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Dengue NS5 Global Consensus Sequence Development to Find Conserved Region for Antiviral Drug Development

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Abstract:

Objective: To draw a representing consensus sequence of each DENV serotype, align all four consensus sequences to draw a global consensus sequence and also study the highly conserved residues. Methods: A total of 376 DENV NS3 sequences, belonging to four serotypes, reported from all over the world were aligned to develop global consensus sequence. Results: The active site residues Met343, Thr366, which are involved in nuclear localization and also interact with the NS3 viral, are highly conserved among all the DENV serotypes. Cys450, Gly466 and Ala468, Arg482 are highly conserved in all the serotypes. Structural zinc (Zn1) sited consist of Cys-446, Cys-449, His-441, and the carboxylate group of Glu-437. This pocket is also found near the functionally important residues Ser-710 and Arg-729, which bind to the incoming rNTP. Meth530, Thr543 Asp597, Glu616 and Arg659, Pro671 are structurally conserved in all serotypes. Identification of four out of six conserved sequence motifs accountable for NTP binding and GDD catalytic active site, located in the palm domain. Leu766, Ala776 residues have high conservancy in all serotypes are observed in consensus sequence analysis. The thumb domain also has Zinc binding site (Zn2) and is synchronized by His-712, His-714, Cys-728 of motif E, and Cys-847. Pharmacological blockage of cavity B could potentially lead to suppression of initiation of the viral RNA synthesis and/or inhibition of NS3/NS5 interaction. Thirteen different peptides from the highly conserved regions of DENV NS5 protein were drawn which can be used to develop peptidic inhibitors. Conclusions: In spite of a high mutation rate in DENV, the residues which are present in the Nuclear localization signal (NLS), Di-valet ion binding

sites, NTP binding, GDD catalytic active site, Thumb domain, priming loop are highly conserved. These are target sites for the development of antiviral agents or peptide vaccines.

Keywords: Pharmacology, Infectious diseases, Consensus sequence, Antiviral drug target

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1. Introduction

Main text paragraph.

Dengue infection has become a major health problem in >100 countries of Africa, Asia, America, Western Pacific and the Eastern Mediterranean [1]. During the recent epoch, dengue infection has caused many endemics in Pakistan and thus, has become a major health issue in Pakistan. The global incidence of dengue infection has increased and estimated 50-100 million cases of dengue infections are reported annually from more than 100 tropical and subtropical countries of the world [2]. Dengue virus has four distinct serotypes and phylogenetically unique dengue viruses (DEN1-DEN4)[3]. DENV-2 serotype was most prevalence circulating serotype in Pakistan. Two types of infections caused by Dengue virus, fluctuating from a dengue fever to a more severe infection that can cause dengue haemorrhagic fever and dengue shock syndrome [1].

The dengue virus belongs to a member of the Flaviviradae that causes a wide range of diseases, including benign febrile illness, dengue fever (DF), plasma leakage syndrome and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS)[4]. The dengue virus is transmitted to humans by Aedes aegypti and Aedes albopictus mosquitoes [5,6]. The dengue virus is an enveloped, ssRNA positive-strand and comprises 11 kilo base long viral genome[7] having three structural proteins C (Capsid , M (Membrane) and E (envelop) [8] and seven Non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 which play an integral part in viral pathology[4,9].

All Non-structural proteins including NS5 are involved in enzymatic reactions which plays an important role in viral replication. NS5 is the most prominent Flavivirus protein due to its high molecular weight is 105 kDa. NS5 proteinsharing the minimum of 67% identity through all serotypes of DENV that's why NS5 served as highly conserved viral domain. It comprises an N-terminal methyl-transferase domain (MTase) domain covers 1 to 296 amino acid residues.

The MTase activity of NS5 is liable for both guanine N-7 and ribose 2_-O methylations and a C-terminal RNAdependent RNA polymerase (RdRp) domain leads 270 to 900 amino acids and it is creditworthy for synthesizing a transient double-stranded replicative RNA intermediate [10-13]. All Flaviviruses has few highly conserved moieties, which lies between amino acid residues 320 and 368 of NS5 and play an important role in binding with beta-impotin and also with NS3[3]. These conserved residues can be imperative in developing unambiguous antiviral agents and inhibitors against dengue virus. In this study by using a unique approach to produce a substantiation consensus sequence NS5 will be helpful in designing anti-peptide to find a possible cure for dengue infection. The present study is designed to draw a global consensus sequence of the NS5 protein of dengue virus and to study DENV NS5 conserved domain function.

Results and Discussion

NS5 protein comprises of two domains, methyl-transferases (MTase) and RNA dependent RNA polymerases (RdRp). The dengue NS5 MTase domain involves in cap formation which recognized by host cell and RdRp domain plays vital role in viral genome replication. Consensus sequences of NS5 protein of all DENV serotypes (DENV 1-4) was drawn in CLC main workbench. Alignment was done to extract the highly conserved peptides among all serotypes which we were studied. Figure 1 shows the alignment of the consensus sequence of all the four DENV serotypes; the global consensus sequence is presented at the bottom. Conserved residues are shown with their corresponding symbols while the highly variable amino acids are denoted by "x" symbol. The alignment of all the consensus sequences will help us to study the considerably conserved residues in the DENV NS5 protein. Short peptides of 9 to 18 amino acids were designed from the highly conserved regions of the DENV NS5 consensus protein sequences; the sequence and position of these peptides are shown in the Table 1. These are the locations which are highly conserved and are the targets to design peptide vaccines or site specific inhibitors.

Dengue infection has become a global risk to human health to all over the world. Dengue virus has four serotypes and because of four different serotypes, there is no successful vaccine developed. NS5 domains become helpful for novel drug designing due to (1) largest DENV protein (2) its high conservancy (3) sharing minimum of the 67% identity through all serotypes of DENV. NS5 is multifunctional domain and has two domains N-terminal methyl-transferase domain (MTase) domain (1 to 296), C-terminal RNA-dependent RNA polymerase (RdRp) domain (270 to 900) [10, 12, 13] and possess the 37-amino acid inter-domain spacer sequence that contains a functional nuclear localization signal (NLS) between amino acid residues (320 and 405). RdRp contains three subdomains finger subdomain with zinc binding sites

Zn1 (Cys-446, Cys-449, His-441, Glu-437), palm domain with GDD catalytic active sites (Asp-663 and Asp-664), thumb domain with Zinc binding sites is Zn2 (His-712, His-714, Cys-728, Cys-847) and six motifs (A, B, C, D, E, F) at C-terminal region of NS5 that contains five amino acid sequence [14]. The consensus sequence analysis shows that Met343, Thr366 are highly conserved among all serotypes.

This conserved region involved in nuclear localization sequences (NLS). The NLS has been divided into alpha-NLS (spanning residues 320 to 368) and alpha/beta-NLS (Residues 369 to 405). The alpha-NLS region thought to interact with the NS3 viral helicase [3]. Interestingly, the NLS domain signatures are dispersed between the fingers and thumb subdomains [15]. The region which binds with beta-importin that recognized NLS and carry protein inside nucleus placed between this amino acid [16]. The consensus sequence analysis shows that Cys450, Gly466 and Ala468, Arg482 are highly conserved in all the serotypes. The base of the fingers domain arrangements a concave surface shaped by the solvent-exposed residues of helices (alpha 6, alpha 14, and alpha 15) near the N terminus of the protein. Structural zinc (Zn1) sited in the fingers subdomains is harmonized by Cys- 446, Cys-449, His-441, and the carboxylate group of Glu-437. The zinc ion Zn2 likely donates to the structural stability of the region adjacent motif E of the DENV polymerase. This pocket is also found near the functionally important residues Ser-710 and Arg-729, which

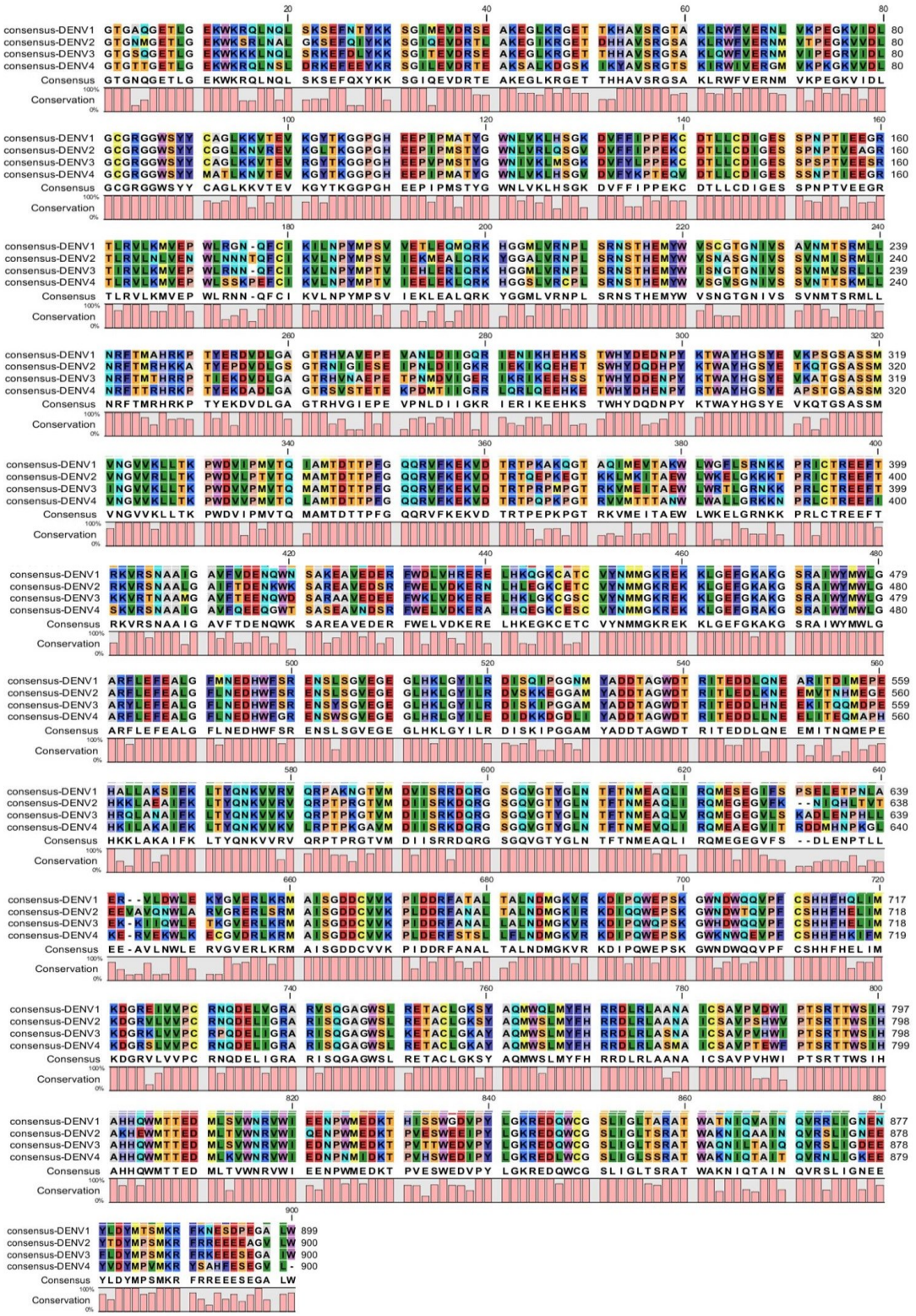
bind to the incoming rNTP [11]. The consensus sequence analysis shows that Meth530, Thr543 Asp597, Glu616 and Arg659, Pro671 are conserved in all serotypes and lay under palm domain. It is the collection of a small antiparallel beta-strand platform, beta 4 and beta 5, surrounded by eight helices (alpha 11 to alpha 13 and alpha 16 to alpha 20). The palm domain performs to be the most structurally conserved among all known polymerases, reflecting the salvation of the design of the catalytic site during evolution. Identification of four of six conserved sequence motifs accountable for NTP binding and catalysis, located in the palm domain [17]. The GDD catalytic active site (motif C, comprising Asp-663 and Asp-664) is placed in the turn between strands beta 4 and beta [11]. Mutation in residue Asp-663 and Asp-664 to any negative charge residue can disrupt the function of RdRp. Leu766, Ala776 is thumb domain and has high conservancy in all serotypes. Thumb domain forms the C-terminal end of the RdRp of DENV, is the most structurally mutable among known polymerase structures. It covers two conserved sequence motifs. Motif E forms an antiparallel beta-sheet wedged between the palm domain and several alpha-helices of the thumb domain. A loop spanning amino acids 782 to 809 forms the priming loop that partially occludes the active site [18]. Three mutations (L328A, W859A, and I863A) reduced de novo RNA synthesis by >=85%. These L328A, W859A, and I863A three mutations Table 1.

Position and sequence of the peptides along with potential domain; which can be used as a peptide vaccine. Position of conserved peptides Sequence of peptides Domain encoding 79–90 No putative conserved domain, 104-113 No putative conserved domain, 141-151 No putative conserved domain, 209-220 No putative conserved domain, 342-363 Functional nuclear localization signal (NLS), 450- 466 N-terminus finger subdomain, 468-482 N-terminus finger subdomain, 530-543 Palm domain, 568-578, 597-616 Palm domain, 659-671 Palm domain, 766-776 Thumb domain, 791-801No putative conserved domain reduced viral replication by decreasing the initiation of RNA synthesis. These three mutations decreased RNA synthesis initiation (L328A, W859A, and I863A) are placed at the same line between the delta 1 loop and remaining RdRp DENV thumb subdomain. The K330A mutation abridged the NS3/NS5 interaction. Thumb domain also has Zinc binding site (Zn2) and is synchronized by His-712, His-714, Cys-728 of motif E, and Cys- 847 of helix alpha 26 and mutation in these residue abolish the activity of RdRp.

Table 1. Position and Sequence of the Peptides along with potential domain; which can be used as a Peptide Vaccine

Position of conserved Peptides	Sequence of peptides	Domain encoding
79 - 90	DLGCGRGGWSYY	No Putative conserved domain
104 - 113	TKGGPGHEEP	No Putative conserved domain
141 - 151	DTLLCDIGESS	No Putative conserved domain
209 - 220	PLSRNSTHEMYW	No Putative conserved domain
342 – 363	MAMTDTTPFGQQRVFKEKVDTRT	**Functional nuclear localization signal (NLS)**
450 - 466	CVYNMMGKREKKLGEFG	**N-terminus finger subdomain**
468 - 482	AKGSRAIWYMWLGAR	**N-terminus finger subdomain**
530 - 543	MYADDTAGWDTRIT	**Palm domain**
568 - 578	IFKLTYQNKVV	
597 - 616	DQRGSGQVGTYGLNTFTNME	**Palm domain**
659 - 671	RMAISGDDCVVKP	**Palm domain**
766 - 776	LMYFHRRDLRLA	**Thumb domain**
791 - 801	PTSRTTWSIHA	No Putative conserved domain

Figure 1. Multiple sequence alignment of consensus sequences of the Dengue NS5 of all serotypes.



3. Materials and Methods

2.1. Drawing consensus sequence of DENV NS5:

A total of 385 sequences of DENV NS5 of all serotypes were retrieved from the NCBI database. All the sequences were imported in CLC main workbench. Consensus sequences of all serotypes were developed by applying multiple sequence alignment feature of CLC main workbench. All four serotypes sequences were retrieved randomly from NCBI protein database. Hundred NS5 sequences of Dengue 1 serotype reported from USA, Indonesia, China, and Australia were used to developed consensus sequence. One hundred sequences of serotype 2 (DENV2) belongs to China, USA, and Taiwan were used to build the serotype two consensus sequence with the support of CLC workbench software. Ninety five sequences of serotype three (DENV3) belonged to the USA, Singapore, and China, were obtained to CLC workbench software to construct consensus sequence. Ninety sequence of serotype four (DENV4) retrieved from NCBI related to Indonesia, USA, China and Thailand were fetched to CLC workbench to produce consensus sequence of DENV4.

2.2. Peptides designing for potential peptide vaccine:

The consensus sequences of all the four serotypes (DENV1-DENV4) were taken up in CLC workbench software. These consensus sequences were aligned in the CLC workbench to develop the global consensus sequence. The consensus sequence was used to study variations in different motifs and domains of the DENV NS5 region. Short peptides from the highly conserved regions of the DENV NS5 protein were selected from the consensus sequence analysis; these peptides are the best targets to be tested as a potential peptide vaccine.

4. Conclusions

Our study suggests that there are certain stretches of amino acids, which take part in binding with divalent cat-ions, RNA Synthesis, ATPase binding, Nuclear Localization, Binding NS5 with NS3 domain and viral replication are highly conserved; and can be used as a potential target for the development of antiviral agents. Pharmacological blockage of cavity B could potentially lead to suppression of initiation of viral RNA synthesis and/or inhibition of NS3/NS5 interaction [19].

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Author Contributions

Both authors contribute equally.

Conflicts of Interest

The authors declare no conflict of interest.

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