Lanka / 1266/ 2000)**	**DENV4 (% Similarity) (strain Dominica/ 814669/1981)**
Capsid protein	40	34	41	38.3838
PrM	40.3614	37.9518	39.1566	41.5663
Envelope	56.5657	52.5253	55.9838	54.1414
NS1	53.9773	54.8295	55.6818	53.6932
NS2A	21.1009	22.9358	21.5596	22.0183
NS2B	36.9231	40.7692	37.6923	38.4615

NS3	65.154	65.316	66.6126	65.9643
NS4A	38.5827	49.6063	40.1575	43.3071
NS4B	48.996	50.4032	50.4032	48.9796
NS5	64.6274	64.5556	64.7778	66.2222

Immunology of infection enhancement

The antibodies that are produced against DENV or ZIKV - E protein was found to be cross-reactive. It is possible that the T-cells are reactive based on the target peptide. Low-level cross-reactivity of the CD4 T-cell between the DENV and ZIKV was identified in DENV/ ZIKV immune human donors. Whereas, T-cell cross reactivity between DENV and ZIKV was observed in normal human and DENV resistant mice after ZIKV infection.

To identify the immunological similarity of the DENV and ZIKV, Structural proteins, capsids and envelop and non-structural protein, NS3 and NS5 were taken. Here, the non-structural protein NS3 and NS5 were primary targets of CD4 and CD8 t-cell response specific to DENV infection. T-cell antigen induced cross reactivity was predicted by modelling potential epitopes from ZIKV proteome that are capable of binding with the HLA class I or HLA Class II alleles or the epitopes capable of binding with the transgenic mice expressing human HLA-B*07:02 and HLA-A*01:01 molecules.

ZIKV and DENV endemic region population innately possessed ZIKV epitopes targeted by CD4 and CD8 T-cells. T-cell response in DENV infection was against the non-structural proteins (NS3, NS4B, NS5) and in ZIKV infection, the T-cells response were against the structural proteins such as E protein, prM and C. Certain unknown regions of DENV and ZIKV were found to be the major targets for cross reacting T-cells.

In sequential DENV/ ZIKV cases cross reactive T-cell dominate response against ZIKV and were identified with $NS5_{2933–07}$ and $NS5_{2973–11}$ amino acids which shares 7 amino acids with DENV1 immune epitope and $NS5_{3253–39}$ showing 67% sequential similarity DENV1 immune epitope [18]. These cross-reactive peptides can stimulate T-cell response towards DENV but not against ZIKV whereas T-cell response of cross reactive ZIKV followed DENV infection is not intense. Thus, it can be interpreted as primary dengue infection enhance following heterologous dengue infection or Zika infection whereas primary ZIKV infection had no enhancement or low enhancement activity towards DENV serotypes.

Neurological complications

Neurological complications associated with ZIKV infection has also been reported with dengue are categorized as neurotrophic, systemic, post-infection [22]. Neurotropic effects associated with DENV infection are Encephalitis, Rhabdomyolysis Meningitis, Myelitis and Myositis. Systemic complications are Stroke (both Ischemic and Hemorrhagic),

Encephalopathy, Papilledema and Hypokalemic Paralysis. Post-Infection impacts include Encephalomyelitis, Acute Disseminated Encephalomyelitis (ADEM), Probable Miller-Fisher Syndrome, Phrenic Neuropathy, Myelitis, Optic Neuritis, Neuromyelitis Optica, Guillain-Barré Syndrome, Long Thoracic Neuropathy, Fatigue Syndrome, Oculomotor Palsy and Maculopathy [20].

Neurological manifestation about Flavivirus encephalitis states that viruses activates the TLR3 pathway in the neural progenitor cells to pro-apoptotic pathway commencement and poor modulation of cell fate decisions [22]. Microcephaly phenotype associated with ZIKV denotes that apoptotic pathways, poorly modulated cell fate and proliferation. TLR3 expression will be more in the early stage and it will decrease with the differentiation of neural progenitor cells and the brain will get mature. ZIKV infection during the early development of the brain denotes the sensitive expression of TLR3 and trimester specific response of fetal brains. TLR3 triggers apoptosis by Ras-ERK and Sonic Hedgehog signaling which plays a part in retinopathy. Identification of candidate genes (Epha3, Ntn1, Adgrb3, Grik2, Slitrk5, Syt11 and Ephb2) were done by comparing the transcriptomic profiles through activation of TLR3 emergence by poly (I:C) and cerebral organoid emergence which is following the microcephaly phenotype by pathway analysis and liable for exhaustion of the neural progenitor population [23]. The results say that the microcephaly phenotype associated with ZIKV denotes the regulation of anti-apoptotic pathway, axonogenesis and cell growth by TLR3 activation of various genetic hub using scalable human cerebral organoid models which are strongly reproducible.

Available diagnosis methodologies

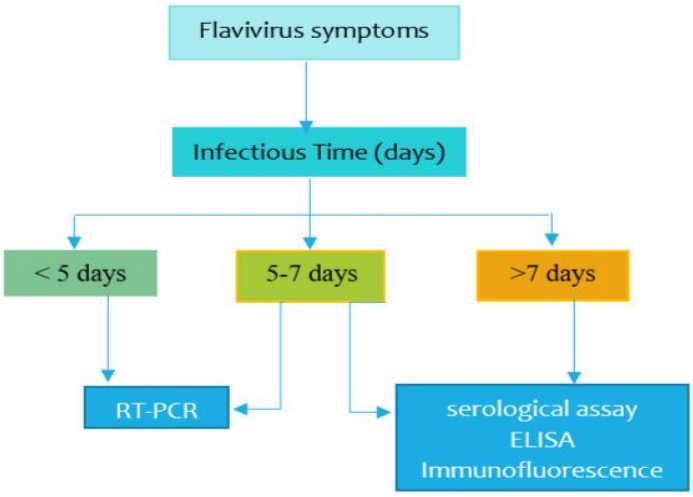

Fig 4. Flavivirus diagnosis protocol

Conventional methods include molecular amplifications (PCR/RT-PCR), Serological testing. Presence of zika virus can be detected by various samples like saliva, urine, amniotic fluids, cerebrospinal fluid, serum can be tested. Conventional testing methods may interpret other viral diseases like DENV. Serological test along with seroneutralization assay (plaque-reduction neutralization test) and MAC-ELISA could specifically distinguish ZIKA and DENV.

Table 3. diagnostic methodologies

Variables	**ZIKV**	**DENV**
Diagnostic methods	Molecular amplification (RT-PCR), Serological assay	PCR, RT-PCR, Serological assay
Specific diagnostic method	Seroneutralization assay (Plaque-reduction neutralization test), MAC-ELISA	ELISA
Samples	Urine, saliva, amniotic fluid, tissue, cerebrospinal fluid, blood.	Blood

Discussion

Zika virus discovered in 1947 wasn't considered as a serious health threat till 2007 yap state outbreak, soon after 2007 it became a serious epidemic with increased reports of Guillain Barré syndrome in 2013 and microencephaly in 2015. Various studies on increased endemic nature of ZIKV which was a dormant pathogen for more than 4 decades after its discovery and abrupt epidemic nature came up with various predictions like mutations happened to virus and seroprevalence of other flaviviruses in epidemic areas. Recent Zika outbreaks were having severe endemic impacts from the earlier outbreaks and these were all hyperendemic dengue areas where people were infected with one or more dengue strains. Structural homogeneity and cross reactivity across flaviviruses can interrogate on protective cross-reactive antibodies as well as enhanced pathogenesis resulting severity of diseases.

In vitro Studies on DENV and ZIKV cross reactivity found that in sequential infection, seroprevalence of DENV can increase ZIKV pathogenesis as well as heterologous DENV pathogenesis by antibody depended enhancement mechanism. Apart from other flavivirus ADE is more relevant in DENV because of its antigenic similarity to different serotypes and uniqueness of NS1 protein and same reason give more relatedness among DENV and ZIKV. In-vitro studies on DENV immune antibodies show less interaction with the fusion loop epitopes ZIKV E protein which indicates less neutralizing activity and thus increasing ADE of ZIKV infection.

T-cell response studies says in normal Zika cases T-cell response is against NS1 protein but with DENV immunity, NS5 peptide will be having more IFN-γ response than NS1 indicating a region of peptides which will be identified by cross reactive T-cells. These cross-reactive epitopes have higher T-cell responses after sequential DENV/ ZIKV infection and results in antibody depended enhancement of secondary Zika infection.

However, there is a trivial argument which states seroprevalence of ZIKV can neutralize heterologous DENV and ZIKV infection, even though discrepancy in the argument can be because of less chance of getting a naïve ZIKV immune donor. Future research on this vital argument can lead to strategy development for infectious disease treatment and vaccine development for various flaviviruses including dengue and zika.

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