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Dengue Fever with emphasis on Vaccines – An update

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ABSTRACT

Dengue virus [DENV] infection is one of the most prevalent diseases transmitted by mosquitoes in tropical and sub-tropical regions. It is considered to be the second-most infectious disease after COVID-19. Approximately 3.6 billion individuals, including over 50% of the global population, reside in regions characterized by a significant susceptibility to Dengue virus (DENV) infections. According to available data, the total number of DENV infections is estimated to be approximately 390 million, with approximately 96 million of these infections resulting in symptomatic manifestations. The infection is a harbinger of non-specific fever, acute febrile viral rash, debilitating headaches, and in extreme cases can cause various physiological afflictions that affects the liver, clotting system, complement mediated immune response, hematopoiesis and the vascular systems. Due to the severity and the illness being widely spread across the globe, vaccine research has been the priority to mitigate the negative effects of this disease. There are various types of vaccines that are being actively researched upon all across the world. The setbacks that have been occurring in vaccine development were mainly due to the occurrence of various serotypes and the problem with the virulence of the dengue virus. However, with the research going on about the virulent parts of the virus, an effective vaccine would come up that can hopefully help tackle the severity of dengue illness.

KEYWORDS: Dengue virus, Vaccine, Serotypes, Pathophysiology, Detection

ABBREVIATIONS:

ADE: Antibody-dependent enhancement

CYD: chimeric yellow fever dengue vaccine

DENV: Dengue Virus

DHF: Dengue Haemorrhagic Fever

DNA: Deoxyribonucleic Acid

DSS: Dengue Shock Syndrome

DTT: Dichlorodiphenyltrichloroethane

DVPS: Dengue Vascular Permeability Syndrome

ELISA: Enzyme-Linked Immunosorbent Assay

GMP: Good Manufacturing Practice

HIV: Human Immunodeficiency virus

HLAs: Human leukocyte antigens

JEV: Japanese encephalitis virus

NIH: National Institute of Health

NVK: Nilavembu kudineer

RNA: Ribonucleic Acid

RT-PCR: Reverse-Transcriptase Polymerase Chain Reaction

THF: Follicular Helper T cells

ULV: Ultralow Volume

WHO: World Health Organization

WNV: West Nile Virus

1.0 INTRODUCTION

The rainy season brings relief to harsh summers but also is a harbinger of various mosquito-borne diseases like dengue, malaria, etc. Among these, viral diseases tend to be the most dangerous to tackle. Dengue virus (DENV) infection is widely recognized as one of the most prevalent mosquito-borne illnesses in tropical and subtropical countries. As per the 2021 WHO report [1], the infected cases of dengue viruses globally per year have reached up to about 100 to 400 million. This virus is endemic

to Africa, Eastern Mediterranean, the Americas, Southeast Asia that includes India, and the Western Pacific[2].

Based on a recent assessment by the World Health Organization (WHO), the escalating incidence of dengue illnesses positions it as the second most contagious ailment, following COVID-19 [1]. The main nations that have reported this virus are the Vietnam, Philippines, Colombia, India, and Brazil[3]. The primary cause of an increase in the total number of dengue instances is rapid urbanization combined with poor infrastructure development, which may result in ineffective vector control management[4].

Also, personal travel and business trips worldwide have led to the spread of these diseases into newer areas [5]. The global situation during the COVID-19 pandemic has been exacerbated by various causes. These include the misdiagnosis of cases, delays in the early and proper treatment of vector-borne diseases, challenges in implementing regular strategies for vector control and prevention, and inconsistencies in government finance support[6].

1.1 ABOUT THE VIRUS

DENV is a Flavivirus that belongs to the Flaviviridae family[7]. DENV have 4 different serotypes which are antigenically distinct. Those serotypes are known as DENV1, DENV2, DENV3, and DENV4 [8]. DENV5 a sylvatic strain has been isolated. DENV is an icosahedrally-enveloped virus with an 11-kilobase-pair positively stranded RNA[9,10]. This encodes for three proteins that are structurally distinct from one another: the capsid protein [C], envelope [E], membrane [M], and seven non-structural [NS] proteins [11]. The assembly of the virus is reliant upon the significance of these structural proteins. The interaction between the C protein and RNA facilitates the assembly of the nucleocapsid [12].

The M protein is important for the formation of the mature virus particle in the E protein which helps in mounting various immune responses against flavivirus infections[13]. Additionally, this protein serves as a surface protein that aids in the attachment and fusion of the virus with the host cell membrane[14].

1.2 NATURE OF ILLNESS AND BURDEN OF THE DISEASE

The classification of dengue illness encompasses two categories: dengue and severe dengue. The sickness can be categorized into three distinct phases: the acute febrile phase, the critical plasma leakage phase, and the convalescent or reabsorption phase. The disease is characterized by two clinical symptoms, namely plasma leakage and aberrant haemostasis, which serve to underscore the severity of the condition[15].

According to this categorization, individuals were classified as either having dengue fever, which represents the most prevalent form of DENV infection and typically presents as a non-specific febrile disease. Dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS) is characterized by the simultaneous occurrence of plasma leakage and coagulopathy, resulting in bleeding that can precipitate a sudden decline in blood pressure, leading to circulatory shock and organ dysfunction. The manifestation of symptoms is contingent upon the specific serotypes of the Dengue virus, namely DENV-1, DENV-2, DENV-3, and DENV-4[8,16].

1.3 GLOBAL AND NATIONAL PREVALENCE

The tropics already had been rampant with dengue infections throughout the eighteenth and nineteenth centuries. The accelerated dissemination over the twentieth and twenty-first centuries can be attributed to the forces of globalization and increased mobility. Multiple viral serotypes started spreading in various areas of the world making them hyperendemic. Hyperendemic means the co-circulation of multiple viral serotypes in the same place[17].

During the 1950s, a dengue epidemic occurred in the South-East Asia. The incidence of the epidemic also had a significant surge during the 1970s and 1980s, resulting in the worldwide dissemination of viruses and mosquito vectors, hence facilitating the extensive transmission of Dengue virus (DENV) throughout tropical and subtropical regions. The broad transmission can be attributed to several factors, namely human population increase, urbanization, modern transportation, global trade, and inadequate mosquito growth control measures. The vector competence of the mosquito population is subject to alteration due to the emergence of novel strains or the decline of herd immunity[18].

Approximately 3.6 billion individuals, including over 50% of the global population, reside in regions characterized by a significant susceptibility to Dengue virus (DENV) infections. According to available data, the total number of DENV infections is estimated to be approximately 390 million, with approximately 96 million of these infections resulting in symptomatic manifestations. Among the symptomatic cases, approximately 2 million individuals experience severe disease, and the annual mortality rate is estimated to be around 21,000 deaths [19].

Children also get affected between 5-15 years of age in Asia, and people belonging to the age group of 19-40 years are infected in the American tropics. In Africa and isolated islands of the Indian and Pacific Oceans, despite rampant infections, most of the cases either under reported or are not reported at all [20].

The economic consequences of dengue are less studied. The magnitude of dengue infection severity is not as high as most viral infections, but the direct and indirect costs are quite high[21].

Examining the context in India, the initial occurrence of a widespread outbreak resembling dengue fever was documented in Madras (now known as Chennai) in the year 1780. Subsequently, the first confirmed epidemic of dengue fever in India, supported by virological evidence, transpired in Calcutta and along the Eastern Coast of India during the period of 1963-1964[22]. There has, however, been an upsurge in infections since the mid-1990s[23].

India accounts for around 34% of the worldwide prevalence of dengue, a significant burden on a global scale[24]. Moreover, the fatality rate associated with this disease in India has been estimated to be 2.6%[25]. A significant proportion of cases remain unreported as a consequence of the extensive population size and the weakening of the public health surveillance system.. Dengue surveillance is conducted by a comprehensive network consisting of over 600 sentinel hospitals, which operate under the purview of the National Vector-Borne Disease Control Programme (NVBDCP) and the Integrated Disease Surveillance Program (IDSP). Additionally, this surveillance system is supported by a network

of 52 Virus Research and Diagnostic Laboratories (VRDLs). Tamil Nadu constitutes a substantial portion of the southern region of India, characterized by abundant rainfall and verdant landscapes. It is noteworthy that this region experiences a considerable load of dengue illness[26].

A separate investigation has conducted an estimation of the force of dengue infection (FOI) within India, revealing a significant FOI among youngsters. This analysis utilized datasets gathered from several locations including West Bengal, Andhra Pradesh, and Mumbai. Although there is need for improvement in data quality, including enhanced monitoring and more comprehensive serotype detection, it is evident that the incidence of dengue cases in India is increasing[27].

This conclusion is supported by another study on age distribution, which indicates that individuals below the age of 10 were the most frequently impacted by dengue [23].

1.4 VACCINES AND TREATMENT OPTIONS

The antibodies protection by the immune system can give lifelong protection to the infection-causing serotype but not the other three serotypes[28].

The neutralizing antibodies target the E protein, however, in some infections, it isn't enough. Naturally, there are various antibody responses like antibody-dependent cell-mediated cytotoxicity and complement fixation. T cell-mediated functions also contribute to natural protection[29].

Vaccine development research has been moving in full swing for ages. Ideally, the vaccine should confer protection against all four serotypes of Dengue virus (DENV) and elicit long-lasting immunity in those who are susceptible to such infections. Some individuals have waning or non-protective immunity due to previous dengue infection.

Live attenuated vaccines work by inducing the immune response and conferring lifelong immune memory. Initially, such vaccines were developed by passaging this virus into two cell lines to modify them to make it less virulent. However, there had been cases of vaccines becoming harsh and stimulating adverse reactions from people leading to such trials being stopped. Mutation was then induced in the DENV RNA and RNA vaccines were developed[30,31].

Subunit vaccines have also been developed but the requirement of adjuvants and several doses are required to overcome its lack of long-lasting immunity. Subunit vaccines target the E glycoprotein. Recently, the Domain III-capsid protein has also been used to induce antibodies and stimulate cell-mediated immunity[32].

In addition to viral interference, inactivated vaccines have been designed to elicit a well-balanced immune response. There exists no potential for viral replication or reversion to the original wild-type virus. Nevertheless, these alternatives exhibit lower efficacy compared to live-attenuated vaccines, necessitating the administration of several doses and the inclusion of adjuvants[33].

DNA vaccines are also gaining traction in this field due to their non-replicative ability, safety, and low reactogenicity. They also come at low cost, ease of production, and storage temperature[33].

Moreover, the absence of a suitable animal model for Dengue further complicates research efforts. This limitation hinders our understanding of the virus's pathogenesis and the development of effective treatments. Cross-protection of vaccines against various flaviviruses adds another layer of complexity, necessitating careful consideration to avoid unintended consequences. Efforts to enhance Dengue treatment effectiveness warrant extensive research.

The setbacks in vaccine development were due to the occurrence of various serotypes and the problem with the virulence of the dengue virus. However, with the research going on about the virulent parts of the virus, an effective vaccine would come up that can hopefully help tackle the severity of dengue illness.

2.0 DENGUE VIRUS

2.1 DISCOVERY

The initial identification of the Dengue virus occurred in 1943 when Ren Kimura and Susumu Hotta effectively isolated the virus from blood samples collected from individuals affected by the dengue outbreak in Nagasaki, Japan. Following a period of one year, Albert B. Sabin and Walter Schlesinger conducted separate investigations that resulted in the isolation of a specific strain of the dengue virus, which is currently identified as the dengue virus-1 [DEN-1][34].

Another serotype DEN-1 along with DEN-2 was later found in Central America and Africa in the 1970s. By 2004, the widespread occurrence was seen. Scientists hypothesize that these viruses evolved from non-human primates and crossed over to humans between 500 and 1,000 years ago.

2.2 INITIAL UNDERSTANDING OF THE DENGUE ILLNESS

Dengue is a severe viral infection that can be fatal due to complications. During the Jin Dynasty (265-420 AD) in China, this phenomenon was once referred to as "water poison" and was linked to the presence of flying insects. The term "Dengue" originates from the Swahili language and is derived from the expression "Ka-dinga pepo," which denotes a seizure characterized by cramp-like symptoms. The initial outbreaks of epidemics primarily occurred within the regions of Asia, Africa, and North America during the 1780s. The inaugural clinical case report dates back to 1789, specifically documenting the 1780 pandemic in Philadelphia, as authored by Benjamin Rush. Due to the symptoms of myalgia and arthralgia, he called it “break-bone” fever. The actual term “Dengue” began getting used only after 1828.

2.3 IMPORTANCE OF SEROTYPES

Dengue illnesses are attributed to four viruses that are closely related, namely DEN-1, DEN-2, DEN-3, and DEN-4. The classification of these entities is attributed to their distinct interactions with antibodies present in human serum. Hence, a person suffering from one serotype infection cannot gain resistance against other serotypes. They are similar, sharing approximately 65% of their genomes

but have some genetic variation. However, these variations result in the same severity of disease and symptoms during infections. They have spread in occurrence across various regions due to many reasons like migration and travel.

Indeed, a recently identified serotype of Dengue virus (DENV) was detected in the region of Sarawak, Malaysia. Initially, the present case was hypothesized to represent a sylvatic dengue infection caused by DENV4, characterized by the transmission between Aedes nivalis mosquitoes and non-human primates[35]. Following an extensive research endeavor and rigorous genetic verification procedures, the novel DENV5 serotype, initially observed in the forests of South-East Asia, was successfully discovered[36]. The precise mechanism of DENV5 transmission remains a subject of ongoing investigation. However, one hypothesis posits that the sylvatic strain undergoes genetic changes, leading to the emergence of human strains [37].

The novel DENV5 serotype exhibits clear phylogenetic divergence from the four pre-existing serotypes[36]. This observation suggests the formation of novel strains of the Dengue virus (DENV) as a result of factors such as zoonotic transmission, temperature fluctuations, and disruptions to ecosystems [38].

2.4 MECHANISM OF INFECTION

The transmission of the dengue virus (DENV) to human hosts occurs via the introduction of the virus into their circulatory system, facilitated by the bites of mosquitoes that are carriers of the virus, such as Aedes aegypti or Aedes albopictus[39]. During the early stages of infection, the dengue virus exhibits a special affinity for dendritic cells and macrophages, designating them as the principal locations for viral entry and attachment. Subsequently, a sequence of intracellular processes ensues, commencing with the fusion of virus and endosome membranes, followed by the liberation of the viral nucleocapsid, synthesis and processing of viral proteins, replication of viral RNA, formation of the nucleocapsid, assembly of viral components, maturation of the virus, and ultimately culminating in the release of fully matured dengue virus particles.

The process of DENV binding to the host cell entails the engagement of mannose receptors, heparan sulfate, and various other molecules present on the cell surface. [40]. Following this, the Dengue virus (DENV) enters the host cell by clathrin-mediated endocytosis, and subsequently merges with the endosome membrane through fusion. As a result, the RNA genome is subsequently released within the cytoplasm. Following this, the RNA genome assumes the role of messenger RNA (mRNA) and subsequently engages in the process of translation, leading to the production of viral proteins. The progression from mild to severe forms of Dengue is associated with a complex interplay of factors, including the individual's immune response, the specific serotype of the Dengue virus, and other host and environmental factors.

2.5 RARE ROUTES OF INFECTION

Another contributing factor to the increase in cases is the occurrence of infrequent instances of non-vector transmission of dengue virus (DENV). The various modes of infection transmission include

mucocutaneous exposure, needlestick incidents during patient care and laboratory accidents, blood transfusion, bone marrow and organ transplantation, intrapartum and perinatal transmission, as well as breastfeeding[41–45]. In a particular instance, a healthcare professional contracted an infection due to a blood splash resulting in mucocutaneous exposure. Numerous neonates have been afflicted with infection by vertical transfer from the placenta or through breastfeeding. Confirmation of aerosol-based and sexual transmission of dengue virus (DENV) in humans is still lacking in academic literature. Understanding the incidence of viral load in different bodily fluids is crucial for assessing the potential for non-vector transmission between individuals who are infected and those who are not. Gaining a comprehensive understanding of the non-vector transmission modes in JENV and ZIKV would provide valuable insights on the uncommon transmission mechanisms of DENV [46].

2.6 STRUCTURE OF THE VIRUS AND EFFECTS

The DENV virus is characterized by its icosahedral envelope structure and possesses a single-stranded RNA molecule with a positive polarity, spanning a length of 11 kilobases[47]. This RNA encodes for three structural proteins namely capsid [C], envelope [E], and membrane [M] and seven non-structural proteins like NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5[11]. Viral assembly is generally overseen by structural proteins. The C protein interacts with the RNA to form the nucleocapsid. E protein and M protein interact to form the mature virus particle which helps generates immune responses against various flavivirus infections[48]. The non-structural proteins and cellular proteins play crucial roles in facilitating several processes of the virus, such as genome replication, translation, encapsidation, and proper folding of viral proteins[10].

2.7 THE MOSQUITO VECTORS

The primary vector *Aedes aegypti* is believed to have originated from Africa and is a ubiquitous feral species there. While this was restricted to this area until the 18th and 19th centuries, they later spread to North America and Europe by river boats, ships, and other means of transportation, leading to the occurrence of the dengue epidemic. The longevity of most mosquito species is facilitated by their capacity to seek appropriate host plants for sugar consumption and to find mates for reproductive purposes. Nevertheless, in contemporary ecological circumstances, their ability to develop resistance against insecticides and adapt to changing environments plays a crucial role in ensuring their continued survival[49].

The species would then highly become adapted to humans and their environment away from the city. The species were probably first adapted to the port cities before they infested the inland cities. Another vector, *Aedes albopictus* is also said to be involved in the spreading of the illness. DENV was also said to be transmitted in sylvatic cycles by *Aedes niveus* [7].

2.8 ETIOPATHOGENESIS [IN CHILDREN AND ADULTS]

The dengue virus gains entry through the skin from an infected mosquito’s bite. Generally, the host immune responses start only after the rapid clearance of viral load from the host organism. So, severe clinical presentation from the disease doesn’t match with the high viral load [50].

2.8.1 PATHOPHYSIOLOGY

Most infections present themselves as either asymptomatic or with non-specific symptoms such as fever, acute viral rash, and various physiological disruptions affecting the liver, blood coagulation, complement, hematopoiesis, and vascular systems.

A) Vascularly Permeability

This is known also as DVPS [Dengue Vascular Permeability Syndrome] and involves abnormalities of the vascular system like capillary permeability and plasma leakage. This often occurs during dengue fever onset[51].

One model of vascular permeability in dengue is associated with a 'cytokine storm' caused by overactive T cells[13].

B) Thrombocytopaenia

Thrombocytopenia occurs due to transient bone marrow suppression during the early convalescent and febrile stages of the disease. The platelet levels can go as low as 5,000 platelets /ml (in comparison with 200,000 platelets/ml in healthy humans). This is seen in acute DENV infections. However, towards the end of the febrile period of a DENV infection, the bone marrow recovers [52].

C) Coagulopathy

The coagulation system interferes with the homeostatic control of clot generation. Viral effectors frequently induce modifications in the levels of anti-coagulant proteins. According to existing literature, it has been postulated that the viral NS1 protein has an affinity for thrombin, leading to the formation of complexes. This phenomenon is generally of a somewhat insignificant nature and typically resolves after a short span of a few days[53].

D) Complement Activation

By interacting with the coagulation system, this system is triggered to control the DENV infection, which leads to pathogenesis. The majority of investigations on complement activation in dengue have focused on individuals with subsequent DENV infections and concluded that complement activation was mediated by circulating immune complexes via the classical pathway. In contrast, research on complement activation in babies with primary DENV infection have suggested an alternate mechanism in complement activation during infection[53].

E) Liver Enlargement

This is a common phenomenon during DENV infection as it is associated also with dengue fever. Children with severe dengue showed signs of hepatomegaly. This occurs either due to vascular permeability or inflammation due to hepatocyte infection. However, jaundice due to dengue illness is rare. Changes in liver enzymes are, however, observable like ALT and AST changes in children with

DHF [dengue hemorrhagic fever] is often seen. Additionally, a study revealed that a subset of patients exhibited elevated levels of serum bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase, with percentages of 7%, 16%, and 83% respectively[54,55].

2.8.2 THE SUSCEPTIBLE POPULATION

While the young and the elderly have less threshold for leakage than adults, primary infections can be asymptomatic in children, but they are more susceptible to vascular leakage and development of DSS. Pregnant women also are susceptible to dengue-linked mortality, however, fetal malformations because of dengue are not yet reported[56–59].

2.8.3 COMORBIDITIES AND COMPLICATIONS

Comorbidities associated with dengue illness are diabetes, sickle cell anemia, and bronchial asthma, including hypertension. Complications include flushed skin and rashes. Musculoskeletal symptoms among adult cases are very common. Myalgia and CK levels tend to be elevated in dengue infections. There also could be complications of the GI tract such as nausea, vomiting, diarrhoea, and pain. Liver dysfunction with increased hepatic transaminase levels is very common [60].

2.9 CO-INFECTIONS WITH OTHER DISEASES

Significant co-infections comprise malaria, chikungunya, enteric fever, scrub typhus, and leptospirosis, which frequently exhibit symptoms that may be mistaken for those of a dengue infection. Malaria, Zika virus, and chikungunya are vector-borne diseases spread by mosquitoes. Consequently, the possibility of co-infection with these diseases is a plausible occurrence[61].

The occurrence of dual illnesses including enteric fever frequently leads to the failure to promptly diagnose and effectively manage cases of dengue shock. The administration of a set of antibiotics during the first treatment of dengue is undertaken with the aim of preventing the occurrence of complications. The occurrence of co-infection with scrub typhus is commonly observed in the majority of cases with dengue illness. There has been a notable rise in the occurrence of coinfection between dengue and leptospirosis in many regions of India, particularly in the southern states and Maharashtra, mostly attributed to the elevated incidence of rainfall and subsequent floods. Stagnant water functions as a conducive environment for the proliferation of mosquitoes, which in turn facilitates the transmission of leptospira, a pathogenic bacterium, through the medium of water and rat urine[62].

In recent times, the COVID-19 pandemic has resulted in an increased prevalence of co-infection morbidities with dengue, owing to the similarity in symptoms exhibited by both diseases and the widespread transmission of the COVID-19 virus. The diagnosis of both conditions is frequently

conducted in order to facilitate the effective treatment of the diseases. Additional uncommon co-infections are Hantavirus infections, hepatitis-A, and typhoid infections[63].

3.0 HOST FACTORS AND ENVIRONMENT

3.1 HOST FACTORS

A few factors of the hosts that play an important role are age, genetics, and immune response. Age is a risk factor in dengue infection severity as the incidences of DSS are high in children and it could be fatal for them. They are more prone to plasma leaks due to more permeable vascular endothelium. Older people also have comorbidities and are prone to many complications and fatalities could be high.

Genetics also can determine susceptibility to dengue infections. People with African ancestry have a reduced risk of dengue illness severity[64]. Human leukocyte antigens [HLAs] have been said to be linked with disease protection. Host immune response also contributes to many clinical presentations. In fact, many complications become severe if patients have a previous history of dengue illness or if children have the maternally obtained anti-DENV antibody [7].

3.2 ENVIRONMENT, CLIMATE AND SPREAD

Mosquitoes responsible for the dengue virus spread tend to inhabit the tropical and subtropical regions of the world. The hot, humid climates tend to help propagate mosquito growth. The Indian subcontinent and most Asiatic regions tend to also have heavy rains that lead to stagnant water remains which is the breeding ground for mosquitoes. The mosquito population initially did not depend on animal blood for nutrition and used to ingest plant resins and discharges.

However, human settlements near forests, deforestation, and habitat loss that occurred subsequently forced the mosquitoes to require human blood for nutrition during the egg-laying season to nourish the larva. Gradually, the loss of forests and artificial water bodies made mosquitoes survive in cities and flourish during the warm and humid rainy seasons. While the virus propagated and used mosquitoes as vectors to enable survival, it also got carried to other places during travel [65].

Climate changes have also expanded the survivability of mosquitoes. Initially, mosquitoes could not survive in cold, dry areas. However, due to global warming and El Nino effects, sudden rains, and unexpected humidities have enabled mosquitoes to survive easily in most areas. This leads to more widespread dengue occurrences and thus, has made it an epidemic.

Climate factors play a crucial role in the lifecycle of *Aedes aegypti* mosquitoes, impacting the rate of viral replication, mortality of affected populations, and mosquito behavior. Numerous studies have identified associations between climate variables and dengue transmission, highlighting the specific climatic conditions in different environments, such as the correlation between rainfall patterns, local temperature, and optimal viral transmission[66,67]. However, some uncertainties remain, such as the

intensity and frequency of climate impacts associated with the La Niña and El Niño. La Niña is a climate phenomenon characterized by the cooling of sea surface temperatures in the central and eastern equatorial Pacific Ocean. It is the counterpart to El Niño, which involves warmer sea surface temperatures in the same region. Both El Niño and La Niña can have widespread impacts on weather patterns around the world, including influencing the transmission dynamics of certain diseases like Dengue fever[68,69]. The relationship between La Niña events and Dengue fever is complex and varies by region. Generally, La Niña tends to be associated with increased rainfall in some areas, creating conditions that favor the breeding of Aedes mosquitoes, the primary vectors of the Dengue virus[67]. The life cycle of the *Aedes* mosquitoes, particularly Aedes aegypti, is closely tied to water availability, and increased rainfall can lead to the creation of more breeding sites. La Niña is often associated with above-average rainfall in some regions. This increased precipitation can create more breeding sites for *Aedes* mosquitoes, as they lay their eggs in standing water. This, in turn, may lead to higher mosquito populations and an increased risk of Dengue transmission. The favorable conditions for mosquito breeding created by La Niña may lead to the expansion of areas at risk for Dengue transmission. Regions that may not typically experience high Dengue activity could see an increase in cases during La Niña events[68,70]. It is important to note that while La Niña may contribute to favorable conditions for Dengue transmission, other factors such as local public health measures, population immunity, and the effectiveness of mosquito control efforts also play a crucial role in determining the actual impact on Dengue transmission. Additionally, the relationship between climate phenomena and disease dynamics is complex and can vary across different regions. Public health authorities and researchers monitor these relationships to better understand and respond to the changing patterns of infectious diseases. Pathogenesis of viral infection is described in figure 3.

4.0 CLINICAL FEATURES [CLASSIFICATION, BASICS]

Dengue infection affects people in three phases: the febrile phase, the critical phase, and the recovery phase [71].

1. The febrile phase occurs the first week with high fever, flu, headaches, vomiting, and joint pain.
2. The critical phase is the potentially fatal phase due to symptoms like plasma leakage and internal bleeding.
3. The recovery phase is where the symptoms get milder due to the recovery of vascular permeability.

There exist three distinct categories of dengue infections, including dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), which is a severe consequence characterized by rapid hypovolemic shock. The normal progression of symptomatic dengue infection involves an initial incubation period of up to two weeks, typically lasting between five to seven days, following a mosquito bite from an infected individual. Subsequently, symptoms manifest in three distinct phases, as previously described, with a duration of up to five days. During the febrile phase, individuals may experience an abrupt start of elevated body temperature, headaches, general

discomfort, feelings of illness, nausea, vomiting, and discomfort in the joints. In addition, symptoms such as cough, rhinorrhea, and sore throat may also manifest[60,72].

The critical phase happens from around 3–6 of illness, and vasculopathy occurs where the permeability of vasculature increases causing capillary leakage syndrome. This can also lead to cardiovascular collapse in hours. In some cases, hemorrhagic manifestation bleeding can also occur in patients with shock. Children who do not exhibit signs of shock may experience minor epistaxis, gum bleeding, and gastrointestinal bleeding. During the critical period, it is common for individuals to experience varying degrees of thrombocytopenia, leukopenia, and disturbed hemostasis[73].

In the recovery phase, all the complications become milder and resolve within 48–72 hours. However, poor function of certain specific organs occurs long after the resolution of infection. Many people have also reported profound tiredness, depression and asthenia as post-infection effects.

5.0 DIAGNOSIS

Diagnosis is extremely important for all research and treatment option development. Direct techniques encompass virus isolation, RNA detection, and antigen identification, while indirect procedures involve serological testing[74,75]. During the incubation period, which typically lasts between 4 and 10 days, viremia may occur, allowing for the isolation of the virus and the detection of viral RNA and sNS1 protein.

There are sensors that allow us to quantitatively assess the viral load in a person which is helpful for early detection. They tend to be specific also[76]. There are also many assays used for DENV diagnosis like Enzyme-Linked Immunosorbent Assay [ELISA] which could be expensive and time-consuming. Virus isolation has also been done but it requires a lot of expertise. Reverse-Transcriptase Polymerase Chain Reaction [RT-PCR] has been used to detect DENV infection at the earlier febrile stage. Immunochromatographic assays that use gold nanoparticle-conjugated antibodies as a capture protein have also been used. Detection of NS1 in immunoassays has been promising as the amount of NS1 in the bloodstream is quite high during infection and remains for a longer time than RNA. High levels of IgM, IgG due to infection can also give insights into the progression of the illness[77].

Many other detection techniques include MALDI-TOF/MS and rapid antigen detection of DENV NS1 in saliva[78]. Co-infections of various serotypes are often identified by various cycles of RT-PCRs with different primers in more than one PCR reaction[79].

5.1 TYPES OF DENGUE DETECTION

A) Acute Dengue Diagnosis

Acute samples are samples taken as early as possible and can be detected with RT-PCR or real-time RT-PCR. NS1 detection can also be done here, along with virus isolation[80].

B) Molecular Diagnosis of Dengue

Serum or plasma might contain DENV RNA along with WBCs and tissues. Western blot or RNA detection can also be done. Nested RT-PCR requires universal dengue primers which can help in initial reverse transcription but can be serotype-specific[81-83].

C) Antigen Detection

Either immunohistochemistry is used or antibodies to detect dengue-specific antigens. The amount of NS1 always correlates with levels in the blood during viremia[84]. As of 2016, the Ministry of Health and Family Welfare (MOHFW), through the National Vector Borne Diseases Control Programme (NVBDCP), made a clear announcement opposing the use of rapid kits in Dengue diagnosis.

D) Early and Late Convalescent Diagnosis

Serological tests are the choices for late and acute convalescent diagnosis. IgM and IgG detection helps in understanding probable and recent infections. IgM levels are seen to increase in secondary infections[85].

E) Viral Isolation and Identification

DENVs are quite tough to isolate but this method is often considered the gold standard. This virus is generally obtained from serum, plasma, peripheral mononuclear cells, and postmortem tissues.

F) Mosquito Inoculation

The intrathoracic inoculation of mosquitoes allowed a new way for DENV isolation. The mosquito species that are bred for this purpose are *A. aegypti, A. albopictus, Toxorhynchites splendens*, and *Toxorhynchites amboinensis*[86].

G) Mosquito Cell Culture

The practice of mosquito cell culture involves the cultivation and propagation of mosquito cell lines for the purpose of facilitating scientific investigations. Three cell lines have been obtained from mosquitoes, namely AP-61 derived from *Aedes pseudoscutellaris*, C6/36 produced from A. albopictus, and TRA-284 derived from *T. amboinensis* [87,88].

5.2 LIMITATIONS OF CURRENT DIAGNOSTIC TESTS

Dengue virus NS1 is a protein produced by infected cells and released into the bloodstream. It plays a role in the replication of the virus and is also detected in the serum of individuals infected with Dengue. Some studies have indicated that during Dengue infection, the immune response may generate autoantibodies that cross-react with NS1. The presence of autoantibodies that cross-react with NS1 can pose challenges in the accurate diagnosis of Dengue infection. Diagnostic tests based on the detection of NS1 may be affected by false positives due to the interference of autoantibodies. The presence of autoantibodies in Dengue infection highlights the complexity of the immune response to the virus and the challenges in developing precise diagnostic tools and effective therapeutic interventions[89].When antibody detection is done, the NS1 regions are highly conserved between

various Flaviviridae that result in cross-reactions with JEV and WNV in more than 65% of the cases. This cross-reactivity with conserved antigens can lead to a lot of false positivity in most cases[90]. The duration of illness can also affect the sensitivity of the diagnostic kits as the kits also are sensitive to the first few days of infection as opposed to the samples collected in the later phases[91].

6.0 PREVENTION AND CONTROL

In diseases like these, good vector control is required to nip the disease in the bud. However, vector control tools are not effectively used yet. Apart from insufficient monitoring, insecticide resistance has also been a significant factor in this. Many methods and indices have been associated with these efforts like maintaining a container index [percentage of water holding containers positive for mosquito larvae], and Breteau index which are containers positive for larvae per 100 houses. Controlling the breeding of mosquitoes by using kerosene, diesel oil, and other products has been used to destroy the larvae. Insecticides like DDT had been used but due to the slowly developing resistance of mosquitoes to methods like these, such practices have now been discontinued. One interesting control method has been the use of natural predators of mosquitoes to kill *A. aegypti*. Larvivorous fishes have also been used to control breeding. Fogging with insecticidal ultralow volume [ULV] aerosols have also been used to kill airborne susceptible insects.

The most interesting strategy has to be genetic-based methods to control vector spread[92]. Here, the vector lifecycle step is disrupted to eliminate the spreading of the virus to humans. There are certain strains of mosquitoes that are designed to either be more susceptible to insecticide or be unable to host the pathogen. However, there has been ethical concerns regarding these as this can cause imbalance of the natural food chain.

7.0 TREATMENT STRATEGIES

Apart from quick treatment and detection which would help in faster treatment and better recovery, managing symptoms and distress of the patient is also extremely important. Symptomatic treatment is essential for recovery from dengue illness. DSS leads to high diastolic pressure and low pulse pressure. In order to achieve prompt replenishment of circulating plasma volume, it is imperative to administer colloidal resuscitation instead of plain saline. The preferred crystalloids for fluid resuscitation in cases of dengue are normal saline with a concentration of 0.9% and Ringer's lactate solution. Blood transfusion is another method to restore low circulating platelets and other blood cells to prevent organ damage[93].

Apart from allopathic medicines, certain Siddha medicines [polyherbal concoctions] can work to prevent viral infection severities. Nilavembu kudineer (NVK) is a polyherbal compound that has been documented for its potential efficacy in the management of chikungunya and dengue. However, without animal testing, it would take a long time for this to reach the market[94].

Certain antibody treatments against DENV can lead to post-exposure prophylaxis due to antibody-dependent enhancement (ADE) of infection which can lead to complications. This also has to be mitigated apart from treating the dengue symptoms[95].

7.1 ANTI-DENGUE DRUG DEVELOPMENT

There are not many antiviral drugs for dengue. The strategy required for anti-dengue effects is that the viral load should reduce such their development of severe dengue is restricted. Chloroquine and Balapiravir are two drugs that have reached human clinical trials [96,97].

Oral drugs are preferred, and they should be inexpensive to manufacture, to help eradicate the dengue epidemic in third-world countries. The drugs have many phases they can target to stop viral propagation: like the viral entry and fusion step, polyprotein translation, replication, and maturation of viral particle steps.

7.2 DENGUE VACCINE DEVELOPMENT

Most viral diseases benefit well from vaccine type of prevention measures. Vaccines can reduce the probability of disease and illness by reducing susceptibility to severe symptoms. Vaccines can also give herd immunity. However, a vaccine for such a serious disease should be a fine balance between immunogenicity and attenuation of DENV pathogenicity such that there is neither under-attenuation nor over-attenuation. A few potential vaccines used are mentioned below.

The CYD vaccine, developed by Acambis/Sanofi Pasteur, utilizes chimeric viruses combining elements of the dengue virus and the yellow fever virus as its molecular backbone. Chimeric dengue vaccinations frequently elicit robust immunological responses in cynomolgus monkeys [98]. NIH Vaccine consists of molecular attenuated viruses which are transcribed from recombinant cDNA with a deletion.

One instance of live-attenuated vaccines involves the cultivation of the virus in mammalian LLC-MK2 cells under normal conditions. However, the virus's growth is significantly impeded when cultivated in C6/36 insect cells, resulting in a variant referred to as "mutant F" or "mutF." The immune responses elicited by these attenuated vaccinations closely resemble those triggered by the wild-type viruses [99]. Serial passaging of the virus can lead to various phenotypic changes which lead to obtaining of vaccine candidates. The tetravalent live-attenuated dengue vaccine, which was produced by The Walter Reed Army Institute of Research, was created through the serial passage of wild-type DENV in PDK cells. Within the chosen passage levels, the rates of seroconversion were seen to be 100%, 92%, 46%, and 58% for a singular administration of DENV-1, -2, -3, and -4, correspondingly[100].

Adenoviruses as vectors can be used to express and present the antigen of all types of the dengue virus and the antibodies obtained from the animals displayed protection in the first three serotypes and moderate protection from the fourth serotype[101].

Recombinant subunit vaccines also exist which use subunits of the DENV like EDIII and NS proteins. However, such subunits have been produced in effective amounts by expression in *E. coli.*, *Drosophila,* yeast, baculovirus in insect cells, and vaccinia viruses[102].

DNA vaccines consist of plasmids, which are circular DNA molecules, that carry dengue genes. These plasmids are present in large quantities within the vaccine and have a eukaryotic promoter and terminator sequence. This specific genetic arrangement enables the transcription of the dengue genes in the receiver of the vaccination. The transcribed RNA molecule serves as a template for protein synthesis within living organisms, while the immune system identifies and generates antibodies targeting this specific RNA molecule[103,104]. Sometimes, immunostimulatory sequences are incorporated into the plasmid and this allows for a more vigorous immune response. DNA-based vaccines are growing in trend as they have many strong points over conventional vaccines like easier generation, high stability, and stable transportation, and with a single vaccine, multiple pathogens can be targeted. A study was conducted by researchers at the US Naval Medical Research Institute to investigate the functionality of two eukaryotic plasmid expression vectors, namely pkCMVint-Polyli and pVR1012. These vectors were utilized to express the PrM protein and 92% of the E protein for DENV-2, specifically the New Guinea C strain [105]. These have led to antibody production in mice that conferred immunity, however, more research is needed in this aspect to make it more effective.

7.3 DENGVAXIA TAK-003 ETC

Dengvaxia is a tetravalent live attenuated vaccination that contains the pre-membrane and envelope genes (prM and E genes) of all four serotypes of dengue virus, as well as the non-structural genes of the yellow fever 17D vaccine strain, resulting in a chimeric yellow fever dengue vaccine (CYD). The chimeric methodology was first devised as a means to counteract the Japanese encephalitis virus[106]. The adoption of this initiative was undertaken by Sanofi Pasteur. DENGVAXIA is the sole vaccine endorsed by the Food and Drug Administration (FDA) for protection against all four strains of the dengue virus. However, its usage is restricted to those aged 6-16 years who have a documented history of dengue infection through laboratory testing, and who reside in countries where dengue is prevalent [107].

The parent strains consist of type1: Thailand PUO-359/TVP-1140, type 2: Thailand PUO-218, type 3: Thailand PaH881/88, and type 4: Indonesia 1228 (TVP-980) and each of these were gotten separately by the recombinant DNA technology[108]. These were all cultured in Vero cells and were later administered as a single vaccine formulation. The genomes were later fully sequenced throughout production to adhere to the good manufacturing practice [GMP] standards as there should be recorded modifications. By the late passages, nine-point mutations were identified. This was observed by electron microscopy and lyophilized. Therefore, Dengvaxia is a sterile and lyophilized pharmaceutical product that is reconstituted before to administration using a sterile solution containing 0.4% sodium chloride. The vaccine and diluent are dispensed in a vial containing a single dose, and the doses are provided with a 6-month interval between each administration. The time points for administration are at 0 months, 6 months, and 12 months.

Despite being classified as a genetically engineered organism, Dengvaxia has undergone comprehensive risk studies[109]. There are no preservatives and adjuvants given with Dengvaxia. It was considered a really effective and safe vaccine[110].

As of September 14, 2018, the Sanofi Pharmacovigilance database recorded a total of 51 reported fatalities. The majority of these instances were seen to transpire in individuals within the age range of nine to thirteen years. The Global Advisory Committee on Vaccine Safety, established by the World Health Organization (WHO), conducted a comprehensive analysis of 14 reported deaths. However, due to the lack of specific criteria to differentiate between vaccine failure and vaccine-related immune augmentation, the committee was unable to definitively ascribe these deaths to either cause. Therefore, it is recommended that instances of this nature be categorized as uncertain, regardless of the duration since immunization[111].

In the Philippines however, the confidence in Sanofi vaccines was diminished completely after Dengvaxia, and the license of this vaccine was revoked. There was a lot of chaos politically, scientifically, ethically, and legally which has led to stricter assessments of further vaccines for diseases like these.

8.0 OTHER DENGUE-RELATED EFFECTS

8.1 ANTIBODY-DEPENDENT ENHANCEMENT (ADE)

ADE or Antibody- Dependent Enhancement is a condition where there is the possibility of infecting cells expressing the complement receptor or the Fc receptor (FcR) rather than the viral receptors. ADE occurs when the Fc receptors, namely the Fc gamma receptor, and/or the complement system facilitate the internalization of the virus-antibody complex into the cellular environment [112].

ADE is of two types: intrinsic and extrinsic ADE. Intrinsic ADE is where when DENV gets internalized, the intracellular antiviral response gets suppressed. The DENV-antibody complexes form and hence, the virus uses the host immune response to propagate [113]. The extrinsic ADE mechanism occurs with the help of Fc receptor (FcR)-dependent ADE and C1q-dependent ADE. This mechanism is commonly seen in HIV, Influenza A, dengue, and Ebola infections. Here, the viral attachment to cell surfaces is enhanced due to virus-antibody complexes, and the antiviral immune responses are repressed. The cells are used for propagating viral particles.

The production of non-neutralizing antibodies during the secondary infection of a different serotype of dengue virus can result in intrinsic antibody-dependent enhancement (ADE), potentially leading to a more severe dengue illness [113]. This particular form of antibody-dependent enhancement (ADE) has the potential to significantly augment viral replication during the first phase of infection in contrast to extrinsic ADE, as it can propagate unrestrictedly without being identified by the interferon pathway[114].

8.2 TFH CELLS AS TARGETS IN BEATING DENGUE SEROSTATUS EFFECT

The development of many vaccines has led to many types of immune responses by individuals. While long-lasting homotypic protection can arise due to infection, having short-term heterotypic protection by a vaccine can enhance the severity of illness during the second infection. The generation of durable antibody-secreting plasma cells is a crucial process in the development of an efficacious dengue vaccine. This process occurs within secondary lymphoid tissues, where germinal centers (GC) are formed. The formation of GCs is facilitated by follicular helper T cells (Tfh), which play a significant role in this immunological process [115]. However, a lot more research is required to exploit this avenue for more effective vaccines.

9.0 EXPANDED DENGUE SYNDROME

The World Health Organization (WHO) formed the term expanded dengue to describe cases which do not fall into either dengue shock syndrome or dengue haemorrhagic fever. This has included many atypical findings of dengue. Dengue virus has been proposed to be an etiological agent in several conditions like encephalitis and Guillain Barre syndrome. The manifestations of dengue are varied, and hence, clearly outlining expanded dengue can help in early diagnosis and effective treatment.

Expanded dengue includes end-organ damage, other organ damage, co-infections and monitoring dengue symptoms in high-risk groups. Central nervous system, hepato-biliary system, cardiovascular and renal systems morbidities occur in most dengue infections. This has to be effectively researched upon and these co-morbidities in high-risk groups have to be managed well to prevent further aggravation and evolution of dengue infections [116].

10.0 CONCLUSION

Dengue virus (DENV) poses a significant ongoing public health challenge, with the COVID-19 pandemic exacerbating the situation, particularly in Asian countries. The repercussions extend to both health and the economy. Complicating matters, cross-reactions have been observed, leading to frequent misdiagnosis and improper treatments. Addressing Antibody-Dependent Enhancement (ADE) is crucial as it can compromise vaccine efficacy. Moreover, the absence of a suitable animal model for Dengue further complicates research efforts. This limitation hinders our understanding of the virus's pathogenesis and the development of effective treatments. Cross-protection of vaccines against various flaviviruses adds another layer of complexity, necessitating careful consideration to avoid unintended consequences. Efforts to enhance Dengue treatment effectiveness warrant extensive research. Promising areas include genetic engineering, nanobody derivation, molecular mimicry, the discovery of antigenic cryptic epitopes, structural studies, and elucidating mechanisms of inhibition. Urgently needed are reliable diagnostic kits for swift and accurate diagnosis. Identifying superior biomarkers for Dengue, detectable in easily obtainable samples, would significantly aid in disease management. This field holds immense potential for research, offering the prospect of improved antiviral treatments that may contribute to better Dengue control in the foreseeable future [117].

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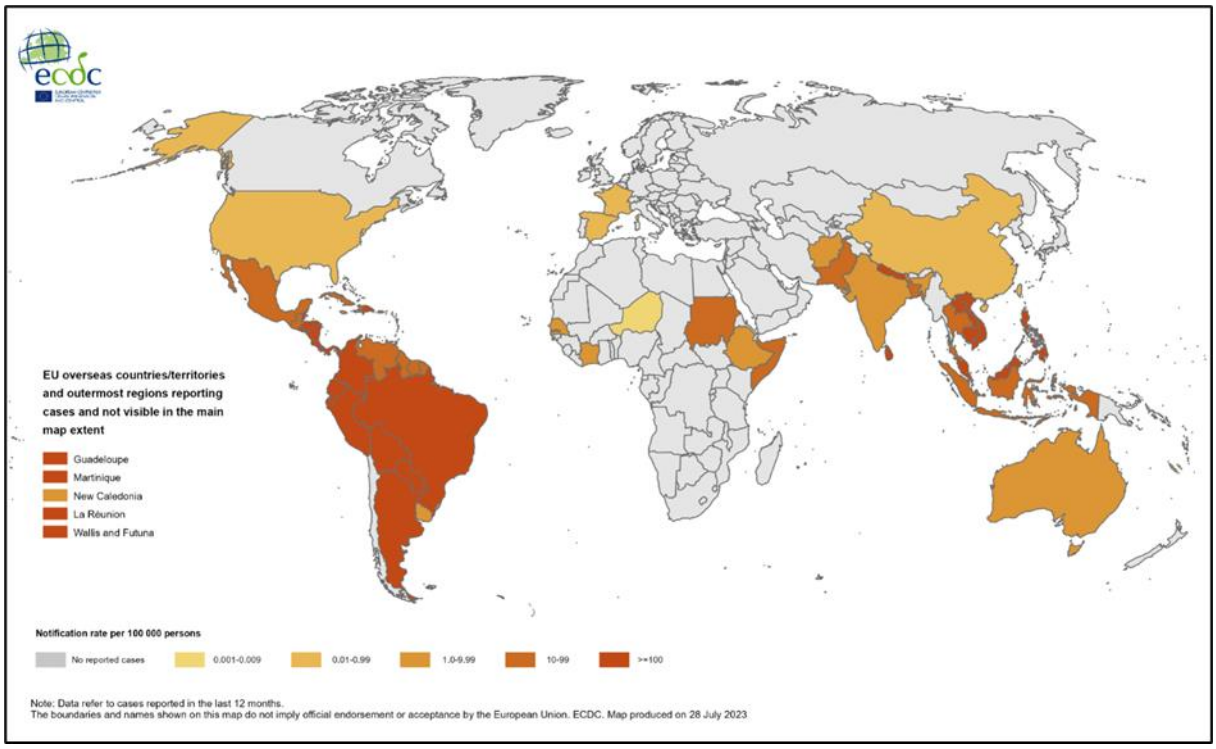

Figure:1 In 2023, and as of 27 July, over three million cases and over 1500 dengue-related deaths have been reported globally. [Communicable Diseases Threat Report. European Centre for Disease Prevention and Control]

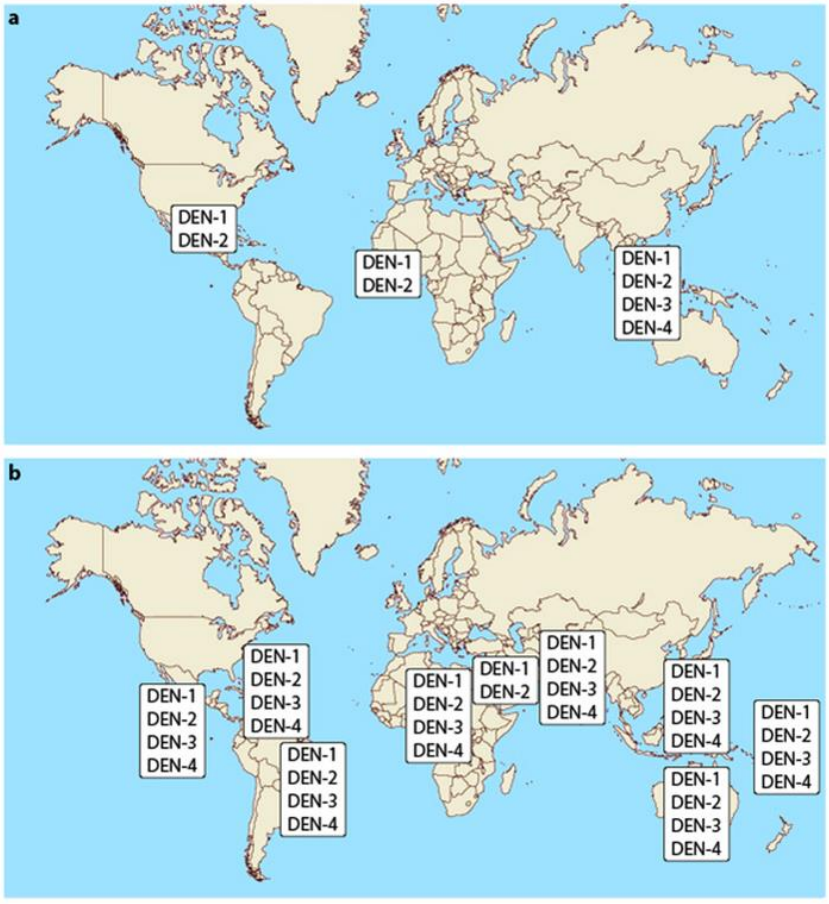

Figure 2: The change in distribution of dengue serotypes in (a) 1970 and (b) 2004

[https://www.nature.com/scitable/topicpage/dengue-viruses-22400925/]

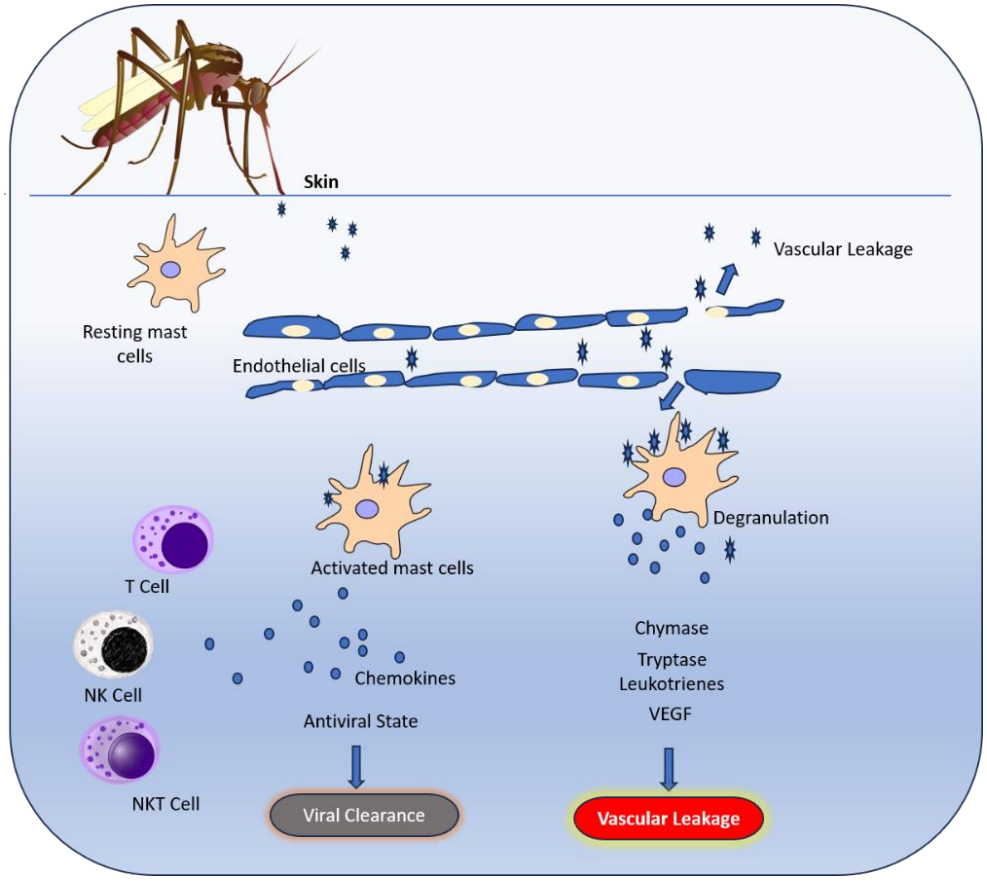

Figure: 3 Pathogenesis of dengue virus infection