A new dimension in the dengue epidemiology with special reference to the genetic diversity of the virus: A review

Probal Basu, Sajal Bhattacharya

Abstract

The severity of dengue is often ascribed to secondary infection with a virus belonging to a serotype distinct from that of the primary infection. Severe pathogenicity of DENV might be regulated at the genetic level and may be associated with unusual mutational and recombination events which are the two major reasons behind the extensive genetic diversity of DENV. The possible emergence of undesired genetically novel variant DENV in the near future could create further complexity and subsequent complications in the pathogenicity of the disease. This review article is an attempt to understand the significance of the extensive genetic diversity of the dengue virus (DENV) in the development of greater magnitude of the viral pathogenicity as well as in the severity of recurrent outbreaks in the context of changing environment and epidemiology. DENV antigens have been detected from mononuclear cells, lymphocytes, Langerhans cells in the skin, neurons, astrocytes, endothelial cells and hepatocytes, heart and skeletal muscle. This altered tropism of the dengue virus in humans might indicate the fitness strategy of the virus in urban areas since human is the only possible source of viremic vertebrate. Remarkably, the RNA virus has developed the ability to recombine with host dsDNA genomes. The extraordinary abilities of RNA virus like DENV may unlock a new vista in dengue research, which encompasses the relevant proposition of a momentous plausibility of crucial genetic exchange between DENV (+) ssRNA genome and dsDNA of the human host and thereby the possible emergence of genetically novel DENV variants associated with altered pathogenicity.

Keywords: Flavivirus, Dengue, Serotypes, Genetic Diversity, Mutation, Recombination.

1. Introduction

The study of arthropod-borne viruses like Dengue virus (DENV) is relevant in the context of public health because they generally impose an enormous disease burden on humans. The plasticity of the genome of RNA virus together with the extraordinary dispersal potential of arthropod vectors is of a keen interest in the study of arboviral diseases like Dengue [1-3]. Dengue fever was initially mentioned as “water poison” linked with flying insects in a Chinese Medical Encyclopaedia in 992 from Jin Dynasty (265-420 AD). The word “dengue” has been acquired from Swahili phrase Ka-dinga pepo, meaning “cramp-like seizure” [4]. Dengue virus (DENV) is a flavivirus and belongs to the family Flaviviridae. The Dengue virus has four antigenically distinct serotypes (DEN 1, 2, 3, and 4) [1]. The first clinically identified dengue epidemics happened almost simultaneously in Asia, Africa, and North America in the 1780s [4]. Dengue fever has re-emerged as a major public health challenge worldwide; with 2.5 billion people at the risk of infection, more than 100 million cases and 25,000 deaths being reported annually [5]. The WHO estimates that almost 2.5 billion people in the world population are at the risk of Dengue infection. This disease is estimated to affect 50-100 million individuals each year in the tropical and subtropical regions. Of these cases, 500,000 develop into severe forms of the disease such as Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Dengue is gradually becoming a global disease. Compared to nine reporting countries in the 1950s, today the geographic distribution of Dengue includes more than 100 countries worldwide. Dengue/DHF is a significant public health problem in India [6]. Dengue has been considered predominantly an urban disease. Presently, however, rural districts have also been affected by this virus. The greater genetic diversity in any virus population provides considerable opportunity to increase viral fitness in a population. RNA viruses have the potential to adapt rapidly via large population size, shorter replication times, and increased mutation rates [1]. This review article is an attempt to understand the significance of the extensive genetic diversity of the dengue virus (DENV) in the development of greater magnitude of the viral pathogenicity as well as in the severity of recurrent outbreaks in the context of changing environment and epidemiology.
2. Morphology of Dengue Virus
The virion of dengue virus is spherical and 40-50 nm in diameter [6]. It consists of a nucleocapsid which is about 30 nm in diameter enclosed in a lipid envelope. The lipid envelope contains a lipid bilayer and an envelope protein (~51000-59000 Daltons) that facilitates attachment, fusion and penetration. A non-glycosylated internal matrix protein (8500 Daltons) is also present in the lipid envelope [6]. Researches demonstrated that mature dengue virion is characterized by a comparatively smooth surface and 180 copies of envelope protein forming an icosahedral scaffold [3].

3. Genome of Dengue virus
The DENV genome is composed of a single-stranded, positive-sense RNA molecule ~10.7 kb in length. It accommodates a single translated open reading frame (ORF) which encodes a precursor polypeptide (~3390 amino acids) that is further processed catalytically by DENV and host proteases into ten viral proteins: three structural proteins (C, capsid; prM/M, precursor of membrane; E, envelope) which are encoded at the 5’ end and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) which are encoded at the 3’ end [6] (FIGURE 1) (Table 1). The ORF of dengue virus is flanked at its 5’ terminus by an untranslated region (UTR) which is about 100 nucleotides in length and a longer UTR at its 3’ terminus which is about 500 nucleotides in length. The 5’ terminus of DENV genome contains a type I cap and there is no polyadenylation at 3’ terminus [6].

Researches also showed the existence of several multifunctional non-structural proteins [6]. The E protein is the most important among the three structural proteins in regard to virus infectivity. It is glycosylated at two specific sites which are Asn-67 and Asn-153 that is responsible for DENV attachments to receptors on host cells and fusion with host cell membrane [6].

Table 1: List of Non-Structural Proteins of DENV and their functions.

<table>
<thead>
<tr>
<th>Non Structural Proteins (NS-Proteins)</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS1</td>
<td>Functions in viral RNA replication complex, soluble complement-fixing antigen</td>
<td>6, 200</td>
</tr>
<tr>
<td>NS2A</td>
<td>Constituent of RNA replication complex.</td>
<td></td>
</tr>
<tr>
<td>NS2B</td>
<td>NS3 protease cofactor</td>
<td></td>
</tr>
<tr>
<td>NS3</td>
<td>Serine protease, RNA helicase, RTPase/NTPase.</td>
<td></td>
</tr>
<tr>
<td>NS4A</td>
<td>Induces membrane alterations required for virus replication.</td>
<td></td>
</tr>
<tr>
<td>NS4B</td>
<td>Blocks IFN α/β-induced signal transduction.</td>
<td></td>
</tr>
<tr>
<td>NS5</td>
<td>RNA-dependent RNA polymerase (RdRp) and Methyltransferase (MTase)</td>
<td></td>
</tr>
</tbody>
</table>

4. Significance of 5’ and 3’ end of DENV RNA genome
The specific structures in 5’ and 3’ end of DENV genome have a profound role in RNA synthesis [9]. The 5’-UTR of dengue virus are 95-101 nucleotides in length which contain two RNA domains that have a distinct role during viral RNA synthesis [9]. The first domain comprised of ~70 nucleotides is believed to fold into a large stem-loop (SLA) structure. DENV SLA is a Y shaped structure and is thought to function as the promoter for viral RNA directed RNA polymerase (RdRp) [9-14]. In addition, three helical regions (S1, S2, and S3) separated by bulges and highly reactive single stranded regions associated with side stem loop and a top loop are present. The second domain of DENV 5’-UTR is thought to produce a short stem-loop (SLB) which possess important sequence for long range RNA-RNA interaction and genome replication [14]. Inside the coding region downstream of the translation initiation codon, AUG, a stable hairpin (cHP) is also found in DENV genome which is necessary for viral RNA replication [13]. The 3’-UTR of DENV is approximately 450 nucleotides in length and can be divided into three specific domains. Domain I is the most variable region and is located immediately after the stop codon [16]. This domain exhibits substantial size distribution between the viral serotypes which usually ranging from more than 120 nucleotides to less than 50 nucleotides [17, 22]. Domain II involves a dumbbell (DB) structure which contains tandemly duplicated nucleotides [17, 20, 22]. These specific domain structures generally function as enhancers of the viral processes [16, 23-26]. Domain III is the most conserved region in the 3’-UTR containing CS1 element which contains a virus cyclization sequence that is complementary to a specific sequence exists in 5’ end of the virus genome and involved in long range RNA-RNA interaction between the virus genome [9, 14]. The terminal 3’ structure bears a short stem loop (shP) which is 14 nucleotides in length, followed by a large stem loop that is 79 nucleotides in length. These two adjacent structures containing a total of 93 nucleotides are collectively referred as 3’SL, which is critical for flavivirus replication [9, 27, 20].

5. Different Serotypes and their genotypes of Dengue virus:
The Dengue virus occurs mainly as four antigenically distinct serotypes (DEN 1, 2, 3, and 4) [1, 29]. All the four serotypes are found circulating in India. There is genetic variation within each serotype in the form of phylogenetically distinct subtypes or genotypes. South-east Asia harbors the greatest genetic diversity of dengue virus, suggesting it acts as a viral ‘source’ population [11, 30]. Recently, a new serotype of dengue virus DEN-5 has been detected from a hospitalized 37 years old farmer in Sarawak state of Malaysia. Researchers demonstrated that this new serotype of dengue virus is genetically similar to other four dengue serotypes, thus thought to be originated from a common predecessor [11]. (Table 2)
Table 2: Different DENV Serotypes and their distribution.

<table>
<thead>
<tr>
<th>Dengue Virus Serotypes</th>
<th>Genotypes</th>
<th>Distribution</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DEN-1</td>
<td>Genotype IV</td>
<td>Nauru, Australia, Philippines and Indonesia.</td>
<td>6, 201</td>
</tr>
<tr>
<td></td>
<td>Genotype V</td>
<td>The America, Africa, Southeast Asia.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DEN-2</td>
<td>Genotype II</td>
<td>Formerly known as subtype II. Encountered in China, Thailand, Vietnam and Latin America.</td>
<td>6, 202</td>
</tr>
<tr>
<td></td>
<td>Genotype V</td>
<td>The America, Africa, Southeast Asia.</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>DEN-3</td>
<td>Genotype IV</td>
<td>Formerly known as genotype IV. Widely distributed including Australia, the Pacific islands, Southeast Asia, the Indian subcontinent, Indian Ocean islands, Middle East, and both East and West Africa.</td>
<td>6, 203</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DEN-4</td>
<td>Genotype II</td>
<td>Indonesia, Malaysia, Tahiti, the Caribbean islands (Puerto Rico and Dominica) and the Americas.</td>
<td>6, 38, 204</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DEN-5</td>
<td></td>
<td>Found and isolated from non-human primates in Malaysia.</td>
<td>31</td>
</tr>
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6. Major Factors behind DENV Genetic Diversity

A. Mutation

The RNA virus population generally associated with high mutation rates and contain genetic diversity [32]. Studies indicated that mutations are not biased toward a specific nucleotide and the mutation frequency of each nucleotide is usually proportional to its occurrence [33]. Point mutations like transitions (A to G, G to A, C to T or T to C) are found most prevalently in the dengue genome (Table. 3). Mutations are also repeatedly observed in the third codon position. Moreover, it has also been observed that the rare variants like deletions and early termination STOP mutations found at low frequencies in the dengue genome during human infections [33]. Variations in the non-structural proteins have also been found in association with increasing dengue severity [34-39].

Table 3: Types of Mutations majorly found in DENV.

<table>
<thead>
<tr>
<th>Genomic Components</th>
<th>Types of Mutations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural Genes</td>
<td>C gene</td>
<td>Nucleotide Substitution</td>
</tr>
<tr>
<td></td>
<td>prM gene</td>
<td>Nucleotide substitution</td>
</tr>
<tr>
<td></td>
<td>E gene</td>
<td>Nucleotide substitution, Deletion</td>
</tr>
<tr>
<td></td>
<td>NS1</td>
<td>Nucleotide Substitution</td>
</tr>
<tr>
<td></td>
<td>NS2</td>
<td>Nucleotide Substitution</td>
</tr>
<tr>
<td></td>
<td>NS4</td>
<td>Nucleotide Substitution</td>
</tr>
<tr>
<td></td>
<td>NS5</td>
<td>Nucleotide Substitution</td>
</tr>
<tr>
<td>Nonstructural Genes</td>
<td></td>
<td>Diagnosis of viral RNA Recombination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Traversal, Transcription, Deletion, Insertion</td>
</tr>
</tbody>
</table>

B. Recombination

Recombination is a momentous impetus behind genomic diversity [40-44]. Recombination events are commonly found in RNA virus, including DENV [45]. Dengue infection with two different strains which is a prerequisite for DENV recombination has been demonstrated in both human and mosquitoes [46-50]. Researchers have demonstrated that recombination can occur in different genetically diverse DENV strains [45, 51-57]. Two major mechanisms have been considered for viral RNA recombination. First one is non-replicative breakage and re-joining and second is replicative template switching [42, 58]. Recombination breakpoint for the

(+ ) ssRNA virus have been found in the non-structural protein and also in the structural protein encoding regions [59, 60]. Researchers reported that three recombination zones in DENV located within the sequences prM-E junction, NS1 and NS3 genes [61]. Interestingly, it has been reported that DENV RNA dependent RNA polymerase (RdRp) exhibits a significant conformational flexibility during the transition from initiation to elongation [62] which might favour the recombination event in virus [61]. Researchers reiterated that RNA recombination events like homologous recombination (at sites with exact sequence match), aberrant homologous recombination (requires sequence homology) and non-homologous
recombination (independent of sequence homology) also occurs in RNA virus [63, 64].

7. Characteristics of Genomic variations leading to the intriguing DENV pathogenesis

Phylogenetic studies indicated that genetic subtypes of the DENV differ (up to 12 %) in the nucleotide sequence of E-gene [65, 66]. Researchers demonstrated that several structural differences are present between two DENV-2 genotypes (such as, the Southeast Asian genotype with DF and DHF and the American genotype with only DF) which results in distinct human clinical presentation [65]. There are six amino acid change differences are encountered, including the pre-membrane protein (prM; amino acid 28 Glu→Lys), the envelope glycoprotein (E; amino acid 390 Asn→Asp), the non-structural protein 4B (NS4B; amino acid 17 Ser→His) and NS5 (amino acid 645 Asn→Asp, 676 Ser→Arg and 800 Lys→Ser). Moreover, the comparative analysis between the American strain and the Thai strains discovered a plausible correlation between the amino acid sequence of the subtypes of DENV-2 strain and the clinical severity of the patients from whom the virus strains were collected [65]. From all DSS cases, three amino acid changes were found in subtype I of DENV-2 strain: D→G change at position 278 in NS1; N→D change at 139 in NS2A; M→I change at 13 in NS3. From all DF cases, five amino acid change differences were found in subtype III of DENV-2 strain I→R change at 16 and T→A at 81 in prM; I→M at 136 in NS2A; A→T at 118 in NS3; and T→M at 337 in NS5 [65].

8. Dengue and RNAi

In insects, RNA interference (RNAi) mechanism is considered as a major antiviral defence mechanism [67-73]. In order to transmit into a suitable host, arbovirus like dengue must escape this anti-viral defence [67, 74]. The DENV 3'-UTR RNA structures are thought to be linked with the generation of subgenomic flavivirus RNA (sgRNA) [75]. SF RNA are produced by the incomplete degradation of viral genome by cellular ribonuclease XRN1 which stalled at pseudoknot structures at the 3'-UTR [75-77]. Interestingly, it is shown that sgRNA also inhibit RNAi pathways in mosquitoes [79].

9. Discussion

The increased incidence of dengue has been observed throughout the world since last three decades [1, 79, 80]. Disease severity of dengue is often ascribed to secondary infection with a virus belonging to a serotype other than that of the primary infection. In this context, evolution of the virus is also considered as a major contributing factor behind the enhanced dengue epidemics [81, 82]. Multi-serotype infection and antigenicity of DENV might have profound effect in augmented dengue epidemics [81, 83]. Mutation rates in viruses with RNA genomes can be six orders of magnitude greater than the viruses with DNA genomes as no proof reading enzymes are available for RNA dependent RNA polymerases, thus exhibiting error frequencies nearly equal to 10^{-4} [1, 84]. High mutation rate associated with RNA polymerase introduces approximately one error per genome during each round of viral replication [1, 84]. According to the “Muller’s ratchet” theory, a high rate of mutation in RNA virus results the accretion of deleterious non-synonymous mutations and such deleterious mutations are then removed by purifying selection [86, 87]. Researchers reiterated that natural recombinants of dengue virus (DENV) carry multiple recombination events [41]. Analysis of the nucleotide sequences from the DEN-1 and DEN-2 serotypes revealed that a networked evolution has occurred in DENV progeny, indicating the strong probability of occurrence of genetic recombination [88]. The inter-virus genetic recombination occurs successfully which could lead to form new virus variants [1, 89]. Interestingly, intra-serotype homologous recombination has also been identified in the non-structural gene region [90]. Surprisingly, studies demonstrated that the high evolving organisms like virus, bacteria exhibit intra-codon recombination event [91, 92], which is a form of genetic recombination where the nucleotide triplets of the same codon engage in sequence exchange via significant breakpoints within the codon [81]. The different serotypes of DENV also exhibit intra-codon recombination event. This indicates that the genetic recombination event within the codon level has a significant effect to maintain the extensive purifying selection [81]. Studies stated that the viral surface protein of arbovirus is also subjected to strong purifying selection [93]. The DENV subtypes or genotypes differ in both fitness and virulence which have poignant significance in the viral population structure [94]. The abrupt change in genetic composition of one genotype of DENV causes the displacement of another genotype in a specific population [38, 39, 95-97]. This is markedly evident from the phenomenon where replacement of low virulence American genotype of DENV-2 virus by high virulence Southeast Asian genotype of the DEN-2 virus. This has been found in Latin America [98-100]. This replacement might be the result of immune mediated natural selection in which an altered genetic composition in one genotype helped it to evade the cross-immunity generated by the other genotype [101]. The four serotypes of dengue virus have been evolved in non-human primate reservoirs and then leaped over to the humans because of clustering of sylvatic strains with the human strains as a result of increased anthropogenic activity [102] (FIGURE 2). Experimental findings suggest that there is no significant adaptive barrier exists for the emergence of sylvatic DENV in human populations, possibly indicating the DENV might be an “opportunistic virus” and has a significant potentiality to infect different primates [102]. Thus, the co-circulation of different dengue serotypes and increased anthropogenic activity enhance the possibility of genetic changes through recombination and thereby increases a significant plausibility for availability of genetically diverse viral strains. These above apprehensions might be considered as potential reasons behind the emergence of a new variant serotype, DEN-5 [31]. Studies suggested that South-east Asia, possibly harbours DENV “source” population [1, 30]. Thus the finding of a new serotype DENV-5 from Sarawak state of Malaysia of South East Asia [31] further increases the importance of thorough study on the emergence of new virus strains and genotypes or subtypes. In this context, it could also be mentioned that global change of climate might become an important factor behind the sylvatic DENV circulation through the territorial expansion of the dengue vectors [103]. Although, DENV infection is mostly dependent on the considerable interaction of vector mosquito and human host [103] but in near future there is a possibility that the newly emerged serotypes such as DEN-5 could result a severe dengue outbreak in human population with an altered pathogenicity which could further complicate the disease situation in the changing environment.
Aedes aegypti has been considered to be the principal vector of dengue virus in the urban transmission cycle while Aedes albopictus serves as a secondary vector or a maintenance vector [104, 105]. It has been reported that these mosquito vectors has aggressively invaded different ecological areas [106-109]. The global expansion of their distribution and introduction to new continents has been contributed by international trade routes through shipping and increased anthropogenic activities [110, 112]. The persistence and the maintenance of the DENV in the nature during inter-epidemic period are results from transovarial transmission of dengue virus by these vector mosquitoes [1]. The outbreak of dengue in India was generally associated with Aedes aegypti but interestingly, in Kerala state Aedes albopictus has identified as the primary vector for dengue [105]. Thus studying the role of two above mentioned Aedes mosquitoes in virus transmission may further elevate our understanding about the vector-virus dynamics in the dengue transmission cycle occurring in natural condition [61]. Aedes albopictus has been included within the hundred most dangerous species in the global invasive species database [1, 113]. The genome of this mosquito possesses extensive repetitive sequence and significant groups of transposable elements. It has found that ~68% of genome representing the repetitive sequence [114]. This high repetitive DNA is associated with large genome size and the length of the entire genome is ~40% more than Aedes aegypti. This large genome size with extensive repetitive sequence confers a prominent status of “Invasive Species” to Aedes albopictus [114]. The DNA sequences related to flavivirus has found integrated in dsDNA genome of these Aedes mosquitoes which indicate the possible DNA synthesis from viral RNA by endogenous reverse transcriptase activity [115-117]. This in turn strongly supports the fact that flavivirus has the potential ability to recombine with the mosquito DNA [118, 119]. A relative complex interaction persists between the mosquito’s nucleic acid metabolism and DENV replication which might facilitate the generation of recombinant virus [60]. Interestingly, research identified that DENV genome harbours a large number of positions which exhibit a high degree of intra host viral diversity [120]. There are seven positions in DENV genome that shows this high degree of plasticity, which are: two in the E gene, one in the NS1 gene, one in the NS3 gene, one in the 2k peptide at the C terminus of the NS4A gene and two in the NS5 gene. The changes of intra-host genetic diversity during the human infection were encountered more extensively in the NS1, NS2A and E-gene. However, in Aedes aegypti, the changes were located in prM, E, NS1, NS3, NS4A (2k peptide) and NS5 genes whereas, in Aedes albopictus, the changes were found in E, NS1, NS4A (2k peptide) and NS5 genes. These differences indicate that the DENV which transmitted through an Aedes aegypti-human cycle may produce genetically different virus from those transmitted through Aedes albopictus-human cycle [120]. Thus, it could be fair to mention that the unique genomic properties and the ecological plasticity of both the Aedes mosquitoes confer a profound effect on the dengue virus global distribution and maintenance of extensive virus genetic diversity in natural condition. This might be associated with unexpected dengue outbreaks and disease complications.

The other members of the Flaviviridae family like WNV and JEV require replication before transmission. Animal hosts such as pigs and birds play an important role in the maintenance and amplification of the virus. These virus survive by virtue of alternation between vertebrate and invertebrate hosts in a “cycle” where man gets the infection tenuantly on accidental intrusion in this pathway [121]. DENV infection requires an invertebrate host (mosquito vector) and a primate host [102, 122]. Interestingly, the flavivirus like WNV, JEV increase their within-host genetic diversity through replication [123]. Albeit, it has been reported that significant purifying selection pressure is also acting on the JEV genome [124, 125] but in vitro studies indicated that a single alternate passage in vertebrate cell culture after sequential passage in mosquito cell culture decrease genetic diversity of the flavivirus [125, 126]. Thus, the infection caused by these viruses to human “dead end” host might result in a conspicuous reduction of the chances of the availability of naturally fit genetically variant virus strains. Moreover, the host alteration reduces virus fitness in comparison to single host viruses [127-131]. The essence of dengue virus evolution relies on the fact that, the mutations (deletions and insertions) in the variable regions of 3'-UTR of the DENV genome [124, 129, 132] and duplication of a RNA structure at 3'-UTR have a profound role in rapid host specific adaptation without significantly reducing virus fitness [78]. In addition, the NS genes are also thought to have an important role in viral fitness [134]. It is demonstrated that a higher recombination rate per nucleotide probably plays a major role in the more effective purifying selection observed in RNA viruses [93]. Thus, the ability of rapid host adaptation of DENV coupled with genetic recombination events may facilitate the extensive genetic diversity which is then might subjected to purifying selection and thereby may produce naturally fit DENV variant strains. Additionally, the significant role of the cis-regulatory region, 3'-UTR of the DENV RNA genome in facilitating the inhibition of RNA interference pathway in host [78] might enlighten and explain the possible strategy of the virus to bypass or avoid the host developed mechanisms against the virus.

The different serotypes of dengue virus also transmitted between non-human primates and mosquitoes in tropical Asia and West Africa. The presence of this silent zoonotic transmission cycle of DENV could explain about the selection of dengue virus variants with altered host range [136]. Interestingly, a large proportion of asymptomatic dengue cases are found in association with symptomatic dengue cases in a group of affected individuals in a population [137]. It has been also reported that mosquitoes might have the possibility of getting the infection from the individuals who are infected by dengue virus but showed no clinical symptoms [138]. This may add a new dimension in the dengue epidemiology, where some human individuals with asymptomatic dengue infection might
Dengue virus shares similarity with the primitive mosquito-borne encephalitis viruses. Interestingly, this virus has significantly evolved a notable biological feature, called “lymphotropism” which segregates them from their more primitive neurotropic ancestors [136]. The virus usually damaged the primate monocyte or macrophage as a principal target cell for replication [141]. Experiments have been revealed that there is a wide range of cellular tropism found in DENV. DENV antigens have been detected from mononuclear cells, lymphocytes, Langerhans cells in the skin, neurons, astrocytes, endothelial cells and hepatocytes, heart and skeletal muscle [142, 143-156]. Researchers reiterated that the C-protein of DENV interacts with the multifunctional host protein Nucleolin (NCL) which facilitates the viral morphogenesis [157]. This altered tropism of dengue virus in humans might indicate the fitness strategy of the virus in urban areas since human is the only possible source of viremic vertebrate. This possibly ensured the sustained transmission of dengue virus in urban situations. The “lymphotropism” phenomenon also provides an opportunity for the survival, maintenance and spread of DENV in urban areas in the absence of any non-human primate reservoir. Moreover, this extraordinary capacity of DENV to replicate in human tissues to high titer level indicates the possibility of emergence of potentially variant strains with altered tropism which could significantly change the disease expression in the host. Therefore, in the context of flavivirus evolution, there is a high possibility that flavivirus like dengue is able to convert itself into a much threatening pathogen by genetic changes which can efficiently capable of causing encephalitis [136], encephalopathy [158] and other neurological complexities. This can be supported by the example which involves the detection of DEN-4 in brain cells and in cerebro-spinal fluid (CSF) of a patient with encephalitis [159, 160]. Interestingly, various neurological manifestations corresponding with DEN-2 and DEN-3 infections have also been found [158]. Thus the possible altered expression of dengue and the burgeoning disease severity might further complicate the existing dengue infection situation in the near future and may create a situation of possible confusion in the disease recognition as well.

Dengue infection with two different strains which is a prerequisite for DENV recombination has been already demonstrated in human [46]. Interestingly, the RNA virus has developed the ability to recombine with the genome of unrelated group of DNA viruses [161]. This further increases the possibility of emergence of entirely new virus strains or subtypes in the environment. Moreover, remarkably researchers reiterated that the RNA virus has also developed the ability to recombine with host dsDNA genomes [162]. Studies indicated that transposon mediated exchanges and the group II intron retro-homing mechanism could facilitate the formation of recombinant virus containing RNA-DNA hybrid genome [161, 163, 164]. The host cell might also use similar related host cell based molecular mechanism to facilitate the formation of recombinant virus. Thus, these extraordinary abilities of RNA virus like DENV may unlock a new vista in dengue research, which encompasses a relevant proposition of a momentous plausibility of crucial genetic exchange between DENV (+) ssRNA genome and dsDNA of the human host. However, any purifying selection within individual hosts may be relatively weak [165], but with the passage of time natural selection might favour the emergence of novel genetically diverse DENV variants, resulted from the crucial genetic exchange between dengue virus genome and the human genome.

Interestingly, it has been demonstrated that the dengue virus can be capable of causing apoptotic cell death in both, in vitro and in vivo conditions [166]. The crucial induction of programmed cell death by DENV may contribute to severe dengue pathogenesis [142]. Studies demonstrated the presence of apoptotic cells in liver, brain, intestinal and lung tissues which were found in the autopsy examination of fatal DHF/DSS cases [167]. It was also indicated that in the neuroblasta cells of mice, the notable aggregation of viral proteins in the ER membrane might responsible for the ER weakening of the host immune response [174, 175]. Moreover, the apoptosis in the dendritic cells could disable innate immune response or could facilitate the viral escape from the immune surveillance which ultimately results in severe dengue pathogenicity [142]. The severity of dengue infection also results in the undesired destruction of neighboring cells (Bystander effect) and thereby leads to the weakening of the host immune response [174, 175]. Moreover, patient with acute dengue infection has been found with viral myositis, which then leads to the development of rhabdomyolysis in the patient [176]. These instances of severe pathogenicity of DENV might be regulated at the genetic level of the virus and may be associated with unusual mutational and recombination events.

The extensive genetically diverse RNA virus population like DENV thought to consist of a group of closely related non-identical genomes which are referred as viral quasispecies [177, 182]. Viral quasispecies might play an important role in the development of the chronic disease [183]. These quasispecies might evolve as a probable result of competition and selection [184]. The accretion of random mutations resulting from the high mutation rate in the RNA genome of DENV might cause the formation of DENV quasispecies [183]. Quasispecies of a flavivirus like the human hepatitis C virus successfully escape from the host immune response when the host immune system has failed to reduce the genetic diversity of the virus [185]. In addition, they could also escape from the pressure of drug treatment [183]. The viral quasispecies contain the genomes with most fitness that is encompassed by a mutant virus spectrum [184]. Moreover, the diverse virus population involves “low-fitness intact viruses” which might show higher fitness in dissimilar environments. The co-infection in association of “high fitness viruses” in disparate environments might be the possible reason in favour of the survival of those “low-fitness intact viruses” [183, 186]. Remarkably, studies indicated that RNA
virus like DENV could carry a memory genome in the virus population at the intra-host and inter-host level. The virus population in the form of quasispecies might also carry a molecular memory genome [183]. The memory genome of DENV could result the different cell tropism of different DENV [187]. The co-infection of different DENV strains or genotypes might be considered as the reason behind the formation of viral quasispecies. The DENV population might contain different small minor populations and these populations might be maintained through infections in both mosquitoes and humans. DENV population may be successful to carry the unique genome and genomic combinations which might in turn result in altered cell tropism [183]. Therefore, the uniqueness of DENV genomic constituents and its unconventional interaction with the host genome could further complicate the pathogenicity and severity of the disease.

The evolution of the mosquito borne virus involves the genetic bottleneck and extinction events [188]. The genetic bottleneck causes the emergence of new virus strains and variants from limited members amongst the majority of co-circulating virus in a particular region. The reintroduction of various DENV subtypes in a particular area has been reported [189, 190]. The intricate mutational events in the virus in association of interaction with the host cells may confer an ability of a notable resistance to the positive sense strand RNA virus like dengue against its extinction [194]. Albeit the vertical evolution through random mutation has been considered as the principal factor in the evolution of RNA virus like DENV, but the admixture of genetic constituents between individuals of different strains of the dengue also directs the mode of DENV evolution horizontally by facilitating horizontal evolution [191]. The unique capability of DENV in the maintenance of extensive genetic diversity by high mutation rates, structural modification of cis-regulatory region of its RNA genome and unusual genetic exchange between its different strains, related and unrelated group of viruses and host ds DNA genome in artificial and natural condition is a possible indication of rapid evolutionary adaptation of the DENV in the changing environment. Thus, these unique genetic strategies might indicate the prospective direction of DENV evolution, which is associated with altered disease expression in the changing climate.

Vaccination might produce an ambiance where reasonably low transmission of natural DENV could be found [34]. The World Health Organization (WHO) suggested that the development of tetravalent vaccine is the most effective approach for prevention of dengue [192]. The most important feature of an effective dengue vaccine must include its ability to provide protection against all dengue virus in different dengue endemic geographical regions [193]. The live tetravalent vaccine development has been initiated in Bangkok, Thailand in late 1970 [194]. In addition to this, other significant approaches like recombinant vaccines have also been developed [195]. Inspite of extensive efforts, a safe and effective dengue candidate vaccine was not developed [192]. However, a world renowned multinational pharmaceutical company, Sanofi Pasteur has recently developed the first dengue vaccine Dengavax™, which has been approved by Mexico [196]. Studies indicated that recombination events either between strains which are presented in multivalent vaccine or between an attenuated vaccine strain and wild type strain might cause the unwanted emergence of new variant virus with unusual properties [197-199]. Thus, in this changing epidemiological situation, the effectiveness of a vaccine in lowering the transmission of several naturally developing genetically variant DENV in different dengue endemic geographical regions is still a matter of conjecture.

10. Conclusion

The evolutionary arms race between host and virus has always played a pivotal role in shaping the virus evolution. The mutational and unique recombination events might be a principal reason behind their extensive genetic diversity. The mosquito vector provides an environment where changes of viral genotype occur and applies selection pressure on the different viral phenotypes. Hence, vectors have a great chance to influence the evolution of such viruses. An inclusive understanding of host-virus interaction is also needed, to fully assess the factors that regulate the potential for host shifts and geographic expansions. Additionally, rapidly increasing size of the human population and globalization may allow natural selection to play an even more important role in viral evolution, especially, arthropod-borne viruses like dengue virus. Therefore a sustained active surveillance, molecular sequencing studies and phylogenetic analysis of different DENV isolates are required to understand the evolutionary trend of dengue virus with reference to genetic diversity in the changing climate.

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12. Conflict Of Interest

There is no conflict of interest.

13. References

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