

Dengue Epidemic is a Global Recurrent Crisis: Review of the Literature

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Abstract

Purpose

This review highlights the global scenario of dengue outbreaks, dengue pathogenesis, symptoms, immune response, diagnosis methods and preventive measures which facilitates the better understanding of the global expansion and concerns relating to the disease.

Recent Findings

A recent study showed that natural killer cells of the infected person become activated soon after the infection which may help in treatment and vaccine development. A research team has also produced synthetically engineered mosquitoes that can prevent the transmission and dissemination of the dengue virus by the activation of an antibody. Furthermore, a mutation in the protein envelope of the dengue virus leads to variation in shapes, developing resistance towards the vaccine.

Summary

The mosquito vectors marked their worldwide distribution through an increasing number of reported cases which was further facilitated by the growth in the shipping and commerce industries. The immune system, through activation of the innate and adaptive immune responses, facilitates the recruitment of an array of leukocytes which help neutralize the virus. Apart from the laboratory standard PRNT method, several other dengue detection methods such as ELISA, RT-LAMP and several optical, microfluidic and electrochemical methods have been developed. The existence of the 4 different viral serotypes makes the secondary infection life-threatening and also leads to difficulties in vaccine development. Since Dengvaxia® (CYD-TDV) has its own set of drawbacks and limitations, several companies have been investing for the production of more potential vaccines that are currently in trial.

Keywords: Dengue Hemorrhagic Fever (DHF); *Aedes Aegypti*; epidemic; pathogenesis; identification; vaccine

1. Introduction

Dengue is one of the most rapidly spreading mosquito borne viral infections in humans leading to about 10,000 deaths annually across over 125 countries in the world [1]. The spherical enveloped dengue virus is a positive single stranded RNA virus consisting of about 10,200 nucleotides which codes for structural and nonstructural proteins [2]. Dengue virus which is a member of the genus Flavivirus (family Flaviviridae) are classified into four serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) [3][4]. Mosquitoes of the genus *Aedes* aid to transmit dengue virus from one human to another [5][6]. These mosquitoes are ubiquitous in the tropical and subtropical regions from 30° north to 20° south latitude. Escalation of dengue infection is associated with climatic changes which leads to an increase in temperature, high levels of precipitation, humidity and vapour pressure. [7]. All these factors along with degree of globalization, trade and travel are coherent thus triggering the vectors for dengue transmission. Initially the person experiences mild flu syndrome known as dengue fever or break bone fever accompanied with skin rash which may later proceed to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) which may prove to be a huge threat eventually leading to death in very critical cases [8]-[11]. With no treatment available, this virus presents a life-threatening health concern for people in many countries. Appropriate measures, proper knowledge, understanding of symptoms, timely diagnosis and supportive treatments are the only options to combat this viral disease.

2. Origin of Dengue Viruses

During the 17th century, there were many cases which had clinical symptoms similar to that of dengue. However, the first confirmed dengue outbreaks were reported simultaneously in Asia, Africa and North America in 1779-1780 [12]. In 1951, an American physician, Benjamin Rush mentioned about the probable dengue fever which occurred in Philadelphia, United States in 1780 [13]. He named this fever as “break bone fever”- term which is synonymous to the pain the patients described they endured back then [14]. Immediately after World War 2, global dengue pandemic emerged in Southeast Asia. This was due to the destruction of water systems during the war, creating many stagnant water containers which provided a suitable environment for the mosquitoes to breed. The movement of the war equipment allowed the vectors to bypass the geographic barrier very easily [15]. Due to the dispersal of the dengue virus throughout, Pacific regions and the Americans experienced DHF outbreaks. Followed by World War 2, in 1975, Southeast Asia experienced DHF endemicity due to the increased degree of urbanization and the ecological setting which was just perfect for the vectors. The countries soon became hyperendemic- all 4 serotypes were present among the human- vector cycle. The second DHF epidemic began in 1980 in Asia which expanded towards India, Pakistan, Sri Lanka, Maldives as well as the People’s Republic of China [16].

3. Vectors and Hosts of Dengue Viruses

Aedes aegypti being the principal vector and *Aedes albopictus*, also known as “Asian tiger mosquito”-the competent vector has a limited ability to transmit dengue virus [17]. They are well adapted to the urban environment and are found both indoors and outdoors close to human abode which makes them suitable vectors for horizontal transmission of dengue virus (mosquito-human).

The primary vector of dengue virus, *Aedes aegypti* from the tropical and subtropical regions of the world are mostly associated with the space occupied by humans for living and feeds on human blood [18]. Female *Aedes aegypti* requires blood before laying eggs. Moreover, *Aedes albopictus* which is an exophilic mosquito even prefers feeding on human blood. Therefore, their strong preference for human blood in order to support their survival makes them an ideal vector for DENV virus transmission [19]. The transmission of dengue virus by a vector begins as a mosquito bites an infected person, carrying dengue virus in his or her blood. The mosquito eventually gets infected and hence becomes a dengue vector which can prove to be a threat to the population since it can horizontally transmit this virus for the rest of its lifespan (two weeks to one month). Although early studies have shown no clear evidence of vertical transmission of DENV virus in *Aedes* mosquitoes, recent studies prove that vertical transmission of DENV virus is attainable both experimentally as well as in nature. In addition to that, some evidence affirms that *Aedes albopictus* are much more efficient than *Aedes aegypti* in terms of vertical transmission [20]. A case of vertical transmission of dengue virus was reported in Guangzhou, China. A 25 years old woman, 39 weeks pregnant was admitted in a general hospital. She experienced fever for 5 days and then gradually developed skin rash. She went into labour and delivered a girl. Results from ELISA dengue virus NS1 antigen test (Wantai, Beijing, China) and dengue virus fluorogenic quantitative PCR test (Liferiver, Shanghai, China) confirmed that both the mother and the baby suffered from dengue [21]. This indicates that vertical transmission can be an alternative mode of dengue transmission.

4. Pathogenesis of Dengue Virus Infection

4.1. Entry of Virus inside Cell and Replication

Once the mosquito feeds on the blood of an infected person, the DENV develops an infection in the midgut from where it spreads and replicates in other tissues of the mosquito. It takes about 8 to 10 days and a temperature of about 25° C for the virus to multiply, mature, migrate and infect the salivary glands and eventually shed in the saliva [22]. While the female dengue vector bites and feeds on the blood of a healthy individual, it injects its saliva in order to prevent blood clotting of the host and facilitates feeding. Thus, infecting the individual by inoculating the virus into the dermis and epidermis as well as injecting some viruses directly into the bloodstream. It is then followed by infection in the most common cell type in the skin- keratinocyte [23]. It infects and replicates inside a specialized immune cell in the skin- Langerhans cell (a type of dendritic cell) [24]. The infection spreads to the lymph node thus activating the recruitment of leukocytes.

The replication of a flavivirus commences as it attaches to the extracellular surface receptor on the host cell (**Figure 01**). The virus is then taken up into the cell by endocytosis and is confined within an endosomal vacuole. Acidification of the endosome causes a change in the E-protein thus facilitating the release of the viral genome into the cytoplasm [25]. The viral genome can engage in any of the two fates-either the released genome can be transported to the ER in order to be translated in a polyprotein which undergoes post translational modification to produce structural and nonstructural protein for virus assembly and maturation. After making multiple copies of virions inside infected cells, they lyse the cells, get outside by endocytosis and continue to infect other healthy cells [26].

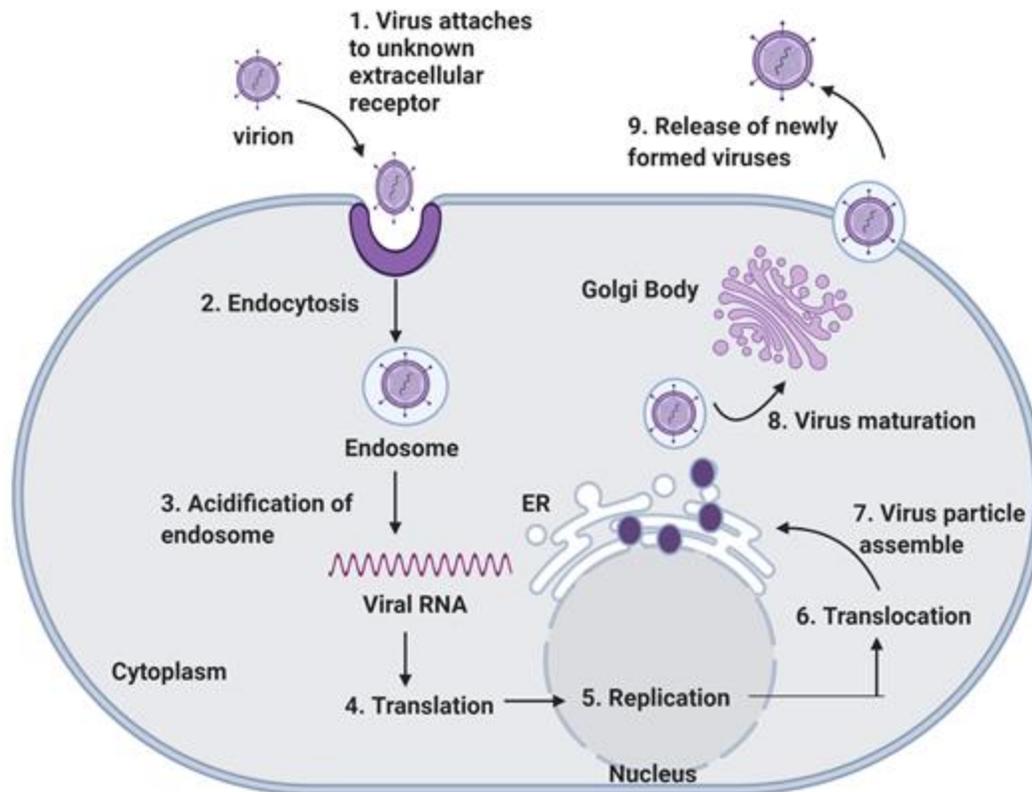


Figure 01: Reproductive Cycle of DENV virus in mediating viral pathogenesis. 1. DENV virus binds to an uncharacterized receptor on the host cell surface. 2. Enters into the cytoplasm by endocytosis. 3. Endosomal acidification causes irreversible trimerization of the viral E-protein thus exposing the viral RNA into the cytoplasm. 4. The viral RNA is translated into polyprotein in the ER. 5. After viral replication complex is synthesized, RNA synthesis starts. This takes place in two steps- positive mRNA is at first copied to negative sense mRNA and then this acts as template for synthesis of multiple stands of positive sense RNA which are then used for translation. 6. Translocation takes place. 7. Virus assembly takes place in the ER membrane. 8. Immature virus particles pass through the Golgi body where they undergo modifications. 9. Virions are released by endocytosis [27-32].

After 4 to 5 days of being bit by a dengue vector, the person develops viremia- a condition in which the level of dengue virus in blood tends to elevate due to replication of the viruses. It can last from

5 to 12 days. If a mosquito feeds on the blood of an infected individual within this time span, the mosquito becomes a dengue vector [33].

Some epidemiological studies show that the development and severity of dengue infection in humans is triggered by many factors. Age is one of them [34]. Other factors include genetic background of the host [35][36], vector, serotype of virus [37], gender, genotype [38][39], environmental condition, immune condition of the infected people, socioeconomic level of the population and secondary infection by heterologous serotype [40].

4.2. Immune Response of Human Body Against Dengue Virus

The immune system is the primary defense system of the body against dengue virus and consists of mainly two parts: The innate immune system and the adaptive immune system. While the innate immune response facilitates the immediate recognition and protection towards any invading pathogen, the adaptive immune response produces cells that specifically and efficiently target the pathogen or infected cell providing a long term immunity, unlike the innate immune system [41].

The cells produced by the adaptive immune system include the antibody-secreting B cells, which are capable of recognizing and binding to foreign cells with high specificity and the cytotoxic T cells which are known to attack the infected cells.

Since the keratinocytes and Langerhan cells are infected through viral replication, the Langerhans, through proper detection, display the antigens from the invading pathogens on their surfaces [42] - [45]. The display of the viral antigens triggers the innate immune response, summoning the white blood cells, monocytes and macrophages for the ingestion and destruction of the dengue virus.

The virus infects these cells instead and spreads throughout the entire body as they travel through the lymphatic system [45] [46]. As the virus spreads throughout, infecting cells of the bone marrow, lymph nodes, spleen, liver or blood, it facilitates the emergence of viremia.

While some of the infected Langerhans travel to the lymph nodes (small glands present throughout the body that are known to fight infections) to trigger the immune response, the remaining secrete proteins called interferons which disrupt the replication process of viruses through the activation of innate and adaptive immune system defenses (**Figure 02**) [24] [47]. They help in the recognition of the infected cells and protection of the uninfected cells. The individuals experience dengue fever (DF) while their body's immune system fights the virus. Antibodies from B cells, immunoglobulin M (IgM) and IgG are secreted into the bloodstream and the lymph fluid in order to neutralize the virus while on the other hand, cytotoxic T cells and killer T cells are used in order to specifically recognize and destroy the virus through adaptive immune mechanisms. The innate immune response further activates the complement system in order to destroy the virus with the help of antibodies and leukocytes, clearly indicating the contribution of both the immune systems, in order to neutralize the dengue infection. [48].

The individual remains protected from the other three serotypes for about 2 to 3 months after recovery from the first dengue infection. However, this provides short term protection only and the usual observation is that a second dengue infection was much worse in individuals infected beforehand than those who were not infected earlier [46] [49]. Even though the memory B cells and memory T cells are normally known to provide protection through adaptive immune response when the virus strikes again, the mechanism fails in case of a second dengue attack. These observations were explained by Halstead through the 'antibody-dependent enhancement of infection' phenomenon which supports the idea that the antibodies present from the first dengue infection or

the newly produced memory B cells facilitate the efficient viral attack of host cells instead of providing protection against the other three serotypes [49] - [54]. This happens since the antibodies from the first dengue infection form a complex with the newly attacking serotype which further binds to the Fcγ receptors (FcγR) present on circulating monocytes [55] - [59]. This results in severe dengue fever, also known as dengue hemorrhagic fever (DHF), since more cells are infected with the help of the pre-existing antibodies. This phenomenon is also active among children who received antibodies for dengue from their mothers while in the womb, putting them at a greater risk of developing severe dengue [60] [61]. Furthermore, it was also seen that the cytotoxic T cells secreted greater amounts of cytokines during the second dengue infection which are known to cause serious inflammations or tissue damage.

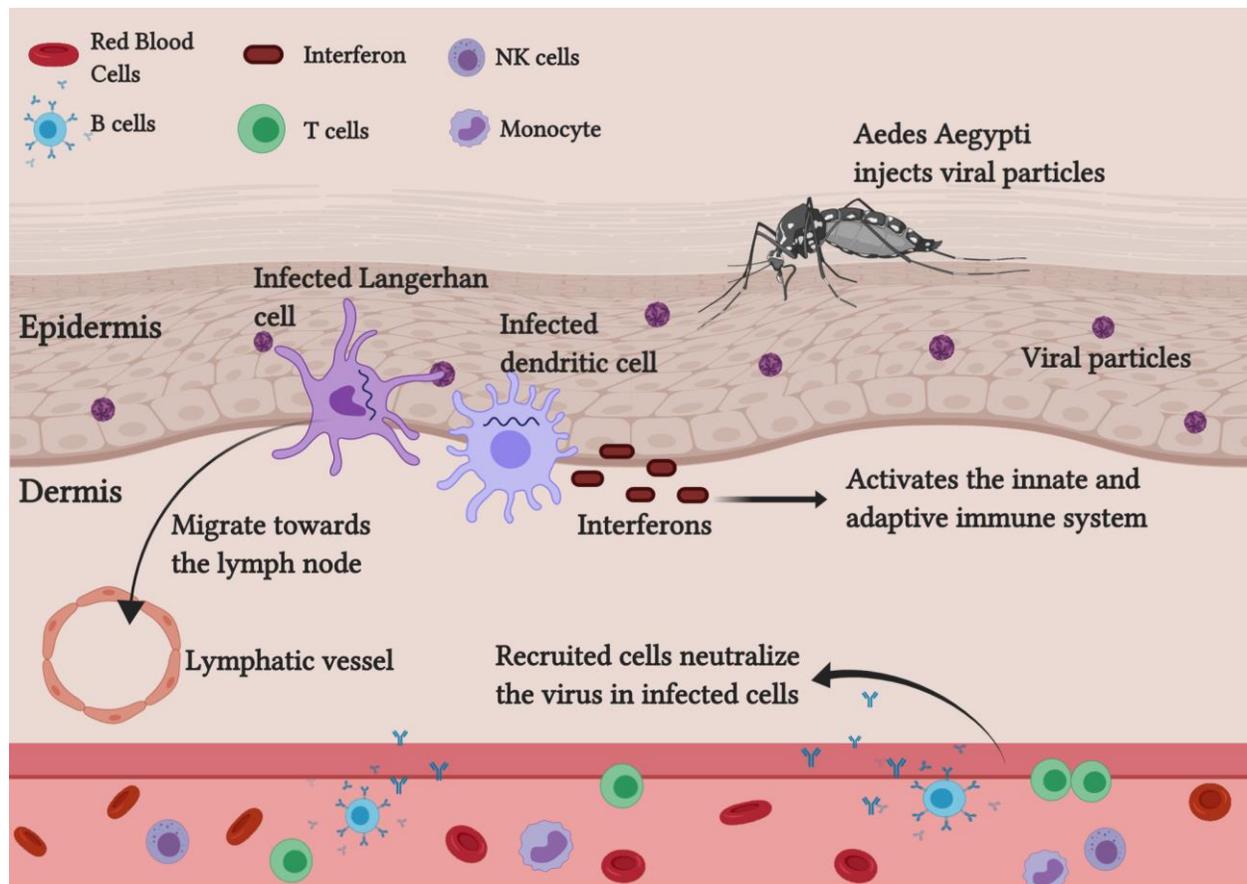


Figure 02: The immune response of human body against dengue virus. *Aedes Aegypti* injects viral particles along with its saliva which infect the keratinocytes and other dendritic cells present near the skin. The infected cells represent the antigens of the pathogens on their surfaces, facilitating the recruitment of leukocytes. The infected cells migrate towards the lymph node to trigger the immune response. Soon after, more and more cells get infected and lead to viremia. The infected cells release interferons which aid the recruitment of B cells that release the antibodies, IgG and IgM and the cytotoxic T cells which target the infected cells. The complement system and NK cells are also recruited to neutralize the virus. The B cells also produce memory B cells in case the patient is infected again however, these memory cells will only be able to help if the patient is infected with the same serotype again [62][63].

5. Symptoms and Clinical Presentation

Symptoms of mild dengue fever may not be visible among children however, if they do make an appearance, they begin 3 to 14 days after the initial infection [64]. Dengue fever (DF) results in a high-grade fever further leading to a number of other symptoms which include headache, retro-orbital pain, muscle pain, vomiting and rash. The symptoms may become worse and life-threatening if the disease is moderate or severe [65] [66]. The symptomatic cases are classified into undifferentiated febrile illness (UF), dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS) and unusual dengue (UD) or expanded dengue syndrome (EDS) [67] [68]. Due to damaged blood vessels, blood plasma leakage and a decreased platelet count, the risk of acquiring dengue hemorrhagic fever (DHF) increases. The symptoms may also progress to massive bleeding, shock, and death; this is called dengue shock syndrome (DSS). The course of dengue infection takes off after the incubation period and is divided into 3 main phases: Febrile phase, critical phase and recovery phase (**Table 01**) [69] - [71].

Phase	Time Interval	Signs and Symptoms	References
Febrile Phase	Day 1-3	<ul style="list-style-type: none"> - High grade fever, usually over 40°C /104 F - Body ache, petechiae, myalgia, arthralgia, headache, anorexia, vomiting - Bleeding of mucosal membrane (nose and gums), gastrointestinal bleeding (rare) and massive vaginal bleeding (in women of childbirth age) - Lasts 2 to 7 days 	[68] [70] [72]-[81]
Critical Phase	Day 4-5	<ul style="list-style-type: none"> - Occurs around defervescence - There is a drop in temperature: 37.5–38°C or less - Blood plasma leakage, leukopenia, plasma leakage - Shock, severe organ impairment, metabolic acidosis and disseminated intravascular coagulation (as a result of shock) - Lasts 1 to 2 days 	[77] [79] [82][83]

Recovery Phase	Day 6 onwards	<ul style="list-style-type: none"> - General feeling of well-being, improvements in appetite, gastrointestinal symptoms abate - Reabsorption of leaked fluids - Hemodynamic status stabilizes and diuresis ensues - Rash with a maculopapular or vasculitic appearance, chronic fatigue - May lead to hypervolemia (in case of excessive intravenous fluid therapy) - Lasts 2 to 3 days 	[72] [77] [82] [84]
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Table 01: The symptoms associated with dengue fever

6. Dengue as a Global Crisis

Dengue is an important arthropod borne viral disease that is known to affect an estimated 2.5 billion people around the globe; approximately 975 million are habitants of the urban areas in tropical and subtropical countries in Southeast Asia, the Pacific and America [4] [85] - [88]. The transmission range also covers the African, the Eastern Mediterranean and the rural communities. The number of cases reported each year includes more than 500 million infected and about 500,000 individuals hospitalized for dengue hemorrhagic fever [6] [89] [90].

The first epidemic of dengue dates back to 1635 in the French West Indies and similarly, a disease outbreak identical to dengue was reported in China during 992 AD [91] [92].

The 1779-1780 dengue outbreaks in Asia, Africa, and North America along with simultaneous outbreaks in three other continents indicated the world-wide distribution of the virus and their mosquito vectors. According to the World Health Organization (WHO), the average annual number of reported cases for DF or DHF includes 925,896 people during the 2000-2004 period which is double that of 1990-1999, indicating an increase in number. Again, from 2000 to 2008, the average annual number of cases reported was 1,656,870 which is almost three and a half times that noted previously [93]. While there were little to no cases in the African or Eastern Mediterranean regions in the 2005-2006 period, countries such as Pakistan, Saudi Arabia, Yemen, Sudan and Madagascar had suspected outbreaks [94]. There was an expansion of the dengue transmission regions worldwide, that included the four existing serotypes of dengue and the leading factors could have been the travelling of individuals from endemic regions acting as carriers, uncontrolled vectors, unprecedented population growth and uncontrolled urbanization [90] [95] - [98].

6.1 Dengue in Asia

After World War II, the global pandemic of dengue emerged in Southeast Asia and almost 75% of the world's dengue burden involved countries such as the Philippines, Indonesia and Thailand [99]. Even though DHF had appeared first as an epidemic in the 1950s, it had become one the leading causes of hospitalizations and death by 1975 [16] [100]. DHF had expanded into Asia during the 1980s and China and Taiwan also reported cases of epidemic DF after an absence for 35 years. After a successful control program, Singapore was able to prevent transmission for over 20 years [101]. Even though only sporadic cases of dengue were reported before 2000, it raised a serious public

health concern during 2000 with 5,551 reported cases and 93 deaths [86] [102] [103]. Soon after dengue had become endemic in several regions throughout the years, by the end of 2016, countries such as China, Malaysia and Singapore had reported about 2,91,964 cases [104]. Also, the cases of dengue had spiked ever since its first outbreak with DEN- 3 during the year 2000, in Bangladesh [105] [106].

6.1.1. Recent Dengue outbreaks in Asia (2019-2020)

By the end of 2019, a total of 101, 354 cases and 179 deaths were reported in Bangladesh and about 263 cases were reported till 16 March, 2020 [107] [108]. While Malaysia reported a cumulative number of 127,407 cases and 176 deaths till December 21st, the Philippines reported 420,453 cases including 1,565 deaths till December 14th and Singapore reported about 15,622 cases on week 51 of 2019 [109]. Dengue transmission was also recorded in Afghanistan for the first time, in 2019. China reported 268 cases in January 2020, Malaysia reported 32,951 cases including 48 deaths from 29th December 2019 till 21st March 2020, Philippines reported 37,058 cases including 112 deaths as of February 2020 and Viet Nam reported 20,673 cases including 4 deaths till 20th March 2020 [110].

6.2. Dengue in America

Dengue has been a major public health problem in America. A campaign by the Pan American Health Organization (PAHO) played a role in the eradication during the 1960s from the central and south American countries [111]. The discontinuation of the campaign in the 1970s further led to the reinfestation of the species. In the 1970s, Den-2 and Den-3 were present in America however, the major epidemic in 1977 was due to the Den-1 virus which had an epidemic period of 16 years. In 1981, Den-4 was introduced along with another strain of Den-2 from Southeast Asia which led to a major DHF epidemic and this new strain caused outbreaks in Venezuela, Puerto Rico, Columbia,

Brazil and Suriname [112]. 14 countries in America had endemic DHF after its emergence in 1995. The failed eradication program led to an increase in cases from 2000-2010 and therefore, over 1.7 million cases were reported, from which 50,235 were severe cases and 1,185 deaths. According to a recent report by WHO, the number of cases increased by 15 fold over the past two decades with an increase from 50,5430 cases in 2000 to 2,400,138 cases in 2010 [113]. According to a report from PAHO, America recorded the largest outbreak of dengue with about 3 million cases in the region till 2019 and the number of cases reported was 320,000 till 9 February 2020 [114].

6.3. Dengue in Australia

The first outbreaks in Australia date back to 1879 in Queensland at Townsville and 1885 in Rockhampton [115]. In 1898, the first cases were reported in New South Wales and during 1925-26 it extended to the south. In the Northern Territory, it was prevalent during 1955 [116] - [118]. The species had soon disappeared from the northern and western parts. There was a reduction in the population during the 1960s in Queensland however, after its disappearance for 25 years, it had reappeared during the 1980s [119] - [121]. From the period 2005-2006 to 2009-2010, the number of dengue cases had increased from 156 to 581. Australia reported a total of 1,419 till 18 December 2019 since the start of the year and about 78 cases till 26 February 2020 [109] [122].

6.4. Dengue in Europe

Dengue was endemic in regions of southern Europe until the 1930s due to the presence of *Aedes Aegypti*. In 1927 and 1928, several outbreaks infected millions and led to a death toll of around 2100 in Greece and Turkey [123]. Disappearance of the disease was noted however, several locally transmitted cases in Croatia, France and Portugal were also reported; *Ae. Albopictus* and *Ae. Aegypti* being the responsible vectors. From the period 2012-2013, a large outbreak in Madeira and Portugal

was reported which not only included more than 2100 cases but also involved the spread of the disease to 14 other European countries [124]. According to a report from WHO, *Ae. Albopictus* had been spreading and was known to have established from Spain to Greece and also to the Eastern countries and the Black sea coast. In the year 2019, over 15,000 autochthonous cases of dengue were confirmed till 11 June including at least 9 deaths by the end of the year [125].

7. Dengue Virus Identification Techniques

The accurate and efficient diagnosis of dengue is significant in order to differentiate dengue from other diseases such as leptospirosis, rubella, and other infections caused by flavivirus to provide clinical care, surveillance support as well as to carry out pathogenesis studies and vaccine research along with the case confirmation of DHF/DSS. There are different DENV identification techniques available but the plaque reduction test (PRNT) has been the most widely used method of identifying the immune response to dengue virus. Although the basis of the test has remained the same, over the years, the methods and materials have been modified in different laboratories [126]. As the PRNT method of detecting DENV is a time-consuming process, several different methods have been developed for fast and efficient detection of DENV. The advantages and disadvantages of those methods have been analyzed by taking into account the like the type of biomarkers, the sensitivity, accuracy, rate of detection as well as the possibility of commercialization, linear range, availability, limit of detection, simplicity, mechanism of detection, and if it can be used for clinical applications. The main methods that have been found are the optical, electrochemical, microfluidic, enzyme linked immunosorbent assay (ELISA), and smartphone-based biosensors methods that detect different serotypes and biomarkers (**Table 02**) [127].

Technique Name	Basis of Test	Advantages	Disadvantages	References
Plaque-reduction neutralization test (PRNT)	Plaques forming units (PFUs) are counted	The most serotype specific test and is the standard for all other tests	Labour intensive and difficult to use in a large-scale basis	[128]
SYBR green reverse transcription polymerase chain reaction (SYBR green RT-PCR)	DENV genome	Detection rate and sensitivity are higher than conventional RT-PCR	NA	[129]
Electron immunomicroscopy (EIM)	Viral Polypeptides	Sensitive	Not more sensitive than	[130]
Optical biosensors	Photons	More accurate and precise compared to conventional methods	NA	[131] [132]
Photonic crystal biosensors	Refractive index	Rapid and sensitive	Highly sensitive to surface contamination	[133] [134]
Vertical-cavity surface emitting laser (VCSEL) method	Detects anti-dengue IgG antibodies	Higher sensitivity than ELISA method	False-positive errors	[135]
Colorimetric biosensors	PNA/DNA hybridization	simple, fast response, acceptable sensitivity, selectivity and is efficient	Unmodified gold nanoparticles are used (Au NPs)	[136] [137]
Surface-enhanced Raman scattering (SERS) biosensors	Dengue virus (serotype 4) oligonucleotide sequences	Highly sensitive optical	Ag-Au bimetallic nano wave chips are used	[138] [139]

		detection technique and a multiplexed readout		
Surface Plasmon resonance (SPR) based biosensors	Antibody detection	Optical label free, cheaper than virus isolation or RT PCR	Low sensitivity and specificity. Temperature, size of biosensor and optics are difficult to regulate	[140] [141] [142]
Fluorometric biosensors	Dengue virus	Accurate and sensitive	Use of Au NPs. High background signals.	[143] [144]
Enzyme linked immunosorbent assay (ELISA)	Antigen/antibody detection	Sensitive and specific	Time-consuming, specific and expensive laboratory equipment and expert technicians are required.	[145] [146] [147]
Sandwich ELISA	Biomolecule	More reliable, sensitive and specific than ELISA method	NA	[148] [149]
Reverse transcription loop-mediated isothermal amplification (RT-LAMP)	DENV genome	Efficient, cheap, provides accurate and reliable results	NA	[150]
Voltammetric biosensors	Current measured based on oxidation/reduction	Cost effective, readily available, and rapid detection. Can also detect low amounts of viral antigens accurately.	Developing portable and simple operation electrochemical devices are a challenge.	[151] [152] [153]
Amperometric biosensors	Current measured based on oxidation/reduction	High selectivity, sensitivity in ultra-dilute samples, and	NA	[154] [155]

		commercial availability.		
Impedimetric biosensors	Current/Voltage ratio	High detection capability and ability to report charge transfer resistance during hybridization process.	NA	[156] [157] [158]
Electrochemical impedance spectroscopy (EIS)	Changes in charge transfer resistance	High sensitivity and ability to report charge transfer resistance	NA	[159]
Conductometric biosensors	DENV nucleic acid	Cheap and high sensitivity	NA	[160]
Metal oxide based electronic biosensors	Nucleic acids, alkali metal ions and proteins	Ultra- low detection capabilities, low assay time, and availability	Debye length, restricting the detection of large biomolecules such as DNAs and RNAs	[161] [162]
Microfluidics system	DENV antibodies	Low consumption of reagents, minimal handling of materials, less time consuming, multiplex analysis, portability, and versatility in design	NA	[163]

Smart phone-based methods	Dengue viruses	Popular, portable, data acquisition and transferring abilities, high-quality camera lenses and cost-effectiveness	NA	[164]
Silicon nanowire (SiNW) biosensor	PNA (peptide nucleic acid)			[165]

Table 02: Different DENV identification techniques. NA: Not allowed

8. Prevention and Treatment Strategy

The DENV is spread by a human-to-mosquito-to-human cycle by the Aedes mosquito [166] thus most strategies to prevent spread of the virus includes preventing the growth of mosquitoes and laying of eggs. The measures to be taken include proper disposal of solid waste, cleaning water storage containers every week and applying insecticides in those containers. Water storage tanks must be covered at all times and appropriate insecticides must be added to the water. Additionally, means of personal protection must also be used such as long-sleeved clothes, window screens, coils, vaporizers etc. During outbreaks, spraying insecticides inside the households and thorough monitoring must be carried out to prevent infection [113] [167] [168].

The life cycle of dengue virus involves the entry of the virus into our body, membrane fusion, RNA genome replication, assembly, and ultimate release from the infected cell. Thus, the treatment of dengue is not specific and remains to be supportive [169]. Supportive treatment includes oral fluid administration and antipyretic treatment with paracetamol along with daily full blood counts. If there is excessive vomiting or diarrhea observed, admission to hospital is necessary to avoid

dehydration. The patient must be administered oral fluid as much as possible [170]. The symptoms of dengue are flu-like and so there is no specific treatment but an early diagnosis and clinical management can reduce the severity of the effects of dengue on the body [113] [171].

Currently, there is no licensed antiviral agent available against dengue as the drug needs to address all four serotypes of the dengue virus. Researchers have been trying to develop drugs by targeting several different steps in the disease pathway in order to interfere with the replication of dengue virus. For example, nucleoside analogue blocks a dengue infection by preventing synthesis of the viral RNA genome so that the dengue virus cannot replicate. Viral replication can be difficult to prevent because there is a short timeline for treatment which is why it is important to target other steps in the disease pathway to prevent dengue at later stages [172].

9. Vaccine for Dengue Virus

Till date, no effective direct vaccine has been discovered that can successfully combat the dengue infection. The vaccines produced must work against all four of the DENV serotypes and must provide lifelong protection. This may be done by incorporating an antigen that is common for all four serotypes. As vaccines with live, attenuated or nonliving viruses usually produce less antibody than an infection with wild-type virus, it is most likely that two doses of the virus will be required. Several other factors need to be taken into account such as the level of symptoms seen after the vaccination, if the vaccine can be used by people of all ages and the cost of the vaccine [166] [173].

Providing lifelong protection against all four serotypes by neutralizing antibodies is difficult due to antibody-dependent enhancement (ADE) or immune enhancement. ADE observed during dengue infection due to heterologous non-neutralizing antibodies may cause dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) during secondary infection by a different serotype.

Additionally, over time, antibody responses below protective levels may increase the possibility of immune enhancement by a natural infection due to wild type DENV. Another major challenge of producing a dengue vaccine is the lack of an appropriate human like animal model for the trials of the vaccines which will allow us to observe the pathogenesis, immune response and clinical course of dengue infection in humans. Inoculation of mouse-adapted DENV strain has caused paralysis or death of that animal [174].

So far only one such vaccine for dengue has been approved, Dengvaxia® (CYD-TDV). Dengvaxia® (CYD-TDV) has been developed by Sanofi Pasteur and it is for people who have already been infected by DENV once. This works on people ages 9 to 45 and must be given three doses in intervals (113, 167, 175). However, a large number of vaccines are now in different phases of clinical trial in different companies (**Table 03**).

Vaccine Name	Company	Phase	References
Dengvaxia® (CYD-TDV)	Sanofi Pasteur	III	[113] [176]
Cell culture passage based live attenuated viruses (Tetravalent vaccine)	Walter Reed Army Institute of Research (WRAIR), GlaxoSmithKline Biologicals	II	[177] [178]
Yellow fever- DENV chimeric viruses (Tetravalent vaccine)	National Institutes of Health (NIH) and St. Louis University Health Science Centre, Sanofi Pasteur	III	[179] [180]
Mixture of cell culture passage based attenuated virus and dengue-dengue intertypic chimeric viruses (Tetravalent Vaccine)	Inviragen Inc.	II	[181] [182]
Mixture of targeted mutagenesis based attenuated viruses and dengue-dengue intertypic chimeric viruses (Tetravalent vaccine)	National Institute of Allergy and Infectious Disease (NIAID), National Institutes of Health (NIH), Butantan Institute	I	[183] [184]

Purified inactivated dengue vaccine (Tetravalent vaccine)	Walter Reed Army Institute of Research (WRAIR), GlaxoSmithKline Biologicals, Oswaldo Cruz Foundation	I	[185]
Recombinant subunit vaccine (Monovalent vaccine)	Merck and Co.	I	[182] [186]
DNA vaccine expressing prM and E protein (Monovalent vaccine)	Naval Medical Research Centre, Walter Reed Army Institute of Research (WRAIR)	I	[187]

Table 03: Progresses in dengue vaccine development in different companies.

Pre – Clinical trials include conducting the research in lab assays or on animals. This includes identification of relevant antigens, creating a vaccine, testing it on lab animals and test tubes, and lastly, using proper manufacturing standards to manufacture the vaccine. Finally, clinical trials are carried out to assess the safety and efficacy of agents under investigation in different sample sizes.

There are four stages of clinical trials (**Table 04**) [188] [189]:

Phases of Clinical Trials	Sample size	Testing
Phase I	10 – 100 people	To check whether it is safe for humans
Phase II	100 – 1,000	The potency of the vaccine against artificial infection as well as vaccine safety, side effects and immune response
Phase III	1,000 – 10,000	The performance of the vaccine against natural infection
Phase IV	Large scale	Post marketing surveillance after the vaccine has been licensed and to find out rare side effects

Table 04: Phases of clinical trial.

10. Current Status and Future Prospects of Dengue Research

A new serotype of dengue virus known as DENV 5 was reported in October 2013 which is similar to DENV 2 and follows the sylvatic cycle rather than the human cycle that the other four serotypes follow. It has been found that DENV-5 constitutes a unique, distinct phylogenetic group, but this discovery requires further confirmation and even though this was thought to be a variant of serotype 4, several studies have shown that rhesus macaque monkeys infected with the serotype 5 produced a different set of antibodies. Developing a tetravalent dengue vaccine that is both cost effective and safe has been a challenge for scientists all over the world and several potential vaccines are being tested after Mahidol University, Thailand and Walter Reed Army Institute of Research (USA) initiated the research [190] [191]. Several other organizations such as Takeda Pharmaceuticals Company Limited, Bill & Melinda Gates Foundation, The Global Health Innovative Technology (GHIT) Fund and many more have invested millions towards dengue research [192 - 195]. A study in Singapore confirmed that right after the infection, the immune cell called natural killer (NK) cells were activated in the blood and researchers hope that this knowledge may be beneficial while developing vaccines and drugs [196]. Moreover, another research team at University of California San Diego has synthetically engineered mosquitoes that will prevent the transmission of dengue virus by the activation of an antibody that prevents the replication of the virus and its dissemination [197]. Additionally, a study has shown that mutations in the protein envelope cause the dengue virus to change its shape and become resistant to vaccines and therapeutics [198].

Taking climate change into account for dengue research is important for the government and public health officials to take actions to protect the public from future dengue outbreaks. As the global climate changes, the global surface temperature will change and so will the patterns of rainfall around the world, affecting the environmental suitability for the survival and growth of dengue

viruses and mosquitoes, which will eventually lead to the change in patterns of dengue globally, nationally, and locally. Having said that, several issues need to be taken into account during the research on DENV such as the sociodemographic factors e.g., travel and demographic change, and other climatic factors in some areas where temperature may not be the most vital aspect influencing the spread of dengue. Moreover, the non-climatic factors affecting the spread of *A. aegypti* and *A. albopictus* must also be explored [199]. Scientists have been investigating dengue pathogenesis in order to gain a better understanding of dengue infection. Improving surveillance of dengue cases by databases such as DengueNet that continuously updates and shares current and historical data on dengue cases by providing early warnings prior to epidemics can help improve the preparedness of public health officials, and help reduce fatality rates [200]. Many experts have said that dengue may increase in the future due to viral evolution, climate change, globalization, travel and trade. Settlement factors and socioeconomic factors also play a role in the spread of dengue. In light of this, it is important to follow the guidelines in WHO Global Strategy for Dengue Prevention and Control, 2012–2020 in order to prevent a huge outbreak as neither a perfect remedy nor a vaccine has been found yet [201].

11. Conclusion

Even though dengue is the most emerging global infection in the 21st century, the adaptation of a successfully planned control program can prove as a preventative measure. Apart from this, our body's immune system also plays a major role in the process of viral neutralization. Growth in population, frequent travels as well as urbanization has shown to affect the re-emergence of the dengue epidemic. The increasing dengue outbreak along with no specific existing treatment for the disease makes prevention our first priority. Some methods of prevention may include the use of insecticides, removal of mosquito vectors or the use of vaccines, mostly in the high burden

epidemiologic areas. Rapid and more efficient dengue detection methods are required for an immediate approach towards treatment. As the factors contributing to the spreading of dengue virus are increasing with the rise in globalization and climate change, the research for dengue requires more attention in order to avoid another devastating outbreak.

Conflict of Interest

Authors declare no conflict of interest regarding the publication of the manuscript.

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