Research in Molecular Medicine

In Silico Design of a Multivalent Epitope Vaccine Against SARS-CoV-2 for Iranian Populations

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ABSTRACT

Background: Because of high genetic variation in human leukocyte antigen (HLA) alleles, epitope-based vaccines do not show equal efficacy in different human populations. Therefore, we proposed a multi-epitope vaccine against SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) for Iranian populations.

Materials and Methods: For this purpose, the proteins without allergenicity and high antigenicity, as well as conservancy levels from SARS-CoV-2, were chosen for computational epitope mapping. The T-cell epitope mapping process was performed based on the most frequent human leukocyte antigen (HLA) alleles in Iran. The B- and T-cell epitopes were determined based on their allergenicity, antigenicity, and hemolytic potential. Then, the epitopes with acceptable features were subjected to the final construct. The screened epitopes were structured in the final vaccine sequence. The secondary and tertiary structures of the proposed vaccine were predicted, and its affinity to HLA-I, HLA-II, toll-like receptor (TLR)-3, and TLR-4 were evaluated by the molecular docking method. Additionally, possible immune responses against the vaccine were predicted through immune simulation.

Results: The final vaccine construct includes six linear B-cell epitopes, eight HLA-I restricted epitopes, and six HLA-II restricted epitopes. The evaluations confirmed that the proposed vaccine is a 60.3 kDa stable, water-soluble, and high antigenic protein with high affinity to the selected target molecules and could elicit both humoral and cellular responses.

Conclusion: Altogether, the study results suggest that the planned vaccine can be an adequate anti-COVID-19 vaccine candidate for the Iranian population.

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Introduction

Although two years have passed since the first report of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) in China, the world continues to face the global pandemic of COVID-19 disease caused by the virus. As of 24 September 2021, the total cases of SARS-CoV-2 confirmed globally by the World Health Organization (WHO) were 230418451, with 4724876 reported deaths.

Fever, cough, tiredness, diarrhea, headache, hemoptysis, dyspnea, acute respiratory distress syndrome, cardiac injury, and lymphopenia are the recognized clinical signs of COVID-19 [1]. The virus is mainly transmitted through droplet infection and contact routes. The high similarity of the SARS-CoV-2 genome sequence with bat coronaviruses suggests its probable origin in bats. The virus proteome includes four structural proteins: S protein (Spike), E protein (Envelope), M protein (Membrane), and N protein (Nucleocapsid). Also, there are six accessory proteins (ORF1ab, NS3, NS6, NS7a, NS7b, and NS8) encoded by a 30-Kb positive-sense and single-stranded RNA molecule [2].

Despite receiving FDA approval of some anti-COVID-19 vaccines, such as Pfizer-BioNTech, Janssen, and Moderna, concern about their long-term safety, fair access to the available vaccines, and effectiveness are compelling reasons to understand the widespread need for new effective vaccines. In addition, with the occurrence of new mutant strains of SARA-CoV-2, especially the delta variant, as well as a substantial reduction in the vaccine efficacies, the introduction of new cross-protective vaccines is a vital need during the COVID-19 pandemic [3, 4].

Epitope-based vaccines (EBVs) are new platforms with unique properties to address obstacles of common vaccine platforms such as low immunogenicity, allergenicity, and strain-restricted activity. Therefore, recently several EBVs have been proposed against Hepatitis C virus (HCV) [5], Crimean-Congo hemorrhagic fever virus (CCHFV) [6], Shigella spp. [7], human immunodeficiency viruses (HIV) [8], Dengue virus [9], foot-and-mouth disease virus (FMDV) [10], influenza virus [11], Saint Louis encephalitis virus (SLEV) [12], Helicobacter pylori [13], the Middle East respiratory syndrome coronavirus (MERS-COV) [14], and many other pathogens. Regardless of the advantages of epitope-based peptide vaccines, so far, a few have been commercialized due to the tedious, expensive, and complexity of the experimental epitope mapping process [7].

Consequently, newly computational epitope-mapping methods have been regarded as complementary attitudes to the experimental epitope identification process. Subsequently, several in silico-designed epitope-based vaccines have been introduced against SARS-CoV-2 [15-20].

However, to date, there is no epitope-based anti-COVID-19 vaccine in receiving the international approval process. The presence of a wide range of human leukocyte antigen (HLA) alleles in human populations with potentially different binding specificities to predetermined epitopes and various allele frequencies in different ethnicities are two significant obstacles to the successful development of EBVs. Therefore, designing the EBVs based on a set of conserved epitopes with high affinity to the most frequent HLA alleles in a targeted population may improve the EBVs' efficacies [21-23].

Here, we proposed a multivalent EBV against SARA-CoV-2 for the Iranian population. To this end, a T-cell epitope mapping process was performed based on the most frequent HLA-I/II alleles in Iran. Then, an epitope screening was done by considering the toxicity potential as well as physicochemical properties of the predicted epitopes. After structural engineering, vaccine efficacy evaluations were also considered by molecular docking and in silico immune simulation approaches.

Materials and Methods

Antigen selection

To achieve safe, conserved, and effective B-and T-cell epitopes from SARS-CoV-2, we started an antigen selection process. For this purpose, the proteins encoded by the virus (Table 1) were retrieved from UniProt and checked for their antigenicity score, conservancy level, and potential allergenicity by VaxiJen [24], multiple sequence alignment through Clustal Omega and AllerTOP v. 2.0 [25], respectively. Finally, the protein antigen(s) with suitable attributes was/were considered for the vaccine design process.

Linear B-cell epitope prediction

The linear B-cell epitopes from the targeted protein were predicted using Immune Epitope Database (IEDB) through BepiPred-2.0 method [26]. The IEDB is an open-access resource that comprises a wide-ranging col-

Table 1. The encoded proteins by SARS-CoV-2

Protein	UniProt Accession Number	Number of Amino Acids
Spike glycoprotein	P0DTC2	1273
Replicase polyprotein 1ab	P0DTD1	7096
Replicase polyprotein 1a	P0DTC1	4405
ORF7a protein	P0DTC7	121
Nucleoprotein	P0DTC9	419
ORF3a protein	P0DTC3	275
Membrane protein	P0DTC5	222
Envelope small membrane protein	P0DTC4	75
ORF8 protein	P0DTC8	121
ORF6 protein	P0DTC6	61
ORF9b protein	P0DTD2	97
ORF7b protein	P0DTD8	43
ORF3b protein	P0DTF1	22
ORF9c protein	P0DTD3	73
ORF3d protein	P0DTG0	57
ORF3c protein	P0DTG1	41
ORF10 protein	A0A663DJA2	38

lection of empirically assessed B- and T-cell epitopes and a set of tools for epitope prediction and analysis. The BepiPred-2.0 predicts linear B-cell epitopes from a protein sequence, using a Random Forest algorithm trained on epitopes and the non-epitope amino acids determined from crystal structures.

Identification of the most frequent HLA-I/II alleles in the Iranian population

To improve the proposed vaccine efficacy and predict effective T-cell epitopes from the targeted antigen, we determined the most frequent HLA-I/II alleles in the Iranian population by allele frequencies server [27]. Thus, the most frequent HLA-I (HLA-A, HLA-B, and HLA-C) and HLA-II (HLA-DRB) were identified based on the percentages of individuals in the studied sample (n=15600) that had the alleles.

T-cell epitope prediction

The HLA-I and HLA-II restricted epitopes from the targeted protein antigen were mapped by IEDB/MHC-I and IEDB/MHC-II binding prediction modules based on the most HLA alleles in Iran. For HLA-I and HLA-II restricted epitopes, the NetMHCpan El 4.1 [28] and IEDB recommended 2.22 [29] prediction methods were applied, respectively.

Epitope screening

The five top-scored linear B-cell epitopes, predicted by each method, and T-cell epitopes were subjected for the evaluation of their antigenicity potential, allergenicity, and hemolytic potential by VaxiJen, AllerTOP v. 2.0, and HemoPred [30], respectively. The epitopes with suitable features were subjected to the final construct.

Construct engineering and physicochemical features evaluation

The screened B- and T-cell epitopes were arranged in the final construct. To this end, the epitopes were joined together by lysine-lysine (K-K) linkers. The merged epitopes were considered the core of the suggested vaccine. The final construct of the vaccine was prepared after integration of the poly-epitope region with cholera toxin B-subunit (CTxB) (UniProt accession number Q57193) and Type II heat-labile enterotoxin (LT-IIc) (UniProt accession number H6W8F2) using EAAAK linker. Furthermore, molecular weight, net charge at pH 7, isoelectric point, half-life in the mammalian reticulocytes, water solubility, and instability indexes of the anticipated vaccine were predicted using ProtParam [31] and PepCalc [32].

2D and 3D structure prediction

The PRABI with the GOR4 method [33] was used for predicting the secondary structure of the planned vaccine. The method applied possible pair frequencies in a 17 amino acid residues segment with an accuracy of 64.4%. Furthermore, the tertiary structure of the vaccine was estimated through the SWISS-MODEL [34]. SWISS-MODEL is a computerized system for modeling the tertiary structure of a protein using homology modeling methods.

3D model improvement and quality appraisement

To achieve a reliable tertiary structure of the vaccine and reduce structural errors in the predicted model, the primary model was further refined using the 3Drefine [35]. Moreover, the quality of the generated model was assessed and grounded on the Ramachandran plot and ERRAT quality factor by ERRAT [36] as well as z-score through the ProSA [37].

Prediction of discontinued B-cell and interferon (IFN)-γ-stimulating epitopes

ElliPro with default parameters was used for predicting probable discontinued B-cell epitopes from the tertiary structure of the planned vaccine [38]. Additionally, interferon(IFN)-γ-stimulating epitopes in the final construct of the vaccine were also predicted via the IFNepitope through motif and support vector machine (SVM) hybrid prediction method [39].

Evaluation of the vaccine efficacy

The vaccine efficacy was assessed by measuring its affinity to HLA-I, HLA-II, toll-like receptor 3 (TLR3), and TLR4 by molecular docking method via ClusPro v. 2.0 [40]. For this purpose, 3D structures of the mentioned proteins and human serum albumin (HSA) as negative control were retrieved from the protein data bank as represented in Table 2. The raw structures of the subjected proteins were optimized in terms of energy and geometry using UCSF Chimera 1.15 software [41]. Additionally, the possible immune responses against the proposed vaccine were predicted by the C-ImmSim [42] with default parameters (random seed: 12345, simulation volume: 10, simulation steps: 100, host HLA: A MHC class I, B MHC class I, DR MHC class II, a time step of injection: 1, adjuvant quantity: 100, and the number of antigenic particles: 1000). The C-ImmSim predicts immune responses through combined systems biology, especial epitope prediction algorithm, and Miyazawa and Jernigan protein-protein potential measurements.

Virtual cloning process

For expressing the vaccine in E.coli (strain K12), a virtual cloning and codon adaptation process were accomplished. For this purpose, firstly, the amino acid sequence of the vaccine was back-translated to the DNA sequence by the Java Codon Adaptation Tool (JCAT) [43] based on the codon usage table of the mentioned

Table 2. List of the selected target molecules for evaluation of the vaccine efficacy by molecular docking

Protein	PDB Entry	Resolution (Ångstrom)
HLA-I (HLA-A0201)	4UQ3	2.10
HLA-II (HLA-DRB1_01:01)	1AQD	2.45
TLR3	2A0Zv	2.4
TLR4	3FXI	3.10
HSA	1ao6	2.50

HLA: human leukocyte antigen; TLR: toll-like receptor; HAS: human serum albumin.

strain and avoid the rho-independent transcription termination, prokaryote ribosome binding site, and cleavage site of restriction enzyme as additional options. Afterward, the codon adaptation index (CAI) and GC content of the sequence were computed, and then suitable restriction sites were added to 5'- and 3'-OH of the vaccine nucleotide sequence by NEBcutter v. 2.0. Lastly, the DNA sequence of the vaccine was cloned into multiple cloning sites (MCS) of the pET-28a(+) plasmid vector through VectorBiulder.

Results

Antigen selection

Achieving safe and effective B- and T-cell epitopes are directly dependent on the physicochemical properties and toxicity potential of their original proteins. Therefore, all encoded proteins by SARS-CoV-2 were evaluated for their conservancy level, allergenicity, and antigenicity. The results demonstrated that most studied proteins, especially ORF3b, membrane protein, and ORF9b, have appropriate potential antigenicity (Table 3). Predicting the potential allergenicity of SARS-CoV-2 encoded proteins showed that only replicase polyprotein 1ab, membrane protein, and ORF9c protein have allergenic potential. Additionally, the results revealed that replicase polyprotein 1a and ORF7b have the most and least conservancy levels among the proteins. Based on the obtained results, three proteins of ORF3a, ORF7a, and spike glycoprotein were subjected to further analysis because of their suitable properties.

Prediction of linear B-cell epitopes

The results of linear epitope prediction are summarized in Supplementary Table 1. The predicted epitopes with a minimum length of 3 amino acids were considered primary epitopes. A total of 31, 7, and 3 linear B-cell epitopes with different lengths and positions

Table 3. The results of antigen selection process

Protein	VaxiJen Score	Allergenicity	Conservancy Level
Spike glycoprotein	0.5683	No	>99.1
Replicase dd 1ab	0.4202	Yes	>99.3
Replicase polyprotein 1a	0.4207	No	>99.7
ORF7a protein	0.5991	No	>97.5
Nucleoprotein	0.5104	No	>99.5
ORF3a protein	0.772	No	>99.2
Membrane protein	0.6996	Yes	>99.1
Envelope small membrane protein	0.4262	No	>93
ORF8 protein	0.4389	No	>98.3
ORF6 protein	0.5014	No	>93
ORF9b protein	0.6474	No	NA
ORF7b protein	0.6055	No	>72.1
ORF3b protein	0.2842	No	NA
ORF9c protein	0.4733	Yes	NA
ORF3d protein	0.5666	No	NA
ORF3c protein	0.5726	No	NA
ORF10 protein	0.4779	No	>84

Table 4. List of most common human leukocyte antigen (HLA)-I and HLA-II alleles in Iranian population

Allele	Type	% of Individuals With the Allele
A*02	HLA-I	31.3
A*01	HLA-I	21.9
A*03	HLA-I	20.9
A*11	HLA-I	17.3
A*24	HLA-I	25
B*35	HLA-I	28.8
B*51	HLA-I	22.7
DRB1*03	HLA-II	16.2
DRB1*04	HLA-II	19.7
DRB1*07	HLA-II	16.9
DRB1*11	HLA-II	32.4
DRB1*13	HLA-II	18.5
DRB1*15	HLA-II	20.4

were collected from spike glycoprotein, ORF3a, and ORF7a, respectively.

Exploration of the most frequent HLA-I/II alleles in Iranian people

Cellular immunity has a crucial role in the recognition and elimination of virus infections. Therefore, identifying effective T-cell epitopes can increase the success of epitope-based vaccines. Because of the significant diversity of HLA alleles in human populations, predicting T-cell epitopes for the most common HLA alleles can increase vaccine effectiveness. Therefore, here most frequent HLA-I and HLA-II alleles in Iranian people were determined. The results show that (A*02) and (DRB1*11) are the most recurrent HLA-I and HLA-II alleles in the Iranian population, respectively (Table 4).

T-cell epitope prediction

The HLA-I and HLA-II restricted epitopes from SARS-CoV-2 ORF3a, ORF7a, and spike glycoprotein are presented in supplementary Tables 2 and 3, respectively. The results show a total number of 22, 8, and 6 potential HLA-I restricted epitopes (with the ability to bind at least two alleles and scores of more than 0.5) in spike glycoprotein, ORF3a, and ORF7a, respectively. Similarly, 13, 6, and 4 HLA-II restricted epitopes (with IC_{50} less than 200 and percentile ranks less than 10%) were determined in spike glycoprotein, ORF3a, and ORF7a, respectively.

Epitope screening

The development of a safe epitope-based vaccine is dependent on determining harmless B- and T-cell epitopes. Therefore, an epitope screening process was considered to disregard the epitopes with possible allergenicity, hemolytic potential, and low antigenicity. The results of the epitope screening process are presented in supplementary Tables 4 and 5. The evaluations of prospective antigenicity of the predicted T-cell epitopes confirmed that, generally, HLA-I restricted epitopes have higher potential antigenicity than HLA-II restricted epitopes. The results also confirm that ORF3a has more antigenic T-cell epitopes than the other proteins. Additionally, predicting the potential allergenicity and hemolytic of the T-cell epitopes reveal that HLA-II restricted epitopes, especially the epitopes with the spike glycoprotein origin, have higher allergenicity and hemolytic potentials. Moreover, the obtained results about the linear B-cell epitopes confirmed that the epitopes from the spike glycoprotein and ORF7a have the most and least potential antigenicity, respectively. Besides, the B-cell epitopes with allergenicity and hemolytic potentials are more frequent than the other antigens. Finally, the B- and T-cell epitopes with

Table 5. Screened B- and T-cell epitopes from spike glycoprotein, ORF7a, and ORF3a, subjected to vaccine designing process

Epitopes	Type	VaxiJen Score	Origin
VYDPLQPEL	HLA-I	0.4525	Spike glycoprotein
LGAENSVAY	HLA-I	0.4173	Spike glycoprotein
LPFNDGVY	HLA-I	0.5627	Spike glycoprotein
VLPFNDGVYF	HLA-I	0.4809	Spike glycoprotein
FPNITNLCPF	HLA-I	1.3964	Spike glycoprotein
RIFTIGTVTLK	HLA-I	1.2278	ORF3a
FTIGTVTLK	HLA-I	2.0317	ORF3a
SVSPKLFIR	HLA-I	0.5973	ORF7a
YSKHTPINL	HLA-II	1.0547	Spike glycoprotein
LIVNNATNV	HLA-II	0.4125	Spike glycoprotein
ITLKKRWQL	HLA-II	1.9347	ORF3a
VRATATIPI	HLA-II	0.7515	ORF3a
LALSKGVHF	HLA-II	1.1343	ORF3a
VKHVYQLRA	HLA-II	0.7008	ORF7a
KHTPINLVRDLPQGFS	B-cell	0.6403	Spike glycoprotein
YNSASFSTFKCYGVSPTKLNDLCFT	B-cell	1.4031	Spike glycoprotein
GDEVRQIAPGQTGKIADYNYKLP	B-cell	1.1017	Spike glycoprotein
VNNSYECDIPI	B-cell	0.6124	Spike glycoprotein
KIITLKKRWQL	B-cell	1.0171	ORF3a
VKHVYQLRARSVSPKLFIRQEEVQEL	B-cell	0.6123	ORF7a

appropriate properties (VaxiJen score more than 0.4 and without allergenicity and hemolytic potential) were selected for the final vaccine construct (Table 5).

Construct engineering and physicochemical features evaluation

As depicted in Figure 1, the selected B- and T-cell epitopes were combined with the adjuvants to form the final

Table 6. The result of tertiary model refinement by 3Drefine Server

Model	3Drefine Score	GDT-TS	GDT-HA	RMSD	RWPlus	MolProbity
1	6583.86	1	1	0.267	-10214.970252	2.371
2	6666.05	1	1	0.242	-10176.306440	2.312
3	6746.64	1	1	0.216	-10111.667991	2.344
4	6850.90	1	1	0.184	-10098.821039	2.399
5	7796.80	1	1	0.140	-10037.793207	2.331

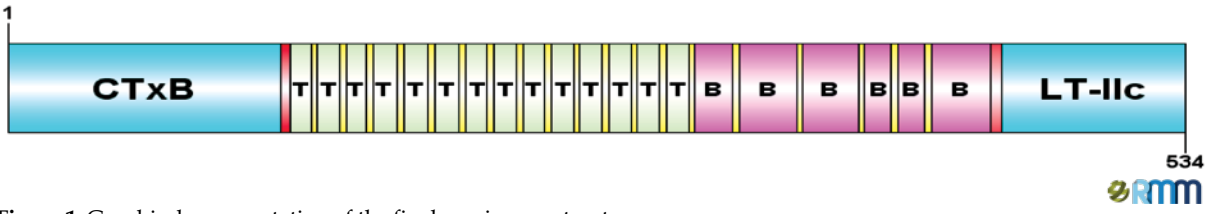

Figure 1. Graphical representation of the final vaccine construct

The yellow and red regions are EAAAK and KK linkers, respectively. B and T are one-letter codes for B- and T-cell epitopes, respectively.

Figure 2. Secondary (left) and tertiary (right) structures of the proposed vaccineh: helix; e: extended strand; c:random coil

vaccine construct. The proposed vaccine comprised six linear B-cell epitopes, eight HLA-I restricted epitopes, and six HLA-II restricted epitopes. The physiochemical properties of the proposed vaccine were also evaluated. The results show that the vaccine is a 60.3 kDa, stable, and good water-soluble chimer protein. The vaccine isoelectric point (pI), net charge, and predicted half-life in mammalian reticulocytes were ascertained to be 10.02, 60.5, and 30 h, respectively.

2D and 3D structure prediction

The secondary and tertiary structures of the suggested vaccine are depicted in Figure 2. The results confirm that 35.21%, 18.35%, and 46.44% of the total of 534 amino acids are structured in alpha-helix, beta-strand, and random coil sequentially. Furthermore, the 3D structure of the vaccine was modeled by SWISS-MODEL through the homology modeling method. The predicted model shows GMQE (Global Model Quality Estimate) and QMEANDisCo (distance constraint) of 0.4 and 0.40 ±0.07, respectively. The parameters indicate an overall model quality measurement between 0 and 1, with higher numbers indicating higher expected quality. Additionally, the predicted model showed a sequence identity of 69.33% in the coverage area with the automatically selected template (with PDB entry of 3D0G).

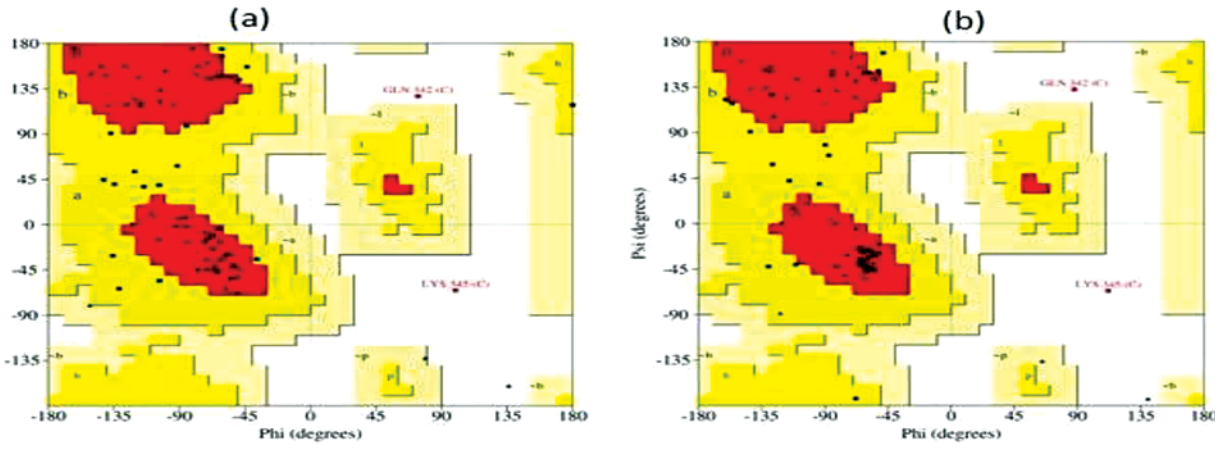

Figure 3. Ramachandran plots of primary (a) and Refined (b) 3D structures of the suggested vaccine

In both structures, 77.1%, 20%, and 2.9% of amino acids were placed in favored, allowed, and outlier regions, respectively.

Table 7. Predicted discontinued B-cell epitopes from 3D structure of the recommended vaccine

Epitope Composition	Number of Residues	Score
P354,K355,K356	3	0.776
S302,K303,N306,S307,A308,S309,F310	7	0.7
K275,V276,K277,H278,Y280,Q281,L282	7	0.671
R283,A284,K285,K287,P290,I291,N292,L293,V294,R295,D296,L297,Q299	13	0.66
R336,Q337,A339,P340,G341,Q342,T343,G344,K345,D348,Y349,N350	12	0.539

Model refinement and quality assessment

To improve the quality of the predicted 3D structure of the planned vaccine, a model refinement was performed by the 3Drefine server. In the process of secondary structure features, loop areas and side-chains were refined. The outputs of the 3Drefine server are five quality improved tertiary structures of primary models, categorized based on potential energy (3Drefine score and RWPlus), similarity score (GDT-TS and GDT-HA), division score, and physical realism score. A refined model with higher GDT-TS, GDT-HA, and RMSD, a lower 3Drefine score, RWPlus, and MolProbity, designate a reliable 3D structure model. Therefore, based on the results of the refinement process (Table 6), the model with appropriate parameters was subjected to further analysis. Moreover, the quality of the improved model was checked by the Ramachandran plot, z-score, and ERRAT quality factor. As illustrated in Figure 3, there is no significant difference between primary and refined models in terms of the presence of amino acid residues that were found in favored, allowed, and outlier regions. So that in both models, 77.1%, 20%, and 2.9% of residues were placed in favored, allowed, and outlier regions, respectively. The ERRAT quality factor and z-score of the primary model were respectively 47.94 and -1.86, whereas mentioned parameters were computed to be 63.79 and -2.32, demonstrating some refining in the geometric quality of the primary model.

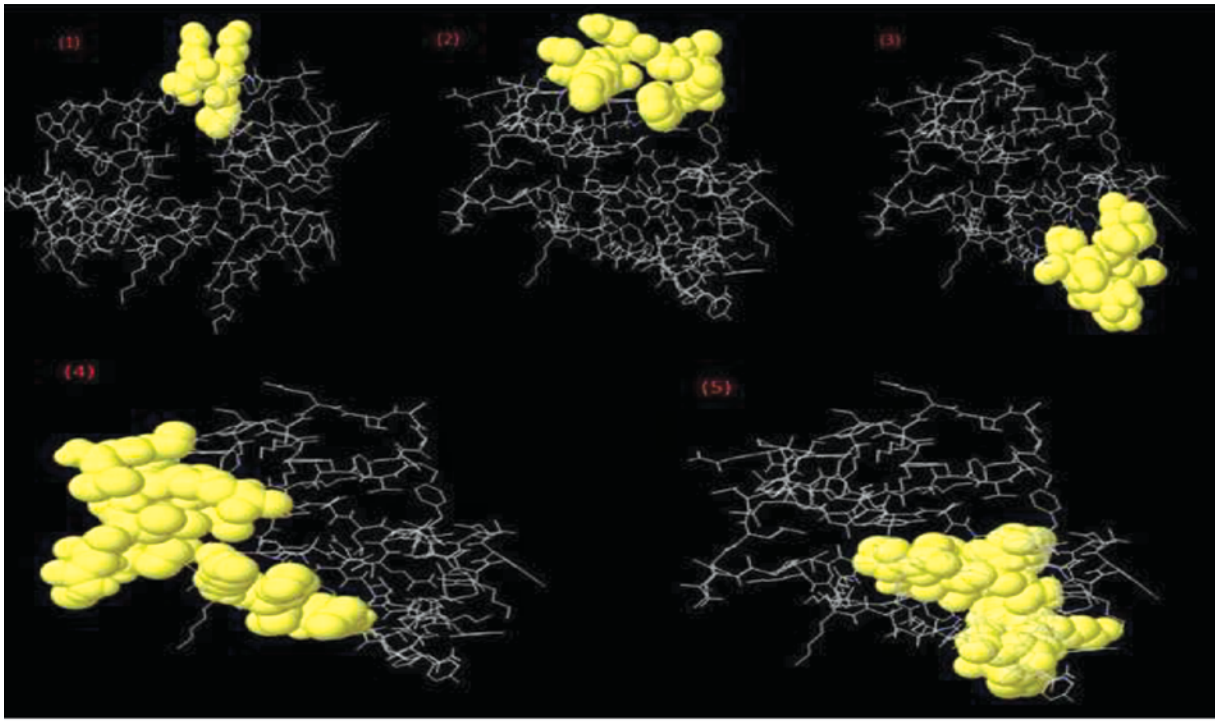

Figure 4. The predicted conformational B-cell epitopes from the proposed vaccine against SARS-CoV-2
Five discontinued B-cell epitopes were identified from the vaccine.

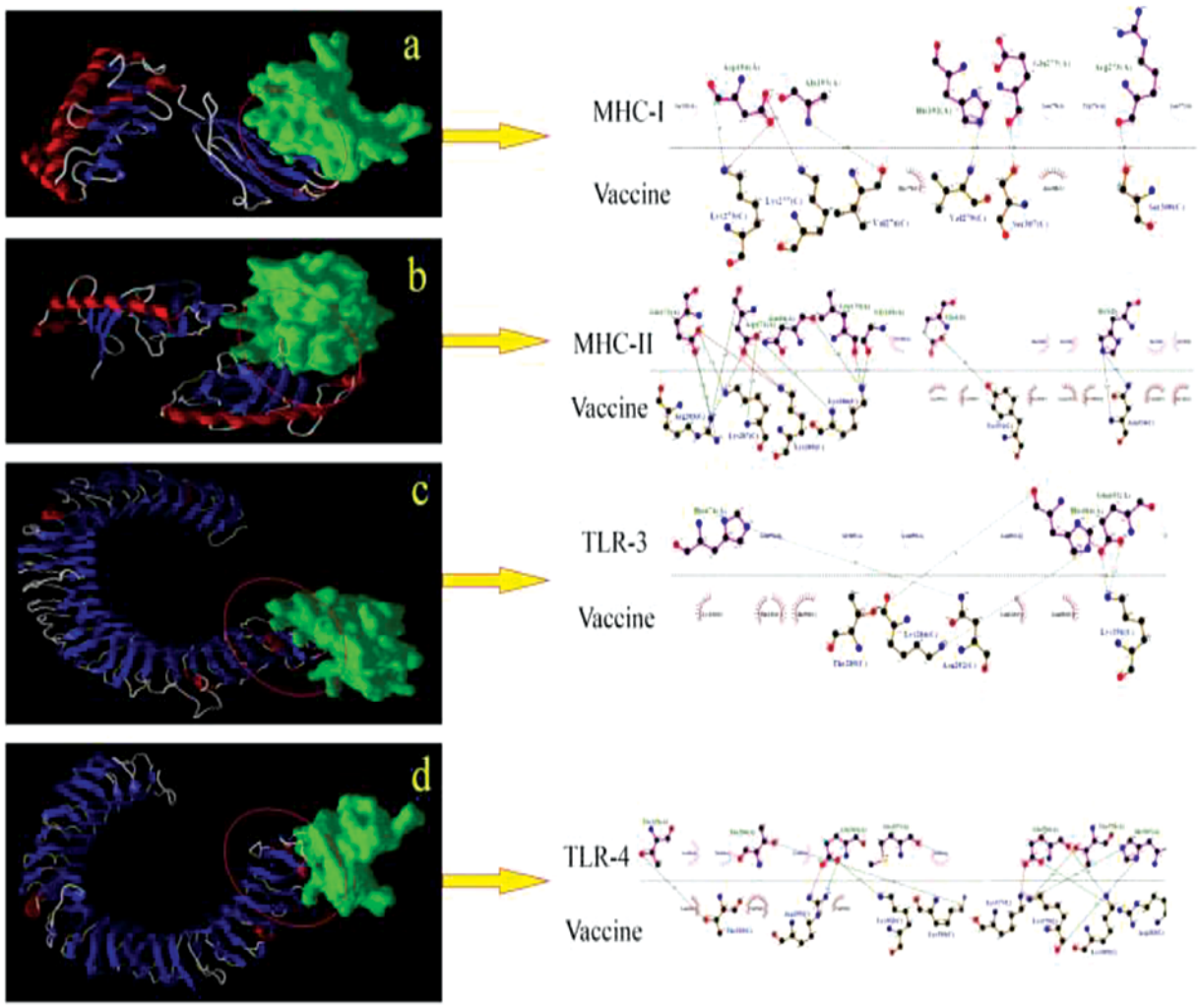

Figure 5. The interactions of the planned vaccine with human leukocyte antigen (HLA)-I (a), HLA-II (b), toll-like receptor (TLR)-3 (c), and TLR-4 (d)

Prediction of discontinued B-cell and IFN-γ-inducing epitopes

Because of the importance of conformational B-cell epitopes and IFN-γ-inducing epitopes in pathogen recognition and antigen neutralization, the probable discontinued B-cell epitopes and IFN-γ-inducing epitopes in 3D and primary structures of the proposed vaccine were predicted. The results showed 5 probable conformational B-cell epitopes in the vaccine (Figure 4). The epitopes were composed of 42 amino acids, and most of them were organized in a particular linear manner (Table 7). Furthermore, searching the amino acid sequence of the vaccine showed 97 probable 15-mer IFN-γ-inducing epitopes with a score of 1 and more (Supplementary Table 6). The epitopes were found in the adjuvants and the multi-epitope region, especially the 130-144 and 260-290 regions.

Evaluation of the vaccine efficacy

The molecular docking evaluations between the proposed vaccine and HLA-I, HLA-II, TLR-3, and TLR-4 were performed to evaluate the vaccine efficiency. The results confirm that the vaccine has a high affinity to the tested molecules, particularly the HLA-II molecule with a docking score of -1038.4 kcal/mol, followed by HLA-I (-766.7), TLR-3 (-628.2), and TLR-4 (-625.2). It was also found that the vaccine affinity to all studied targets was more than HSA as negative control (-622.2 kcal/mol). The vaccine interactions with the selected target molecules are depicted in Figure 5. Additionally, the probable immune responses to the proposed vaccine were predicted using in silico immune simulation. The results confirm that the vaccine could successfully elicit both humoral and cellular responses (Figure 6). The results also demonstrate that the IFN-γ level increased after the vaccine administration.

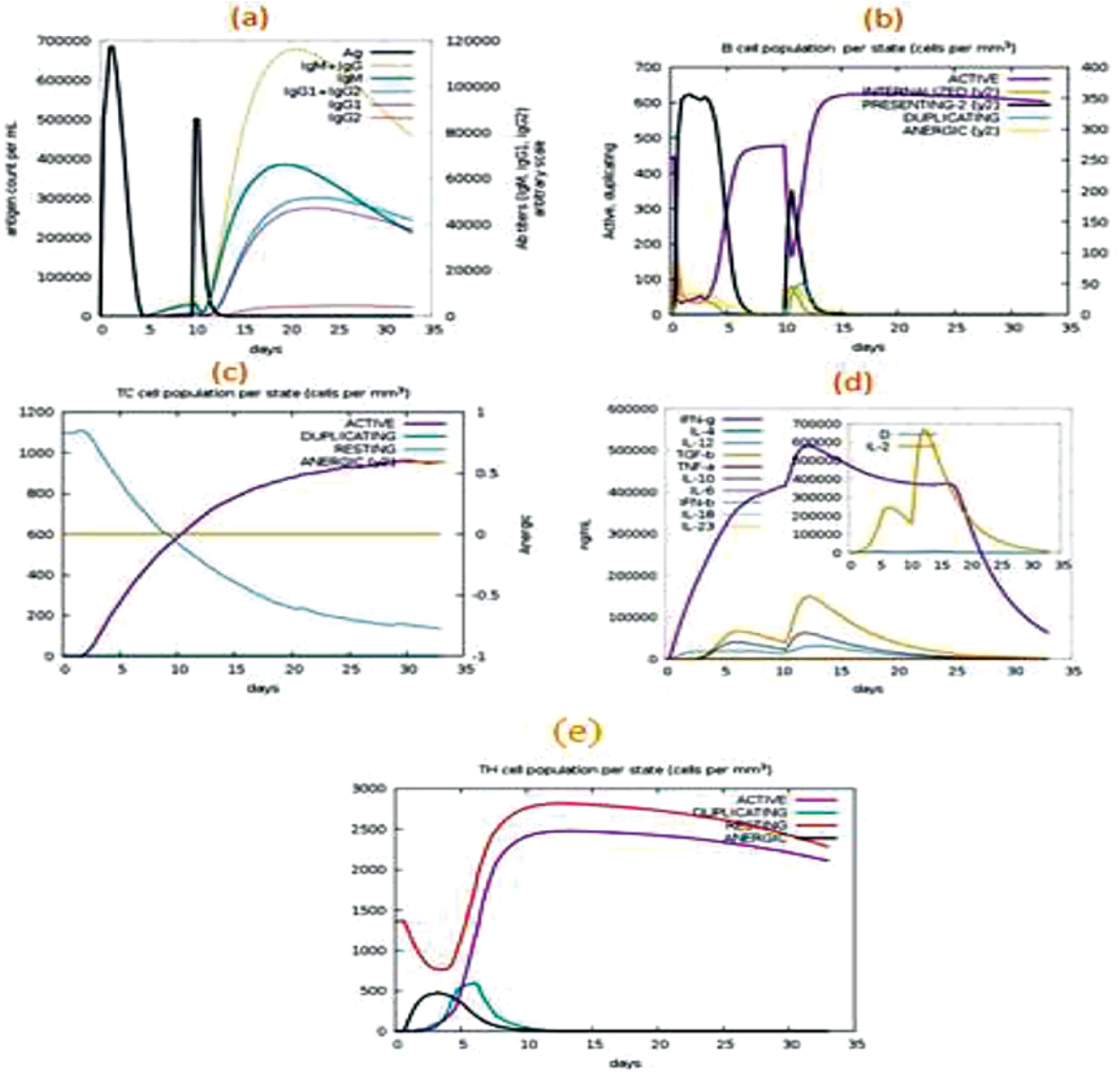

Figure 6. The results of immune simulation using the proposed vaccine

Increasing the IgG and IgM antibodies (a), B lymphocytes population (b), T cytotoxic lymphocytes population (c), the quantity of interferon-gamma (d), and T helper lymphocytes population (e) after the vaccine administration reflects the significant efficacy of the selected epitopes and the vaccine effectiveness.

In silico cloning

A virtual cloning process was performed to evaluate the expression potential of the proposed vaccine in a bacterial host. For this purpose, the back-translated sequence of the vaccine was codon-optimized, and its key features, including CAI and GC content, were computed. The recombinant expression vector is depicted in Figure 7. The CAI and CG content were calculated to be 0.95 and 45%, presenting the probability of good expression in the E. coli host because the ideal ranges for CAI and GC are 0-1 and 30%-70%, respectively [7].

Discussion

Since the first report of SARS-CoV-2, many investigations have been conducted to introduce effective vaccines against COVID-19. Computationally designed multi-epitope vaccines are highly regarded because of their safety, engineerability, serotype independency, and high immunogenicity properties. Despite the advantages of the multi-epitope vaccine, high variation in HLA alleles is a serious limitation against their practical administration [44-48]. Therefore, we proposed a multi-epitope vaccine based on the most common HLA-I/II alleles in the Iranian population.

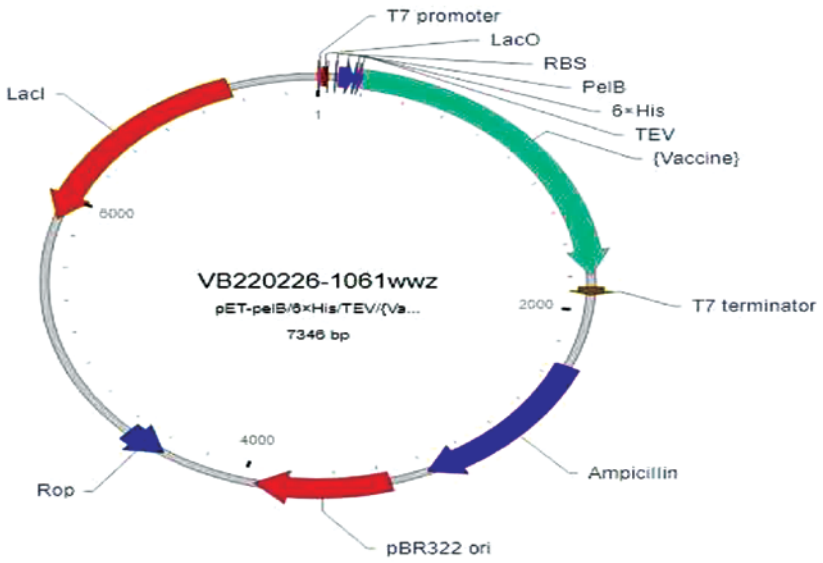

Figure 7. The pET-28a(+) plasmid vector, containing final nucleotide sequence of proposed vaccine against SARS-CoV-2 (green region)

Previous studies confirm the importance of T-cell responses in COVID-19 severity and protective immunity [49, 50]. Subsequently, considering T-cell epitopes that target the most frequent HLA alleles may improve an epitope-based vaccine. Here, we proposed a multi-epitope vaccine, including B- and T-cell epitopes from the ORF3a, ORF7a, and spike glycoprotein of SARS-CoV-2 using a multi-step screening and immunoinformatics approach.

Several in silico-designed multi-epitope vaccines have already been proposed against COVID-19, primarily engineered based on only one antigen. To our knowledge, the spike glycoprotein has been subjected to vaccine design in most studies [51-53]. However, high variation in the spike glycoprotein may restrict vaccine effectiveness and cross-reactivity. Moreover, recent studies demonstrate that other protein antigens from SARS-CoV-2, including the M protein, N protein, non-structural proteins, and accessory proteins, can be considered target antigens for vaccine development [51, 54]. Therefore, in this study, an antigen screening process was performed to evaluate the antigenicity, allergenicity, and conservancy level to achieve safe, conserved, and effective B-and T-cell epitopes from SARS-CoV-2. We selected ORF3a, ORF7a, and spike glycoprotein of SARS-CoV-2 based on the mentioned parameters. ORF3a is a viral ion channel contributing to inflammasome activation, apoptosis, and autophagy inhibition. Recent research has confirmed that deletion of the protein reduces SARS-CoV-2 titer and morbidity rate in mice, suggesting that it is an effective target for vaccine development [55]. ORF7a encodes a 121-amino acid long protein with almost 86% sequence identity to the ORF7a of SARS-CoV-1. The protein is expressed on the surface of infected cells and can provoke both humoral and cellular immune responses. Therefore, ORF7a is a suitable target antigen for vaccine design [56, 57].

The forecast of linear B-cell epitopes from the screened antigens showed that the spike glycoprotein has the most epitope density, followed by ORF3a and ORF7a. The predicted epitopes were found in different regions of the origin proteins. To achieve safe and effective linear B-cell epitopes, an epitope screening process was carried out based on the epitopes' antigenicity, allergenicity, and hemolytic potential. A total of 6 linear B-cell epitopes were subjected for the final vaccine extract. Regarding the selected linear B-cell epitopes, some epitopic regions in the predicted epitopes, such as "VNNSYECDIPI" [58], "KHTPINLVRDLPQGFS" [59], "KIITLKKRWQL" [44], and "VKHVYQLRARSVSPKLFIRQEEVQEL" [60] have previously been confirmed as effective linear B-cell epitopes.

Analysis of the HLA data in the Iranian population showed that HLA-A*02 and HLA-DRB1*11 are the most frequent HLA alleles. Furthermore, previous reports confirmed the association of the alleles with viral diseases [61, 62]. Therefore, in the most suggested epitope-based

vaccines against COVID-19, the alleles have been considered in the T-cell epitope prediction process. Similar to linear B-cell epitopes, a screening process was performed for the predicted T-cell epitopes. The screened epitopes organized in the final vaccine construct included six linear B-cell epitopes, eight HLA-I restricted T-cell epitopes, and six HLA-II restricted T-cell epitopes, as well as LT-IIc and CTxB as natural adjuvants. Unlike HLA-I restricted T-cell epitopes and linear B-cell epitopes, most of the screened HLA-II restricted T-cell epitopes in the final structure had an ORF3a origin. The high T-cell density of the ORF3a has been demonstrated in other studies. In this regard, Hisham et al. showed that the ORF3a had a high $CD4^+$ and $CD8^+$ epitope density with 0.92 allele coverage [63]. Peng et al. confirmed significant SARS-CoV-2-specific T cell responses against the ORF3a [64]. Grifoni et al. demonstrated that the ORF3A was responsible for approximately 7% of total immune responses in convalescent COVID-19 patients [65].

As common approaches in in silico vaccine design studies, molecular docking [66] and immune response simulation [42] methods were considered for evaluating the proposed vaccine efficacy. The molecular docking between the vaccine and HLA-I/II molecules revealed its high affinity to the receptors and efficacy of the selected T-cell epitopes. Furthermore, because of the important role of TLR-3 and TLR-4 in recognizing viral patterns [67], the probable interactions between the vaccine and the molecules were also evaluated. The results demonstrate that the vaccine has a high binding affinity with almost equal energy bindings. Moreover, simulation of possible immune responses to the vaccine by C-ImmSim shows that both humoral and cellular responses can be expected from the vaccine. Because of the importance of immune response type in reinfection rate and severity of infectious diseases, especially in COVID-19, changes in different lymphocyte populations, including B lymphocytes, T cytotoxic lymphocytes, and T helper lymphocytes, were monitored over 35 days. Our results demonstrate that both B- and T helper lymphocytes trailed a logarithmic phase in the first ten days, followed by a decreasing phase with a gentle slope.

In contrast, decreasing phase in the T cytotoxic lymphocytes diagram was not observed during the evaluation time. In this regard, previous studies [68, 69] also show that a high level of cytotoxic T-cell responses could be detected in patients with mild COVID-19. Furthermore, the related works [43, 70] showed that SARS-CoV-2 specific antibody and T helper responses are measurable in all patients when cytotoxic responses may not be elicited and may lead to severe disease. Therefore, a specific vaccine with the ability to stimulate strong and stable B and T lymphocyte responses can be considered a promising weapon against SARS-CoV-2 infection.

The final vaccine construct includes conformational B-cell epitopes and IFN-γ-inducing epitopes, which can affect the vaccine efficacy and memory of SARS-CoV-2. To evaluate the successful expression of the designed vaccine in a bacterial host, codon optimization was performed. Hopefully, both CAI (0.95) and GC content (45%) were appropriate for high-level expression of the vaccine in bacteria. However, despite promising results, more in silico, in vitro, and in vivo evaluations are needed to consider the proposed vaccine as a candidate against COVID-19 in Iran.

Conclusion

Because of the high genetic variation in HLA alleles, epitope-based vaccines do not produce equal efficacy in different human populations. Therefore, in this study, we proposed a multi-epitope vaccine based on the B- and T-cell epitopes from the ORF3a, ORF7a, and spike glycoprotein of SARS-CoV-2 considering the most frequent HLA alleles in the Iranian population. The results confirmed that the vaccine has appropriate physicochemical properties and high affinity to HLA-I, HLA-II, TLR-3, and TLR-4. Despite promising results, more in silico, in vitro, and in vivo evaluations are required to consider the proposed vaccine as an effective anti-COVID-19 for the Iranian population.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article.

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Authors contribution's

Data collection and analysis, draft preparation, review, and editing: Faezeh Soltanveis; Conceptualization and supervision: Mokhtar Nosrati.

Conflict of interest

The authors declared no conflict of interest.

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