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REVIEW ARTICLE

A BRIEF SCENARIO OF DENGUE VIRUS

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ABSTRACT

Dengue is one of the most emerging vector-borne viral diseases. Prevalence has enlarged 30-fold in the last 50 years with the increased geographic expansion to new countries. An estimated 50 million dengue infection occurs annually and approximately 2.5 billion live in dengue endemic countries where more than 21,000 deaths occur each year. Dengue is anticipated to rise due to factors as contemporary modification of climate, travel, globalization, trade, viral evolution, socioeconomics and settlement. Poor disease scrutiny, misdiagnosis, limited public knowledge, low levels of reporting have found it hard to establish the accurate impact of dengue internationally. Dengue disease with accessible statistics possibly underestimates the pathophysiological, economic, social and ecological problems. Target of numerous vaccines now in progress is to bring out defensive neutralizing antibody responses are going on through clinical evaluation. The need of balanced immune response against all four DENV serotypes with a single vaccine is the main challenges encountering by the developers. The mortality can be reduced with prompt case detection, appropriate clinical management, and reporting but a safe and effective vaccine is probably the only long-term solution. Hence there is a need to build up an efficient, low-cost and safe vaccine that can target all the four serotypes of dengue virus.

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INTRODUCTION

Dengue is documented and recognized as the most important acute mosquito-borne viral infection of humans placing a significant socioeconomic and disease burden in urban areas in tropical and sub-tropical countries in Southeast Asia, the Pacific and the United states (**Guzman and Kouri, 2002; Gubler 2011**). The first probable recorded dengue fever (DF) was referred as “water poison” in a Chinese medical encyclopedia during the Jin dynasty (265-420 AD) (**Whitehorn et al., 2010**). Dengue is originated from the word ‘Swahili’ meaning “break bone fever” associated with myalgia and arthralgia (www.globalmedicine.nl/index.php/dengue-fever). The four distinct dengue virus serotypes referred to as DV-1, DV-2, DV-3 and DV-4, originated from the family Flaviviridae and genus Flavivirus (**Whitehorn et al., 2010**). The four serotypes has augmented to cause a wide spectrum of illness from mild asymptomatic illness to severe fatal as associated with Dengue fever (DF), Dengue haemorrhagic fever/ Dengue shock syndrome (DHF/DSS). An attempt to understand the evolutionary relationship of the four serotypes has been carried out (**Rodriguez-Roche et al., 2005**). DV comprises of three structural protein genes and is a positive-stranded RNA genome which encodes — the nucleocapsid or

core (C) protein, membrane-associated (M) protein, envelope (E) glycoprotein — and seven non-structural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) (Halstead, 2008). (Figure 1) They are transmitted and highly adaptable mainly by the bite of infected mosquitoes *Aedes aegypti* and *Aedes albopictus*. The early clinical manifestations marked by continuous high fever lasting 2-7 days, malaise, headache and rash which in the later stage progress with symptoms such as bleeding, thrombocytopenia (platelet count <100,000 platelets mm⁻³), plasma leakage manifested by haemoconcentration, persistent vomiting, adynamia (loss of strength or vigor), fainting, ascites, severe and continuous pain, etc (**PAHO, 1994; Nimmannitya, 1987**).

The main objective of this paper will provide an update to review the history, transmission cycle, also have an impact upon the diagnosis and control measures and the prospective behind the progression to be made for development of dengue in the upcoming future.

Historical background

The epidemic that resembled clinical dengue-like illness with its widespread occurred as early as 1635 and 1699 in the West Indies and Central America.

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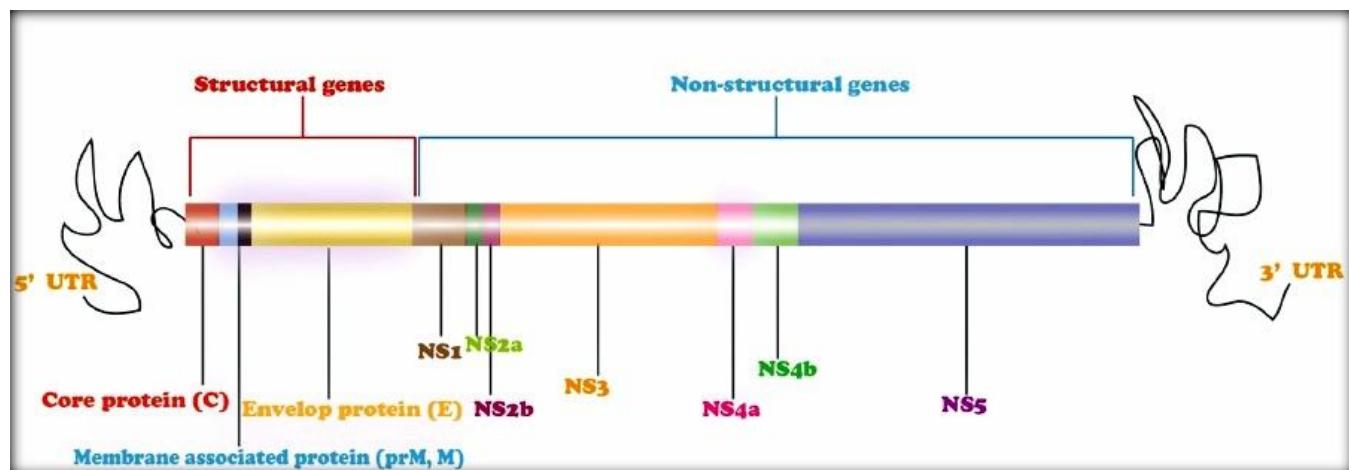


Figure 1. Dengue Virus showing 3 structural proteins genes (C, M and E) and 7 non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5)

In Philadelphia a foremost outbreak occurred in 1780 followed by a quick global spread of epidemics which became common in the USA into the early 20th century (Gubler, 1997; Wilder-Smith and Gubler, 2008). Due to the commercial expansion of the shipping and development of port cities in the 18th and 19th centuries the mosquito vector, *A. aegypti* and the dengue viruses extended steeply to new geographic areas. The transmission cycle for the vector along with humans completes with the help of the shipping vessels to the tropical coastal sites around the world (Wilder-Smith and Gubler, 2008; WHO, 2011; Gubler, 2002). During the World War II the extension of transmission became keen and more sweeping by the employment of the modern transportation connecting countries (Wilder-Smith and Gubler, 2008). Following the end of the war, rapid urbanization in Southeast Asia led to increased transmission and hyperendemicity with abrupt appearance of the consequence of the dengue (Gubler, 2011).

The operation as conceded by the Pan American Health Organization (PAHO) controlled the spread of the disease all through the American continent (WHO, 2011; Shepard *et al.*, 2011). The discontinuation of the operation urge once again to broaden the disease in 1970s as the break in dengue was brief. The escalation of the incidence has amplified over the last five decades and reports of cases have also prolonged (WHO, 2013).

The former outbreak of clinical dengue-like illness in India was recorded in Madras (now Chennai) in 1780s and the prevalence of outburst of dengue fever (DF) occurred in Calcutta (now Kolkata) and Eastern Coast of India in 1963-1964 (Sarkar *et al.*, 1964; Chatterjee *et al.*, 1965; Carey *et al.*, 1966). The secluded dengue virus was observed by inoculation of serum in suckling mice in Japan in 1943 (Kimura and Hotta, 1944). The serum samples of US soldiers were carried out to isolate the virus at various parts of the world including Calcutta (now Kolkata) in 1944 (Sabin and Schlesinger, 1945). Dengue haemorrhagic fever (DHF) occurred in India in 1996 linking areas around Delhi (Dar *et al.*, 1999 and Lucknow Agarwal *et al.*, 1999) and subsequently it extend to all over the country (Singh *et al.*, 2000; Shah *et al.*, 2004).

Transmission cycle

The key vector *A. aegypti*, a well reclaimed tropical mosquito, is a small, black-and-white that put down its eggs usually in and around the human vicinity, for e.g. old automobile tires, open septic tanks, flower vases, buckets that collect rain water, cement cisterns, and wastes of all-purpose. The adult mosquito can be identified by the white bands or scale patterns on its legs and thorax. They prefer to nourish on humans in the early morning (for 2 to 3 h after daybreak) and the late afternoon (several hours before dark). An epidemic transmission cycle may occur by the inoculation of the virus on humans with the bite of the female mosquito saliva. The virus replicates and localizes in various target organs as local lymph nodes and the liver which further spread out via blood to contaminate white blood cells and other lymphatic tissues. The virus circulating in the blood undergoes an incubation period of 3 to 14 days (average 4 to 7 days), results in viraemia that lasts for about five days. During the viraemic period, an uninfected female Aedes aegypti mosquito bites the person and ingests blood containing dengue virus. Further replication takes place within the mosquito undergoes an extrinsic incubation period of 8 to 12 days and when the mosquito bites another human, the cycle continues. Being nervous feeders, these female mosquitoes get distracted in the feeding process at the least movement, merely to revisit to the same or a different person to continue feeding process. The reason for these deeds habitually feed on several persons during a single blood meal and, if infective, may convey dengue virus to numerous persons in a small time (Gubler and Rosen, 1976; Platt *et al.*, 1997; Putnam and Scott, 1995; Scott *et al.*, 1997). It is not exceptional to see numerous members of the same household become ill with dengue fever within a 24 to 36-h time edge, signifying that all of them were infected by a single infective mosquito.

Clinical manifestations

Dengue infection may be asymptomatic or may cause undifferentiated febrile illness (viral syndrome), dengue fever (DF), or dengue haemorrhagic fever (DHF) including dengue shock syndrome (DSS).

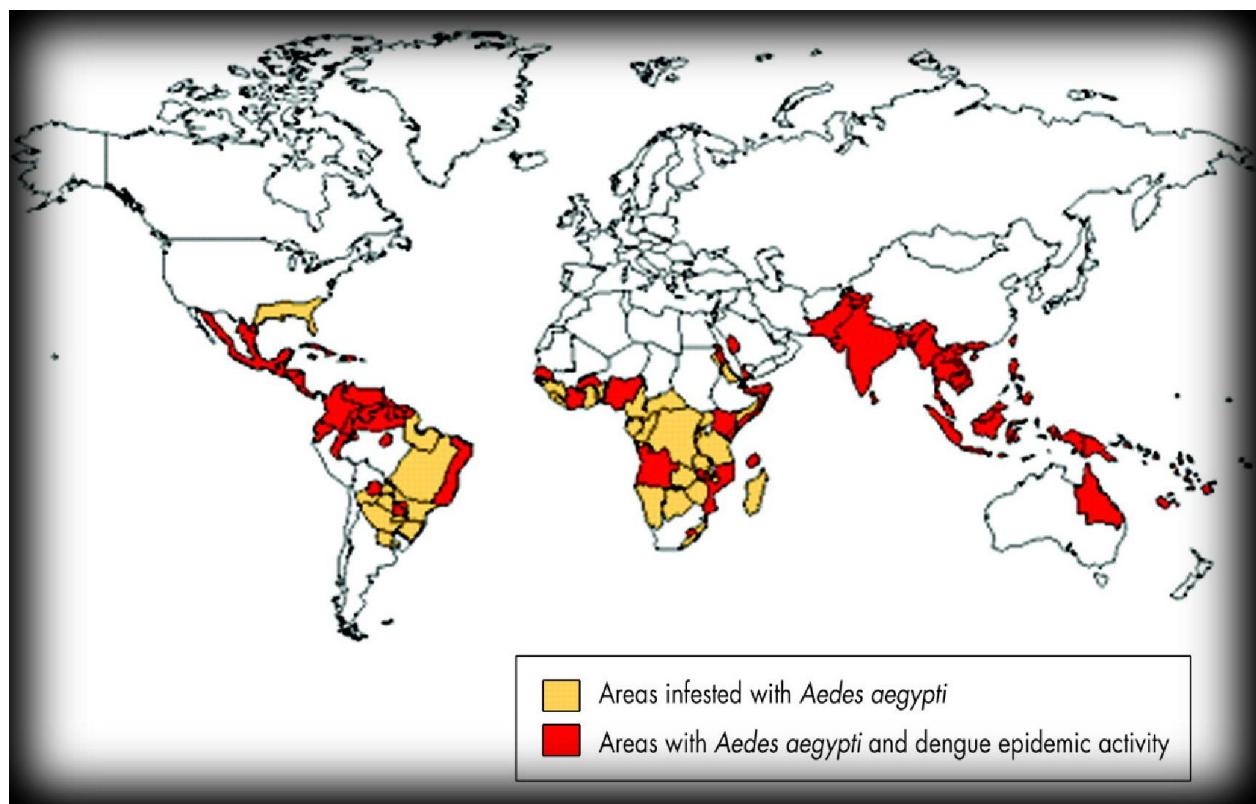
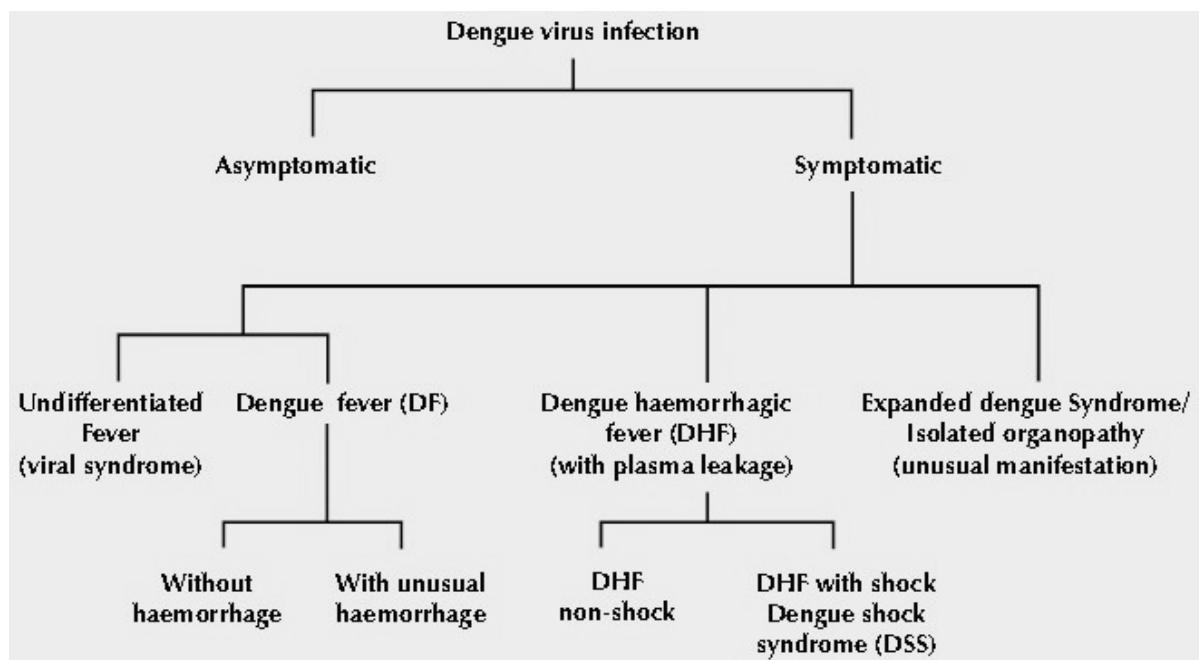


Figure 2. Global Dengue Risk (Based on combined reports from the World Health Organization, the Centers for Disease Control and Prevention)

Infection with one dengue serotype gives lifelong immunity to that particular serotype, but there is only short-term cross-protection for the other serotypes. weakness, and rash (Waterman and Gubler, 1989; Siler *et al.*, 1926; Hayes and Gubler, 1992).



Typical Dengue fever (DF) is primarily a disease characterized by the sudden onset of fever and a variety of nonspecific signs and symptoms, including frontal headache, retro-orbital pain, body aches, nausea and vomiting, joint pain, abdominal pain,

Beside these reports of patients as may be anorexic, have altered taste sensation, and have a mild sore throat. Constipation, diarrhea and respiratory symptoms are infrequently reported and may be due to concurrent infections.

The feasible predictive marker of development towards severe disease as associated with symptoms like conjunctival congestion, rash and abdominal pain, melaena and haematemesis were the most common manifestations in (Dengue haemorrhagic fever) DHF. The clinical presentation reported in neurological manifestation of dengue infection are encephalitis, meningitis, acute motor weakness, seizures, optic neuritis, Guillain Barre syndrome and acute viral myositis (**Misra et al., 2006; Verma et al., 2011**).

The Global renaissance of DF/DHF is accountable for unplanned and uncontrolled urbanization, exceptional population growth, augmented air travel, lack of valuable mosquito control programme and worsening of Public Health infrastructure. The hazard hauling with the amplified mass of the mosquito are due to warm and humid climate, water storage outline in houses, debris in open spaces, socio-economic,

case suggests a probable dengue infection and is detected by Capture-ELISA. Artificial NS1 receptors as a reuseable microchip can confine and recognize NS1 instantly and may be used for bedside diagnosis of dengue infection (**PAHO 1994; Tai et al., 2006; TDR/WHO 2009**). The laboratory criteria for confirmed and probable dengue infections are

Confirmed Dengue infection	Probable Dengue infection
Virus isolation	IgM positive
Genome detection	Elevated IgG titre (that is, 1,280 or greater by haemagglutination inhibition test)
Antigen detection	
IgM or IgG seroconversion	

MAC-ELISA

IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) was developed for dengue by the Armed Forces



Figure 3: Hemorrhagic Manifestations of Dengue Infection. (A. Typical rash, B. Bleeding at the site of injection, C. Macular rash, D. Proximal nail fold hematoma in male patient with Hemorrhagic Dengue).

settlement factors etc. In view of the above enlighten factors accountable for disease, it is crucial to reveal on arrangement and strategic track that attempts to trim down the impact of further spread of the disease.

Laboratory diagnosis

The broad spectrum of Dengue infection ranging from mild febrile illness to several severe syndromes makes precise analysis complex. The diagnosis is conceded out by isolation of the virus using infant mice or in tissue culture or by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The presence of NS1 antigens in blood during viraemia is detected by ELISA. An IgM or IgG antibody is the standard for serologically confirming a dengue infection. The presence of IgM or IgG in acute serum collected from a suspected dengue

virus-infected cell culture supernatants or infected suckling mouse brain preparations) is used for the assay (**Cardosa et al., 1992**). IgM in the test serum of dengue patient is detected by former capturing all IgM using human-specific IgM bound to a solid phase. MAC-ELISA has better sensitivity and specificity of 90% and 98% than the other tests present (PAHO 1994). Beside the serum of infected patient, dengue specific IgM also can be detected in saliva and in whole blood samples. The limitation behind the MAC-ELISA lies with the false-positive results due to dengue-specific IgG and cross reactivity with other flaviviruses.

IgG ELISA

Dengue-specific IgG ELISA test can be used to confirm a dengue infection in paired sera. The assay is carried out by the utilization of the serum dilutions to titre dengue-specific IgG or by using the ratio of IgM to IgG (**Vazquez et al., 1997; Falconar et al., 2006**). The mixture of four dengue antigens as MAC-ELISA correlates with results from the haemagglutination inhibition assay. IgG response to the prM membrane glycoprotein is specific to individual flaviviruses as no cross reactivity was observed in sera collected from dengue infected individual or Japanese encephalitis virus (**Cardosa et al., 2002**). The combination of recombinant polypeptide of the envelope protein shows high specificity in dengue-specific IgG patient. The sero-epidemiological studies for the identification of past dengue infection is practical with the IgG assays (**Baretto dos Santos et al., 2004**).

IgM:IgG ratio

IgM and IgG capture ELISAs assay can be used to distinguish primary from secondary dengue virus infections. The process can distinct dengue infection as a primary infection if the IgM:IgG OD ratio is greater than 1.2 (using sera of patient at 1:100 dilution) or 1.4 (using sera of patient at 1:20 dilution). Similarly for the secondary infection if the ratio is less than 1.2 or 1.4 (**Kuno et al., 1991**).

Reverse transcriptase PCR (RT-PCR)

RT-PCR assay as one of the nucleic acid amplification test (NAAT) has been developed for the diagnosis of the dengue which aim different genes and utilize different amplification events. The method is rapid, sensitive, simple and reproducible if properly controlled and can detect viral RNA in human samples, autopsy tissues, or mosquitoes (Guzman and Kouri, 1996). The technique employed in the PCR reaction involves an initial reverse transcription and amplification step. It is followed by using of dengue primers that aim a preserved region of the virus genome with a second serotype specific amplification step. Further electrophoresis on an agarose gel is performed allowing the dengue serotypes to be differentiated on the basis of size (Raengsakulrach *et al.*, 2002).

Real-time RT-PCR

This is a single-step NAAT assay which has the advantage to determine viral titre early in the dengue illness in approximately 1.5 hours (Vaughn *et al.*, 2000). Real-time RT-PCR developed are singleplex (detecting one single serotype per reaction), multiplex (identifying all four serotypes from a single sample) (Kong *et al.*, 2006). The different assays of nucleic acid amplification tests (NAATs) developed for the diagnosis of the dengue infection has not been commercialized to date and quality assurance equipments are not broadly accessible to guarantee the excellence of the outcome.

Dengue control and prevention strategies

With the mounting geographic distribution and amplified disease prevalence in the past 20 years, a worldwide approach for dengue prevention and control has become more vital (**Monath, 1994**). Undoubtedly, the prominence ought to be on

disease prevention if the trend of growing disease is to be inverted. Efforts made to aim on active laboratory-based surveillance based on a comprehensive health information system, emergency response, education of the medical community to ensure valuable case management, community-based integrated mosquito control, and effective use of vaccines when they become available (**Gubler and Casta-Velez, 1991**). The three primary aspects to focus on as supervision for planning and response, dropping the disease burden and altering behaviours to improve vector control has been carried out. (**WHO, 2000**) Still, with time, recognition and appropriate clinical management, includes maintaining proper fluid balance, NSAIDs (paracetamol) for fever and discomfort, blood transfusion in case of decreasing hematocrit, the case fatality of severe dengue can be lower. Development of several innovative enhanced or validated tools and strategies for dengue control and prevention have been developed in the recent years which are accessible to public health practitioners and clinicians.

Vector Control

Vector control programme serve as one of the good technique to minimize or reduce dengue virus transmission in most endemic countries; but its delivery by public health practitioners is often inadequate, unproductive or both. *A. aegypti*, the commonest vector of dengue virus specify that it is well established in peri-urban areas. A mixture of vector-control methods, remarkably environmental supervision and chemical control methods (anti larval substances) is required for the avoidance of primary vector *A. aegypti* (TDR/WHO 2009). International Programme on Chemical Safety (IPCS) has assessed the active ingredients of larvicides to determine their safety. Several reports from India have confirmed resistance of mosquito vector with anti larval substances like DDT & Dieldrin but susceptibility to malathione is reported. Organophosphate substances like Temephos are comparatively more effective in controlling *A. aegypti* followed by fenthion, malathion and DDT (**Tikar et al., 2008**). Larvivorous fish and copepods (Biological control agents) have a comprehensible role in controlling *A. aegypti* (**Nam et al., 2000**). Plant based repellent (*Poncirus trifoliate*) have various actions against different life stages of *A. Aegypti*. Leaf extract of *Eclipta alba* have shown potential for controlling *A. aegypti* mosquito. Nanosilica (Hydrophobic) is also proved to be effective against mosquito species (**Govindarajan et al., 2011; Barik et al., 2012**).

Environmental supervision plays as an indispensable module of dengue prevention and control. Source reduction, 'clean up' campaigns, targeting for cleaning and emptying not only household's containers but also public spaces (green areas, schools and cemeteries), solid waste management, installation of water supply systems and urban planning falls under the criteria of environmental management. Access to secure and dependable water supplies and solid waste disposal systems requires huge funds in infrastructure. Beside this introduction of metered water, actually support the household collection and storage of roof catchment rainwater, which can be harvested at no cost. Furthermore, forthcoming tools as window curtains and water container covers (treated with long-lasting

insecticide) are being tested with controlled release larvicides that supply several months of control following a single application to targeted containers. Profitable products for personal and household protection have a huge potential for pest control (**Focks and Alexander, 2006; Kroeger et al., 2006**). Dengue transmission programme will encompass an impact upon the society with the improved organized control services using new tools and partnership strategies, based on the values of integrated vector management, role of public health workers and commitment towards prevention and control (**TDR/WHO 2009**).

Vaccine development

With the ongoing stretch and increasing intensity of dengue, transformation of awareness and investment in dengue vaccine development took place, making secure, valuable and reasonable tetravalent dengue vaccine worldwide for public health precedence. Dengue vaccines have been under progress since the 1940s, but we do not have a valuable vaccine against it, which indicates problems in its progress. Problems include the complex pathology of the illness, need to control the four virus serotypes concurrently, pre-existing heterotypic dengue antibody as a risk factor for DHF and inadequate investment by vaccine developers have hindered the progress (**Hombach, 2007**). The secondary infection associated with DENV poses a extraordinary challenge in the progress of dengue vaccine indicating for such vaccine to provoke a vigorous immune response against the four serotypes in naive as well as in earlier immune individuals (**Hombach et al., 2007**). Development of tetravalent vaccine which concurrently provides long-term protection against all DV serotypes is round the corner (**Guy et al., 2011**). Tetravalent formulation that retains the immunogenicity of all serotypes has proven complication requiring the inclusion of multiple immunization regimens (**Chaturvedi et al., 2005**). Several vaccines candidates under progress have focused mainly on live attenuated virus vaccines, live chimeric virus vaccines, inactivated virus vaccines, subunit virus vaccines, vector and DNA vaccines (**Murrell et al., 2011**).

Live attenuated virus vaccines

Live virus vaccines resultant from an attenuated DENV ought to tentatively provoke robust humoral and cellular immune response to both structural and non-structural (NS) proteins. Subsequent to vaccination, symptoms as fever, headache and arthralgia seen in natural dengue disease would not be acceptable. Beside this, asymptomatic or sub-clinical signs of infection as mild rash, mild liver enzyme elevations and transient leucopenia would be acceptable as they are part of the normal response to a replicating DENV (**Guirakhoo et al., 2006**). The ongoing attenuated DENV vaccine is carried by means of access in tissue culture cells for each serotype of DENV by the investigators as at Mahidol University in Bangkok, Thailand and the Walter Reed Army Research Institute (WRAIR) group in the USA separately (**Bhamarapravati and Sutee, 2000; Sun et al., 2003**). Thai adults and children were taken into consideration for Phase I and II clinical trials by the Mahidol group. Some of the volunteers among them showed unacceptable reactogenicity

(**Sabchareon et al., 2002; Sabchareon et al., 2004**). Similarly problems of unbalanced immunogenicity and reactogenicity was also faced by the WRAIR-produced tetravalent dengue vaccine formulation (**Sun et al., 2003**). Further evaluation is to be carried for the new formulations for its efficacy as a safe and immunogenic one (**Thomas et al., 2013**). Attenuation of the viral genome is carried out on the basis of site-directed mutagenesis as with the deletion of nucleotides to retain the immunogenicity in animal models and humans (**McArthur et al., 2008; Murphy and Whitehead, 2011**).

Live chimeric virus vaccines

Sanofi Pasteur's ChimeriVax platform has been used by Acambis (Cambridge, USA) for the tetravalent chimeric vaccine (CVD1-4) with the utilization of the licensed YFV 17D vaccine as genetic backbone by substituting the genes for the prM and E proteins from each of the four DENV serotypes (**Guirakhoo et al., 2001**). The tetravalent vaccine is genetically and phenotypically stable as confirmed in pre-clinical studies, appeared to be safe with low viraemia, less neuro virulent than YFV 17D and immunogenic (**Barrett et al., 2005; Guirakhoo et al., 2004; Capeding et al., 2011**).

Inactivated virus vaccines

Its advantages over live attenuated virus vaccines lies on its safety, as it is not possible for inactivated vaccines to revert to a more pathogenic phenotype and induction of a balanced antibody response. Challenges linger as lack of immunity to NS proteins, necessity of adjuvant for enhancement of immunogenicity, multiple booster doses are required to provide long-term immunity and also they can be costly to manufacture as DENV does not raise to high titres in tissue culture cells (**Simmons et al., 2006**). A purified, inactivated DENV2 vaccine showing immunogenicity and protective role in mice and rhesus monkey might be useful as a military or traveler's vaccine or as a part of a prime-boost approach with live or replicating vaccines (**Simmons et al., 2006; Putnak et al., 1996**).

Live recombinant, DNA and subunit vaccines

Dengue vaccine efforts in current scenario of molecular biology progress by means of live recombinant, DNA and subunit vaccines. The main immunogen here serve as the dengue antigens, primarily E proteins. Beside this, immunogenicity and protective efficacy in animal models have been developed with the recombinant E proteins (from yeast and insect cells) (**Jaiswal et al., 2003; Clements et al., 2010; Coller et al., 2011**). Monovalent DENV-2 or DENV-4 truncated E proteins using alum as an adjuvant have been developed and immunized in monkeys found to achieved partial protection (**Guzman et al., 2003**).

The flow in the publication on the progress of dengue vaccine with the incorporation of biotechnology techniques denotes the deep curiosity and necessity for combating the dengue disease. So as such new vaccine candidates have been close to, or

currently in, clinical evaluation have been mentioned in the Table 1.

interventions that are presently available (**Schneider et al., 2004; Jessie et al., 2004**).

Table 1. List of dengue vaccine candidates that are under development

Vaccine approach	Vaccine developer(s)	Status
Live attenuated tetravalent chimeric YF-DEN	Sanofi Pasteur	Phase III (Kitchener et al., 2006)
Live attenuated tetravalent viral isolate	WRAIR and GSK Biologicals	Phase II (Edelman et al., 2003; Sun et al., 2006)
Live attenuated chimeric DEN2	CDC and Inviragen	Phase I (Huang et al., 2003)
Recombinant E subunit	Merck	Phase I (Putnak et al., 2005)
Live attenuated tetravalent vaccine comprising 3' deletion mutations and DEN-DEN chimeras	US NIH LID and NIAID	Phase I/II (Blaney et al., 2006)
Subunit recombinant antigen (domain III)	IPK/CIGB	Preclinical
Live attenuated chimeric YF-DEN	Oswaldo Cruz Foundation	Preclinical
Tetravalent DNA	US NMRC and GenPhar	Preclinical
Purified inactivated tetravalent DNA Monovalent	WRAIR and GSK	Preclinical (Putnak et al., 2005)
	Navy Medical Research Center	Phase I (Raviprakash 2003)

An ideal dengue vaccine ought to induce life-long protection against infection with any one of the four DENV serotypes, reasonable, should be free of important reactogenicity (**Whitehead et al., 2007; Rothman, 2004**). Assessment of the vaccine candidates ought to be based on population efficacy trials in diverse geographical settings including Asia and the Americas experiencing different patterns of dengue spread intensity and dengue virus circulation (**Hombach, 2007**). CDC, Centers for Disease Control and Prevention; CIGB, Center for Genetic Engineering and Biotechnology; GSK, GlaxoSmithKline; IPK, Pedro Kouri Tropical Medicine Institute; NIAID, National Institute for Allergy and Infectious Diseases; US NIH LID, United States National Institutes of Health Laboratory of Infectious Diseases; US NMRC, United States Naval Medical Research Center; WRAIR, Walter Reed Army Institute of Research; YF, yellow fever.

Conclusions and future perspectives

Being a worldwide risk, dominance of dengue virus is almost exaggerating countries which are poor and developing enduring to engross newer areas, newer populations and is mounting in magnitude, epidemic after epidemic. The presently observed and predicted expansions are multifactorial. The problem is massive in these countries compounded by the vast population, poor medical and diagnostic facilities, inadequate mosquito control, socioeconomic factors, global travel and trade and all the ground environment that favour growth of the vector (**Wilder-Smith and Gubler, 2008; Astrom et al., 2012**). The need for the enhanced estimates of the true burden of the dengue disease globally is highlighted by the WHO Global Strategy for Dengue Prevention and Control, 2012–2020 (**WHO 2012**). Further inspection and coverage is vital for effective dengue control and precise quantification of the impact of dengue worldwide will permit enhanced political, financial, and research prioritization as well as informed decision making and better modeling (**Shepard et al., 2013; Bhatt et al., 2013; Beatty et al., 2010**).

Hard work to solve the problems and minimizing the human sufferings can be extended by building up improved laboratory-based scrutiny systems predicting looming dengue epidemics and also aware the public to take action and physicians to diagnose and treat appropriately. As in ongoing progress with new tools such as vaccines, antiviral drugs and enhanced diagnostics, better utilization should be made of the

Conflict of interest statement

The author does not declare any conflict of interest.

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