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Developing a multiepitope vaccine against dengue virus in Bangladesh using immunoinformatics approach

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ABSTRACT

Dengue fever is a devastating mosquito-borne illness that has claimed the lives of countless people. The virus responsible for this disease is a member of the Flaviviridae family, which produces positive-stranded RNA. Dengue fever is an exquisite viral fever caused by the bite of an Aedes mosquito carrying one of four serotypes of dengue virus. This virus is transmitted via a vertical route utilizing a comprehensively unique system. Unfortunately, no effective vaccine has yet been developed to eradicate this disease. This study employed computational methods to design and propose a multi-epitope vaccine against dengue virus in Asia. This study utilized various immunoinformatics databases to predict potent epitopes on the envelope protein of the dengue virus using in silico methods. We identified a total of 14 epitopes from the target envelope protein by assessing their ability to induce both innate and acquired immunity through T- and B-lymphocyte-mediated responses. Because dengue virus is an RNA virus, epitope conservation was considered, and all selected epitopes were 100 percent conserved. The antigenicity of the final component of the multi-epitope vaccine was 0.7055. To improve the stability of the vaccine protein, disulfide engineering was performed in a region with high mobility. Additionally, codon adaptation and in silico cloning ensure that the proposed subunit vaccine will be expressed at a higher level in *E. coli*. In order to evaluate the binding free energy and stability of the combination, the vaccine protein and TLR-4 receptor were subjected to a molecular docking simulation. In order to establish active immunity against the dengue virus, the proposed in silico vaccine must be tested for safety and immunogenicity.

INTRODUCTION

Dengue is a serious disease transmitted by mosquitoes that has the potential to spread as a pandemic. Endemic dengue is a causative agent and it is a grave health warning for many developing tropical countries. They have been established in all tropical regions of the planet for more than six decades [1]. Southeast Asia is identified as the region with the highest prevalence of this disease in numerous tropical areas. The awe-inspiring hemorrhagic pattern of dengue fever has become the most dangerous cause of death in southeast Asia. This infection has also been documented in non-tropical regions of Asia, including East Asia and China [2]. Dengue occurs irregularly in Bangladesh, where the virus caused a widespread epidemic in 2000 and persisted until 1964. At that time, we discovered dengue in Bangladesh, where it was first identified, and identified factors beneficial to future dengue hemorrhagic fever epidemics [3]. Most likely, the outbreak started when a strain of the dengue virus came from a nearby country where it was common, and probably it was Thailand. In addition to the end of dichlorodiphenyltrichloroethane (DDT) spraying, the spread of disease was caused by weather, population, and lifestyle [4]. Even though it increased every other year, the highest number of cases was reported in 2002. Thus, we reduce the number of

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notifications that may be an artifact of the surveillance system [5]. Poll-based serological observation suggests that transmission of dengue is typical. Without intelligent interventions, future dengue risk will be exacerbated by unplanned urbanization, environmental decline, rising population mobility, and financial factors. Therefore, there is an urgent need to develop a vaccine for these high-risk regions. As part of this research, we need to use immunoinformatics to make a vaccine against the Asian-associated dengue virus that will work in Bangladesh. A flow chart discloses the full procedure of the study in Figure 1.

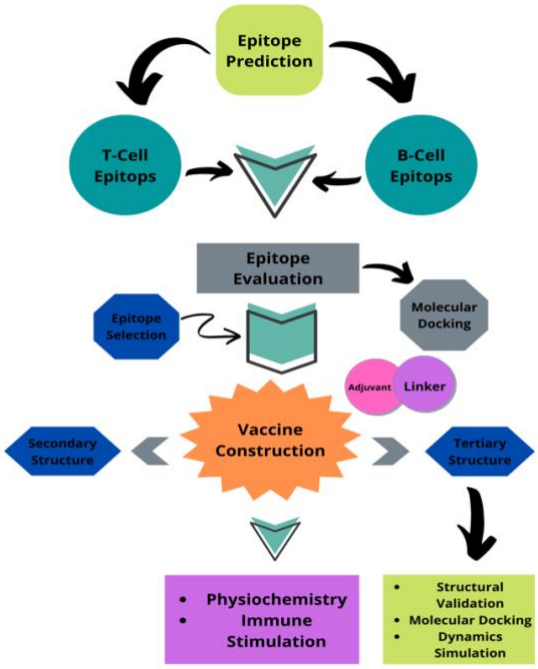

Figure 1. Schematic depiction of our workflow procedure.

METHODS AND MATERIALS

Proteome salvation and antigen extracts

We selected probable HCMV proteomes from the viprbrc website database in order to pick antigens [6]. On the HCMV surface membrane, spike proteins were deposited. They collaborated with this host-binding protein to enter the human genome [7]. We measured the HCMV spike protein for the multiepitope vaccine strategy with the precise link between glycoproteins and disease. First, we selected the protein sequence of the dengue virus, which had been downloaded as a fasta file. The ddg-pharmfac database website was used to analyze the collected antigens having a threshold value of 0.4 [8]. Lastly, the spike protein with the highest antigenic score was chosen for further investigation.

Forecast and evaluation of helper T-lymphocyte epitopes

Helper T-cells (HTLs) are an essential component of adaptive immunity that recognizes different antigens and initiates B and cytotoxic T-cells, resulting in the destruction of the pathogen. Furthermore, B-cell and T-cell epitopes were predicted from dengue virus, as well as SARS coronavirus, which became disease and could be pandemic. To determine the HTL epitopes, we utilized the IEDB's MHC class II essential allele prediction tool. The HTL epitopes were chosen using the Agreement method to support

a percentile level of fifty [7]. These epitopes underwent additional testing and demonstrated antigenicity using Vaxijen server version 2.0 [9].

Forecast and evaluation of cytotoxic T-lymphocyte epitopes

Cytotoxic T lymphocytes are able to kill phagocytes via this mechanism directly [10]. Therefore, we utilized the NetCTL v1.2 server [11]. The collected epitopes were tested once more using the Vaxijen v2.0 [10], Toxiprod [13], and Allerrtop v2.0 [9] servers. All parameters are left at their defaults for all forecasts. A prediction threshold of 0.75 was established for CTL epitope identification [13].

Forecast and evaluation of linear B Lymphocyte Epitopes

B cell epitopes are needed to safely give humoral or antibody medication [14]. For this purpose, the online portal iBCE-EL (http://www.thegleelab.org/iBCE-EL/) utilized this with default levels [15].

Modelling of multi-epitope vaccine

The vaccine was produced using the selected CTL epitope, HTL epitope, and LBL epitopes, a complete adjuvant, and the pertinent linkers [14; 16]. As an adjuvant for viral glycoprotein recognition, we used TLR4 agonist here [17; 18]. Therefore, 50S ribosomal protein (NCBI ID: P9WHE3) was valued as an adjuvant to enhance the immunogenicity of the candidate vaccine. The adjuvant was linked to the linker EAAAK. In contrast, the selected CTL was linked with (AAY) linkers, the HTL was linked with (GPGPG) linkers, and the LBL was linked with (KK) linkers [14; 16]. The AAY linker was utilized to affect protein equilibrium [19; 20]. The linkers effectively separate two epitopes to ensure that each epitope maintains its optimal function [13].

Physicochemical and immunological evaluation

The functional characteristics of the vaccine were predicted using the ProtParam database (http://web.expasy.org/protparam/) [21]. ProtParam is a program that calculates the various physical and chemical parameters of a protein sequence, such as its molecular weight, theoretical pI (isoelectric point), amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average hydropathicity (GRAVY). Again, we used Vaxijen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [22] to evaluate the vaccine's immune properties by measuring its MHC-1 immunogenicity [9; 11] using Allertop, Biosoland, and SOLpro [8].

Secondary construction forecast

The SOPMA server (https://npsa-prabi.ibcp.fr/NPSA/npsa seccons.html) and PSIPRED v4.0 server (http://bioinf.cs.ucl.ac.uk/index.php?id=779) identified the vaccine model's two-dimensional basic characteristics, such as alpha-helix and random coils, when given the vaccine model [23]. SOPMA produces a secondary structure prediction accuracy of greater than 80% [13].

Homology modelling, 3D construction clarification and validation

We uploaded the created vaccine to the I-TASSER server (https://zhanggroup.org/I-TASSER) [24] in order to construct the structure prognosis. Then, to breed vaccine composition, we refine the vaccine from the Galaxyweb server (https://galaxy.seoklab.org/) [25]. The complete structure was downloaded from the portal, and the chosen structure was subsequently named based on the highest RMSD rate and effectiveness number. Using the PyMOL v2.3.4 software, we could observe the refined and refined formation practiced imaginatively. The ProSA-web accessory and Procheck demonstrated the significance of the Ramachandran plot and Z-point [26].

Molecular docking investigations

It highlights the key connections between protein model units and receptor units. For this docking study [27], we uploaded the completed vaccine model as ligand and the TLR4 protein as a receptor molecule to the ClusPro v2.0 site. The TLR4 receptor (PDB ID: 3W3M) was picked from the PDB website.

Molecular dynamics simulation study

The simulation of molecular dynamics was used to examine the physical motions of atoms and molecules and biophysical systems. This would allow us to assess the dynamics and safety of the vaccine-receptor fear [28]. The illusion had been removed from the iMODS website (http://imods.chaconlab.com).

Exempt Protected rejoinder simulation

The whole construct was sent to the C-IMMSIM server (www.cbs.dtu.dk/services/C-ImmSim-10.1/) to see how the vaccine might affect the immune system [29]. As mentioned, we agreed that a 30-day break between application submissions would be the minimum acceptable gap [30].

Codon adaptation and in silico cloning Technique

As the appearance of an alien gene in an organism is problematic, organism-specific codon optimization is required more frequently. Depending on the codon change, the JCat server released the construct. The modified course was evaluated based on the codon adaptation ratio (CAI) preference and guanine-cytosine content. In SnapGene v4.2, the body in silico cloning strategy was successfully implemented.

RESULTS

Best antigenic protein selection

Using UniProt server, different structural and non-structural protein sequences were retrieved to construct the vaccine. Based on antigenicity, the design protein scored with an antigenic point of 0.7055 (Vaxijen). Further analysis of these proteins' amino acid sequences was done in order to determine CTL and HTL epitopes.

Possible HTL epitopes

A total of 195 epitopes, consisting of 15 amino acids, were identified using the IEDB server for mouse MHC-II alleles (IAb, IAd, IAs, IEb, IEd and IEs). We only selected among top five HTL epitopes based on antigenic scores to construct the final vaccine (Table 1).

Table 1. The selected HTL epitopes for the final vaccine construction.

HTL epitope	Percentile rank	Adjusted rank	Antigenicity	Allergenicity	Toxicity
LLTWLGLNSRSTSLS	2.50	2.50	1.4511(Probable ANTIGEN)	PROBABLE NON-ALLERGEN	Non-Toxin
ILLTWLGLNSRSTSL	3.30	3.30	1.4712 (Probable ANTIGEN)	PROBABLE NON-ALLERGEN	Non-Toxin
LTWLGLNSRSTSLSM	3.50	3.50	1.9214 (Probable ANTIGEN)	PROBABLE NON-ALLERGEN	Non-Toxin
GILLTWLGLNSRSTS	6.00	6.00	1.3691 (Probable ANTIGEN)	PROBABLE NON-ALLERGEN	Non-Toxin
LLTWLGLNSRSTSLS	2.50	2.50	1.4511 (Probable ANTIGEN)	PROBABLE NON-ALLERGEN	Non-Toxin
ILLTWLGLNSRSTSL	3.30	3.30	1.4712 (Probable ANTIGEN)	PROBABLE NON-ALLERGEN	Non-Toxin
LTWLGLNSRSTSLSM	3.50	3.50	1.9214 (Probable ANTIGEN)	PROBABLE NON-ALLERGEN	Non-Toxin
GILLTWLGLNSRSTS	6.00	6.00	1.3691 (Probable ANTIGEN)	PROBABLE NON-ALLERGEN	Non-Toxin

Possible CTL epitopes

From NetCTL 1.2, server, structural, and non-structural protein CTL epitopes were predicted on the basis of antigenicity, allergenicity, toxicity and C. score. A number of 170 epitopes, where each consist of a length of nine amino acids CTL were predicted from the sorted out of spike protein. We took top five CTL epitopes based on antigenicity score (Table 2).

Table 2. The selected CTL epitopes for the final vaccine construction.

CTL epitope	C. Score	Antigenicity	Allergenicity	Toxicity
TSEIQLTDY	3.1296	1.6089	PROBABLE ALLERGEN	Non-Toxin
LTDYGALTL	2.1842	1.0464	PROBABLE NON- ALLERGEN	Non-Toxin
IVQYENLKY	1.5782	0.7699	PROBABLE ALLERGEN	Non-Toxin
QTSGTTTIF	1.2389	0.0235	PROBABLE ALLERGEN	Non-Toxin
GTVLVQVKY	1.1348	1.4341	PROBABLE NON- ALLERGEN	Non-Toxin

Possible LBL epitopes

All structural and non-structural proteins of linear B-cell epitopes were predicted from the iBCE-EL server, where they were shortlisted based on the prediction score. We chose only one out of a total of 36 B-cell epitopes that was shown to be antigenic, non-toxic, and non-allergenic (Table 3).

Table 3. The selected B cell epitopes for the final vaccine construction.

B cell epitope	Start position	Score	Antigenicity	Allergenicity	Toxicity
TTDSRCPTQGEATL VEEQDANFVCRRT FVDRGWGNG	156	1	0.7	Non- Allergen	Non-Toxin

Vaccine construct and fundamental premises

The final vaccine was constructed with the selected 11 epitopes, which belong to three different classes, for example, 5 CTL, 5 HTL, and 1 LBL (Figure 2). All epitopes were joined by the AAY, GPGPG, and KK linkers. The TLR4 agonist 50S ribosome added in the beginning played the role of extra support to the immunogenicity to construct these 178 amino acid vaccines.

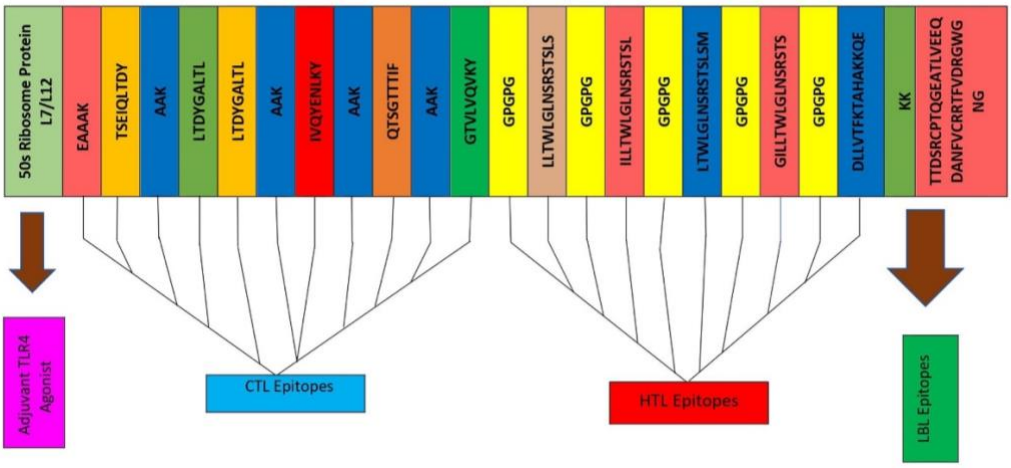

Figure 2. Graphical outline of the expressed multi-epitope vaccine assembles where it linked with adjuvant, CTL epitopes, HTL epitopes and LBL epitopes (Left to right). Here the adjuvant and CTL were linked by EAAAK linker (pink), CTL epitopes were linked by AAK linker (Blue), HTL epitopes were linked by GPPG (Yellow) and finally LBL epitopes were linked by KK linker (Green).

Physicochemical characteristics and immunological assessment

The physicochemical properties of the constructed vaccine were analyzed and documented, as shown in (Table 4). The constructed vaccine's molecular weight was 18822.22 Da. In addition to the 178 amino acids, the theoretical isoelectric point (pI) was 9.36. Simultaneously, the vaccine had the formula $C_{834}H_{1319}N_{231}O_{259}S_3$, an instability index of 35.33, an aliphatic index of 75.67, and a grand average of hydropathicity of -0.337. In addition, the constructed vaccine's antigenicity was 0.7055, it was non-toxic, and its solubility was 0.614 out of 1 (Figure 3) indicating that it is highly soluble, and the windowed charge score and fold propensity score were depicted in Figure 4.

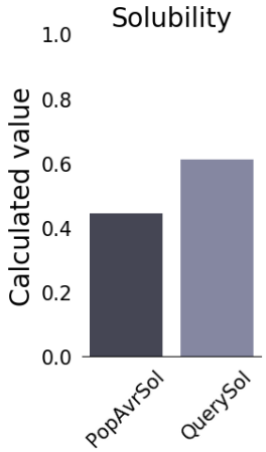

Figure 3. Solubility value by protein sol server.

Figure 4. A) Windowed charge score per amino acid. B) Windowed fold propensity score per amino acid. C) Secondary structure prediction report.

Table 4. Physicochemical characteristics of the construct.

Characteristics	Findings	Remarks
Number of amino acids	178	
Molecular weight	18822.22	
Theoretical pI	9.36	
Total number of negatively charged residues	12	
Total number of positively charged residues	17	
Formula	$C_{834}H_{1319}N_{231}O_{259}S_3$	
Total number of atoms	2646	
The estimated half-life	10 Hours	
The instability index	35.33	thermostable
Aliphatic index	75.67	stable
Grand average of hydropathicity	-0.337	Hydrophilic
Antigenicity	0.7055	
Allergenicity	No	
Solubility	0.614	

The a-helix, b-strand, and random coil secondary structures of the vaccine were evaluated using the SOPMA and PSIPRED servers (Table 5 and Figure 5A).

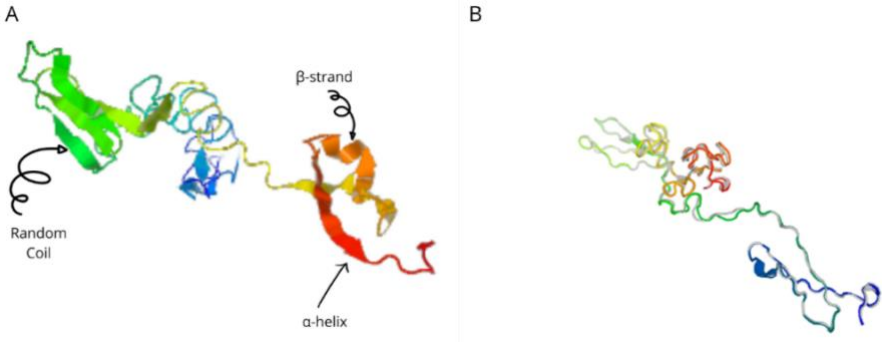

Figure 5. A) Tertiary structure of designated vaccine. Different secondary structure of amino acids is identified as different color, like a-helix as red color, b-strand as yellow color and random coil as green color. B) Refined Tertiary structure of designated vaccine.

Tertiary structure, sophistication and evaluation

The I-TASSER server (https://zhanggroup.org/I-TASSER) was used to obtain the best homology model (PDB id:01) among the top five modes. As advised by the server, we selected the lowest C-score (-3.38). Figure 5B depicts the designed vaccine's tertiary structure. The vaccine demonstrated in the Ramachandran graph that it exceeds 93.8 percent in the significant region, with a GDT- score, RMSD, MolProbity 2.047, 1.276 Clash 1.3, and rotamers score of 0.0. This was determined by refining the model that we developed (Figure 6). The average Z score for the vaccine is -8.81 according to the Procheck online site, which was provided with Table 6, an additional file containing all findings, and the ProSA web server.

Table 5. The secondary structural features of designated vaccine.

Features	Findings
Alpha helix (Hh)	23 is 12.92%
310 helix (Gg)	0 is 0.00%
Pi helix (Ii)	0 is 0.00%
Beta bridge (Bb)	0 is 0.00%
Extended strand (Ee)	56 is 31.46%
Beta turn (Tt)	11 is 6.18%
Bend region (Ss)	0 is 0.00%
Random coil (Cc)	88 is 49.44%
Ambiguous states (?)	0 is 0.00%
Other states	0 is 0.00%

Table 6. The protein structure and overall structure geometry.

Features	Findings
Ramachandran plot	92.1% core 5.0% allow 1.4% gener 1.4% disall
All Ramachandrans	8 labelled residues (out of 176)
Chi1-chi2 plots	1 labelled residues (out of 86)
Sidechain params	5 better 0 inside 0 worse
Residue properties	Max. deviation: 4.0, Bad contacts: 0
Bond len/angle	5.0 Morris et al class: 1 1 2
G-factors Dihedrals	-0.01 Covalent: -0.01 Overall: -0.01

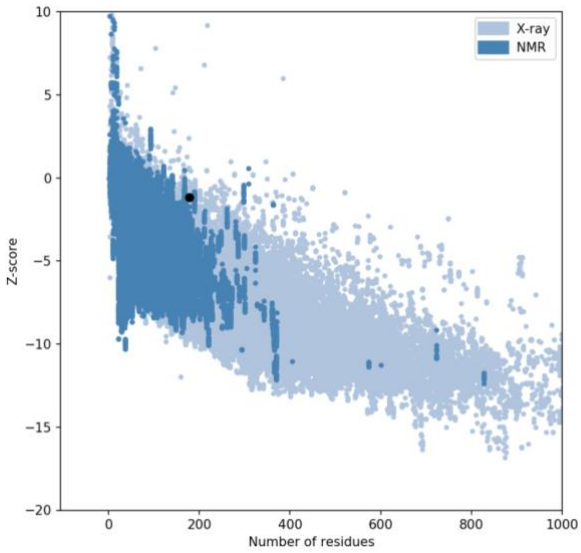

Figure 6. Validation of the tertiary structure of the vaccine.

Molecular docking analysis

We evaluated the interaction between the refined model and immune receptor TLR-5 using a software-based simulation Clus Pro v2.0 site. It docked 30 models in various positions where the minimum energy value was determined using an additional file. Consequently, we placed model 2 in the dangling position with an energy score of -1401.2% (Figure 7).

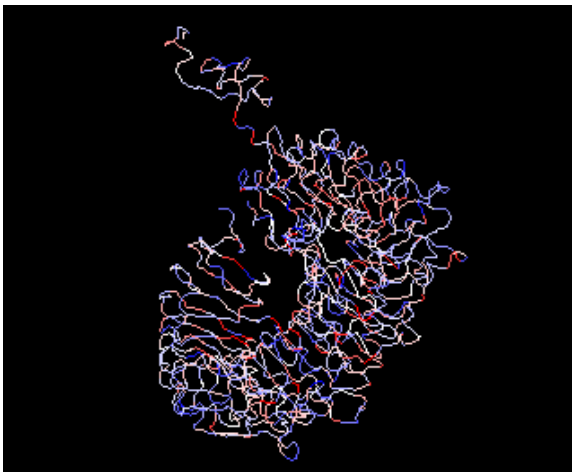

Figure 7. Constructed vaccine after molecular docking.

Molecular dynamics simulation experiment

Docked complex was subjected to molecular dynamics using the iMODS server to examine stable interactions between ligand molecule and receptor (TLR-5) at the microscopic level. The average life B- factor map, Eigenvalues, Variance, Covariance map, and Elastic network were displayed on Figure 8A-F, respectively.

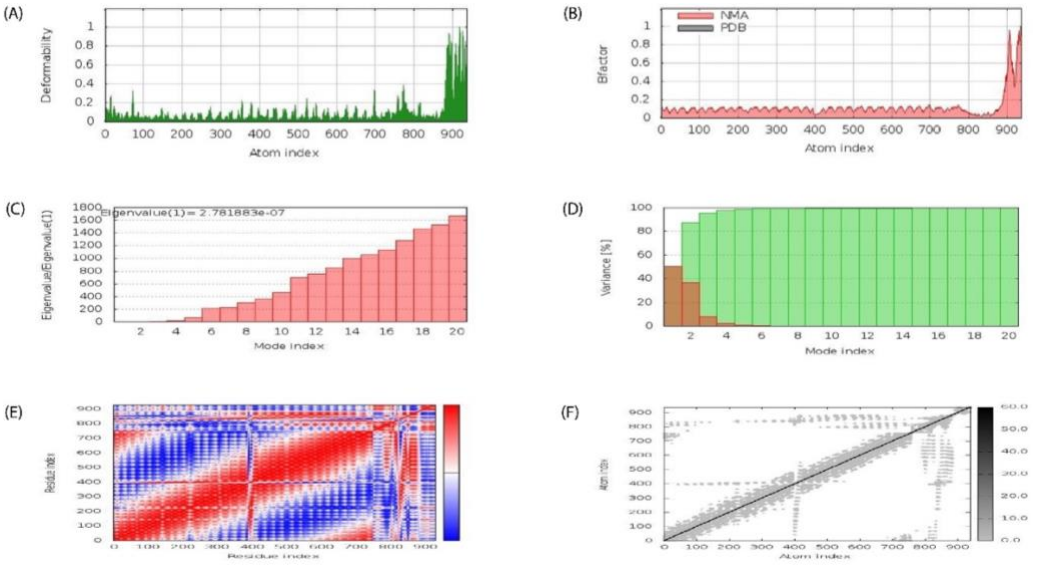

Figure 8. Molecular dynamics simulation of the vaccine. Here, different MD simulation plots show (A) Molecular deformability on molecular dynamic simulation; (B) B-factor/mobility on molecular dynamic simulation; (C) Eigenvalues on molecular dynamic simulation; (D) Variance on molecular dynamic simulation; (E) Covariance map on molecular dynamic simulation; (F) Elastic network on molecular dynamic simulation.

Exempt rejoinder simulation

Special pathogens expressed produced actual immunological aspects confirmed (Figures 9A-G). Antigen and immunoglobulin values showed in Figure 9A, B lymphocytes cell; IgM and IgG in Figure 9B, plasma B lymphocytes count as sub-divided per isotope (IgM, IgG1, IgG2) in Figure 9C. The other value showed in Figure 9C to 9G.

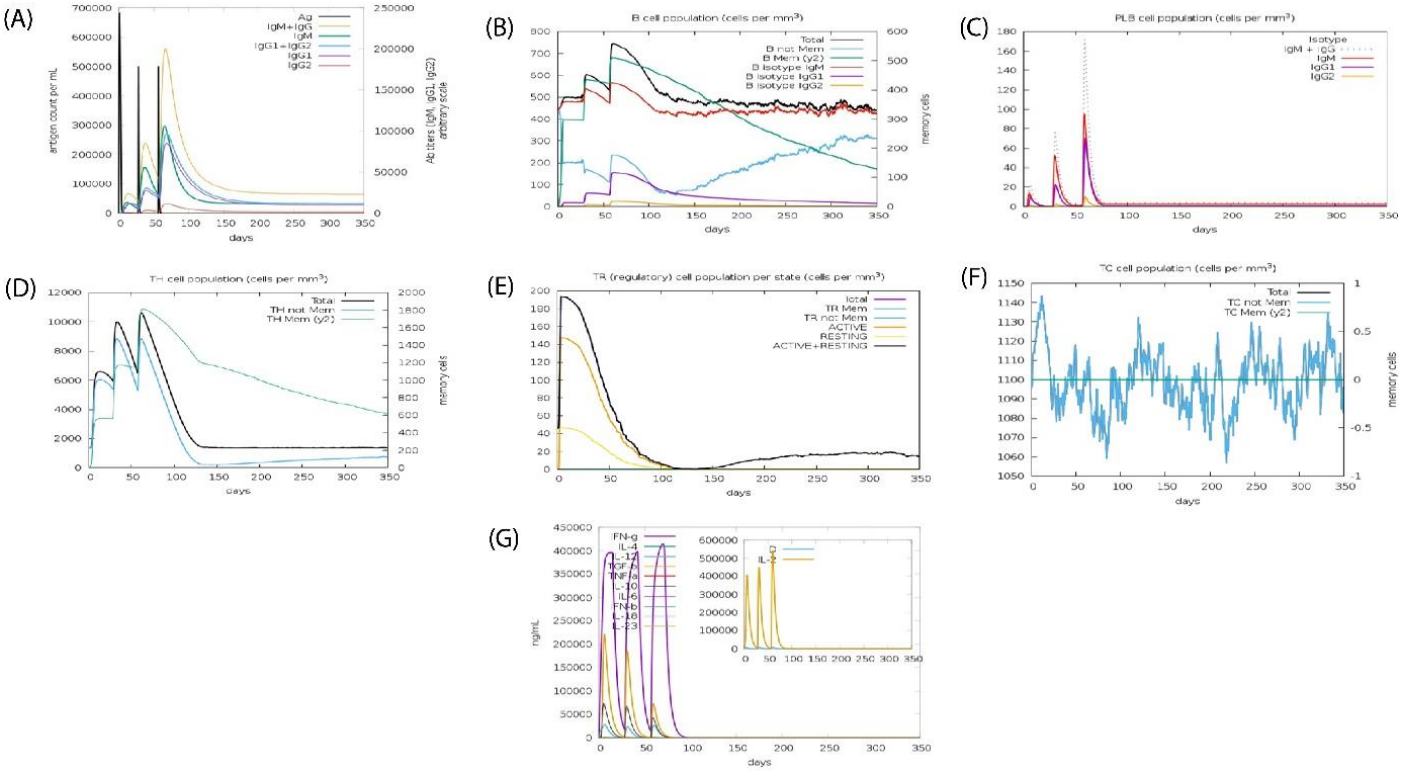

Figure 9. Immune response stirred up by the designed vaccine where the graph shows (A) Antigen and immunoglobulins. Antibodies are divided per isotype; (B) B lymphocytes: total measure using selected epitopes; (C) Plasma B lymphocytes measure using the isotype (IgM, IgG1 and IgG2); (D) CD4 T-helper lymphocytes count. The plot shows total and memory counts; (E) CD4 T-regulatory lymphocytes count. Both total, memory and per entity-state counts are plotted here; (F) CD8 T-cytotoxic lymphocytes count. Total and memory shown; and (G) Cytokines Concentration of cytokines and interleukins. D in the inset plot is danger signal.

Codon evolution and in silico cloning

The codons in the created vaccine were optimized to develop their key factor according to the *E. coli* bacteria in JCat site. Lastly, the formed size of the vaccine cloning product is 5907 base pairs, and the vector was 5369, insert 546 base pairs (nucleotide base pair). Figure 10 depicts the cloned product that was manufactured.

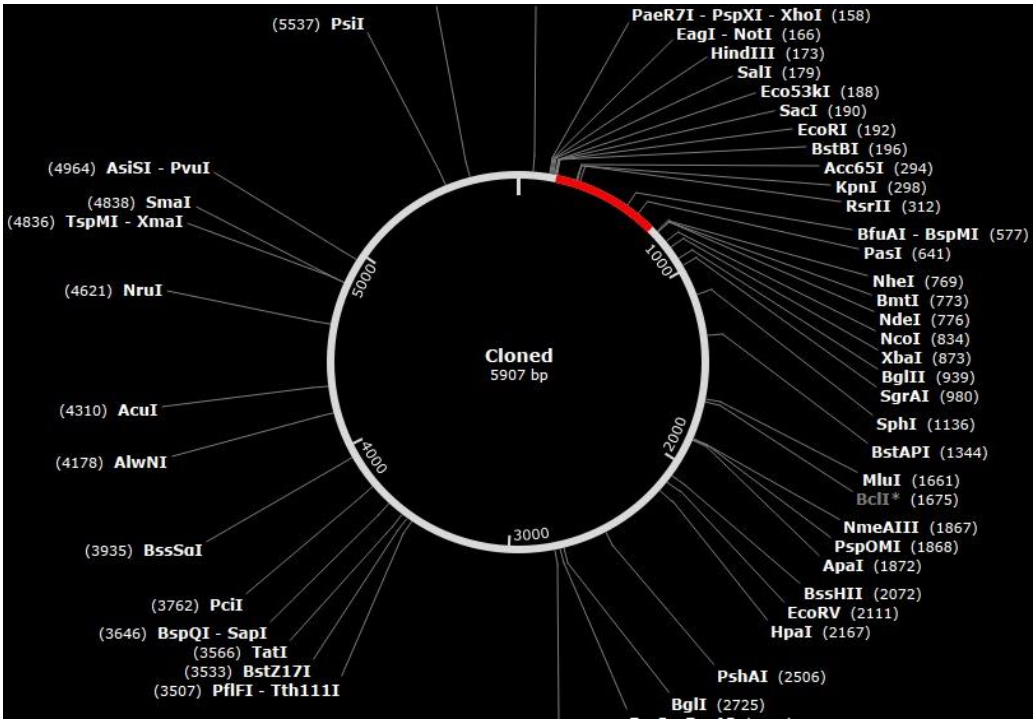

Figure 10. Constructed vaccine after cloning. The red section indicates the codon-optimized multi-epitope vaccine that has been introduced into the pET-28a (+) expression vector.

DISCUSSION

An estimated 3.9 billion people worldwide are susceptible to dengue infection. In 129 nations, Asians shoulder the lion's share of the burden or nearly 70 percent of the total [31].

In recent decades, millions of dengue cases have been reported annually, claiming the lives of a substantial section of the tropical and subtropical populations. In addition, the disease's rapid global spread [13] makes dengue an ever-worsening global problem. dengue is a virus transmitted by mosquitoes, and different DENV serotypes can cause the disease. These serotypes (DENV-1-4) exhibit immunological cross-reactivity [31]. dengue treatment should stray as far as possible from "eliminating the pathogen and reducing confusion" [32]. Yet, no specific vaccination has been discovered due to the lack of an effective vaccine and the goal to limit vaccine adverse effects. A vaccine must be developed immediately to eradicate dengue from the human body. Various types of candidates are already available in the field of research, but none has yet been established. In this study, however, we introduce a multi-epitope-based vaccine based on computational methods for the Asian region. This novel strategy is required to combat this life-threatening public health condition. The S protein contributes to the viral host range, infectiousness, and human-to-human transmission. Therefore, we had to design the vaccine to target the S protein to reach the virus's surface. We selected the CTL, HTL, and LBL epitopes in this study. We determined that the HTL was associated with the production of humoral and cellular immune responses and the HTL's role in extending immunity and eliminating virus-infected cells.

Refining with the highest antigenic number, we selected one B-cell epitope from the top five models between HTL and CTL epitopes. The CTL, HBL, and LBL epitopes were attached to linkers to build the suitable vaccine. The constructed vaccine consists of 178 amino acids, and its physiological properties were established utilizing web resources. As the vaccine's solubility was measured at 0.614 on a scale of 1, it was more soluble

and would easily enter the *E. coli* host. Based on the vaccine's physiological features, the theoretical PI value was determined to be 9.16 and fundamental. In addition, the physiochemical values of several metrics showed a higher possibility of effectiveness against the dengue virus. Web-based programs were used to analyze and forecast the homology modeling and three-dimensional structure of the manufactured vaccine. Using the PROCHECK server, the PDB file containing the final 3D structure of the vaccine was validated [31]. For the Ramachandran plot, we discovered a good overall predicted value of Z-score (-8.81) and the characteristics of the most preferred, accepted, and disallowed regions.

It is essential to establish the molecular basis of the TLR4 receptor's effective immune response to develop the most efficient vaccination against MHC alleles (HLA-DRB1*04-01). Molecular docking between the peptide vaccine and the virus glycoprotein-binding favorable receptor of TLR4, which had the lowest energy score of -1401.2, suggested that the vaccination may inhibit infection. Furthermore, it revealed a potential close interaction between the modeled vaccine ligand and the TLR5 receptor surface [31]. Codon optimization was conducted to stabilize the built vaccine within the host for optimal multi-epitope vaccine generation [31], and codon optimization was performed. In silico cloning was performed using the JCAT web service [31] to optimize codons in a pET28a (+) vector to prevent codon bias. As long as we maintain the greatest degree of safety tools and procedures, we hope that the dengue virus will be eradicated if this vaccination instruction is followed in the laboratory.

CONCLUSIONS

Dengue is currently one of the most significant and life-threatening diseases in the world, and it is rapidly spreading. Bangladesh is one of the countries with the highest incidence of dengue transmission. Over the past few decades, dengue fever incidence has increased at an alarming rate. There is currently no effective and permanent treatment for dengue disease. Different approaches have been taken over the past few years, but none of them has yielded a definitive solution. In this study, a computational method was utilized to create a multi-epitope-based vaccine against the dengue virus.

Our designed vaccine possessed a high level of immunity and could bind with the immune receptor TLR4 to produce the greatest response against the dengue virus. As a result of this study, we hope that the designed vaccine may play a significant role in eradicating this rapidly spreading virus.

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AUTHOR CONTRIBUTIONS

SRA, MIH and MSA designed the study. SRA, MIH and MSA performed the experiments, analyzed, and interpreted the data. SRA and MIH prepared the manuscript. MSA reviewed the manuscript. All authors approved the final version of the manuscript.

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CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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