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Design of Epitope- based vaccine for Dengue virus using Immunoinformatic approach

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ABSTRACT

Dengue, a flavivirus, transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes, are a reason for incredible worry to public health in India. It is the quickest developing mosquito-borne viral infection and is common in India. Dengue fever and dengue hemorrhagic fever are vital arthropod-borne viral diseases. Vomiting, fever, thrombocytopenia and leukopoenia were the normal highlights for clinical presentation of Dengue fever. Consistently, a huge number of people are affected and contribute to the burden of health care. In this work, Vaxijen tool was used to predict antigenic proteins and Propred1 was used to identify the epitopes binding to MHC class I molecules. The 3D structures of alleles were generated using Modeller or Crystal structures. Structures of predicted epitopes were generated by PepstrMod server. The selected epitopes were further evaluated for binding against HLA class I molecules by docking method. Two epitopes KRGLRTLIL and VRPTFAAGL bind to HLA-B*2705 allele was found to be the most potential T cell epitopes. Binding affinity of epitopes KRGLRTLIL and VRPTFAAGL with HLA-B*2705 allele were found to be -5.1 kcal/mol and -4.24 kcal/mol.

Keywords: Dengue; epidemiology; MHC class I Alleles; T-cell epitope; Molecular docking

1. INTRODUCTION

Dengue is an infectious disease caused by any of the four dengue virus serotypes: DENV1, DENV2, DENV3 and DENV4. It is a mosquito-borne disease and is primarily transmitted to people by the female Aedes mosquito. Infection with Dengue Virus brings about

fluctuating degrees of pathological conditions, extending from mild asymptomatic dengue fever (DF) to serious dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which may turn very fatal [1]. Dengue virus genome consists of 10,696 nucleotides of which 10,173 nucleotides encodes for the single open reading frame which relates to 3,391 amino acids [2]. The disease is mainly found in tropical and subtropical regions, putting about 33% of the human population, around the world, in danger of infection [3, 4]. An dramatic overall development of the Dengue infection has happened because of fast urbanization, increment in worldwide travel, absence of viable mosquito control estimates utilize and globalization of world [5]. As per the World Health Organization report, 397 million new cases happen every year around the world [6]. A sum of 128 nations in Asia, Pacific, America, Africa, and the Caribbean stay influenced by dengue fever [7]. The South-East Asia Region of WHO includes 10 nations, in particular, Bangladesh, Bhutan, India, Indonesia, Democratic People's Republic of Korea, Maldives, Myanmar, Nepal, Sri Lanka and Thailand, with an aggregate population of 1.45 billion [8].

In India, National vector born disease control programmed directorate general of health services, Ministry of health and welfare announced that Dengue cases and deaths in the nation in 2013 cases 75808 deaths 193, in 2014 cases 40571 deaths 137, in 2015 99913 cases 220 deaths, in 2016 cases 129166 deaths 245, in 2017 cases 36635 deaths 58 (http://nvbdcp.gov.in). Dengue cases in India have been expanded by 5 fold in five years [9].

2. MATERIALS & METHODS

2.1 Sequence retrieval and selecting the novel antigens

The amino acid sequences of the Dengue virus were retrieved from protein database of NCBI. The screening of whole genome was performed by Vaxign Software to analyse the potential antigens in designing a vaccine [10]. Vaxign is the most precise programming that predicts vaccine targets dependent on a few parameters. The adhesion properties of the host are fundamental for bacterial colonization and speak to the potential focuses for the vaccine advancement. The cut off value is 0.51, thus the antigens showing value >=0.51 are adhesins and were selected as novel antigens.

2.2 Antigenicity and allergecity prediction of selected antigens

The antigenicity of the filtered proteins were analysed by Vaxijen server [11]. Allergenicity of the proteins was predicted by using Allergen FP tool and non allergens target proteins will be considered. Proteins candidates having allergic properties have been excluded from our research.

2.3 Prediction of T cell Epitope for MHC Class I Alleles

T-cell epitopes induces the immune response, which play an important role in vaccine designing. Identification of the T cell epitopes binding with higher affinity with HLA class I alleles were examined by using Propred I tool [12]. It predicts the potential epitope binding to MHC Class-I HLA alleles supertypes A and B, can be recognized by $CD8^+$ T-

cells. The threshold for the analysis was set at 4%. The most crucial step involve in the development of an effective T cell peptide-based vaccine is the selection of a good epitopes that induces the best immune response. High scorer epitopes were shortlisted for the toxicity prediction. To check the toxicity of the peptides Toxin Pred was used, toxic peptides cannot be manipulated in vaccine designing.

2.4 Structure base modeling

The tertiary structure of the potential epitopes was modeled by using PEPstrMOD, a server that predicts the three dimensional structures of the small peptide [13] and their corresponding HLA alleles by Modeller 9.18 software. All the HLA sequences were taken from IPD-IMGT/HLA Database [14].

2.5 Molecular docking

The interaction between the epitope and MHC Class I alleles were done by molecular docking tool AutoDock 4.2 [15]. Docking is mainly used for the further verification of peptides that bind to MHC class I molecules. Autodock uses a scoring function to calculate the free energy of epitope with corresponding HLA allele. By default, Autodock tool generates 10 docked complexes that were ranked based on binding energy, torsion energy, geometry and electrostatic energy. The best outputs were finalized based on lower binding affinity. During the docking procedure a Lamarckian Genetic Algorithm (LGA) were used for flexible peptide rigid allele docking calculation.

2.6 Protein antigenicity determination

To predict the protective antigens as vaccines, the sequences were then analyzed with VaxiJen [11] with default parameters to find out antigenicity.

3. RESULTS & DISCUSSION

Despite of the clear evidence of the dengue outbreaks in the recent years, till date there is no potential vaccine that provides protection against it. Here, an attempt is made to design an epitope based vaccine which could be tested for wet lab analysis for their ability to induce an effective immune response. Dengue virus proteins were subjected to Vaxijen tool which identifies the best antigenic proteins, which has the ability to trigger the desired immune response on the basis of threshold value. To check the allergenicity of the selected proteins, AllergenFP algorithm prediction method was performed. Proteins which fulfill the criteria of Antigen as well as non allergen were selected as immunogen candidates to carry out the peptide based vaccine design (table 1).

Table 1: Proteins analysed by AllergenFp and Vaxijen server

Protein Name	**Accession No.**	**Allergen FP**	**Vaxijen**
Poly	JQ955624	Allergen	Antigen

2k	JQ955624	Non-Allergen	Non-Antigen
NS5	JQ955624	Non-Allergen	Antigen
E	JQ955624	Non-Allergen	Antigen
prM	JQ955624	Non-Allergen	Antigen
NS2a	JQ955624	Non-Allergen	Antigen
NS2b	JQ955624	Non-Allergen	Antigen
NS4b	JQ955624	Non-Allergen	Antigen
NS3	JQ955624	Non-Allergen	Antigen
Anchored capsid	JQ955624	Non-Allergen	Antigen
C	JQ955624	Non-Allergen	Antigen
NS1	JQ955624	Non-Allergen	Antigen

Peptides binding to MHC class I molecules have proven to be very useful in triggering an adaptive immune response, therefore characterization of immunogenic T cell epitopes by computational screening methods from a proteome of Dengue virus. Screening of MHC class I restricted epitopes have been done by using immunoinformatics tools. With the help of Vaxign server [16], the complete proteome of Dengue virus strain was filtered for immunogenic proteins. It works on the following parameters: adhesion probability, subcellular location prediction, transmembrane domain prediction and sequence similarity to host proteome which results in the identification of proteins at a threshold value of 0.51 (table 2).

Table 2: Screening of shortlisted protein by Propred I for identifying the most promiscuous epitopes binding to MHC class I allele and further epitopes were presented to vaxign server at threshold of >0.51.

Protein Name	**Accession No.**	**Start Position**	**Peptides /epitope**	**Allele**	**Real Score (**Propred I**)**	**Vaxign score**
NS5	JQ955624	476	YMWLGARFL	HLA-A*0201	4089.38287	0.4460
		511	GLHKLGYIL	HLA-A*0201	1930.068	
		481	ARFLEFEAL	HLA-B*2705	10000	
		769	RRDLRLAAN	HLA-B*2705	10000	
		476	YMWLGARFL	HLA-B*2705	10000	
		29	KRSGIQEVD	HLA-B*2705	3000	
E	JQ955624	470	SRSTSLSVS	HLA-B*2705	9000	0.6725
		33	TMAKNKPTL	HLA-B*2705	3000	
		467	GMNSRSTSL	HLA-	2000	

				B*2705		
prM	JQ955624	150	QRVLIFILL	HLA-A2	2811.68073	0.5826
		150	QRVLIFILL	HLA-B*2705	6000	
		15	SRQEKGKSL	HLA-B*2705	2000	
		33	MCTLMAMDL	HLA-B*5102	2420	
NS2a	JQ955624	83	VRPTFAAGL	HLA-B*2705	2000	0.7851
		93	LRKLTSKEL	HLA-B*2705	2000	
NS2b	JQ955624	390	KNEEEEQTL	HLA-B*2705	3000	0.6941
NS4b	JQ955624	213	KRGLRTLIL	HLA-B*2705	30000	0.6480
		426	RRCMKPVIL	HLA-B*2705	15000	
		538	RRGDLPVWL	HLA-B*2705	6000	
		208	VREAIKRGL	HLA-B*2705	6000	
NS3	JQ955624	213	KRGLRTLIL	HLA-B*2705	30000	0.5745
		426	RRCMKPVIL	HLA-B*2705	15000	
		538	RRGDLPVWL	HLA-B*2705	6000	
		208	VREAIKRGL	HLA-B*2705	6000	
Anchored capsid	JQ955624	40	GRGPLKLFM	HLA-B*2705	2000	0.3792
		84	FRKEIGRML	HLA-B*2705	2000	
		27	QQLTKRFSL	HLA-B*2705	1800	
		38	LQGRGPLKL	HLA-B*2705	1800	
C	JQ955624	40	GRGPLKLFM	HLA-B*2705	2000	0.3593
		84	FRKEIGRML	HLA-B*2705	2000	
NS1	JQ955624	34	FQPESPSKL	HLA-B*2705	2000	0.6029
		262	TQTAGPWHL	HLA-B*2705	2000	
		116	KTWGKAKML	HLA-B*2705	2000	

		78	ILSENEVKL	HLA-B*2705	2000	

Filter epitopes were subjected selected for the toxicity prediction. Toxin Pred method was used to predict the toxicity of peptides/epitopes (table 3).

Table 3: Toxicity prediction of the peptides by Toxin Pred

Peptide Sequence	**SVM Score**	**Prediction**	**Hydrophob-icity**	**Hydropathi-city**	**Hydroph-ilicity**	**Charge**	**Mol. Wt**
FQPESPSKL	-1.24	Non-Toxin	-0.21	-1.01	0.28	0.00	1032.28
GMNSRSTSL	-0.73	Non-Toxin	-0.27	-0.64	0.07	1.00	952.17
ILSENEVKL	-1.08	Non-Toxin	-0.10	0.12	0.29	-1.00	1044.35
KNEEEEQTL	-0.89	Non-Toxin	-0.51	-2.42	1.47	-3.00	1119.28
KRGLRTLIL	-1.21	Non-Toxin	-0.26	0.21	0.16	3.00	1069.50
KTWGKAKML	-0.40	Non-Toxin	-0.21	-0.69	0.18	3.00	1062.47
LRKLTSKEL	-1.08	Non-Toxin	-0.38	-0.66	0.72	2.00	1087.46
QRVLIFILL	-1.42	Non-Toxin	0.19	2.16	-1.09	1.00	1114.59
RRCMKPVIL	-0.89	Non-Toxin	-0.29	0.27	0.18	3.00	1115.59
RRGDLPVWL	-1.09	Non-Toxin	-0.24	-0.40	0.06	1.00	1111.44
SRSTSLSVS	-1.14	Non-Toxin	-0.24	-0.13	0.09	1.00	923.10
SRQEKGKSL	-0.65	Non-Toxin	-0.57	-1.94	1.22	2.00	1032.29
TMAKNKPTL	-0.36	Non-Toxin	-0.25	-0.76	0.20	2.00	1003.35
TQTAGPWHL	-1.00	Non-Toxin	-0.02	-0.60	-0.76	0.50	1010.25
VREAIKRGL	-0.66	Non-Toxin	-0.34	-0.28	0.71	2.00	1041.39
VRPTFAAGL	-0.97	Non-Toxin	0.04	0.80	-0.47	1.00	1.00
MCTLMAMDL	-0.94	Non-Toxin	0.14	1.49	-0.71	-1.0	1028.45
NPITLTAAL	-1.10	non-toxin	0.14	1.02	1.02	0.00	913.21

The 3D structure prediction of the epitope with their corresponding HLA alleles was performed by using Modeller or Crystal structure (table 4).

Table 4: Template structures were retrieved from protein data bank for 3D structures modeling of alleles by modeller.

Sl. No	Allele	Template (PDB ID)	Crystal Structure / Model
1	HLA-B*2705	2BSR	Crystal structure
2	HLA-A*201	4 WUU	Crystal structure
3	HLA-A2	4 WUU	Crystal structure
4	HLA-B*5102	1E27	Model

Using Autodock 4.2, the molecular docking between the identified epitopes with their respective alleles was performed (table 5).

Table 5: Best identified epitopes binding with HLA alleles by using autodock4.2.

Peptide	Allele	BE	IME	IE	TorE	VdwE	EE
FQPESPSKL	HLA-B*2705	-0.98	-10.23	-7.65	9.25	-8.8	-1.43
GMNSRSTSL	HLA-B*2705	0.52	-9.03	-7.45	9.55	-6.87	-2.16
ILSENEVKL	HLA-B*2705	2.29	-8.74	-7.77	11.04	-8.71	-0.04
KNEEEEQTL	HLA-B*2705	3.16	-9.07	-7.62	12.23	-7.98	-1.09
KRGLRTLIL	HLA-B*2705	-5.1	-16.44	-5.31	11.34	-12.69	-3.75
KTWGKAKML	HLA-B*2705	5.27	-5.77	-8.04	11.04	-5.97	0.2
LRKLTSKEL	HLA-B*2705	0.96	-10.97	-7.8	11.93	--7.07	-3.89
QRVLIFILL	HLA-B*2705	0.66	-10.38	-10.71	11.04	-8.72	-1.66
RRCMKPVIL	HLA-B*2705	-1.72	-12.75	-6.98	11.04	-11.28	-1.48
RRGDLPVWL	HLA-B*2705	-2.92	-12.77	-9.91	9.84	-9.88	-2.89
SRSTSLSVS	HLA-B*2705	-2.17	-11.12	-4.93	8.95	-9.57	-1.55
SRQEKGKSL	HLA-B*2705	0.45	-11.18	-8.15	11.63	-10.21	-0.97
TMAKNKPTL	HLA-	-0.77	-10.62	-7.08	9.84	-8.98	-1.64

	B*2705						
TQTAGPWHL	HLA-B*2705	-1.88	-9.94	-7.55	8.05	-8.54	-1.4
VREAIKRGL	HLA-B*2705	-1.88	-12.92	-6.71	11.04	-10.58	-2.34
VRPTFAAGL	HLA-B*2705	-4.24	-11.99	-8.78	7.76	-10.29	-1.7
MCTLMAMDL	HLA-B*5102	1.32	-8.82	-6.01	10.14	-8.62	-0.21

BE: Binding Energy; IME: Intermolecular Energy; IE: Internal Energy; TorE: Torsional Energy; VdwE: Vdw-lbDesolv Energy; EE: Electrostatic Energy.

The binding affinities of the best predicted peptide KRGLRTLIL and VRPTFAAGL to the HLA-B*2705 were observed to be -5.1 kcal/mol and -4.24 kcal/mol, respectively. Simillar work was performed by Kamthania and Sharma on Nipah virsus [17, 18].

4. CONCLUSION

Peptide vaccines are a safe and economical technology compared to traditional vaccines made of dead or attenuated pathogens, inactivated toxins, and recombinant subunits. The result obtained from our report clearly shows that the screening of the complete proteome through computational method led to the identification of 2 promiscuous epitopes has the capability to induce a immune response. The two best epitopes having good binding scores can be constructed as a potential vaccine candidates.These epitopes KRGLRTLIL and VRPTFAAGL can be suggested for further experimental analysis for the utility of vaccine.

5. REFERENCES

1. Murphy BR, Whitehead SS. Immune response to dengue virus and prospects for a vaccine. Annual Review of Immunology, 2011; 29:587–619.

2. Osatomi K, Sumiyoshi H. Complete nucleotide sequence of dengue type 3 virus genome RNA. Virology, 1990; 176:643–647.

3. Malavige GN, Fernando S, Fernando DJ, Seneviratne SL. Dengue viral infections. Postgraduate Medical Journal, 2004; 80(948):588–601.

4. Chen R, Vasilakis N. Dengue-Quo Tu et Quo Vadis?. Viruses, 2011; 3(9):1562–1608.

5. Gubler DJ. Dengue/dengue haemorrhagic fever: history and current status. Novartis Foundation Symposium, 2006; 277:3–16.

6. World Health Organization, Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control, WHO, Geneva, Switzerland, 2013.

7. Outbreak Notice – Dengue in Madeira, Portugal [webpage on the Internet] Atlanta, GA: Centers for Disease Control and Prevention (CDC); 2013. [cited March 9, 2013]. Available from: http://wwwnc.cdc.gov/travel/notices/outbreak-notice/dengue-madeira-portugal.htm.

8. World Health Organization. Global Strategy for Dengue Prevention and Control, 2012–2020, Geneva (2017).

9. Brady OJ, Gething PW, Bhatt S et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Neglected Tropical Diseases, 2012: 6(8), Article ID e1760.

10. Yongqun H, Xiang Z, Harry, Mobley LT. Journal of Biomedicine and Biotechnology, 2010; Article ID 297505.

11. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics, 2007; 8:4.

12. Singh H, Raghava GPS. Propred I: prediction of HLA class-I binding sites. Bioinformatics, 2003; 19:1009-1014.

13. Kaur H, Garg A, Raghava GPS. PEPstr: A de novo method for tertiary structure prediction of small bioactive peptides. Protein Pept Lett., 2007; 14:626-30.

14. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SGE. The IPD and IMGT/HLA database: allele variant databases Nucleic Acids Res., 2015; 43 (D1): D423-D431

15. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry, 2009 16: 2785-91.

16. Xiang Z, He Y. Vaxign: a web-based vaccine target design program for reverse vaccinology. Procedia in Vaccinology,2009, 1(1): 23-29.

17. Kamthania M, Sharma DK. Screening and structure-based modeling of T-cell epitopes of Nipah virus proteome: an immunoinformatic approach for designing peptide-based vaccine.3 Biotech, 2015, 5(6):877–882.

18. Kamthania M, Sharma DK. Epitope-based peptides prediction from proteome of nipah virus. International Journal of Peptide Research and Therapeutics, 2016, 22 (4), 465-470.