IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
January 2023 Vol.:26, Issue:2

Predicting Severity *via* Biomarkers and Prevention of Dengue in the Era of COVID-19

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Yashik Bansal[1], Nidhi Singla[2*], Varsha Gupta[2]

[1]*Department of Microbiology, ESIC Medical College Hospital, Alwar, Rajasthan, India*

[2]*Department of Microbiology, Government Medical College Hospital, Chandigarh, India*

Submitted: 25 December 2022
Accepted: 31 December 2022
Published: 30 January 2023

Keywords: Dengue, Biomarker, Vaccine, Dengue Diagnosis, Dengue Vaccine

ABSTRACT

Dengue, though a disease of tropics, is a worldwide threat. Dengue fever is caused by the dengue virus which belongs to flaviviridae family and affects about 390 million people worldwide. In the era of the COVID-19 pandemic, substantial public health effort has been diverted to contain the pandemic, and the second wave of COVID-19 in India severely affected the available infrastructure for the management of other entities. The year 2020 saw a reduction in the number of reported dengue cases across India that could be attributed to factors such as resource constraints during the pandemic and under diagnosis coupled with under reporting due to overlapping clinical features. The presence of dengue and COVID-19 co-infection can worsen the prognosis and it is important to raise awareness, especially among the primary health care physicians, who are often the first point of contact in the community. Various biomarkers that predict the severity of dengue and the dengue vaccines currently licensed and in development are discussed in this review.

INTRODUCTION

Dengue fever (DF) is a viral illness affecting about 390 million people worldwide [1]. The disease is endemic in tropical countries but is a threat worldwide. Dengue fever is caused by the dengue virus which belongs to flaviviridae family [2]. It is transmitted by the bite of mosquito belonging to genus *Aedes*, most commonly by anthropophilic *A. aegypti* and by *A. albopictus* with certain other species such as *A. polynesiensis, A. scutellaris* and *A. niveus* capable of transmitting the virus to humans [3]. Other modes of transmission through blood transfusion, organ transplant and vertical transmission [4] have also been recognized over the years. Disease manifestations vary from simple dengue illness to severe dengue where patients have bleeding tendencies and some may even present with expanded dengue syndrome unusually manifesting as involvement of heart, kidney and brain.

The mortality rate ranges from 0.3% to 1.4% (**Figure 1**). Morbidity rate in the patients is considerably high. There is no specific antiviral drug available and the treatment is supportive and symptomatic. Vaccines are yet in preliminary phase with a single licensed vaccine available as of 2021, i.e. CYD-TDV [5]. As significant population gets involved during seasonal epidemic phase every year in many endemic countries, significant resources are needed for diagnosis, treatment and control of the infection. Lots of research is going on in these areas and many recent developments have occurred in our understanding of the disease as well as its prevention and control. Dengue is a huge economic burden for the countries involved during its outbreak and for the world otherwise [6].

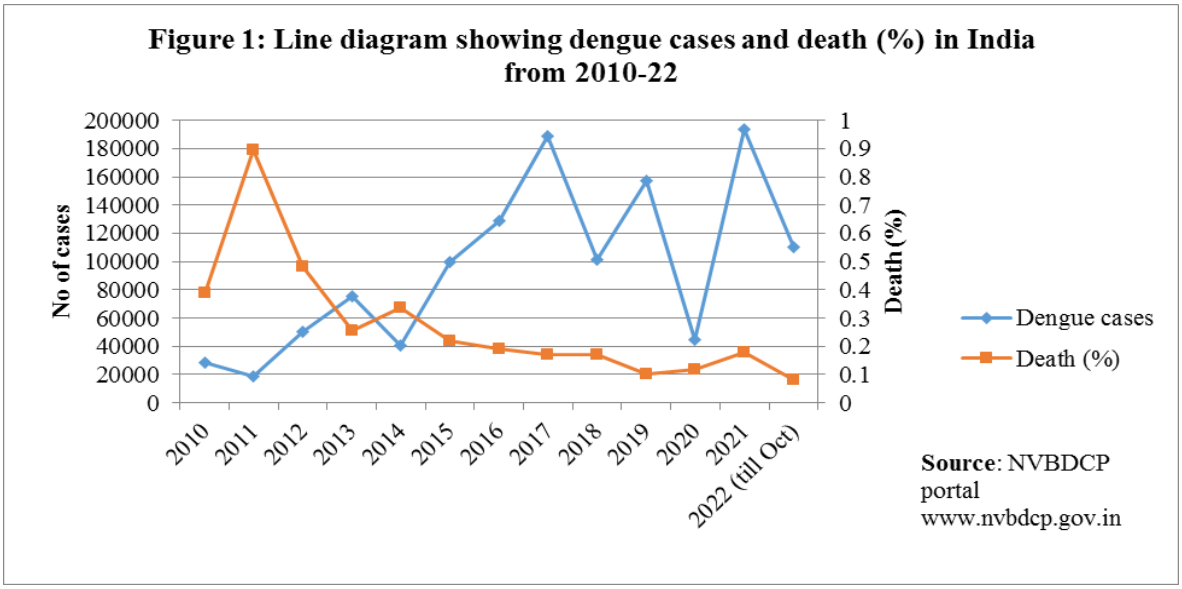

Figure 1: Line diagram showing dengue cases and death (%) in India from 2010-22.

Dengue virus is a positive sense, single stranded RNA virus and has four antigenically different serotypes Dengue 1-4. Recently, a fifth serotype has also been recognized [7]. Although it is yet to be established in routine, its presence can further jeopardize an already difficult situation. The infection with one serotype does not protect against other serotypes, rather the disease process is severer due to the phenomenon of Antibody Dependent Enhancement (ADE) [8]. World Health Organization (WHO) has given the criteria for classification of patients as a) dengue without warning signs b) dengue with warning signs and c) severe dengue [9].Virus, vector and host factors, all three play an important in determining the pathogenesis, severity and progression of disease in an individual.

In the era of the COVID-19 pandemic, substantial public health effort has been diverted to contain the pandemic, and the second wave of COVID-19 in India severely affected the available infrastructure for the management of other entities. The year 2020 saw a reduction in the number of reported dengue cases across India that could be attributed to the above mentioned factors (resource constraints during the pandemic) and under diagnosis coupled with under reporting due to overlapping clinical features [10]. Additionally, there have been reports of co-infection of dengue and COVID-19 [11]. Such cases of co-infection could have a potential adverse outcome although such data is limited at this stage [12]. Identifying such co-infections require high index of suspicion and moreover, it is difficult to distinguish the two because of overlapping clinical presentations. Nevertheless, diagnosis of dengue becomes more important during COVID-19 outbreak since some of the treatment options used for the management of COVID-19 (e.g. low molecular weight heparin) can increase the risk of complications in dengue, such as bleeding risk in the presence of thrombocytopenia [12].

Considering the above factors, it could be beneficial to sensitize and raise awareness among the primary health care physicians, who serve the majority population and are often the first point of contact for most patients [13]. Besides having a high index of suspicion, optimum utilization of the diagnostic laboratory can help identify cases of dengue and co-infections. There is an urgent need to develop biomarkers which can safely predict the individuals likely to proceed to severe dengue [14]. We did a literature search to discuss new literature in biomarkers and vaccine development since there is an urgent need for prophylactic measures, with vaccine being an important aspect.

Biomarkers for Dengue

An ideal biomarker should help us in identifying individuals who are at risk of developing severe dengue. Traditionally, platelet count had been a biomarker with thrombocytopenia as potential marker for progression of disease. For practical purposes, a platelet count of 60,000 cells/c.mm serves as a better cut-off in identifying more severe cases [15]. Thrombocytopenia within the first four days of illness has been identified as a risk factor for progressing to severe disease [16]. In a resource constraint, developing country like India with a huge population at risk of dengue, it is important to consider other causes of febrile thrombocytopenia especially in view of the use of immunochromatography (ICT) based card tests in smaller labs that can give false positive results owing to their lower specificity [17]. This can lead to misdiagnosis of the patient [18, 19]. Antiplatelet IgM antibodies have been found to be increased in patients of severe dengue [20]. Thrombopoietin is a cytokine which regulates megakaryocytopoiesis and gets activated when platelet count is decreased to regenerate the platelets. Serum TPO levels are thus a marker in dengue for megakaryocytopoiesis and its levels are inversely proportional to the platelet count and so, denotes dengue severity [21].

Since severity of the disease is related to ADE, it is augmented inflammatory response and cytokine storm which proceeds severe dengue [22, 23]. Immunopathology can be considered the basis and so many host immune response components including cells, cytokines complements and other cellular mediators can be chosen to serve as biomarkers of severe disease. Increased levels of IFN-γ, TNF-α, IL-1β, IL-4, IL-6, IL-7, IL-8, IL- 10, IL-13, IL-15, IL-17, IL-18, macrophage migration inhibitory factor (MIF) and chemokines CCL2, CCL4, CCL5, and CXCL10 (IP-10) have been seen in patients with Dengue Hemorrhagic Fever (DHF) [24-27]. In dengue patients, activation of mast cells leads occurs which leads to substantial increase in plasma levels of chymase and tryptase [28]. Mast cell activation also leads to increased levels of urinary histamine and the levels have been found to correlate with disease severity. It further has an advantage of ease of obtaining the sample, urine [29].

Total and dengue-specific IgE antibody levels have been found to be higher in patients with severe dengue in comparison to DF [30]. Also, the anti-endothelial antibodies are elevated which might be used as biomarkers of severe dengue disease. Other endothelial marker could be angiopoietin-2, whose serum levels are normally less than angiopoietin-1 (Ang-1) but exceeds Ang-1 during severe dengue manifestations [31]. Soluble factors are more stable. A

number of soluble factors are raised during severe dengue namely sTNFRII, sCD4, sCD8, sCD163, sCD 25 and sIL-2R [32]. Out of these, sCD163 present on macrophages efficiently differentiates between severe dengue and only dengue fever [33]. Serum Nitric oxide (NO) levels in DHF patients have been shown to be significantly lower than those of the DF patients. Liver involvement associated with severe dengue disease results in increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase, alkaline phosphatase, and serum albumin concentrations. However, liver enzyme levels tend to peak late in the disease course (typically during the second week) which limits their usefulness as prognostic markers [34].

In children, there is no correlation between platelet count and bleeding manifestations. Therefore, other markers are being explored and exploited. Hyperferritinemia in children is associated with severe disease and is, thus, currently used as a marker of severity [35].Children with acute dengue infection have elevated vWF, higher in Dengue Shock Syndrome (DSS) patients [36]. Serum level of sTM is proposed as diagnostic and prognostic marker of endothelial activation and dysfunction with others being sICAM and sVCAM-1. Levels of total plasma cholesterol, High-Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) have also been found to be significantly decreased in children with the severe dengue especially when severity is very high [37]. The Inter-α Inhibitor Proteins (IaIp) belong to a family of serine protease inhibitors and its concentrations in pediatric patients suffering from severe DENV infection were significantly lower than in patients with mild DF and healthy controls [38].

Other than these, if facilities are available, many genes loci can be analyzed namely LOC286087,SLC4A4, PSPH, MYOM2, CACNA2D3, CD244 molecule and SMAD5 which may possibly predict severe dengue since their expression profile vary significantly as disease progresses [39].A recent study established vitronectin (Vtn, 55.1 kDa), hemopexin (Hx, 52.4 kDa), and serotransferrin (Tf, 79.2 kDa) as markers which best differentiated between dengue and severe dengue [40]. Similarly dysregulated MiRNA profiles has been identified as potential markers for dengue [41]. Another study has predicted that galectin-9 and galectin-3BP might be critical inflammatory mediators in acute dengue virus infection [42].

Dengue serotypes and genotypes

Dengue serotypes and genotypes have to be taken into consideration to understand the various aspects associated with dengue vaccine development. Dengue has four serotypes with

another serotype been recently discovered. Dengue viruses depend upon viral RNA dependent RNA polymerase for their replication. This enzyme lacks proof reading capability resulting in very high substitution rates. These led to accumulation of intra serotypic genetic variations forming distinct genotypes among serotypes. Although the whole viral genome is liable to genetic diversity, it is the envelope gene which is the most important. Envelope gene encodes for the envelope protein which is responsible for virus attachment and its entry into host cell and membrane fusion. Envelope protein is main protein involved in formation of neutralizing antibodies and eliciting an immunological response.

DENV 1 has five genotypes (I to V) identified presently with DENV2 having 6 genotypes (Asian genotype 1, Asian genotype 2, Asian/American, American, Sylvatic and Cosmopolitan) and DENV3 and DENV4 with four genotypes each (I to IV) [43].Most of the genotypes are based on E gene (envelope gene) sequencing of these serotypes. All these serotypes and genotypes are distributed worldwide in specific regions. In America, it is DENV 1 genotype V, DENV 2 genotypes native American and Asian American along with DENV 3 genotype III. DENV 3 genotype I was recently found in Brazil, Colombia and Ecuador. In Africa, DENV 1 genotype I, DENV 2 genotype cosmopolitan, DENV 3 genotype III.

Mostly these genotypes are stable but for South East Asia where increased global travel, commercialism and tourism has led to ever changing epidemiology. In India and its neighboring countries, it is DENV 1 genotype I, II, DENV 2 genotype Asian II, cosmopolitan, DENV 3 genotype I, II, III and DENV 4 genotype I, III [44]. In other regions of South East Asia, it is DENV 1 genotype I, IV, DENV 2 genotype cosmopolitan, DENV 3 genotype I, III and DENV 4 genotype I and II. However, it is worth mentioning that no country is exempt from imported cases and so, new genetic establishments can occur. Also, DENV 1 genotype III represents proposed Malaysian sylvatic 1972 (Monkey) and 2005 (human) strains [45]. Another recent study has proposed DENV 1 genotype VI which includes DENV 1 Brunei 2014 strain which is a highly divergent genotype from the ones previously known [46].

Further, these genotypes have many lineages within themselves [47]. This intra serotype genetic diversity is detrimental for preventive measures especially vaccine development. Moreover, severity rate of the disease has also been found to be varied depending upon the serotype involved in the disease.

Dengue vaccines

Challenges in development

It has been more than a century since the research in the field of vaccine production for dengue was started way back in 1920 [48]. With no animal models available and complexities such as ADE and four dengue serotypes jeopardizing the situation, the path for dengue vaccine development has been very challenging. For a successful vaccine to be produced, a robust immune response against all serotypes should be generated following vaccination [49]. Further, the monotypic immune response thus generated against each serotype needs to be strong enough to exert a protective response. This has been difficult to achieve during the vaccine development. Many vaccines types including DNA vaccines, inactivated/ killed vaccines and live attenuated vaccines were in development. However, only live, attenuated vaccines could proceed to phase III trials [49]. The other challenge with dengue vaccine is the exhibition of ADE following immunization that leads to severe dengue following natural infection in vaccinated individuals.

The licensed vaccine: CYD-TDV

As of 2021, only one licensed vaccine, i.e. CYD-TDV, marketed as Dengvaxia (Sanofi, Pasteur, France) is available for use in 20 countries till date [50]. It is a live attenuated vaccine against all four serotypes of dengue. This is a recombinant vaccine which was registered in December, 2015 in Mexico [51]. It is a chimeric vaccine and has 17D yellow fever vaccine virus as a backbone with envelope and pre-membrane proteins from each of the four wild dengue viruses. This candidate vaccine (CYD-TDV) cleared phase III trials which included more than 30,000 participants aged 2 to 16 years. The vaccine efficacy was 59.2% against confirmed dengue following the primary series vaccination schedule of 0/6/12 months. The vaccine efficacy varied with strains, with higher efficacy of 75% & 77% exhibited against serotype 3 and 4respectively whereas efficacy of 51% and 34.0% was shown against serotype 1 and 2 respectively [52]. It has been licensed to be given in age group 9-45 years.

Vaccination campaigns were undertaken by some countries such as the Philippines and Brazil but the campaign was stopped by Philippines following warning by the manufacturer in 2017 that the vaccine should not be given to seronegative individuals owing to risk of development of severe dengue following natural infection subsequently [53]. This occurred due to

development of ADE as a result of generation of antibodies that were serotype cross reactive instead of being type specific neutralizing antibodies [5].

Taking view of above results, WHO in December 2017 recommended that countries with high dengue seroprevalence should include vaccination as part of their dengue control program with vaccinating only dengue-seropositive persons. The US FDA licensed the use of this vaccine in people with previous laboratory confirmed dengue, aged 9-16 years in areas with high dengue prevalence [54]. However, it is pertinent to note that there is no point of care test for detecting dengue serostatus [53].The vaccine has been far from perfect but it has role in severe dengue and decreases hospital admissions significantly [55].

Newer second generation vaccines

Two other promising candidates which are in phase 3 trials are TAK-003 or DENVax (Inviragen/Takeda) and TV003/TV005. TAK-003 is a whole, live attenuated serotype 2 in primary dog kidney cell lines forming a backbone with chimeric serotype 1, 3 and 4 replacing the pre-membrane (prM) and envelope (E) proteins [56]. The TV003/TV005 is live attenuated, tetravalent vaccine of wild-type dengue strains with genetic deletions. Both these candidates have been developed by National Institute of Allergy and Infectious Diseases (NIAID) and National Institutes of Health (NIH) in partnership with Brazil's Butantan institute. Important vaccine trials in phase 2are TDEN-PIV (purified inactivated type) and a prime boost vaccine PIV+LAV. Vaccines in phase 1 trials include V180 (recombinant subunit type), TVDV (DNA vaccine) and D1ME100 (DNA) [57]. Recently, KD-382 and Dengusiil have entered phase 1 trials. The list of vaccine candidates in different phases of clinical trials are enumerated in **table 1** [58].

Table 1: Dengue vaccines in clinical development phase

Name of vaccine	Sponsor	Type of vaccine	Dosage
Licensed vaccine(s)			
CYD-TDV	Sanofi Pasteur Institute	Recombinant live attenuated vaccine	3 doses given at 0, 6, 12 months
Phase 3			
TAK-003 (TDV)	Takeda	Recombinant live attenuated vaccine	2 doses (3 months apart)
TV003/TV005	NIAID, NIH	Recombinant vaccine	Single dose
Phase 2			
TDENV-PIV	GSK, WRAIR	Inactivated virus vaccine	2 doses (1 month apart)
PIV and LAV F17	USAMRMC	Prime boost: inactivated vaccine (PIV) + live attenuated vaccine (LAV) formulation 17	2 doses (1 month apart)
Phase 1			
V180	Merck	Recombinant subunit vaccine	3 doses given at 0, 1, 2 months
TVDV	USAMRMC	DNA vaccine	3 doses given at 0, 1, 3 months
KD-382	KM Biologics	Live attenuated vaccine	Single dose
Dengusiil	SII India	Live attenuated vaccine	Single dose

Future vaccines in pre-clinical development

The recent spread of COVID-19 pandemic across the globe saw the development of mRNA vaccines to fight the pandemic on global levels [59]. Earliest mRNA vaccines were studied against Tick-Borne Encephalitis Virus (TBEV) in 1990s [60]. These mRNA vaccines GeneGun of gold particles coated with RNA [61]. Similarly, mRNA vaccines are being evaluated for dengue virus as well and three vaccine constructs have thus far been documented [62-64]. These vaccines utilize mRNA-LNP (lipid nanoparticles) design for mRNA delivery via the intramuscular route.

A dengue vaccine DSV4 is being jointly developed by Sun Pharma and the International Centre for Genetic Engineering and Biotechnology (ICGEB) [65]. The vaccine is based on the Virus Like Particle (VLP) platform provided by Hepatitis B surface antigen (HBsAg) co-assembled together with a second protein called DS protein to form a mosaic VLP. The vaccine demonstrates little ADE potential. Uno et al [66] recently developed a vaccine construct based on Computationally Optimized Broadly Reactive Antigen (COBRA) methodology in which the surface of Subvirion Viral Particles (SVPs) expressed COBRA and wild type E antigens. NIAID is studying the early and late innate immune responses to the saliva of *Aedes aegypti* in the human skin and it hopes to use this as the basis of future vaccine development and research [67].

Future challenges to dengue vaccines

Although vaccine research is need of hour and is progressing worldwide, we should not forget one important aspect of dengue and that is Sylvatic dengue. The sylvatic dengue strains have the potential to become reservoir for dengue especially in primates like green monkeys and baboons. These stains of sylvatic dengue are distinct genetically from human dengue strains but it has been seen in many experimental assays that sylvatic dengue has a good potential of transmission and so, chances of becoming a human pathogen [68].

REFERENCES:

1. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol. 2013;5:299-309. doi: 10.2147/CLEP.S34440.
2. Singla N, Chaudhary P, Thakur M, Chander J. Dengue: An Analysis of Epidemiological Pattern Over a Six Year Period. J Clin Diagn Res. 2016;10(12): DC12-4. doi: 10.7860/JCDR/2016/22482.9011.
3. Kumar CS, Sharma SK. Denguevirus infection.Natl Med J India. 2016; 29(2):61-3.
4. Singla N, Arora S, Goel P, Chander J, Huria A. Dengue in pregnancy: an under-reported illness, with special reference to other existing co-infections. Asian Pac J Trop Med. 2015;8(3):206-8. doi: 10.1016/S1995-7645(14)60316-3.
5. Wilder-Smith A. Dengue vaccine development: status and future. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2020;63(1): 40-4. doi: 10.1007/s00103-019-03060-3.
6. Lee JS, Mogasale V, Lim JK, Ly S, Lee KS, Sorn S *et al*. A multi-country study of the economic burden of dengue fever based on patient-specific field surveys in Burkina Faso, Kenya, and Cambodia. PLoS Negl Trop Dis. 2019;13(2):e0007164. doi: 10.1371/journal.pntd.0007164.
7. Mustafa MS, Rasotgi V, Jain S, Gupta V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Med J Armed Forces India. 2015;71(1):67-70. doi: 10.1016/j.mjafi.2014.09.011.
8. Shukla R, Ramasamy V, Shanmugam RK, Ahuja R, Khanna N. Antibody-Dependent Enhancement: A Challenge for Developing a Safe Dengue Vaccine. Front Cell Infect Microbiol. 2020;10:572681.doi: 10.3389/fcimb.2020.572681.
9. World Health Organization. Geneva, Switzerland: WHO. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control 2009.

10. Phadke R, Mohan A, Çavdaroğlu S, Dapke K, Costa ACDS, Riaz MMA *et al.* Dengue amidst COVID-19 in India: The mystery of plummeting cases. J Med Virol. 2021;93(7): 4120-1. doi: 10.1002/jmv.26987.
11. Saddique A, Rana MS, Alam MM, Ikram A, Usman M, Salman M et al. Emergence of co-infection of COVID-19 and dengue: A serious public health threat. J Infect. 2020;81(6):e16-e18. doi: 10.1016/j.jinf.2020.08.009.
12. National Vector Borne Disease Control Programme, Ministry of Health and Family Welfare, Government of India. National Guideline for Dengue case management during COVID-19 pandemic.
13. Pathak VK, Mohan M. A notorious vector-borne disease: Dengue fever, its evolution as public health threat. J Family Med Prim Care. 2019;8(10): 3125-9. doi: 10.4103/jfmpc.jfmpc_716_19.
14. Malavige GN, Rostron T, Rohanachandra LT, Jayaratne SD, Fernando N, De Silva AD et al. HLA Class I and Class II Associations in Dengue Viral Infections in a Sri Lankan Population.PLoS ONE. 2011; 6: e20581.doi: 10.1371/journal.pone.0020581.
15. Pawitan JA. Dengue virus infection: predictors for severe dengue. Acta Med Indones. 2011;43:129–35.
16. Sangkaew S, Ming D, Boonyasiri A, Honeyford K, Kalayanarooj S, Yacoub S et al. Risk predictors of progression to severe disease during the febrile phase of dengue: a systematic review and meta-analysis. Lancet Infect Dis. 2021; 21(7): 1014-26. doi: 10.1016/S1473-3099(20)30601-0.
17. Bansal Y, Priyadarshi K, Kombade SP, Nag VL. Diagnostic dilemma in a case of *Salmonella* Typhi sacroiliitis. J Clin Orthop Trauma. 2020;11(Suppl 4):S657-9. doi: 10.1016/j.jcot.2020.04.011.
18. Bansal N, Bansal Y, Ralta A. Thrombocytopenia in COVID-19 patients in Himachal Pradesh (India) and the absence of dengue false positive tests: Insights for patient management. J Med Virol. 2021; 93: 606-7. doi: https://doi.org/10.1002/jmv.26373.
19. Bansal Y, Maurya V, Aggarwal N,Tak V, Nag VL, Purohit A et al. Thrombocytopenia in Malaria patients from an arid region of western Rajasthan (India). Tropical Parasitology. 2020; 10(2):95-101.doi: 10.4103/tp.TP_68_19.
20. Kumar P, Charaniya R, Ghosh A, Sahoo R. Intravenous Immunoglobulin Responsive Persistent Thrombocytopenia after Dengue Haemorrhagic Fever. J Clin Diagn Res. 2016;10(4):OD10-1. doi: 10.7860/JCDR/2016/17770.7605.
21. De Azeredo EL, Monteiro RQ, de-Oliveira Pinto LM. Thrombocytopenia in Dengue: Interrelationship between Virus and the Imbalance between Coagulation and Fibrinolysis and Inflammatory Mediators. Mediators of Inflammation 2015; 313842. doi:10.1155/2015/313842.
22. Marin-Palma D, Sirois CM, Urcuqui-Inchima S, Hernandez JC. Inflammatory status and severity of disease in dengue patients are associated with lipoprotein alterations. PLoS One. 2019;14(3):e0214245. doi: 10.1371/journal.pone.0214245.
23. Patro ARK, Mohanty S, Prusty BK, Singh DK, Gaikwad S, Saswat T et al. Cytokine Signature Associated with Disease Severity in Dengue. Viruses. 2019;11(1):34. doi: 10.3390/v11010034.
24. John DV, Lin Y-S, Perng GC. Biomarkers of severe dengue disease – a review. Journal of Biomedical Science 2015; 22: 83. doi: 10.1186/s12929-015-0191-6.
25. De-Oliveira-Pinto LM, Gandini M, Freitas LP, Siqueira MM, Marinho CF, Setúbal S et al. Profile of circulating levels of IL-1Ra, CXCL10/IP-10, CCL4/MIP-1β and CCL2/MCP-1 in dengue fever and parvovirosis. Mem Inst Oswaldo Cruz. 2012;107(1):48–56.doi: 10.1590/s0074-02762012000100007.
26. van de Weg CA, Pannuti CS, de Araújo ES, van den Ham HJ, Andeweg AC, Boas LS et al. Microbial translocation is associated with extensive immune activation in dengue virus infected patients with severe disease. PLoSNegl Trop Dis. 2013;7(5):e2236.doi: 10.1371/journal.pntd.0002236.
27. Rathakrishnan A, Klekamp B, Wang SM, Komarasamy TV, Natkunam SK, Sathar J et al. Clinical and Immunological Markers of Dengue Progression in a Study Cohort from a Hyperendemic Area in Malaysia. PLoS One 2014; 9(3): e92021.doi: 10.1371/journal.pone.0092021.
28. Rathore AP, Mantri CK, Aman SA, Syenina A, Ooi J, Jagaraj CJ et al. Dengue virus-elicited tryptase induces endothelial permeability and shock. J Clin Invest. 2019;129(10):4180-93. doi: 10.1172/JCI128426.
29. Furuta T, Murao LA, Lan NT, Huy NT, Huong VT, Thuy TT et al. Association of mast cell-derived VEGF and proteases in Dengue shock syndrome. PLoS Negl Trop Dis 2012;6(2):e1505.doi: 10.1371/journal.pntd.0001505.

30. Koraka P, Murgue B, Deparis X, Setiati TE, Suharti C, van Gorp EC et al. Elevated levels of total and dengue virus-specific immunoglobulin E in patients with varying disease severity. J Med Virol. 2003;70(1):91-8. doi: 10.1002/jmv.10358.
31. Yacoub S, Lam PK, Vu LHM. Association of Microvascular Function and Endothelial Biomarkers with Clinical Outcome in Dengue: An Observational Study. The Journal of Infectious Diseases 2016; 214(5): 697-706. doi: 10.1093/infdis/jiw220.
32. Arias J, Valero N, Mosquera J, Montiel M, Reyes E, Larreal Y et al. Increased expression of cytokines, soluble cytokine receptors, soluble apoptosis ligand and apoptosis in dengue. Virology. 2014;452-453:42-51. doi: 10.1016/j.virol.2013.12.027.
33. Ab-Rahman HA, Rahim H, AbuBakar S, Wong PF. Macrophage Activation Syndrome-Associated Markers in Severe Dengue. Int J Med Sci. 2016; 13(3): 179-86. doi: 10.7150/ijms.13680.
34. Yacoub S, Wills B. Predicting outcome from dengue. BMC Medicine.2014;12:147. doi: 10.1186/s12916-014-0147-9.
35. Van de Weg CAM, Huits RMHG, Pannuti CS, Brouns RM, van den Berg RWA, van den Ham HJ et al. Hyperferritinaemia in Dengue Virus Infected Patients Is Associated with Immune Activation and Coagulation Disturbances. PLoS Neglected Tropical Diseases. 2014; 8(10):e3214. doi: 10.1371/journal.pntd.0003214.
36. Djamiatun K, van der Ven AJAM, de Groot PG, Faradz SM, Hapsari D, Dolmans WM et al. Severe Dengue Is Associated with Consumption of von Willebrand Factor and Its Cleaving Enzyme ADAMTS-13. PLoS Neglected Tropical Diseases. 2012 ;6(5): e1628.doi: 10.1371/journal.pntd.0001628.
37. Biswas HH, Gordon A, Nuñez A, Perez MA, Balmaseda A, Harris E. Lower Low-Density Lipoprotein Cholesterol Levels Are Associated with Severe Dengue Outcome. PLoS Neglected Tropical Diseases. 2015;9(9):e0003904. doi: 10.1371/journal.pntd.0003904.
38. Koraka P, Lim Y-P, Shin MD, Setiati TE, Mairuhu AT, van Gorp EC et al. Plasma Levels of Inter-α Inhibitor Proteins in Children with Acute Dengue Virus Infection. PLoS ONE. 2010;5(4):e9967.doi: 10.1371/journal.pone.0009967.
39. Sun P, García J, ComachG, Vahey MT, Wang Z, Forshey BM et al. Sequential waves of gene expression in patients with clinically defined dengue illnesses reveal subtle disease phases and predict disease severity. PLoS Negl Trop Dis. 2013;7(7):e2298.doi: 10.1371/journal.pntd.0002298.
40. Poole-Smith BK, Gilbert A, Gonzalez AL, Beltran M, Tomashek KM, Ward BJ et al. Discovery and Characterization of Potential Prognostic Biomarkers for Dengue Hemorrhagic Fever. Am J Trop Med Hyg. 2014; 91(6):1218-26. doi: 10.4269/ajtmh.14-0193.
41. Ouyang X, Jiang X, Gu D, Zhang Y, Kong SK, Jiang C et al. Dysregulated Serum MiRNA Profile and Promising Biomarkers in Dengue-infected Patients. Int J Med Sci. 2016;13(3):195-205. doi: 10.7150/ijms.13996.
42. Liu K-T, Liu Y-H, Chen Y-H, Zhang Y, Kong SK, Jiang C et al. Serum Galectin-9 and Galectin-3-Binding Protein in Acute Dengue Virus Infection. Int J Med Sci. 2016;17(6):832.doi: 10.3390/ijms17060832.
43. Shi Y, Li S, Li X, Zheng K, Yuan S, Huang J. Epidemiological and molecular characterization of dengue viruses imported into Guangzhou during 2009-2013. Springerplus. 2016;5(1):1635. doi: 10.1186/s40064-016-3257-3.
44. Usme-Ciro JA, Méndez JA, Laiton KD, Páez A. The relevance of dengue virus genotypes surveillance at country level before vaccine approval. Human Vaccines & Immunotherapeutics. 2014;10(9):2674-8.doi: 10.4161/hv.29563.
45. Teoh B-T, Sam S-S, Abd-Jamil J, Abu Bakar S. Isolation of Ancestral Sylvatic Dengue Virus Type 1, Malaysia. Emerg Infect Dis. 2010;16(11):1783-5.doi: 10.3201/eid1611.100721.
46. Pyke AT, Moore PR, Taylor CT, Hall-Mendelin S, Cameron JN,Hewitson GR et al. Highly divergent dengue virus type 1 genotype sets a new distance record. Scientific Reports. 2016;6:22356.doi: 10.1038/srep22356.
47. Waman VP, Kolekar P, Ramtirthkar MR, Kale MM, Kulkarni-Kale U. Analysis of genotype diversity and evolution of Dengue virus serotype 2 using complete genomes. Peer J. 2016; 4: e2326.doi: 10.7717/peerj.2326.
48. McArthur MA, Sztein MB, Edelman R. Dengue vaccines: recent developments, ongoing challenges and current candidates. Expert Rev Vaccines. 2013;12(8):933-53. doi: 10.1586/14760584.2013.815412.

49. Thomas SJ, Yoon IK. A review of Dengvaxia®: development to deployment. Hum Vaccin Immunother. 2019;15(10):2295-2314. doi: 10.1080/21645515.2019.1658503.
50. Shim E. Cost-effectiveness of dengue vaccination in Yucatán, Mexico using a dynamic dengue transmission model. PLoS One. 2017;12(4):e0175020. doi: 10.1371/journal.pone.0175020.
51. Rosa BR, Cunha AJLAD, Medronho RA. Efficacy, immunogenicity and safety of a recombinant tetravalent dengue vaccine (CYD-TDV) in children aged 2-17 years: systematic review and meta-analysis. BMJ Open. 2019;9(3):e019368. doi: 10.1136/bmjopen-2017-019368.
52. The Lancet Infectious Diseases. The dengue vaccine dilemma. Lancet Infect Dis. 2018;18(2):123. doi: 10.1016/S1473-3099(18)30023-9.
53. Henein S, Swanstrom J, Byers AM, Moser JM, Shaik SF, Bonaparte M et al. Dissecting Antibodies Induced by a Chimeric Yellow Fever-Dengue, Live-Attenuated, Tetravalent Dengue Vaccine (CYD-TDV) in Naive and Dengue-Exposed Individuals. J Infect Dis. 2017;215(3):351–358. doi: 10.1093/infdis/jiw576.
54. Centres for Disease Control, Atlanta, United States of America. Dengue vaccine.https://www.cdc.gov/dengue/prevention/dengue-vaccine.html.
55. World Health Organization. Immunization, Vaccines and Biologicals: Questions and Answers on Dengue Vaccines. https://www.who.int/immunization/research/development/dengue_q_and_a/en/
56. Osorio JE, Velez ID, Thomson C, Lopez L, Jimenez A, Haller AA et al. Safety and immunogenicity of a recombinant live attenuated tetravalent dengue vaccine (DENVax) in flavivirus-naive healthy adults in Colombia: a randomised, placebo-controlled, phase 1 study. Lancet Infect Dis. 2014;14(9):830-8. doi: 10.1016/S1473-3099(14)70811-4.
57. Kirkpatrick BD, Whitehead SS, Pierce KK, Tibery CM, Grier PL, Hynes NA et al. The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model. Sci Transl Med. 2016; 8(330): 3–4. doi: 10.1126/scitranslmed.aaf1517.
58. World Health Organization. Vaccine pipeline tracker. https://www.who.int/immunization/research/vaccine_pipeline_tracker_spreadsheet/en/
59. Huang Q, Zeng J, Yan J. COVID-19 mRNA vaccines. J Genet Genomics. 2021;48(2):107-114. doi: 10.1016/j.jgg.2021.02.006.
60. Wollner CJ, Richner JM. mRNA Vaccines against Flaviviruses. Vaccines (Basel). 2021;9(2):148. doi: 10.3390/vaccines9020148.
61. Mandl CW, Aberle JH, Aberle SW, Holzmann H, Allison SL, Heinz FX. In vitro-synthesized infectious RNA as an attenuated live vaccine in a flavivirus model. Nat. Med. 1998;4: 1438–40. doi: 10.1038/4031.
62. Roth C, Cantaert T, Colas C, Prot M, Casademont I, Levillayer L et al. A Modified mRNA Vaccine Targeting Immunodominant NS Epitopes Protects Against Dengue Virus Infection in HLA Class I Transgenic Mice. Front Immunol. 2019;10:1424. doi: 10.3389/fimmu.2019.01424.
63. Zhang M, Sun J, Li M, Jin X. Modified mRNA-LNP Vaccines Confer Protection against Experimental DENV-2 Infection in Mice. Mol Ther Methods Clin Dev. 2020;18: 702–12. doi: 10.1016/j.omtm.2020.07.013.
64. Wollner CJ, Richner M, Hassert MA, Pinto AK, Brien JD, Richner JM. A Dengue Virus Serotype 1 mRNA-LNP Vaccine Elicits Protective Immune Responses. J Virol. 2021:JVI.02482-20. doi: 10.1128/JVI.02482-20.
65. Swaminathan S, Khanna N. Dengue vaccine development: Global and Indian scenarios. Int J Infect Dis. 2019;84S: S80-6. doi: 10.1016/j.ijid.2019.01.029.
66. Uno N, Ross TM. Universal Dengue Vaccine Elicits Neutralizing Antibodies against Strains from All Four Dengue Virus Serotypes. J Virol. 2021;95(4):e00658-20. doi: 10.1128/JVI.00658-20.
67. National Institute of Health. US National Library of Health. Characterization of Skin Immunity to Aedes Aegypti Saliva in Dengue-endemic Participants in Cambodia. https://clinicaltrials.gov/ct2/show/NCT04350905.
68. Althouse BM, Lessler J, Sall AA, Diallo M, Hanley KA, Watts DM et al. Synchrony of sylvatic dengue isolations: a multi-host, multi-vector SIR model of dengue virus transmission in Senegal. PLoS Negl Trop Dis. 2012;6(11):e1928. doi: 10.1371/journal.pntd.0001928.