Frontiers in Chemical Sciences (FCS) 2021, 2(2); 133-145

A Systematic Review on the Use of Different Therapeutic Agents to Control Dengue Virus & other Associated Symptoms

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Abstract
Dengue is an infectious viral disease transmitted by the bite of an infected Aedes mosquito. Signs and symptoms associated with dengue vary with the severity of the disease. Dengue fever is a leading cause of serious illness and death among children in some Asian countries, including Pakistan. Increasing cases of dengue have been reported every year since 2006 . The present study aims to inquire about different therapeutic strategies used for the treatment of dengue. We investigated the effectiveness of different remedies such as fluid treatment, immunological treatment, antiviral treatment, herbal treatment, plant extract, and vaccines. This review focuses on the nature of dengue fever, the transmission of dengue virus, a possible mechanism of action of papaya, antiviral agents, vaccines, immunological treatment, and plant extracts. Fluids were found to be effective in the maintenance of normal blood volume. C. papaya leaf extract and capsules are effective in decreasing thrombocytopenia associated with dengue fever, increasing WBCs, RBCs and stabilizing normal hematocrit levels and other effects. Anti-viral agents play a role in inhibiting the process of activity of dengue viruses by inhibiting them at different levels e.g. translation, replication, etc. Different vaccines are developed for the prevention of dengue fever, but there is one vaccine that is licensed in some particular countries where dengue is originating. Vaccines having different efficiencies and safety against all dengue virus strains are under the process of development in various institutes.

Keywords: Dengue virus, therapeutic agents, infection, *Ades aegypti*, vaccine

1. Introduction
Dengue is a mosquito-borne infectious disease caused by any of four related types of Dengue virus (DENV*I*, DENV *II*, DENV *III*, DENV *IV*) (Benelli, 2016; Dawson, 1998; Hettige, 2008). It is the most prevalent disease infecting over 50 million people each year (Kroeger, Nathan, & Hombach, 2004). Pakistan has been suffering from dengue outbreaks for decades. Its climatic condition and subtropical location are suitable for the expansion of vectors as a result many vectors borne diseases may exist in this region. In Dengue fever, some patients experience intense muscle and joint pain that feels like bones are breaking that is why this disease was also called to be break-bone fever. Dengue is mainly found in tropical and subtropical regions around the world (Dawson, 1998). Transmission of Dengue fever occurs by the bite of an infected *Ades aegypti* mosquito. A person after the bite of an infected mosquito shows disease-related symptoms in about 5-7 days. As dengue has four serous types so a person may get infected by the dengue multiple times (Morens & Brody, 2008). But only once by the same type (Dawson, 1998). According to the guideline of World Health Organization (WHO) 1997, dengue patients can be classified into three types including dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. Based on WHO guideline 2009, the patients suffering from dengue can be further classified on the extremity basis that includes dengue patients with no warning signs, dengue patients with few warning signs, and severe dengue patients (Giang et al., 2018). Primary Symptoms of dengue fever are shown in Figure1. Secondary symptoms are associated with severe dengue hemorrhagic fever and are characterized by a low level of platelets and leakage of blood plasma and sometimes it is also referred to as Dengue Shock Syndrome (Pigili & Runja, 2014).

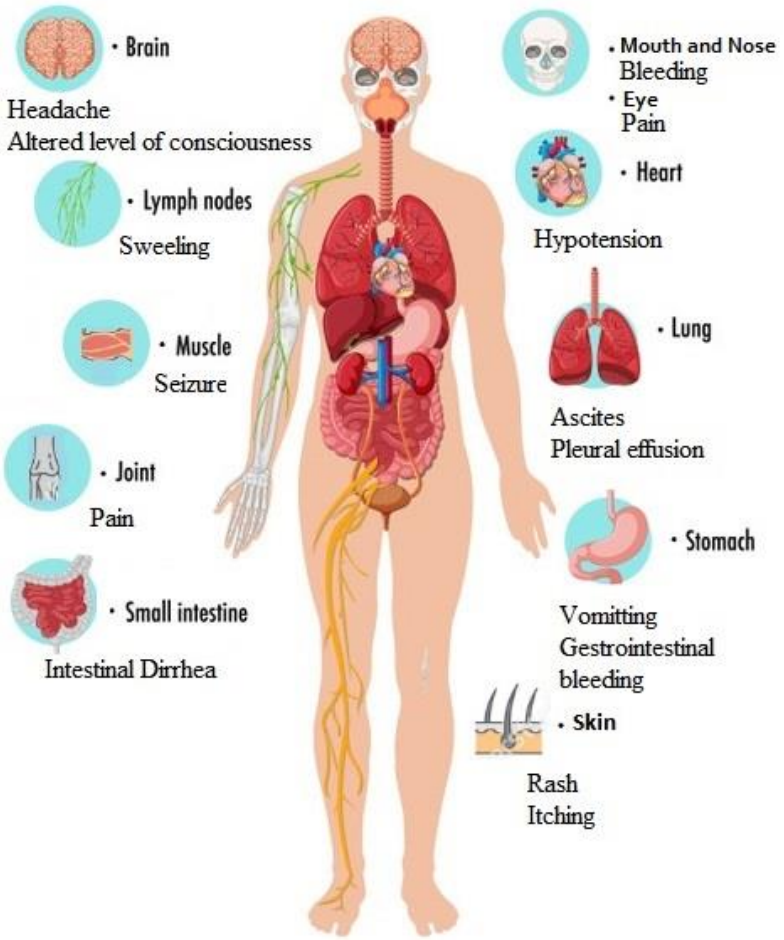

Figure 1 Symptoms associated with Dengue. The symptoms of dengue include fever, headache, severe body and joint pain, and a rash. Other symptoms of dengue fever include a decrease in the number of white blood cells and a low level of platelets in the blood. Patients with dengue fever may have skin hemorrhages (bleeding under the surface of the skin) that appear as red or purple spots on the body. Dengue fever can also cause bleeding from the skin, nose, and gums.

1.1 Dengue Virus Infection

Dengue Virus (DV) is a lipid-enveloped virus. A large polyprotein precursor is encoded by viral RNA. Three structural proteins E (envelope) glycoprotein, C (core) and prM (M protein intracellular precursor), and two non-structural proteins NS1 to NS5 are produced by protease processing of this precursor (Mukhopadhyay, Kuhn, & Rossmann, 2005; Weaver & Barrett, 2004). Endocytic pathway of DV particles is directed by E protein binding to cellular receptors. Conformational changes in E protein are triggered by an acidic environment in the endosome. This leads to the entry of the virus into the host cell by fusion of host and viral cell membrane (Weaver & Barrett, 2004).

The first cells that are believed to be targeted by DV are immature Langerhans cells (LCs) and dendritic cells (DCs) (Wu et al., 2000). The factor that helps in the entry of the virus is Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) dependent mechanism, present on dendritic and macrophages. It is a C-type tetrameric lectin that binds to several viral glycoproteins through its C-terminal carbohydrate recognition(van Kooyk & Geijtenbeek, 2003). E protein is the only glycoprotein exposed on the surface of the Dengue virus and it is responsible for virus entry into the host. Moreover, two N-linked glycosylation sites are distinctively used by four serotypes of DV. It is believed that after binding to DC-SIGN, DV is directed towards the endosome (having an acidic environment) where membrane fusion takes place. Various studies indicated that the use of anti DC-SIGN antibodies strongly inhibited DV infection. In addition to this pH rise within endosomes is also suppressed by DV infection as shown in figure 2 (Ludwig et al., 2004; Tailleux et al., 2003).

1.2. Mechanisms fueling DENV-associated thrombocytopenia

Thrombocytopenia is a common feature in both types of dengue syndrome i.e. mild and severe dengue syndromes and corresponds with clinical outcome (Bozza et al., 2008; Krishnamurti et al., 2001). These mechanisms include diminished thrombopoiesis (Krishnamurti, Peat, Cutting, & Rothwell, 2002; La Russa & Innis, 1995) and/or peripheral platelet impairment. In the latter mechanism, there is the involvement of enhanced interaction of platelets with endothelium or leukocytes, (Tsai et al., 2011) antibody-induced platelet clearance, and activation of platelet as they come in contact with the dengue virus (Ghosh et al., 2008; Noisakran et al., 2009). Recent studies propose that the interaction of platelet with virus-infected endothelium cells leads to the activation of these platelets. Moreover, DENV itself can interact with platelets inducing changes in their ultra-structure (Hung et al., 1999; Kuhn et al., 2002). Various studies stipulate activation of platelets during dengue illness however fundamentals of this process and its clinical outcomes remain unknown. In activated platelet, mitochondria are known to maintain apoptotic pathways (Leytin, Allen, Mykhaylov, Lyubimov, & Freedman, 2006; Mason et al., 2007). It was found that platelet activation was notably increased in dengue patients particularly patients with thrombocytopenia. Platelets of dengue patients showed diminished mitochondrial function with triggered apoptotic pathways. Similar activation responses were also observed by in vitro dengue virus infection along with mitochondrial function impairment and apoptosis through DC-SIGN dependent mechanism. Other than fluid therapy and supportive measures there is no specific treatment for dengue. Various therapeutic measures have been evaluated with minimum success over the last 50 years (Nanda et al.; Rajapakse, 2009). There are several difficulties regarding the management of Dengue such as lack of facilities and resources in developing countries, besides, the exact pathophysiology of dengue is not known which created hurdles in adequate evidence-based management procedures aimed at the specific pathophysiological occurrence of the illness (Thomas, Strickman, & Vaughn, 2003). In the last few decades certain preventive and therapeutic measures have been investigated to decrease the disease burden of dengue. This review provides an update regarding several therapeutic procedures for dengue fever and their positive consequences in the management of dengue fever.

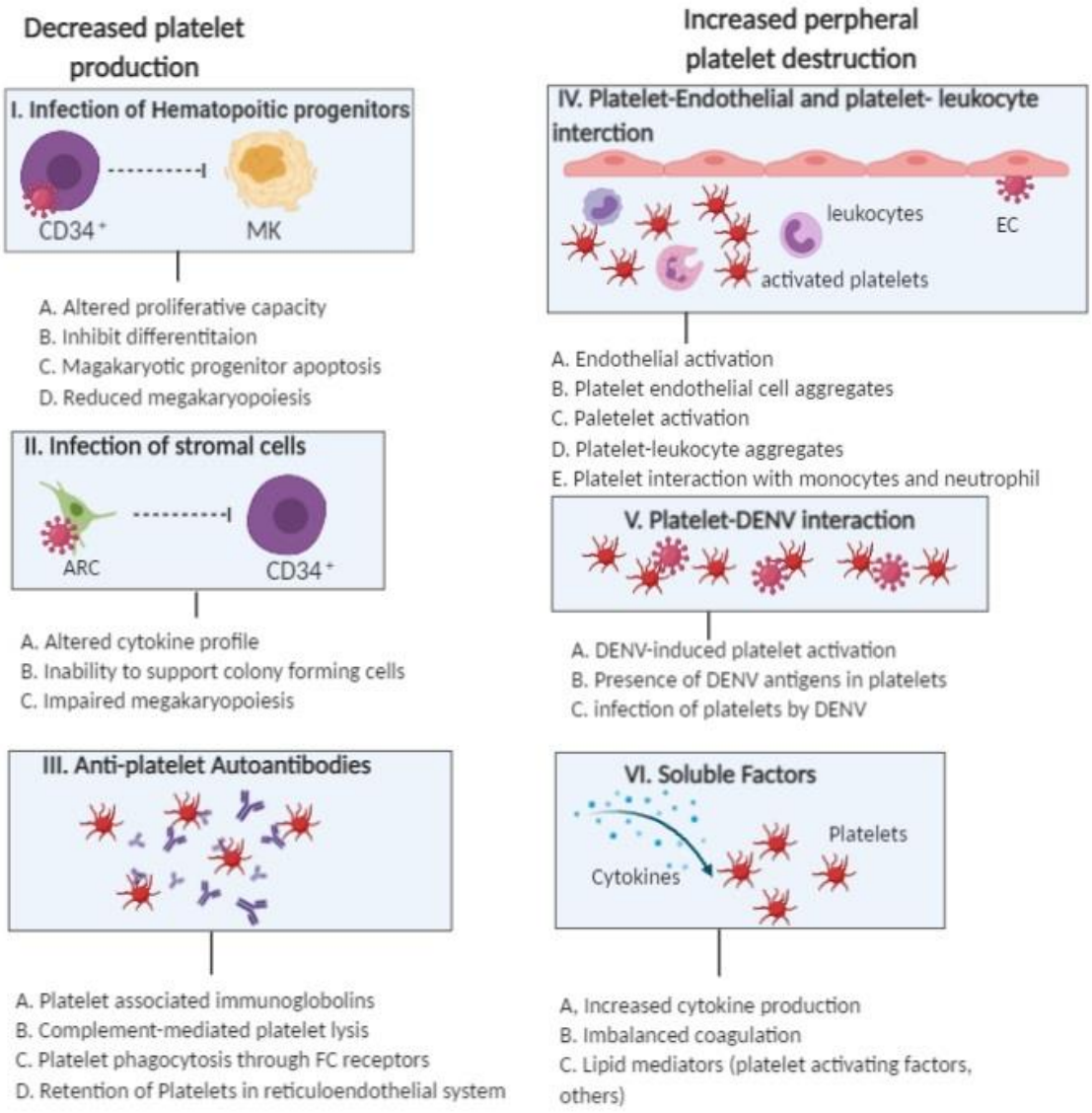

Figure 2. Molecular mechanism describing Dengue-induced thrombocytopenia

2. Management of DV

2.1. Phases in dengue infection

The natural infectious process of DV is quite straightforward. After the inoculation of the virus the incubation period is of 4-7 days (Heilman, De Wolff, Beards, & Basden, 2014). There are three phases of the symptomatic phase i.e, the first phase is febrile phase, second phase is critical phase and third phase is recovery phase (Organization et al., 2009). The fibrile phase is characterized by retro-orbital headache, nausea, malaise, and vomiting and lasts for 5-7 days. Over 2-3 days platelet count drops rapidly which leads to fever development. After 5 days of the onset of fever, there is a detectable amount of IgM antibodies (Heilman et al., 2014). The start of the critical phase is at any time from 3-7 days after the start of fever. The critical phase is characterized by the capillary permeability and extravasation of fluid (Stephenson, 2005). 48–72 hours following the critical phase there would be the re-entering of extravasated fluid in intravascular compartments there is a chance of developing pulmonary edema or heart failure if there had been extra administration of fluid (Premaratna, Liyanaarachchi, Weerasinghe, & de Silva, 2011).

2.2. Symptomatic management

There is a recommendation of antipyretic treatment with paracetamol and liberal oral fluid administration during a febrile phase. With daily full blood, count the patient can be managed at home if he or she has a nearby health care facility. But if a patient is suffering from diarrhea, severe prostration, dehydration he or she should be admitted to the hospital for close observation (Organization et al., 2009).

2.3. Critical phase management

The main problem in the management of the critical phase is the accurate identification and prediction of the beginning of this phase. The critical phase has already been started several hours ago before the appearance of its clinical symptoms (ascites or effusion). A rise in hematocrit above the baseline and platelet count lower than 100,000/ μL are also signals that the patient is in danger of entering the critical phase (Gamakaranage et al., 2012). During the critical phase, wise administration of fluid to the patient is a key management strategy. There are limited testified results regarding the amount and choice of fluid that should be administered. For volume expansion fluid used are ringer lactate, plasma substitutes, Glucose 5% diluted (1:1 or 1:2 in normal saline). Crystalloids and colloids are also being used and in Vietnam, a series of studies compared the use of the colloids and crystalloids.

It has not been contented in the trials the perfect dose and time duration after which dose should be re-administered, and guidance is based on treatment practice in different centers. With effective use of fluid mortality rate and dengue, shock state has been reduced to 0.2 % (Gamakaranage et al., 2012; Organization et al., 2009). Guidelines have been published by World Health Organization(WHO) and endemic countries local health authorities with exact details and recommendations. It is not the aim of this review to re-explain them. However, the principles of fluid therapy can be summarized as follows:

- As much as possible oral administration of fluid is necessary. When a patient is not able to take fluids orally, then intravenous supplementations are compulsory.
- During leakage phase, enough fluid in the vascular system should be maintained to avoid hypovolemia. But the patient should not be overloaded with too much fluid.
- Crystalloids (0.9 % saline) are recommended as first line intravenous fluid (Organization et al., 2009).
- During critical phase, there should be a stepwise increase or decrease in the rate of intravenous fluid administration along with 4-6 hourly hematocrit monitoring (Organization et al., 2009).
- Immediate sustainment with 20mL/kg boluses is referred until blood pressure becomes recordable.
- When low blood pressure is not maintained by boluses of intravenous crystalloids then colloids are recommended as second line therapy.
- There is a need for more fluids when hematocrit level increases as a result of hemo-concentration due to leakage. However, internal bleeding and extravasated fluid reabsorption may also lead to a drop in

hematocrit level. In cases of suspected bleeding, the management strategy is transfusion of fresh whole blood.

- Some guidelines recommend the calculation of a fluid quota for the entire critical period.
- Fluid administration should be followed by frequent monitoring and intravenous volume assessment during the critical stage(Gamakaranage et al., 2012; Organization et al., 2009).

2.4. Blood product used

Patients who have developed serious hemorrhagic symptoms or have low platelet counts are transfused with platelets. Although there is no defined platelet count at which platelet should be given. In patients with shock syndrome transfused platelets survived for a very short period (Isarangkura & Tuchinda, 1993). The amount of platelet transfused varies inversely with the degree of shock and directly with an increase in the number of circulating platelets after transfusion. Although the related effects of plasma transfusion have not been studied yet in the controlled clinical trial (Sellahewa, Samaraweera, Thusita, & Fernando, 2008). Moreover, blood transfusion is required in patients with severe hemorrhage but no published data is available on its use.

2.5. In the phase of recovery

As the patient recovers, there is no need to restrict fluids. Oral fluid therapy could be continued. But special monitoring is necessary to overcome other complications in patients with hypertension, congenital heart disease, diabetes, and congenital heart disease (Weerakoon et al., 2011).

3. Treatments

3.1. Treatment targeting the immune system

It is proposed that cytokines including interleukin-2 (IL-2), interferon-gamma, IL-8, IL-6 TNF alpha are highly elevated in the severe form of dengue and cause damage to capillaries endothelial cells resulting in fluid leakage. So the treatment involving the suppression of immunological overactivity has shown little success (Kurane, 2007).

In some studies, corticosteroids and intravenous immunoglobulins have shown some positive effect of treatment effect while in others no significant effect was found.

3.2. Treatment of anti-Viral agents

Frequently, treatment is done by the process of increased intake of fluid and by controlling pain and fever by taking paracetamol instead of aspirin because it may enhance bleeding. Some of the herbal remedies and antiviral agents are known to show better action against DF (dengue fever). The genome of this virus contains RNA, which is single-stranded. The genome contains five non-structural and three structural proteins. Anti-viral agents are still under the process of development, depending on the protein they act. Based on the protein, they act like NS5, NS3, and capsid (Fusco & Chung, 2014) they are categorized as is shown in figure 3. Anti-viral agents Balapavir and Celgosivir are in the process of clinical testing of phase 1 as NCT01096576 and NCT02569827 correspondingly (Low, Ooi, & Vasudevan, 2017; Nguyen et al., 2013). Research is going on to check the efficiency of Sofosbuvir for the medication of dengue fever. Sofosbuvir is already used in the treatment of hepatitis. An active metabolite of Sofosbuvir (GS-461203) was evaluated by the analyses of molecular docking. It performs its action on NS5, a non-structural protein that functions as RNA-dependent RNA polymerase for duplication of the dengue virus.

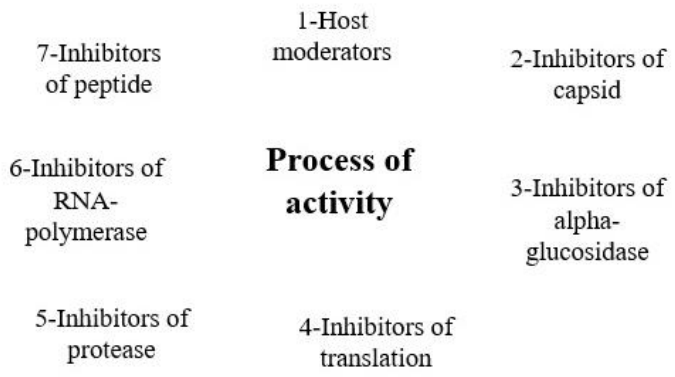

Figure 3: Categorization of anti-viral agents based on their mechanism of action. Antiviral agents can be divided into 7 types

3.3. Herbal remedies

Alkaloids, steroids, saponins, glycosides, carbohydrates flavonoids, and terpenoids are found in the extracts of the unripe papaya (Ayoola & Adeyeye, 2010). Anti-oxidants improved red blood cell membrane stabilization (Ranasinghe et al., 2012), and free radical scavenging properties (Okoko & Ere, 2012) of papaya leaf extract, as well as hematological and anti-viral effects, are used in the treatment of dengue. Flavonoids from leaf extract are used to inhibit a protease enzyme, involved in the assembly of the virus (Senthilvel et al., 2013). Thrombocytopenia associated with the condition of dengue is treated by the use of papaya plant. It is shown by the research that leaf extract of Papaya shows immunoregulatory effects because it is involved in the regulation of Th1 immune process, so plays a role in the prevention of immune shift to Th2 type immune reaction (Chaturvedi, Agarwal, Elbishbishi, & Mustafa, 2000). Effects of treatment of different studies based on the use of *C. papaya* are described in table 1.

Parida et al organized an in-vivo and in-vitro study on the leaves extract of *A. indica*. Positive effects of this plant contrary to the dengue virus particularly for serotype 2 are shown by his study (Parida, Upadhyay, Pandya, & Jana, 2002). *A. paniculata*, belongs to Acanthaceae family, is an erect herb with an extremely bitter taste. Methanolic extract of *A. paniculata* showed antiviral activity on dengue fever as reported by in vitro studies of Tang et al., (Tang, Ling, Koh, Chye, & Voon, 2012). It was found to be a powerful anti-dengue agent, specifically towards DENV-1 serotype.

C. austral is a member of Fabaceae family. Anti-viral activity of castanospermine has been investigated by Whitby et al (Whitby et al., 2005). Castanospermine, a natural alkaloid, is derived by in vitro assay from the tree *C. australae*. Studies on the mechanism of action of castanospermine suggest that it can hamper the deletion of terminal glucose units on N-linked glycans in viral proteins and thus disturb the folding of proteins in the dengue virus.

B. diffusia is a member of *Nyctaginaceae* family. Anti-dengue effects of the plant of *B. diffusa* Linn & the stem of *Tinospora cardifolia* Miers were studied by Rajashree Sinha & Priyank Bharati. Anti dengue effects were examined by providing the Ayurvedic mixture of *Boerhaavia diffusa* & *Tinospora cardifolia* to dengue patients (2-3 times each day) (Meshram, Itankar, & Patil). *A. herbaceous* perennial flowering plant, *Houttuynia cordata* is a member of family saururaceae. The principal bioactive compound, hyperoside, is a chief component of this plant and was found to play a role in inhibition action against DENV-2 (Leardkamolkarn, 2012).

Recently potential ovicidal properties of the plant extract pure metabolites and essential oils have been proposed against the mosquito vector. Only five herbal extracts are included because of their clinical/preclinical evidence. Herbal extracts (0.1 ppm) made by using *P. glabra* (karanj) and (*A. indica*) neem oil showed mortality of eggs (100%) against A. mosquito. In the hexane leaves extract of *Limonia acidissima* (2 ppm) protolimonoid nilocitin is present which exhibits 83.2% of ovicidal activity as described in the study of Benelli carried out in 2015 (Benelli, 2016).

4. Vaccines

4.1 CYD-TDV Dengvaxia

Sanofi Pasteur's CYD vaccine is a recombinant tetravalent live attenuated vaccine. The vaccine contains the enveloped and pre-membrane proteins isolated from a wild-type virus similar to four serotypes are placed into the 17D vaccine backbone of yellow fever. Evaluation of vaccine strain (serotype 2) was done in the first clinical trial in healthy adults, in which a strong antibody neutralizing response against DENV2 was drawn out. CYD is the first licensed vaccine, which is recently registered in 15 countries. It has a three-dose schedule of immunization which is administered subcutaneously after 6 months (Ravel et al., 2017). In seronegative children, vaccination with Dengvaxia can mimic early infection in the first step in the development of antibody-dependent enhancement (ADE). Vaccine protection is not equal for all serotypes and is incomplete, so if natural infection with virus occurs later in life, it can complete all sequence of events, causing serious life-threatening fever and ADE (Dans, Dans, Lansang, Silvestre, & Guyatt, 2018). It should be given after 0, 6, and 12 months schedule (three-dose series). Each country should define the target age for routine vaccination, purposed for a planned facility of targets belonging to a specific age group, and maximize the impact of vaccination (Pinheiro-Michelsen et al., 2020).

4.2 DENVax

TDV type is a tetravalent live dengue vaccine, which is build-up based on serotype 2 strain (TDV 2). TDV 2 is an attenuated molecularly proposed strain. Bypassing through 53 serial passages of the DENV-2 wild type in PDK (primary dog kidney) cells, the PDK-53, which is a DENV-2 virus was achieved initially. Based on clinical trials which were conducted in Thailand and United States, PDK-53 virus has been confirmed to be antigenic, well-tolerated, and safe. By exchanging the enveloped and pre-membrane structural genes of respective DENV type (strain) within the attenuated backbone of TDV 2 three recombinant strains (TDV 4, 3, and 1) were engineered (Ravel et al., 2017). As it contains the distinctive enveloped and pre-membrane proteins for all serotypes, TDV is constructed to enhance cellular and humoral immune responses against all serotypes of the dengue virus. DENV 2 used as a structural backbone can induce additional safety against dengue.

4.3 TV005 & TV003 Dengue vaccine

To identify types of vaccines with highly acceptable safety, immunogenicity profile, and infectivity various tetravalent and monovalent candidates of dengue vaccine were tested by the Laboratory of infectious diseases. From these, TV003 is a mixture of four live attenuated recombinant candidate viruses of the dengue vaccine (Kirkpatrick et al., 2016). To optimize strains of the dengue vaccine, numerous monovalent types were tested initially (Phase 1 trial). It was found that serotype 2 is a chimeric virus based on serotype 4 strain vaccine having replaced structural proteins by serotype 2, while serotype 1, 4, and 3 are vaccine viruses depending on complete viruses. Against each serotype above 90% seroconversion rates are elicited by a single dose of TV005.

4.4 DPIV tetravalent purified inactivated vaccine

A type of tetravalent live-attenuated vaccine against dengue virus was made by WRAIR (The Walter Read Army Institute of Research) in co-operation with GSK (GlaxoSmithKline). It consists of four live strains of the virus, depicted for four DENV serotypes. Bypassing these strains in a serial passage in PDK cells (Thomas et al., 2013), they were attenuated. A tetravalent DNA vaccine (TVDV) having an adjuvant known as Vical's Vaxfectin and genes which encode the envelope and pre-membrane proteins for all types of dengue viruses is constructed by NMRC (Naval medical research center) of US Navy. Both adjuvants formulated and non-formulated vaccines are recently evaluated in the first phase of human testing. A well-accepted safety profile in immunocompromised hosts

as well as through a broad age range is provided by inactivated vaccines. These can also be administered concurrently with other vaccines. This type of vaccine can be used to provide the feasibility of rapid immunization and shortened vaccination scheme. For such reasons, an efficient and safe tetravalent dengue virus PIV could be appropriate for national programs of immunization across baseline health status and wide age ranges. It is also suitable as an option of active immunization for military persons and travelers and a potential instrument for outbreak reaction (Martinez et al., 2015).

4.5 Recombinant protein and chimeric vaccines

Another approach for the development of a recombinant chimeric vaccine for dengue is molecular genetics. This is done by the use of the backbone of correlated flavivirus by the substitution of PrM-E gene with a correspondent DENV gene. The goal is to integrate the dengue antigenicity while retaining the attenuation properties of the backbone viral vaccine. The NIH (National Institute of Health) in the USA & NIAID (National Institute of Allergy & Infectious Diseases) had employed different procedures that depend on infectious cDNA clones derived from DEN4 814669 strain, to attenuate the dengue virus. Reverse genetics is employed for the mutation of 3′ UTR (untranslated region) in the cDNA clone. The deletion of thirty nucleotides from DENV-4, -1 resulted in a successful balance between attenuation & immunogenicity in both humans & monkeys. This exclusive method as in the possession of high virus titer & envelope genes is considered to be essential while decreased viral replication is guaranteed by the deletion mutation. The DENV E-protein has been chosen as the primary antigen, for the development of recombinant protein vaccine, where correct expression & folding of E-protein is assisted by PrM chaperone protein (Zahid et al., 2020).

5. Conclusion

To find more efficient and less toxic drugs against the dengue virus, the formation of new anti-dengue products from biologically active compounds is necessary. Plant extracts and anti-viral agents are showing inhibitory effects against different stages of viral strains. Good efficacy of fresh juice from the leaves of Carica papaya has been reported against the dengue virus. So, it can be used as a promising therapy to treat this disease in the future. Still, it would require a proper standardization and well-established pharmacodynamics and pharmacokinetic and studies for this extract or juice. Only one dengue vaccine Dengvaxia is registered for use against the dengue virus. Other vaccines are in different phases of the clinical trial. So by this study, we concluded that all therapies have some positive consequences. But no clinically standardized dosage of these therapeutic agents and well-defined administration procedures are yet available. Further studies are needed to discover more therapies against different dengue virus serotypes as well as standardize the available therapies.

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