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Research Article

Immunoinformatic Guided Rational Design of a Next Generation Multi Epitope Based Peptide Vaccine against Marburg Virus Disease Combined with Molecular Docking Studies

Mujahed I. Mustafa[1], Shaza W. Shantier[2]

[1]Department of Biotechnology, Faculty of Industrial and Applied Science. University of Bahri, Khartoum, Sudan.

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, Khartoum, Sudan.

Abstract:

Marburg virus disease (MVD) is a hemorrhagic fever and cause death up to 88% of people and is the same as Ebola virus, It is transmitted through skin contact or mucous membrane in the eyes, nose or mouth with blood or body fluids like urine, saliva sweat, feces and by object contaminated with body fluids. Despite these facts, yet there is no approved vaccine have been developed for the eradication of Marburg virus infections. Therefore, this study described a multi epitope-based peptide vaccine against Marburg virus viral protein 35, using several immunoinformatics tools combined with molecular docking studies. Utilizing Vaxijen 2.0 server, the V35 protein revealed to be antigenic with a score of 0.4316. Prediction of the T-cell and B-cell epitopes was then conducted. RTFDAFLGV epitope was found to be the most promising one with binding affinity to the highest numbers of MHC I alleles, a positive score in the Class I immunogenicity study and non-allergen. These results were further confirmed by the good interaction of RTFDAFLGV to the groove of HLA-A*02:01 with binding energy of -8.1 kcal/mole. Finally, the vaccine was cloned *in silico* to ensure its validity and efficiency of expression. To ensure its safety and immunogenic profile, *in-vitro* and *in-vivo* bioassays are recommended to confirm these findings.

Keywords: Marburg virus disease (MVD); Vaccine design; Immunoinformatic; Molecular Docking Study.

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1.Introduction:

The Marburg virus (MARV), a single-stranded RNA virus that causes Marburg virus disease (MVD) in humans, was first recognized in 1967 following simultaneous outbreaks in Marburg and Frankfurt, Germany [1, 2]. Uganda, in particular, experienced multiple MARV outbreaks in 2012, 2014, and 2017. On August 9, 2021, Guinea recorded the first-ever case of MARV. The Marburg virus is a negativesensed, unsegmented enveloped RNA virus with a filamentous structure and a snail-like number 6 or spiral shape that can be branched on occasion [2, 3]. MARV is transmitted through mucosal surfaces, skin abrasions, and parenteral encounters. Various bat species have been proposed as reservoir hosts [2, 3]. Spread of the virus between people has occurred in close environment and among direct contacts. Laboratory exposures can also occur when lab staff handle live Marburg virus [4].

The incubation period is 2 – 21 days, after which symptoms such as fever, chills, headache, and myalgia appear [4]. From day 5-7, the infection becomes more severe. Patients experience persistent fever, conjunctivitis, maculopapular rash, and finally hemorrhagic fever symptoms such as petechiae, mucosal bleeding, and blood in the vomitus, stool, and venipuncture sites [5].

The laboratory diagnosis of the Marburg virus disease is difficult because many of the signs and symptoms are similar to those of other infected diseases such as malaria, typhoid fever, or Ebola. Antigen capture enzyme linked immunosorbent assay (ELISA) testing, polymerase chain reaction (PCR), and IgM capture are all used in the diagnosis. Within a few days of the onset of symptoms, ELISA can be used to confirm a case of the virus [5, 6].

The Marburg virus belongs to the filoviridae family, which is further subdivided into three types: Marburgvirus, Ebolavirus, and Cuevavirus. The genus Marburgvirus contains only one species, Marburg Marburgvirus, which is represented by two distinct viruses, Marburg virus (MARV) and Ravin virus (RAVV)[5].

The structural proteins encoded by MARV genomes are Nucleoprotein (NP), envelope glycoprotein (GP), RNA-directed RNA polymerase L (L), matrix protein VP40 (VP40), polymerase cofactor VP35 (VP35), minor nucleoprotein VP30 (VP30), and membrane-associated protein VP24. The four proteins NP, VP35, VP30, and L work together to form a nucleocapsid complex that encircles the viral genome [7].

MARV VP35 is required for the synthesis of nucleocapsids, which play important roles in the viral replication cycle, including the production of type I interferon (IFN), dendritic cell maturation, and protein kinase R activation (PKR) [8-11].

In this research, we investigated The VP35 protein to scrutinize prospective antigenic and immunogenic epitopes that prompts not only humoral (B-cell) immune response but also cell-mediated (T-cell) immune response. We have taken potential epitopes from the selected structural proteins into account and construct a multi-epitope vaccine. We expect the present study to facilitate MARV vaccine development; experimental work will be performed further to validate our current findings.

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2. Material and Method:

2.1. Sequence retrieval:

The sequence of VP35 protein was obtained from the national center of biotechnology information (NCBI) (https://www.ncbi.nlm.nih.gov/). The sequence applied in different software to select the epitopes that can generate human immune response and can design a vaccine against Marburg virus. The selection and prediction of the epitopes depend on both cellular and humeral immune response that B and T cell response.

2.2. Antigenicity prediction:

To confirm the immunogenic character of all epitopes fragments Vaxijen 2.0 server (http://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [12] was used. It is based on the alignment independence method which predict antigenicity using physiochemical properties and ACC methods for antigenicity assessments peptide fragment with a threshold greater than 0.4 were marked as potentially antigenic.

2.3. B-cell epitope prediction:

B-cells must be stimulated by certain antigens/epitopes in order to produce antibodies against a disease. The BCPRED server (http://ailab.ist.psu.edu/bcpred/) was used to search for promiscuous linear B-cell epitopes in the designated antigenic protein of the Marburg virus. With a Fasta sequence as input, the BCPRED service creates 20mer epitopes with a default specificity of 75% for B-cell receptors. The epitope with the best score was chosen for continued development of a multiepitope vaccine [13].

2.4. T-cell epitope prediction tools:

To predict the interaction with different MHC I alleles, the Major Histocompatibility Complex class I (MHC I) binding prediction tool on the IEDB http://tools.iedb.org/mhcI [14] was used. It services distinct approaches to measure the binding affinity of selected sequence to a definite MHC class I molecule. The half maximal inhibitory concentration (IC50) values of peptide binding to MHC class I molecule was calculated by artificial neural network (ANN) approach. All alleles having a binding affinity of IC50 that are equal or less than 100 nM were selected for further analysis.

CTL epitope predictions are also essential for building a coherent vaccine. This was accomplished using NetCTL1.2 36, an internet-based server designed to detect human CTL epitopes in a target protein. TAP transport efficiency, proteasomal cleavage, and MHCI molecules binding affinity data were added to get the overall score. The parameter was set to 0.5, which has 0.89 and 0.94 sensitivity and specificity, respectively.

To further check the immunogenicity of the selected epitopes, class I Immunogenicity server available at IEDB (http://tools.iedb.org/immunogenicity/) was utilized.

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2.5. Physiochemical and allergenicity prediction:

ProtParam server available at (https://web.expasy.org/protparam/) was used to anticipate the physicochemical features of the protein under study and to understand the fundamental nature of the vaccine [15].

AllergenFP v.1.0 [16] and AllerTop v 2.0 [17] servers were also used to evaluate the allergenicity of the predicted peptides.

2.6. Tertiary Structure prediction, refinement and validation:

SWISS-Model server was used to construct the VP35 protein's tertiary structure, while Discovery studio 2020 was utilized to display the most promising peptides for vaccine design [18]. QMEAN was used to confirm the predicted 3D structure [19].

GalaxyRefine applies repetitive structure perturbation to side-chains, secondary structure elements, and loops, followed by molecular dynamics simulation to achieve overall structural relaxation [20], while the ProSA-web calculated the energy plot as well as the Z score value, which indicates the overall quality score of the target vaccine design model [21]. Furthermore, by Ramachandran plot, the Ramachandran Plot server estimates the residue-by-residue stereochemical characteristics validation of the vaccination model [22].

2.7. Secondary structure prediction:

Using the reference sequences as an input, PSIPRED was used to predict the secondary structures of the designed vaccine. PSIPRED is a straightforward and accurate 2D structure prediction method that employs two feed-forward neural networks to analyze PSI-BLAST output [23].

2.8. Molecular docking analysis:

In order to estimate the binding affinities between the proposed epitopes and molecular structure of T cells, in silico molecular docking was used. Sequences of proposed epitopes were selected from Ebola virus reference sequence using Chimera 1.10 and saved as PDB file. The obtained files were then optimized and energy minimized. The 3D structures of MHC I allele HLA-A*02:01 (PDB ID: 4UQ3) was retrieved from RCSB PDB (protein data bank) database. Swiss PDB viewer V.4.1.0 software was used for structure optimization and energy minimization.

Molecular docking was then performed using AutoDock 4.0 software [24]. The active residue of the protein was selected, the results less than 1.0Å in positional root-mean-square deviation (RMSD) were then considered ideal and clustered together for finding the favorable binding [25]. The highest binding energy (most negative) was considered as the ligand with maximum binding affinity. The 3D and 2D interactions of the resultant docking files with poses showing the lowest binding energies were visualized using Chimera and DS Visualizer Client 2020.

2.9. Stability enhancement of the vaccine construct by using Disulfide engineering

The vaccine construct was examined for disulfide engineering once the tertiary structure was refined, using the Disulfide tool of the Design 2 website, which may be found at

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(http://cptweb.cpt.wayne.edu/DbD2/) [26]. The residues for disulfide engineering were chosen based on Cα-Cβ-Sγ, chi3angles, and lowest energy. The Eris server (https://dokhlab.med.psu.edu/eris/) was used to investigate the influence of disulfide engineering on the vaccine construct's stability [27].

2.10. Codon Optimization and Computational Cloning

For high-level expression of the vaccine sequence in E. coli K12 strain, Java Codon Adaptation Tool (JCAT) was used to optimize the vaccine construct's codons. [28]. *In silico* cloning was carried out using the pET28b (+) expression vector. In this case, the SnapGene 5.1.7 restriction cloning tool was used to complete in silico cloning.

3. Results:

3.1. Antigenicity prediction

The antigenicity was tested using the VaxiJen server, which supplied an antigenic score for each protein, which was obtained from NCBI. The VaxiJen score threshold for viruses is 0.4, which means that proteins with a score greater than 0.4 are deemed antigenic, whereas proteins with a score less than 0.4 are considered non-antigenic. The VaxiJen score for Marburg Virus's tested proteins was more than 0.4 (0.4316).

3.2. B-cell epitope prediction:

The BCPRED of B cell proposed 7 epitopes, and all epitopes were found 100% conserved using IEDB conservation tools, and thus can generate immune response (**Table 1**)

Table 1: List of linear B-cell epitopes with their location and score by using the BCPRED server:

Position	Epitope	Score
150	AYLNEHGVPPPQPAIFKDLG	0.995
302	AVPPNPTIDKGWVCVYSSEQ	0.979
277	LGVVPPVIRVKNFQTVPRPC	0.965
128	LAKYDHLVISTGRTTAPAAA	0.964
31	EKLYKRRKPKGTVGLQCSPC	0.935
171	AQQACSKGTMVKNATTDAAD	0.857
52	MSKATSTDDIIWDQLIVKRT	0.795

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3.3. T-cell epitope prediction:

Using ANN server for MHC class I, 74 epitopes were predicted for allele HLA-A, B and C. and all with 100% conservancy.

Tables 2: summarizes the most promising peptides bound to MHC I along with their immunogenicity and allergenicity predication:

Peptide	Alleles	Allergenicity	IC50	Immuno-genicity	COMB score*
RTFDAFLGV	HLA-A*02:06,HLA-A*68:02,HLA- A*30:01.HLA-A*02:01.HLA-C*15:02	Non-allergen	2.91	0.219	0.647
TLADLLIPI	HLA-A*02:01,HLA-A*02:06,HLA- A*68:02	Non-allergen	3.89	0.119	0.548
RAVPPNPTI	HLA-C*03:03,HLA-B*58:01,HLA- C*12:03	Probable allergen	5.97	-0.001	0.425
MLAKYDHLV	HLA-A*02:01,HLA-A*02:06.HLA- A*68:02	Non-allergen	6.49	-0.166	0.873
KLYKRRKPK	HLA-A*03:01,HLA-A*30:01,HLA- A*31:01	Non-allergen	6.54	-0.307	0.406
TLSEVTTRV	HLA-A*02:01,HLA-A*68:02,HLA- A*02:06	Allergen	11.85	0.186	0.561

*Combined score of peptide MHC class I binding, proteasomal C terminal cleavage score and TAP transport efficiency.

3.4. Physicochemical properties:

The physicochemical properties of the protein were assessed as shown in Table 3.

Table 3: The obtained physicochemical properties:

Characteristics	Finding	Remark
Number of amino acids	326	Suitable
Molecular weight	36.13 kDa	Average
Theoretical pI	8.96	Slightly basic
Chemical formula	$C_{1609}H_{2599}N_{437}O_{476}S_{14}$	-
Extinction coefficient (at 280nm in H_2O)	25690	-

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Estimated half-life (mammalian reticulocytes, in vitro)	30h	-
Estimated half-life (yeast-cells, in vivo)	>20h	-
Estimated half-life (E. coli, in vivo)	>10h	-
Instability index of vaccine	39.84	Stable
Aliphatic index of vaccine	90.73	Thermostable
Grand average of hydropathicity (GRAVY)	-0.167	Hydrophilic

3.5. Tertiary structure prediction, refinement and validation:

SWISS-Model sever was used to construct the VP35 protein's tertiary structure, while QMEAN was used to confirm the predicted 3D structure **(Figure 1)**.

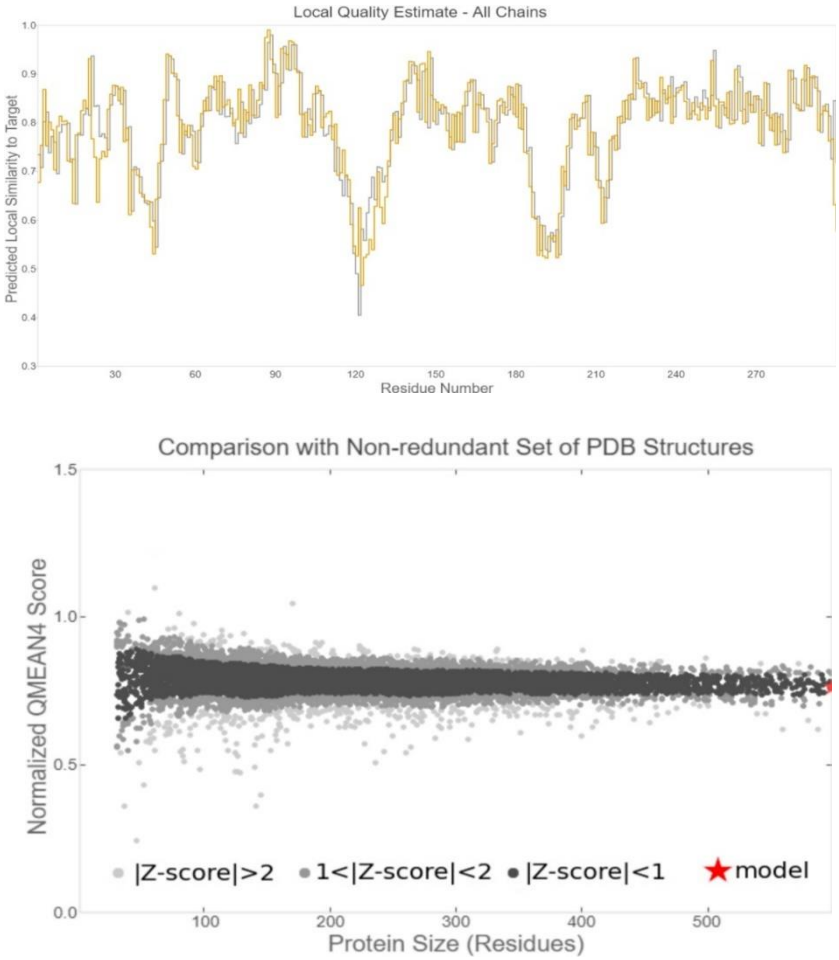

Figure 1: Evaluation of structural superiority by QMEAN server.

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3.6. The refinement of our predicted 3D structure was done with GalaxyRefine server, based on quality score the best refined structure was used as ligand molecules in molecular docking studies **(Figure 2)**.

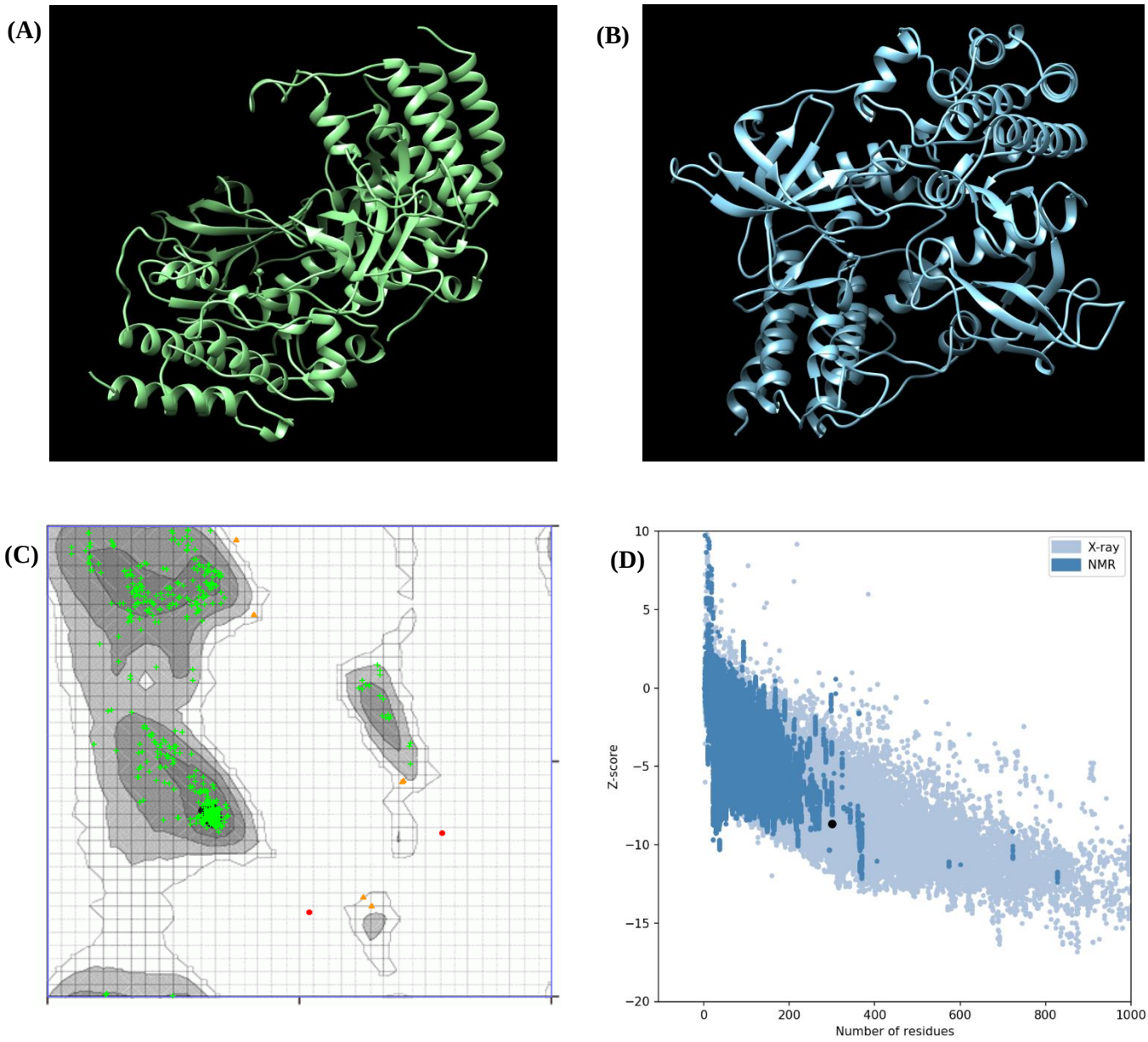

Figure 2: demonstrates multi epitope vaccine design 3D modeling, refining, and validation. (A) 3D modeling by SWISS-Model server. (B) refined 3D structure by GalaxyRefine. (C) Ramachandran plot analysis of the refined 3D structure. (D) ProSA-web shows Z-score of −8.67.

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3.7. Secondary structural prediction of the designed vaccine:

PSIPRED predicted the secondary structure of the designed vaccine. There are no structural deformities in the structures, which are made up of coils, helixes, and strands.

Figure 3: The secondary structures of the predicted Marburg virus vaccine using PSIPRED.

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3.8. Molecular docking study:

The epitopes that share the maximum number of MHC class I supertypes and provided the positive result in the Immunogenicity analysis with no allergenicity were applied to study their molecular interaction. The obtained results are shown in Figure 4.

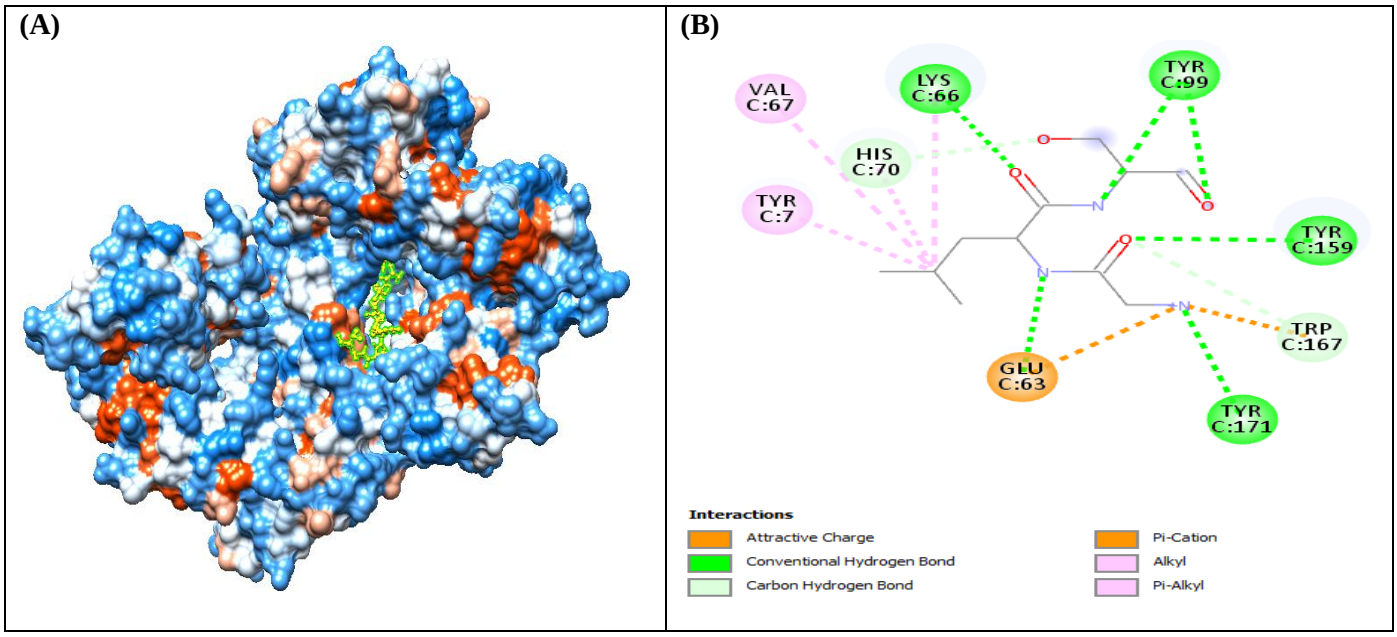

Figure 4: Docking analysis of the predicted epitope RTFDAFLGV with HLA-A*02:01. **(A)** 3D surface structural view, **(B)** 2D structural view.

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3.9. Stability enhancement of the vaccine construct by using Disulfide engineering:

The obtained results disulfide engineering are summarized in (**Figure 5, Table 4**).

Figure 5: Represents the Engineered disulfides bonds along with the residue pair ALA239 and PHE248 used.

Table 4: Selected Residue pair ALA239 and PHE248:

Res1 Chain	Res1 Seq #	Res1 AA	Res2 Chain	Res2 Seq #	Res2 AA	Chi3	Energy
A	239	ALA	A	248	PHE	-86.74	1.23

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3.10. Codon adaption and *in silico* cloning of the constructed vaccine:

Java Codon Adaptation Tool was used to check codon optimism of vaccine candidates in Escherichia coli (strain k12) expression system. It revealed that multi-epitope vaccine construct composed of 2178 nucleotides. The Codon Adaptation Index (CAI) was 0.98. The GC content of VC1 found to be 52.19%. The obtained values indicate that the vaccine candidate was having cloning efficiency. SnapGene tool was used for in-silico insertion of adapted codon sequence of vaccine construct into pET32a+ vector using the EcoRV and MscI restriction sites and clones were obtained successfully **(Figure 6)**.

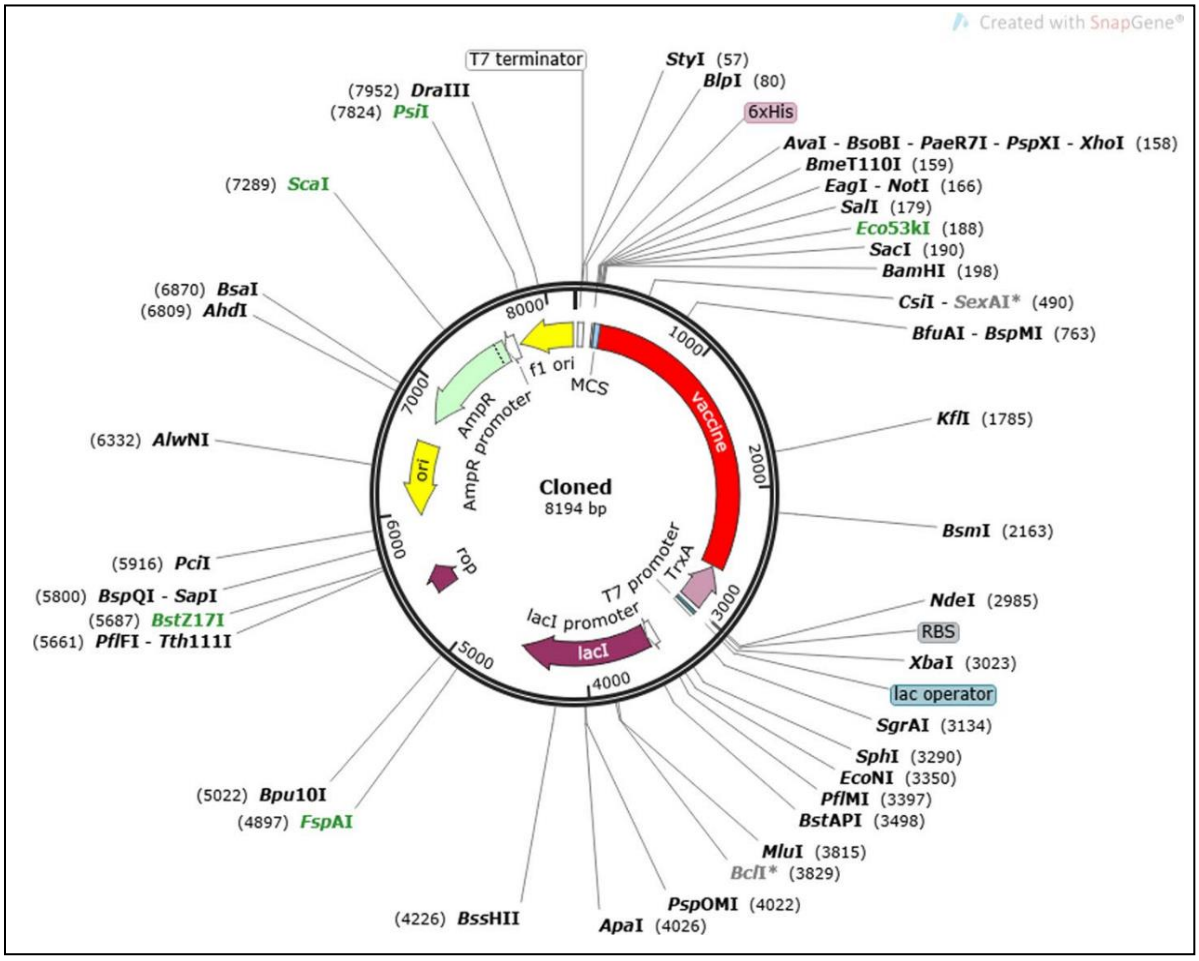

Figure 6: *In silico* cloning of vaccine candidate to ensure the authenticity of the construct's expression efficiency. The vaccine sequence (the red section) *was in silico* restriction cloned into the pET32a+ vector using the EcoRV and MscI restriction sites.

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4. Discussion:

Immunoinformatics studies to the development of effective vaccines against a variety of microorganisms, particularly viruses, are becoming more widely accepted as a first stage of vaccine design. Recently, immunoinformatics-guided methods have been used in the development of several multi epitope-based peptide vaccine for many pathogens [10, 29-36].

The use of computational approaches aids in the dramatic drop of in vitro trials [37]. It also saves time and resources, and improves the chances of a successful vaccine design [38]. For the prevention and treatment of viral or tumor infections, a multi-epitope vaccine containing a series of peptides that activate humoral and adaptive immune responses is an ideal strategy [30, 39-44].

The lack of specific antiviral drugs or approved Marburg Virus vaccines has made infection control and prevention even more difficult. There has been a lot of work done to raise awareness about the Marburg Virus's vulnerability and pathogenesis [45]. Therefore, the current research is focused on the development of an MVD multi epitope-based peptide vaccine. The fundamental benefit of an epitope-based vaccine is that it can elicit targeted immune responses against epitopes, improve binding affinity with target receptor molecules, and hence prevent non-epitope selection [46-48]. The immune response with the target molecules for the next step of cellular cascades is elicited by the identified common epitopes [49]. However, Sami et al. reported a Multi-epitope vaccine against the structural proteins of Marburg virus [50]. They predicted both B and T-cell epitopes for designing a multiepitope-based peptide vaccine candidate against MVD. Now, using a new Immunoinformatics guided rational design of a next generation multi epitope-based peptide vaccine combined with molecular docking studies, we chose distinct antigenic proteins (VP 35) to design a multiepitope-based peptide vaccine. T-cell epitopes derived from B-cell epitopes were found in this study for the formation of both humoral and cytotoxic immune responses. Following that, both MHC-I and MHC-II epitopes were effectively predicted using multiple web tools.

A multi-epitope peptide vaccine has the advantage of eliciting both cellular and humoral immunity over a monovalent vaccine [51]. Predicting B-cell and T-cell epitopes is a critical step in the vaccines development [52]. Therefore, a multi-epitope vaccine developed with guided rational approach could be a valuable tool in the fight against MVD.

In order for a peptide to function as an effective vaccination, it must include a variety of physiochemical features. Molecular weight is one of these physiochemical parameters. The peptide's half-life inside the body is inversely related to its molecular weight, although lymph node exposure is directly proportionate [53]. As a result, peptides larger than 50 kDa are regarded suitable vaccination alternatives for maximum half-life and lymph node exposure for active immune response production. Furthermore, the vaccine construct's hydrophobicity and hydrophilicity have a significant impact on its efficiency. Thus, to evaluate the physiochemical properties, the ProtParam tool available at the Expasy server was utilized. The molecular weight was predicted to be 36.13kDa with a predicted half-life of 30 hours in mammalian reticulocytes. ProtParam classified the vaccine construct as stable in nature with the instability index of only 39.84 (**Table 3**)

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B-lymphocytes are a key component of the humoral immune system, producing a wide range of pathogen-specific antibodies that result in antigen neutralization and viral load elimination. Thus, antigenic proteome was evaluated using the BCPRED service to incorporate epitopes with the potential to activate B-cells in the vaccine design. The obtained results showed 7 conserved epitopes with high scores (**Table 1**).

The MHC class I epitopes activating the Cytotoxic T-cells, in turn, incite the adaptive immune system. Therefore, prediction of MHC class I epitope is essential for vaccine development.

The class I Immunogenicity tool on the IEDB server was also used to examine the immunogenicity and propensity of activating Cytotoxic T-cells for the identified MHC class I binding epitopes. The 9-mer epitopes are analyzed using the Class I immunogenicity tool, which assigns a positive or negative score. The higher the score, the more likely it is to activate an immunological response in the host. Besides the binding with the MHC molecules and immunigenicity, the predicted peptides must be non-toxic and non-allergen, hence, their safety was predicted using AllergenFP v.1.0 and AllerTop v 2.0 servers. As a result, only epitopes (RTFDAFLGV) with binding affinity to the highest numbers of MHC I alleles, a positive score in the Class I immunogenicity study and non-allergen was considered for further analysis, while those with a negative score were discarded (**Table 2**).

The anticipated peptides must bind efficiently with the MHC I molecules in order to trigger immunological responses [54]. Because of their variety and immunogenic connection, the allele HLA-A*02:01 was chosen as target for molecular docking to explore its binding affinity [55, 56].

The 3D structure of a vaccine candidate was created using the SWISS-Model online server (**Figure 2A**), while QMEAN was used to confirm the predicted 3D structure **(Figure 1),** refined using GalaxyRefine (**Figure 2B**), and validated using Ramachandran plot server (**Figure 2C**), and ProSA-web (**Figure 2D**). In this study, the Z score and energy plot were assessed in order to determine the appropriate folding of the protein. The amino acid residues of vaccine candidate were plotted using the Ramachandran method. We predicted a negative result using Z score analysis, indicating that the 3D structure is valid.

PSIPRED predicted the secondary structure of the designed vaccine. There are no structural deformities in the structures, which are made up of coils, helixes, and strands. Epitopes are frequently found in coils and must be exposed (**Figure 3**).

The creation of H-bonds in the protein-ligand complex is a crucial metric for determining the stability of the conformation across the simulation period [57]. To view the 2D interactions and bonding with MHC molecules, the Discovery studio was employed. RTFDAFLGV was bond to the groove of HLA-A*02:01 with binding energies of -8.1 kcal/mole. RTFDAFLGV established four hydrogen bonds with Lys66, Tyr99, Tyr 159, and Tyr171; three Pi-alkyl bonds with Tyr7, Lys66, and Val67. It was formed two carbon hydrogen bond with His70 and Trp167; and two Pi-cation bonds with Glu63 and Trp167 (**Figure 4 A, B**).

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Following that, the effect of disulfide bonds on the constructed vaccine's stability was studied. The addition of new disulfide bonds to proteins improves their stability by lowering conformation entropy and thereby raising the free energy of the macromolecule in its denatured state [26]. Disulfide engineering was used to better stabilize the vaccine construct using the Disulfide tool accessible on the Design 2.0 site. The chi3 angle between the disulfide bonds spans from -87 to +97 degrees, with an energy of less than 2.2 kcal, according to torsion angles analyses of several proteins [27, 58]. The residue pair ALA239 and PHE248 were chosen for disulfide engineering based on these two requirements (**Table 4, Figure 5**). The ERIS tool was also used to test the influence of the newly generated unique disulfide bonds on the vaccine construct's stability. Eris is used to investigate how a mutation in the native structure affects protein stability in terms of $\Delta\Delta G$. The mutation would have a stabilizing impact when $\Delta\Delta G < 0$. $\Delta\Delta G$ owing to the formation of this disulfide bond was determined to be -1.24 kcal/mol, indicating that disulfide engineering had a stabilizing impact on the vaccine design [27].

Finally, using the EcoRV and MscI restriction sites within the pET32a+ vector, the SnapGene cloning tool was used to amplify the desired vaccine sequence within the pET32a+ vector (**Figure 6**).

As a result of these interesting findings, developing a vaccine using the suggested peptides considered to be highly promising, with the potential to be widely used as a universal epitope-based peptide vaccine against MVD.

A multi epitope-based peptide vaccine, on the other hand, has some limitations. For example, one of the major drawbacks of a multi-epitope vaccine that most epitope prediction tools overlook is the need to distinguish proper antigen processing sites that can lead to the prediction and presentation of predicted epitopes. Because the composition of antigen processing mechanisms varies depending on proinflammatory signals and can differ between cell classes, current prediction algorithms may not be suitable for assessing the processing effectiveness of viral antigens in an infected target cell [59]. Yet, Immunoinformatics combined with Molecular Docking Studies would mean a major breakthrough in medical field and therefore stronger efforts made in this path would definitely pay back.

Conclusion:

Immunoinformatics guided rational design of a next generation multi epitope-based peptide vaccine was used in this study, along with molecular docking studies, to develop potential vaccine candidates against Marburg virus disease, taking into account their ease of use in experimental studies. Because the only effective defense against Marburg virus infection is vaccination, a potential vaccine was developed and predicted to evoke specific immune responses in MVD patients. The study's findings are based on in silico approaches. As a result, *in-vitro* and *in-vivo* bioassays is recommended to confirm these findings to ensure its safety and immunogenic profile.

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