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Original Article

Computational Approaches To Design Multi Epitope-Based Vaccine Designing of Dengue virus -2 Enveloped Protein For Dengue Virus

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ABSTRACT

Dengue Fever (DF) is a viral disease transmitted by mosquitoes and is a global concern. A successful vaccine for dengue should induce both neutralizing antibodies and cell-mediated immunity. However, no vaccine currently exists for DF. A multi-epitope vaccine offers a promising strategy for preventing such infections. **Objective:** To create a dengue virus-2 strain multi-epitope vaccine that is safe, non-allergic, and stimulates a strong immune response. **Methods:** Leveraging in silico tools, we retrieved and analyzed Dengue virus-2 protein sequences, determining antigenicity using VaxiJen version 2.0 and assessing allergenicity using AllerTop. T-cell epitopes were identified via Immune Epitope Database (IEBD) server for Major Histocompatibility Complex -I (MHC-I) and Major Histocompatibility Complex -II (MHC-II) binding and B-cell epitopes were anticipated through IEBD Linear Epitope Prediction Tool v2.0. Analysis of population coverage estimated the prevalence of MHC alleles interacting with the identified epitopes. A multi-epitope vaccine construct integrated adjuvants, universal linkers, and epitopes, evaluated for physicochemical properties, toxicity, secondary, and tertiary structures. **Results:** Antigenicity analysis identified highly antigenic Dengue virus-2 protein sequences with low allergenicity. T-cell epitopes revealed multiple epitopes with diverse MHC-I and MHC-II affinity, encompassing conserved regions for potential universal vaccine development. Nine non-toxic, non-allergenic B-cell epitopes were identified. Population coverage analysis demonstrated over 71% prevalence of MHCs binding to identified epitopes across diverse populations. Physicochemical assessments revealed favorable characteristics, including immunogenicity and stability. Tertiary structure prediction illustrated the vaccine's 3D arrangement, validated through Ramachandran plots, exhibiting high-quality protein structure. **Conclusions:** This multieiptope based vaccine is more immunogenic but further in-vitro and in-vivo study is required for its clinical use.

INTRODUCTION

A significant human infection caused by viruses spread by mosquitoes, dengue (DEN) affects many subtropical and tropical countries in the Caribbean, Africa, the Americas, the Pacific, and Asia. The Flaviviridae family of flaviviruses includes the dengue virus [1]. An estimated 50–100,000,000 cases of dengue fever have been reported globally. The disease spread to over 125 countries, including those in the Americas, the Eastern Mediterranean, South Asia, East Asia, Central America, and Africa [2]. The single-stranded RNA-positive Dengue virus (DENV), a member of the Flaviviridae family, has a genome of roughly 10.7 kb in size. DEN-1, DEN-2, DEN-3, and DEN-4 are the names of the four distinct DENV serotypes that have been identified by researchers. It's interesting to note that although these serotypes have similar genetic backgrounds, their antigenicity varies [3]. One polyprotein & seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) makes the DENV genome. Viral RNA

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is enclosed in the capsid protein, called protein C, which is essential for virus budding, fusion of membrane, and nucleocapsid assembly [4]. To stop the initial assembly of envelope proteins, there is another viral protein called peptide PR attached to the envelope protein E in the trans-Golgi at pH 6.0 [5]. The prM protein is essential for hiding and deactivating the envelope protein E fusing peptide, as it is the only mature viral protein in the network of trans-Golgi [6]. The envelope protein E is made up of three domains: envelope domain-1, which is in charge of structural organization; envelope domain-2, which is in charge of host cell fusion; and envelope domain-3, which is in charge of viral budding. The envelope protein E comprises a transmembrane region and an ectodomain. In addition to transporting viral genetic information into host cells, the envelope protein acts as an antigen, assisting human immune cells in recognizing and killing the virus. This envelope protein is an essential element in the production of vaccines and serves as an ideal target for neutralizing and suppressing viruses [7]. Dengue fever requires careful monitoring by physicians and appropriate medical attention to be treated; it cannot be prevented or cured with specific medications. Vaccines, on the other hand, not only protect against infections but also support the cure of vector-borne viral infections [1,8]. Although the first dengue vaccination was created in 1929, many vaccines have been investigated in clinical trials but none have shown effectiveness in combating the DENV [9]. The development of Sanofi Pasteur's live attenuated tetravalent dengue vaccine, Dengvaxia, is illustrated in a recent study. It was initially approved for use in December 2015 in Mexico to treat endemic area residents between the ages of 9 and 45. However, there is a significant chance that the attenuated vaccination strain will revert to a more deadly virus strain because of its recombinant live attenuated nature. Furthermore, clinical investigations have shown that Dengvaxia is not effective against the strain of DENV-2 [10]. As an alternative to the traditional laboratory-based vaccine production method, immunoinformatics provides an in-silico method to develop a dependable and multi-epitope vaccine in a shorter amount of time and at a lower cost [1,11]. Because there are different DENV serotypes, vaccination against the virus has grown more difficult. As mentioned, there is only one vaccine that has been approved, called Dengvaxia, however, it is ineffective against all DENV serotypes [12]. As a result, there are currently no successful therapies or preventative measures for this illness. To lessen the dengue epidemic, effective patient's management techniques and alternative mosquito (vector) control have been implemented. It is therefore extremely time-consuming to find a novel vaccine candidate that is effective on all dengue serotypes [13].

The primary aim in this research was to design an epitope-based vaccine targeting the DENV-2 enveloped protein, focusing on eliciting an enhanced and broad-spectrum immune response against dengue virus. By employing in-silico techniques and leveraging epitope prediction, both T cell epitopes and B cell epitopes will be found in order to build a novel peptide-based vaccine. The study intends to assess the immunological potency and safety profile of this proposed vaccine, aiming for a more cost-effective and expedited production process compared to conventional vaccine development methods. Ultimately, the goal was to contribute to the development of a comprehensive and efficacious vaccine that can confer robust protection against multiple DENV serotypes, addressing the pressing necessity of an efficient and universal dengue vaccine. Further exploration and validation of epitope-based vaccine's efficacy through preclinical and clinical trials could pave the way for its potential application as a comprehensive and universally effective solution against various DENV serotypes, addressing the global health burden of dengue fever.

METHODS

Sequence Retrieval, Antigenicity, and Allergenicity Prediction

The UniProtKB Exation number was P18356. With a threshold value of 0.4 for DENV-2 protein sequences, the representative DENV-2 protein FASTA sequences were examined for antigenicity analysis utilizing VaxiJen v2.0 online server 15 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). The tool predicted antigenicity amount for a protein sequence. Identified a potential vaccination target; the most antigenic sequences were examined. Using the AllerTop web server (https://www.ddg-pharmfac.net/AllerTOP/index.html), the allergenicity of the DENV-2 protein was evaluated. Protein from DENV-2 was non-allergic.

T-cell epitope Identification

The most antigenic DENV-2 proteins were subjected to T-cell epitope identification by *IEBD* (http://tools.iedb.org/immunogenicity/) *MHC-I*. On the IEBD server, the conversancy evaluation was carried out [14]. The pre-configured parameters were employed to predict immunogenicity, and peptides with positive values were picked for further research [15]. The sequence of the peptides was maintained throughout all identified variation sequences to develop broad-spectrum peptide-based vaccines [16]. Table 1 and table 2 illustrated MHC-I and MHC-II immunogeneicity analysis of non-alergic and antigenic T-cell epitopes.

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Table 1: MHC-I binding prediction result

Allele	Peptide	iC50	Antigenicity
HLA-B*15:01	IMLIPTVMAF	3.94	0.7284
HLA-B*15:01	MLIPTVMAF	4.07	0.5139
HLA-B*15:01	IQMSSGNLLF	5.38	0.6255
HLA-A*02:03	LMAMDLGEL	7.11	1.064
HLA-A*02:06	MIIMLIPTV	7.21	0.4429

Table 2: MHC-II binding prediction result

Allele	Peptide	iC50	Antigenicity
HLA-DRB1*01:01	SAGMIIMLIPTVMAF	75.9	0.4727
HLA-DRB1*08:02	AGMIIMLIPTVMAFH	2.3	0.5277
HLA-DRB1*11:01	LRHPGFTIMAAILAY	23.8	0.4302
HLA-DRB1*08:02	GMIIMLIPTVMAFHL	2.3	0.6083
HLA-DRB1*11:01	RHPGFTIMAAILAYT	19.6	0.6979

Epitopes with iC50 values under 100 had stronger affinity for Human leukocyte antigen (HLA), whereas those with iC50 values under 500 had moderate affinity. In this research, we looked at the most conserved epitopes to determine their relative HLA binding at an iC50 value of less than 100. Then, utilized the Vaxigen2.0 server to profile a chosen T-cell epitope's antigenicity and AllerTop to assess its allergenicity

Identification of B cell epitope

For the prediction of B-cell epitopes, only three epitopes were analyzed. SGEEHAVGNDTGKHG, FLDLPLPWLPGADTQ, and WDFGSLGG by using IEBD Linear Epitope Prediction Tool v2.0.

Population coverage prediction

The human population coverage was determined using the potential epitopes, which interacted with different major histocompatibility complex alleles for all putative epitopes. The MHC alleles that bind the chosen epitopes were predicted to be present in a proportion of the global population using an analysis tool for population coverage by IEDB. For the entire world population, the overall coverage of the population for our discovered epitopes was determined to be 71.28%. This tool determines the mean quantity of epitope pairings that the population of various geographic distributions had agreed to.

Multi epitope based Vaccine Construction

The adjuvant Hp91 protein was employed to bind to the vaccine's N terminal. PEAK, GPGPG, and AAY were the three major types of universal linkers that were utilized. Adjuvant was first added, followed by a linker, MHC-I epitopes, a linker again, MHC-II epitopes, a linker, a B-cell epitope, and finally 6x his tag protein. The following phase involved analyzing antigenicity using oxygen, allergenicity using allerTOP, and toxicity using ToxinPred.

Evaluation of physicochemical properties of multi-epitope vaccine constructs

The ExPASy Protparam tool (https://web.expasy.org/cgi-bin/protparam/protparam) was used to analyze the physiochemical data to assess the physiochemical properties of multiple epitopes in the complete vaccine build. The calculated parameters included the atomic composition, estimated half-life, theoretical pI, extinction coefficient, molecular weight, amino acid composition, instability index, and the overall average of hydropathicity (GRAVY).

Evaluation of Toxicity of Multi Epitope Vaccine Construct

In order to figure out how the vaccination interacted with the host body habitat [17], TonxinPred was used (https://webs.iiitd.edu.in/raghava/toxinpred/). An SVM model is used to classify molecules into two categories: toxic or nontoxic. By inserting the FASTA-formatted sequence of those identified epitopes in a search query, only nontoxic peptides were chosen [18]. All of the peptides are non-toxic.

Secondary and Tertiary structure prediction

PsiPreD (http://bioinf.cs.ucl.ac.uk/psipred/) predicts secondary structure, in addition PHYRE 2(http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) predicts tertiary structure after the PDB file has been predicted. then use Chimaera to visualise

Ramachandran Plot

Select the PDB file downloaded from Galaxy Refine and uploaded it to this server. The Ramachandran plot was then evaluated to determine which residues were present in the favorable and unfavorable regions. In this step, the protein structure was validated.

RESULTS

Prediction of Threshold value, FASTA sequence, and interaction of epitopes with MHC alleles

With a threshold value of 0.6603 for protein sequences, DENV-2 protein FASTA sequences were assessed aimed at antigenicity predictions utilizing the internet serve VaxiJen v2.0. This server predicted the amount of antigenicity for a protein sequence. The most antigenic sequences were examined to identify a potential vaccination target. The AllerTop web server was utilized to assess the allergenicity of the DENV-2 protein.Protein from DENV-2 was not an allergen. Coverage of the population of humans to all of the putative epitopes was calculated using the potential epitopes that interacted through different Major Histocompatibility Complex (MHC) alleles. As shown in figure 1.

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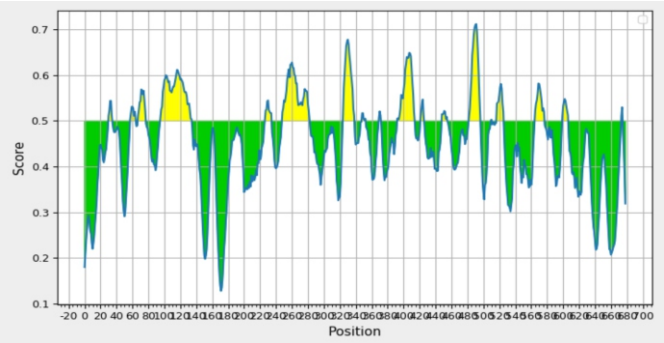

Figure 1: A 0.05 score shows a borderline yellow color, indicating the epitopes of B-cell in protein. The epitope will not be considered if the score is less than 0.05

Prediction of population coverage:

The coverage of the population is analyzed by the tool IEDB for estimating what proportion of the global population possesses the MHC alleles necessary to bind the chosen epitopes. The determined epitope's combined population coverage was found to be 71.28% of the global population respectively, as shown in figure 2.

Poputation: World

MHC class	Coverage	Average hit	PC90
combined	71.28%	3.14	0.35

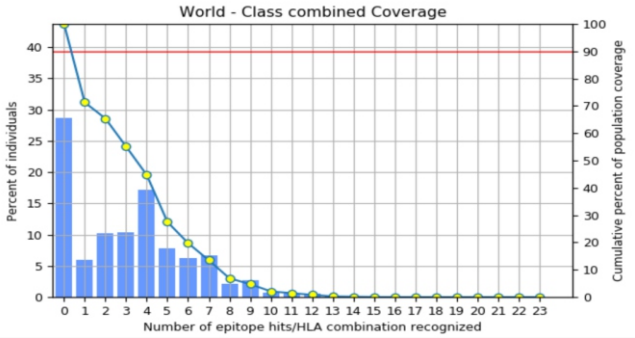

Figure 2: This graph shows the combined coverage of Major histocompatibility complex class I & class II. The percentage of combined coverage in the overall world is 71.28%

Physiochemical properties assessment

Utilizing the ExPASy ProtParam server(https://web.expasy.org/protparam/), the physiochemical characteristics of the vaccine construct were assessed for 7 parameters. The vaccine protein's molecular weight of 20108.27 kDa was discovered, which will increase the vaccine construct's antigenicity23. Its slightly acidic nature was indicated by the theoretical pI of 6.01, and there were a total of 24 positive and negative charge residues, respectively. In vitro, the estimated half-life for mammalian reticulocytes was 3.5 hours; in vivo, it was 10 minutes and longer for yeast and E. coli. Assuming that all cysteine residues decrease, the coefficient of extinction in 280 nm calculated in water has been determined to be 20970 M-1 cm-1. The vaccine construct is stable, as evidenced by the instability index score of 22.88. Grand average of hydropathicity (GRAVY) was 0.852 as well as the aliphatic index was 109.63, accordingly Because a higher aliphatic index value corresponds to greater thermo stability24, the projected amount of aliphatic index indicates the thermostable nature of the designed subunit vaccine. Conversely, the hydrophilic character of vaccine24 is represented by a negative GRAVY value for its input subunit vaccine.

Toxicity

The averaged epitope/HLA combinations accepted from the population across various geographic circulations are calculated using this tool. The vaccine's toxicity design was evaluated utilizing ToxinPred program. The peptides were all non-toxic, as shown in figure 3.

Query Peptides

Peptide ID	Peptide Sequence	SVM Score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt
	HPEAAAKGPGPGAAVIMLIPTVMAFMLIPTV	-0.86	Non-Toxin	0.16	0.85	-0.61	0.50	3166.33
	MAFIQMSSGNLLFLMAMDLGELMIIMLIPTV	-1.49	Non-Toxin	0.22	1.51	-0.86	-2.00	3445.84
	SAGMIIMLIPTVMAFAGMIIMLIPTVMAFHL	-0.88	Non-Toxin	0.32	1.99	-1.13	0.50	3335.81
	RHPGFTIMAAILAYGMIIMLIPTVMAFHLRH	-0.66	Non-Toxin	0.13	1.07	-0.86	3.50	3523.88
	PGFTIMAAILAYTSGEEHAVGNDTGKHGFLD	-1.32	Non-Toxin	0.01	-0.00	-0.19	-2.00	3221.03
	LPLPWLPGADTQGSNWIQKWDFGSLGGHIST	-0.95	Non-Toxin	0.00	-0.29	-0.48	-0.50	3380.27
	AG	-0.80	Non-Toxin	0.21	0.70	-0.25	0.00	146.16

1/1 50

Figure 3: Toxicity evaluated by Toxin Pred serve and evaluated the score of the peptide sequence, hydrophobicity, hydrophilicity, and molecular weight

Prediction of Secondary structure of vaccination

The arrangement of amino acids and other molecules within an antigen responsible for triggering an immune response is known as the secondary structure of a vaccination. A vaccine antigen's secondary structure is crucial to how well it performs since it dictates the antigen's shape and, in turn, how well it can interact with immune system cells, antibodies, and T cells, as shown in Figure 4.

Prediction of 3D structure and Refine structure

A vaccine's three-dimensional arrangement of its component molecules, often proteins or protein fragments, is called its tertiary structure. It is essential to comprehend the tertiary structure because it affects the vaccine's overall design and how it interacts with the immune system to produce an immunological response. A vaccine antigen's efficacy depends on its tertiary structure. The antigen presents its active areas because of the protein's proper folding. As shown in figure 4.

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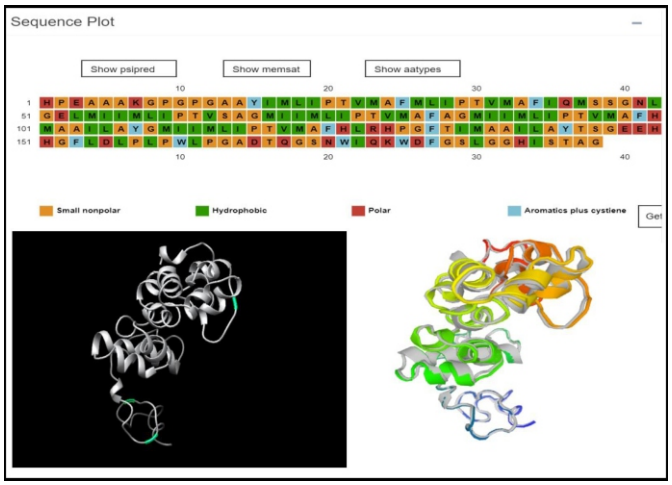

Figure 4: Secondary and tertiary structure (by chimera visualize tool) and Refine structure (by Galaxy WEB) of vaccine

Protein validation

Ramachandran plot for 3D structure generated from PROCHECK shows that it has 87.6% residues in most favored core regions, 9.8% in further permitted areas, and 1.3% in restricted areas. The model's quality parameter was examined through the ERRAT, which is around 91%, whereas a quality factor greater than 80.00 indicates an acceptable structure model.

Molecular Docking:

For molecular docking first we download the receptor from PDB (https://www.rcsb.org/) which is **2Z63** (The TV8 is a hybrid of homo sapiens TLR4 as well as hagfish VLRB61 crystal structure.).Then for molecular docking use ClusPro (https://cluspro.bu.edu/login.php). The clusPro show many results but the best one is the structure which is shown in the Figure 5 because it contain lowest energy -1031.8. visualized these structure by chimera and discovery studio docking.

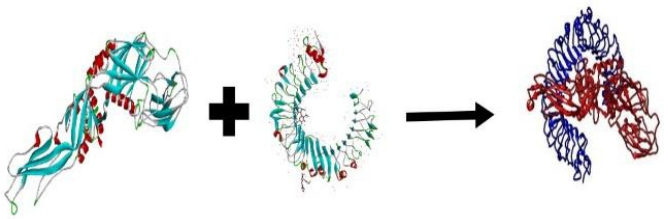

Figure 5: Molecular Docking between MHC1 and Multi-Epitope Vaccine Construct.

DISCUSSION

It is becoming more and more common to employ immunoinformatics techniques as the initial step in the development of effective vaccines against a variety of microorganisms, particularly viruses. Recently, methods guided by immunoinformatics have been used to design SARS-CoV-2 epitope-based subunits [19-26]. Additionally, T-cell epitope vaccine candidate for parasitic helminth infection and potential vaccine candidates against Theileria parasites were identified by Kar *et al* [27, 28]. Separate DENV serotypes (DENV-1–4) result in dengue fever, a virus that is spread by mosquitoes. These serotypes cross-react immunologically with one another. Approximately 96 million cases of DENV infection take place every year among the almost three billion individuals who are susceptible to the virus globally [29]. Because of antibody-dependent improvement, individuals who have had a primary infection are more likely to develop dengue hemorrhagic fever and dengue shock syndrome during a secondary infection [30,31]. Vaccination is the primary preventive measure to lower the disease's burden, as there is currently no specific treatment for dengue fever. Therefore, the goal of this study was to use computational methods to design novel multi-epitope-based DENV vaccine candidates that can stimulate immune responses in DENV-infected individuals [32]. In an antigenicity test, an epitope with a threshold value of 0.6603 is considered to be highly antigenic and likely to elicit an immune response. Although epitopes with a higher affinity for MHC are preferred, population coverage must also be taken into account because different people may have distinct MHC molecules. Regarding DENV-2, a possible target for vaccine development is the over 71% of the human population that carries MHCs with varying affinity towards the discovered epitopes [33].The toxicity and secondary structure of the reported epitopes should be considered in conjunction with their MHC affinity and antigenicity. It is more likely that epitopes with stable secondary structures and low toxicity will be appropriate for vaccine development since they can improve the vaccine's immunogenicity and stability. According to a study, the structural and non-structural proteins of the dengue virus include highly conserved epitopes that could be targets for a universal vaccine [34]. The IEBD-MHC data's yellow hue indicates that no more epitopes have a greater affinity for the Major Histocompatibility Complex in our study. Moreover, these antigenic viral components were used to predict the B and T-cell epitopes. Three antigenic, non-toxic, and non-allergenic epitopes were predicted for the B-cells, compared to ten antigenic and non-allergenic epitopes for each of the two MHC classes. The development of long-lasting immunity that can eradicate infected cells and circulating viruses is made possible by the CTL epitopes [35]. But the good thing is when we look at population coverage results, the affinity is diverse as more than 71% of the human population possesses these MHCs. Toxicity results are also crystal clear. contributing to saving time and money. Nonetheless, the next phase is to carry out in Immunoinformatics approaches are extremely useful to perform in silico studies and can guide laboratory

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experiments vitro immunological assays to validate the predicted vaccines, determine their immunogenicity, and further devise challenge-protection preclinical studies to eventually certify these approaches.

CONCLUSIONS

The conclusion of this epitope-based vaccine design study for DENV-2 illustrated a promising approach in leveraging computational tools and immunoinformatics to identify potential vaccine candidates. By targeting the enveloped protein of the dengue virus, this study had shown a comprehensive method in predicting antigenic epitopes for both T and B cell immune responses. This multiepitope based vaccine is more immunogenic as compared to other available vaccines. In vitro and in-vivo studies will be crucial to confirm the vaccine's ability to induce a robust immune response against DENV-2 while ensuring its safety and efficacy.

Authors Contribution

Conceptualization: HT,
Methodology: MM, MZ, ZB
Formal analysis: TA
Writing-review and editing: MM, TA, HMHA, NH, MA, HS, HM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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