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Original Article

Comparative Evaluation of Dengue Virus (DENV) Serotypes Infections in Human (*Homo sapiens*)

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ABSTRACT

Background: Dengue virus is transmitted by the mosquito species *Aedes aegypti* and causes dengue fever. The single positive-stranded RNA virus of the family *Flaviviridae* belongs to the genus *Flavivirus*. There is four dengue virus (DENV) serotypes. (DENV-1, DENV-2, DENV-3, and DENV-4). Each has altered interactions with the antibodies in the human blood serum. **Materials and Methods:** In the study, we reported that the comparative study of dengue serotypes along with multiple sequence alignment, domain organization, structure modeling, secondary structure prediction, epitope peptide identification, including the phosphorylation sites. **Results:** A significant objective for future research will be to completely comprehend the auxiliary and proper angles and clarify the various jobs. **Conclusion:** We present a comparison of Dengue virus serotypes in this study (DENV1, DENV2, DENV3, and DENV4). We looked into dengue serotype differences in this study.

Keywords: Sequence, Amino acid, Dengue virus, Structure, Epitope, Peptides, Phosphorylation.

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INTRODUCTION

The dengue virus genome (single-stranded positive-sense RNA of ~10,700 bp) encodes seven non-structural proteins and three structural proteins. Dengue virus (flavivirus) causes dengue, a mosquito-borne viral disease. Dengue is spread by the female *Aedes aegypti* mosquito, which acts as a vector, and these are found in tropical and subtropical throughout the world.[1] The capsid, precursor membrane, and envelope glycoprotein are the three structural protein components that make up a virus particle; seven non-structural proteins, such as NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5, are only found in infected host cells and are necessary for viral replication.[2] Dengue virus serotypes 2 and 3 have six genotypes each. There are four genotypes in dengue virus-3 and four in dengue virus-4. Dengue shock syndrome, dengue fever, and dengue hemorrhagic fever can be caused by any serotype (DENV1, DENV2, DENV3, and DENV4) alone or in combination with other serotypes.[3]

NS5-DENV proteins have an N-terminal methyltransferase domain and a C-terminal RNA-dependent RNA polymerase. The methyltransferase (MTase) domain (NS5) protein of Dengue virus has N7 and 2'-O-methyltransferase activity, involved in viral RNA cap production.[4-6] When cap guanine is methylated at its N7 position, 7MeGpppA-RNA is produced, and when it is methylated at its 2' -O location, 7MeGpppA2' OMe-RNA is produced.[7,8] S-adenosyl methionine, which acts as a methyl donor and produces S-adenosyl homocysteine as a by-product, is required for methylation at both sites. The capping and methylation processes are vital because they shield mRNA from 5′ exoribonuclease destruction and improve mRNA translation.[9-11] In this study, we report the comparison of Dengue virus serotype (DENV1, DENV2, DENV3, and DENV4). In this study, we investigated dengue serotype-specific differences.

MATERIALS AND METHODS

Multiple Sequence Alignment

The amino acid sequence of NS5 methyltransferase domain of DENV-1 (262 amino acid), DENV-2 (263 amino acid), DENV-3 (262 amino acid), and DNV-4 (263 amino acid), retrieved from the NCBI (genome database), and BLASTp analysis was done by using multiple sequence alignment of all four Dengue virus NS5 methyltransferase amino acid sequences of similar length. On the other hand, we also identified the conserved domain such as the Tellurite resistance protein, the Methionine biosynthesis protein, Methyltransferase small domain, and Fts-J like methyltransferase.

Domain Organization and Phylogeny Analysis

The FASTA format of amino acid sequences of DENV-1, DENV-2, DENV-3, and DNV-4 was applied for domain organization analysis. For domain organization using the different available bioinformatics based tools such as Pfam, Prosite, InterProScan, SMART, Pfam, Prosite program. Using the maximum likelihood method, an arrangement of other domains organization, we have done a phylogenetic tree among all four NS5 methyltransferase dengue serotypes. The numbers in the tree show the distance between the dengue serotypes.[12-14]

Structure Modelling and Secondary Structure Prediction

The amino acid sequences of the NS5 methyltransferase domain (DENV-1, DENV-2, DENV-3, and DNV-4) were uploaded on the SWISS-MODEL server, and the program was used to select a suitable homology-based template. The structural models were obtained using these templates and Pdb files of the predicted models. The expected structure of the NS5 methyltransferase domain and the ribbon diagram of the template predicted structure was prepared using SWISS-MODEL

online available workspace server, and images were superimposed for the analysis.[15] In the other hand we also identified the secondary structure perdition of all four dengue virus NS5 methyltransferase domain which contains several helix residues, strand residues and coil residues (Phyre 2 and http://bioinf.cs.ucl.ac.uk/psipred/&psipred_uuid=f159bed4-5c34-11e9-b6a7-00163e100d53). It is sound that strands, α-helix, and coil are available in NS5 methyltransferase region.

Predicting of Antibody Epitope from Protein Sequences

The amino acid sequences of the NS5 methyltransferase domain (DENV-1, DENV-2, DENV-3, and DNV-4) were uploaded on the SWISS-MODEL server, and the program was used The BepiPred-2.0 server predicts B-cell epitopes from a protein succession, utilizing a Random Forest calculation prepared on epitopes and non-epitope amino acids decided from precious structures.[16] A successive forecast smoothing is performed subsequently. The fanfares with scores over the edge (default esteem is 0.5) are anticipated to be a piece of an epitope and shaded in yellow on the chart (where Y-tomahawks delineates build-up scores and X-tomahawks build-up positions in the grouping) and set apart with "E" in the yield table.[17] The E values of the scores are not influenced by the chosen edge.[18] The table beneath demonstrates the connection between chosen sides and the affectability/explicitness of the expectation technique (http://tools.immuneepitope.org).[19,20]

Prediction of Phosphorylation Sites

Post-translational modification (PTMs) happens on practically all proteins dissected to date. These modifications regularly firmly influence the capacity of an adjusted protein. In this way, expanded learning about the potential PTMs of an objective protein may build our comprehension of the sub-atomic procedures in which it partakes.[21] The retrieved sequence of all four-dengue virus NS5 methyltransferase protein submitted to NetphosK 3.1a (http://www.cbs.dtu.dk/services/NetPhosK/) was utilized to anticipate the capability of kinases destined to phosphorylate Ser, Thr, and Tyr.[22]

RESULTS

In this study, we have reported the *in-silico* analysis of all the primary domains of dengue serotypes and its comparison.

Identification and Multiple Sequence Alignment and Domain Organization

The alignment of dengue serotypes NS5 methyltransferase domain was done by using bioinformatics tool CLUSTAL W2 program (http://www.ebi.ac.uk). The alignment was done using CLUSTALW2 program (http://www.ebi.ac.uk). The signs '*' means that the residues in that column are the same in the alignment, ':' means that conserved substitutions have been observed and '.' means that semi-conserved substitutions are observed. The conserved motifs are boxed in different colors, and the name of each motif is written. The result reveals that the Tellurite resistance protein domain is denoted with green color box, the Methionine biosynthesis protein domain denoted with yellow color Methyltransferase small domain with black color box, and Fts-J like methyltransferase with pink color box (Figure-1A). Similarly,

The amino acid sequence of the NS5 methyltransferase domain organization of Dengue serotypes (DENV1, DENV2, DENV3, and DENV4) with their specific motifs and position in the protein obtained by *in silico* tools such as SMART, Panther, Scan Prosite, and Interpro using bioinformatics based available facility. The motifs are boxed in different colors, and the name of each motif is written (Figure 1B-E). The phylogenetic tree of Dengue serotypes (DENV1, DENV2, DENV3, and DENV4) was obtained using the maximum likelihood method. The numbers in the tree show the distance between the dengue serotypes (Figure 1F).

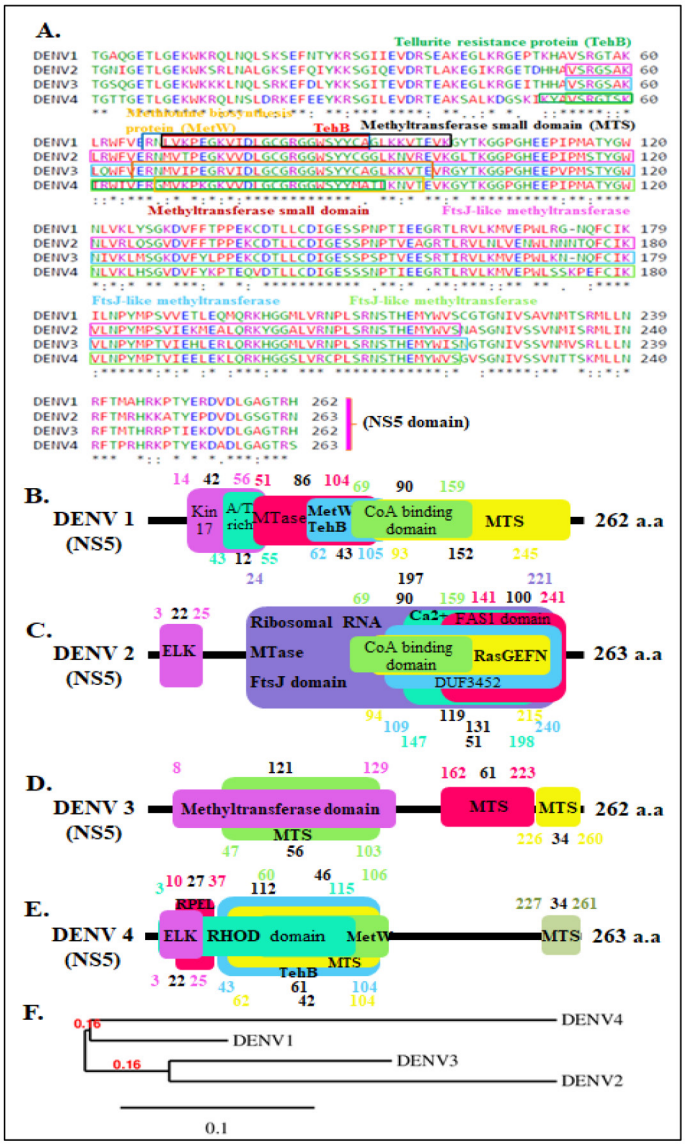

Figure 1: (A) Comparison of the amino acid sequence of the NS5 domain of Dengue serotypes such as DENV1-NS5 (262 amino acid), DENV2-NS5 (263 amino acid), DENV3-NS5 (262 amino acid) and DENV4-NS5 (263 amino acid). **(B-E)** Detailed graphical domain (NS5) organization of Dengue serotypes (DENV1, DENV2, DENV3, and DENV4) with their specific motifs position in the protein obtained by in silico tools such as SMART, Panther, Scan Prosite and Interpro. **(F)** The phylogenetic tree of Dengue serotypes (DENV1, DENV2, DENV3, and DENV4) was obtained using the maximum likelihood method. The numbers in the tree show the distance between of the dengue serotypes.

Structure Modelling

In this study, we have performed further analysis of the modelled structure of Dengue serotypes (DENV1, DENV2, DENV3, and DENV4). The sequences were submitted to the Swiss model server (https://swissmodel.expasy.org/), and the structures were obtained. The molecular graphic images were produced using the UCSF Chimera and Superimposed image (Figure 2). I.DENV-1 (NS5); ii. Template (5ikm.1); iii. Superimposed image; iv. DENV -2(NS5); v. Template (2p41.1); vi. Superimposed image; vii. DENV-3(NS5); viii. Template (5ccv.2); ix.

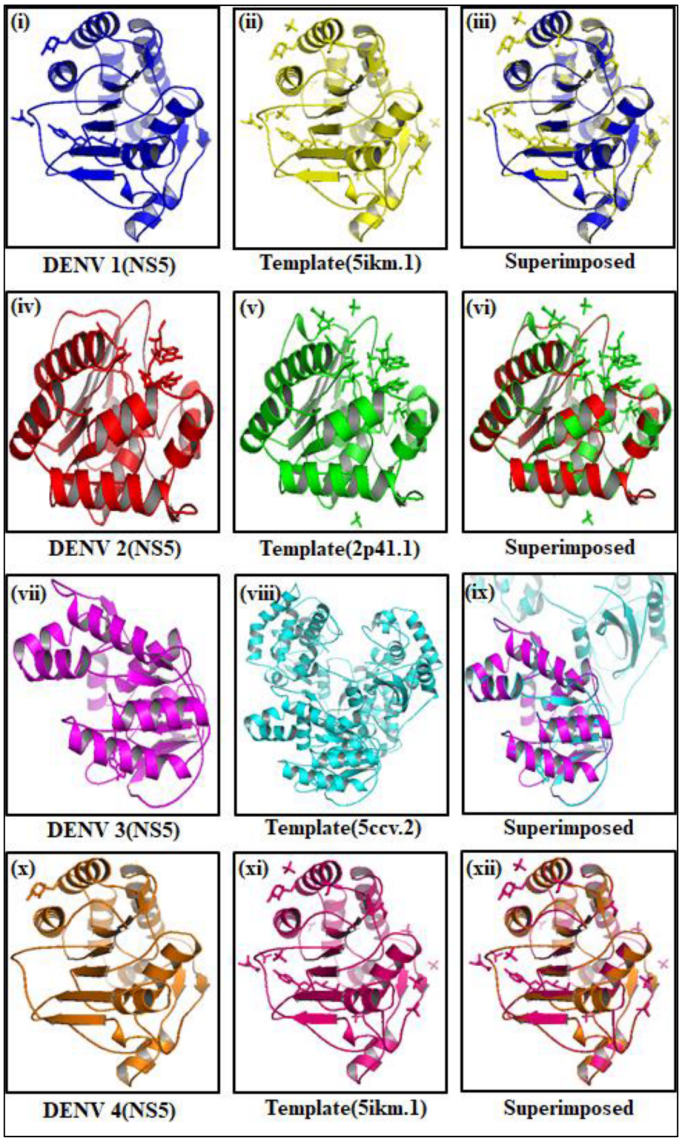

Figure 2: Structure modelling of the NS5 domain of Dengue serotypes (DENV1, DENV2, DENV3, and DENV4). The sequences were submitted to the Swiss model server (https://swissmodel.expasy.org/), and the structures were obtained. The molecular graphic images were produced using the UCSF Chimera. i. DENV1-NS5; ii. Template; iii. Superimposed image; iv. DENV2-NS5; v. Template; vi. Superimposed image; vii. DENV3-NS5; viii. Template; ix. Superimposed image; x. DENV4-NS5; xi. Template; xii. Superimposed image.

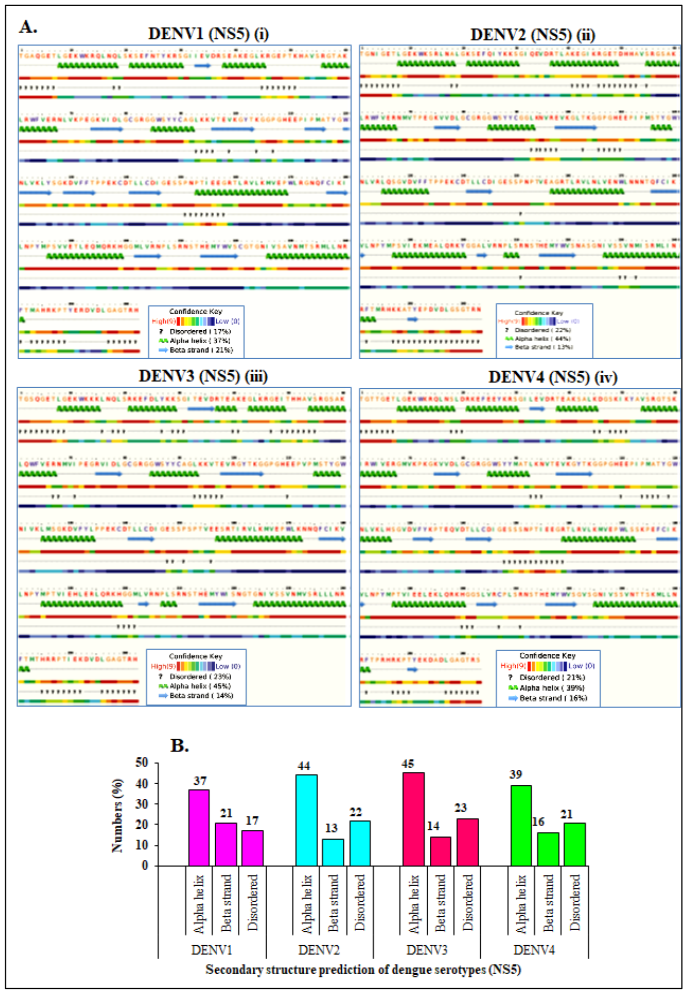

Figure 3: (A) i-iv Secondary structure prediction (Alpha helix, Beta strands, and disordered) of the NS5 domain of Dengue serotypes (DENV1, DENV2, DENV3 and DENV4. **(B)** The Bar diagram with different color pink (DENV1), cyan (DENV2), red (DENV3), and green (DENV4) with Alpha helix; Beta strands and disordered which are present in the structure.

Superimposed image; x. DENV-4(NS5); xi. Template (5ikm.1); xii. Superimposed image.

Secondary-structure Prediction

They assessed the prediction techniques. The general secondary structure examination shows that beta strands, α-helix, and disordered are available in the NS5 methyltransferase domain of dengue (DENV-1, DENV-2, DENV-3, and DENV-4) (Figure 3A i-iv). On the other hand, the Bar diagram with different colors pink (DENV1), cyan (DENV2), red (DENV3), and green (DENV4) with Alpha helix; Beta strands and disordered which are present in the structure (Figure 3B). DENV-1 NS5-MTase domain contains (α helix 37, beta-strand 21, and disordered 17), DENV-2(α helix 44, beta-strand 13 and disordered 22), DENV-3 (α helix 45, beta-strand 14 and disordered 23), and DENV-4 (α helix 39, beta-strand 16 and disordered 21) (Figure 3B). Primary data can be utilized to characterize an underlying theory, for example, secondary or tertiary structure predictions. Data on the folds obtained by biophysical techniques functional data can also be exploited to base an initial approach.

Epitope Prediction Bar Graph

BepiPred programming was utilized to deliver a Linear Epitope Prediction graph (Figure 4A-D). BepiPred examines every amino acid autonomously and assigns it a score.[22] Interestingly, the analysis showed that a total of eleven antigenic epitope peptides were predicted in the NS5 methyltransferase domain (MTase) of dengue serotypes 1, 2, 3, and 4. However, three of these peptides were demonstrated to be antigenic (Figure 4A-D). Predicted antigenic B-cell epitopes were found to be highly conserved and demonstrated strong potentials, which probably predict regions of virus-cell interaction. Further, the analysis showed that the many associated B-cell peptides had spike scores, respectively.[23-24] The bar diagram reveals that the epitope peptide sequence and its length of amino acid (Figure 4A-D).

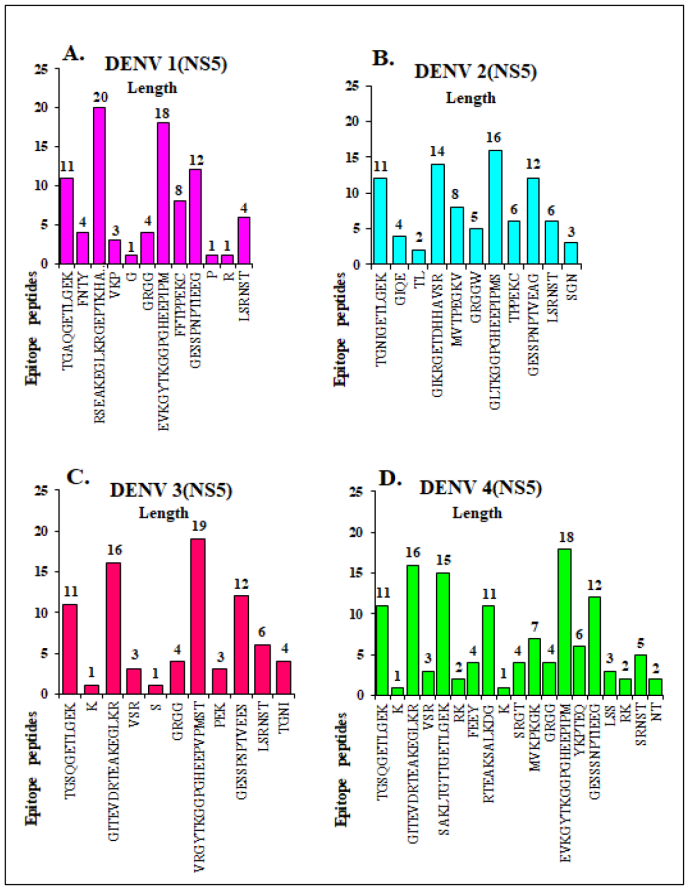

Figure 4: (A-D) The Bar diagrams of DENV1 (Pink), DENV1 (cyan), DENV3 (red), and DENV4 (green) NS5 domain with short epitopes peptides.

PTM-Phosphorylation Analysis

Post-translational modifications (PTMs) are a resource of known and predicted functional associations between interacting proteins.[25] It contains seven PTMs that are Phosphorylation, Ubiquitination, N-linked glycosylation, Acetylation, Nitrosylation, Methylation, and oxidation. We focused on the phosphorylation type of post-translational modification. Prediction results of human Dengue Virus (DENV-1, DENV-2, DENV-3, and DENV-4) NS5 domains methyltransferase domain by using online available software Netphos 3.1a of Ser, Thr, and Tyr are given in Figure 5A-D. These results show a similar phosphorylation potential in DENV-1, DENV-2, DENV-3, and DENV-4. The consequences of NetphosK 3.1a, for foreseeing the capability of kinases with the NS5 methyltransferase proteins auto phosphorylation locales, demonstrate that NS5 methyltransferase proteins are the central potential kinase to adjust these sites.

DISCUSSION

By way of modifications in the global epidemiology of dengue over the last 50 years, the number of countries reporting dengue greater than before, and has the number of undecorated diseases.[26] Dengue fever is a most important mosquito-borne viral disease in India. Dengue infections are affected by four closely related viruses known as DENV-1, DENV-2, DENV-3, and DENV-4. These four viruses are serotypes because they interact differently with antibodies found in human blood serum. The current data presented in this manuscript impact the comparative analysis of NS5 methyltransferase domain of DENV (Dengue) serotypes.

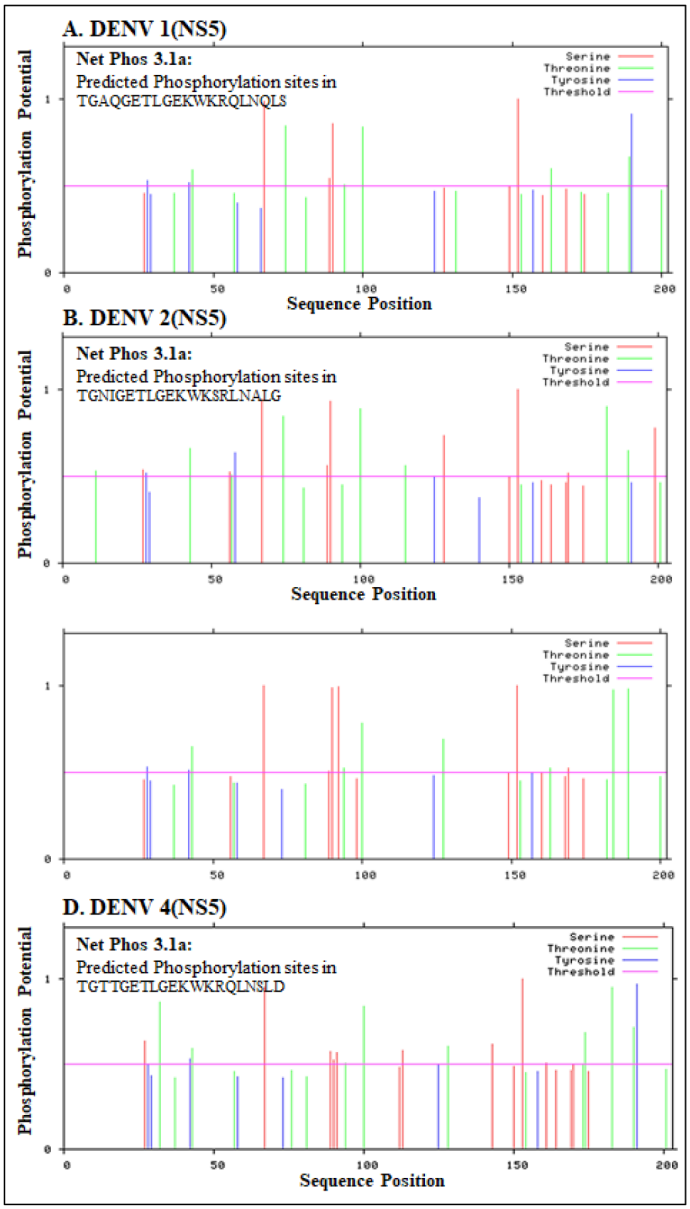

Figure 5: (A-D) The detailed appearance of the phosphorylation potential alteration in human Dengue Virus (DENV-1, DENV-2, DENV-3, and DENV-4) NS5 domains. The pink line denotes the threshold for alteration potential. The red lines reveal the phosphorylated Ser residues; the green lines show the phosphorylated Thr residues; the blue line describes the phosphorylated Tyr residues.

The MTase is in charge of one after the other capping the nascent genomic RNA using S-adenosylmethionine as the methyl donor via sequential methylation on the N7 atom of the cap guanine and the 2'O atom of the ribose of the genome's first strictly conserved adenine.[27- 28] Viral capping, approximating eukaryotic cell 5' capping of mRNA, thwarts degradation and develops interaction by means of the ribosome for translation.[29] Crystal structures of the MTase domain revealed the same / fold as DENV, Japanese encephalitis, and the most recent ZIKV virus structures.[30-32] Although several efforts, there is currently no safe and real dengue vaccine. Cumulative evidence suggests that T cell-based immune responses against DENV may play a defensive role.[33]

Since all four DENV serotypes are known to co-circulate in endemic areas, and for the reason that heterotypic infections increase the risk

of severe dengue, an effective vaccine should elicit protective T cell responses against most, if not all, serotypes. The DENV proteome are exceedingly conserved from corner to corner the majority of the four DENV serotypes and immunogenic enough for T cells to be trained to recognize them. We investigated the NS5 domain with short epitopes peptides. After translation, these modifications are catalysed by enzymes from the kinase/phosphatase, methyltransferase/demethylase, and acetyltransferase/deacetylase families. These modifications generally change the surface charge or serve as a binding site for other proteins to interaction. These are well-known to play roles in cellular pathways such as replication, metabolism, signaling, and immune pathways.[34-39]

Phosphorylation (PTMs) is unique of the supreme common PTMs. On proteins, the phosphate group is added to amino acids (serine, threonine, or tyrosine). It is a reversible reaction that have need of adenosine triphosphate (ATP) and consist of protein kinases and protein phosphatases.[40] The phosphorylation of viral and cellular proteins in a host cell can ominously influence viral infection, replication, and cytotoxicity. These modifications are hand-me-down by eukaryotic cells to control the functional inventory of proteins. Furthermost viral proteins mimic perilous regulatory factors to take over the host machinery and stimulate well-organized viral outcomes. Protein phosphorylation is vital for protein interactions, protein stability, signal transduction, transcription regulation, intracellular localization, cell cycle progression, and apoptosis. Lots of intracellular obligate pathogens depend on on protein phosphorylation to create a productive infection cycle.[40]

Figure 5A-D depicts Thr and Tyr. DENV serotypes (DENV-1- DENV-4) all have a similar phosphorylation potential, allowing to these conclusions. The NetphosK 3.1a results for predicting the aptitude of kinases by way of the NS5 methyltransferase proteins auto phosphorylation locales illustration that NS5 methyltransferase proteins are the principal potential kinase to amend these sites. An imperative aim for yet to come research will be to fully grasp the auxiliary and proper angles and clarify the various functions of PTM, particularly phosphorylation of dengue serotypes.

CONCLUSION

Dengue infections are initiated by four thoroughly related viruses named DENV-1, DENV-2, DENV-3, and DENV-4. These four viruses are called serotypes because each has different interactions with the antibodies in the human blood serum. In the study, we reported that the multiple sequence alignment and their conserved domain are aligned manually with the colors box and the position of amino acids. We have done structure modelling and superimposed with a stable template to identify the stability of all four dengue serotypes. We also predict secondary structure via bioinformatics to determine the number of alpha-helix, beta-strand and disordered. In parallel, we also focused on the epitope peptides for antigen binding. A significant objective for future research will be to completely comprehend the auxiliary and proper angles and clarify the various jobs of PTM, especially phosphorylation of dengue serotypes.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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