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Protein E-Peptide Driven Vaccine for Novel Coronavirus: Immunoinformatics

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ABSTRACT

Background: The COVID-19 pandemic is a red alarm for global health, so researchers around the world are working on it to design an effective vaccine against it. Protein is one of the candidates for vaccine development which plays an important role in virus pathogenesis. Accordingly, this study was done to evaluate the critical characteristic of this protein as a vaccine candidate using in-silico analysis.

Methods: The sequence of SARS-CoV-2-associated E protein was recruited from NCBI and subjected to the IEDB software to evaluate the most potent epitopes. The capacity of the interactions of HLA-I and HLA-II molecules with selective peptides was studied using IEBD tool kit. The E protein sequence was subjected to B cell and T cell tests to realize the most promising peptides that could act as COVID-19 vaccine.

Results: Among the tested peptides for the T cell-test, this study found two interesting epitopes: VSEETGTLI and LTALRLCAY that exhibit high binding affinity as a strong indicator to HLA-I and HLA-II alleles together. The results of the analysis demonstrated that some epitopes in the E protein have a relatively higher immunogenicity score based on interaction with HLA-II, such as SEETGTLIVNSVLLF, TLIVNSVLLFLAFVV, LAFVVFLLVTLAILT, LAILTALRLCAYCCN, and SVLLFLAFVVFLLVT. Furthermore, two sequences (FVSEET and PSFYVYSRVKNLNSSRVP) were reported as the selective linear epitopes for B cell, on the surface of SARS-CoV-2 E protein and being Immunogenic.

Conclusion: Since E protein can stimulate favorable immune responses, T and B- cell responses, its evaluation in patients with COVID-19 is of a great importance.

Keywords: SARS-CoV-2, E protein, Bioinformatics, Vaccine, Epitope

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Introduction

SARS-CoV-2 is an enveloped, single-stranded RNA virus with 29,903 nucleotides in the genome. It is associated with coronavirus disease (COVID-19). The infected individuals with COVID-19 experience mild to severe respiratory complaints, however, people with underlying health conditions such as cancer, heart diseases, diabetes, or chronic respiratory diseases are more susceptible to developing serious symptoms than the others. The rapid spread of COVID-19 along with the emergence of mutant strains made it a red alarm on public health (1). During the COVID-19 pandemic, varieties of vaccine formulations have been proposed, however, most of them considered the critical proteins of the virus in different strategies (2). According to WHO estimates in January 2021, 63 candidate vaccines have been entered into the human clinical trials and more than 172 candidates are in the preclinical phase worldwide (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines).
Among 30 known proteins encoded by the virus genome, nucleocapsid protein (N), membrane glycoprotein (M), Envelope (E), and spike glycoprotein (S), are four crucial proteins during the virus life cycle and in attention for Vaccine development (3). The N and M proteins are essential molecules to form viral nucleocapsid. The S protein plays an important role in viral attachment and fusion to the host cell (4). Envelope (E) protein is a small integral membrane protein involved in envelope formation and virus assembly (5-8). During virus replication, the E protein is highly expressed inside the host cells, however, only a small fragment of E protein is integrated into the virus envelope. The present study aimed to compare the antigenicity of four structural proteins of COVID-19 (S, M, E, and N) as potential targets for vaccine development through in silico analysis.

Methodology

Study type

The present study is an in silico analysis which was ethically approved by the Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (IR.AJUMS.REC.1399.563).

Evaluation of the antigenic potential of N, S, M and E proteins in SARS-CoV-2

VaxiJen v2.0 webserver (http://www.ddg pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) was used to predict the antigenicity of 3 critical SARS-CoV-2 proteins (N, S, M, and E). The protein sequences of all proteins were retrieved from the National Center of Biotechnology Information (*NCBI*) (https://www.ncbi.nlm.nih.gov/) and integrated into the VaxiJen v2.0 as the query. The score 0.4 was considered as the target protein for further analysis (9).

Prediction of T-cell epitopes.

To predict the HLA-I/II T-cell epitopes, The ANN (http://tools.iedb.org/mhci/) and NetMHCII (http://tools.iedb.org/mhcii/) web servers were applied (10, 11). Conserved immuno-dominant peptides with *IC50 < 100* were selected for the next analysis.

The HLA Binding site prediction

The *IEDB package* (http://tools.iedb.org) was used to predict the binding of *HLA* class I/II to the E protein. The antigenicity methods of Class I immunogenicity (http://tools.iedb.org/immunogenicity/) and CD4 T cell immunogenicity prediction (http://tools.iedb.org/cd4episcore/) were proposed to determine the sites of antigenic epitopes to *HLA class I and II*, respectively (12, 13).

Prediction of CTL epitopes in E-protein

The NetCTL (http://tools.iedb.org/netchop/help/#netctl) was used to predict CTL epitopes in E protein (14).

Prediction of E-protein proteasomal cleavage site

Proteasomal cleavage sites in the E protein sequence of the SARS-CoV-2 virus were predicted by means of the *NetChop* webserver (https://services.healthtech.dtu.dk/). The prediction is based on the location of acid amine residues (15, 16).

Probability of the antigenicity of E-protein

Both *NetCTL* and *NetChop* webservers can predict the probability of the antigenicity of E-protein based on E pritein specific sequence in each virus.

Antibody Epitope Prediction

To evaluate the antibody epitope, *IEBD*-antibody epitope prediction toolkit analysis was performed through Parker, Emini, Kolaskar, as well as Tongaonkar gateways.

Parker Hydrophilicity Prediction was used to analyze the hydrophilicity of the predicted epitopes with the default threshold (17). Besides, Emini surface accessibility prediction was used to evaluate the probability of being surface based on the sequence position with the threshold value of 1.000 (18). Kolaskar and Tongaonkar antigenicity scale were also performed to predict the peptide antigenicity by using physicochemical properties of residues and their frequencies in known B-cells epitopes (19). Default threshold was set at 1.00 in both.

Tertiary structure (3D) Modelling of SARS-CoV-2 E protein

The amino acids sequence of E protein was queried into the RaptorX web server (http://raptorx.uchicago.edu/Structure Prediction/). RaptorX predicts the 3D protein structure based on protein secondary and tertiary structures as well as solvent accessibility and disordered regions (20).

Results

Based on the *VaxiJen*-extracted data, the E protein showed a better antigenicity than M, N, and S proteins (Probability of antigenicity: 0.6025 vs. 0.5102, 0.5059, and 0.4646, respectively).

Table 1 shows the E protein-originated peptide sequences which were processed in the *HLA-I and HLA-II* pathways. All the sub-classes of *HLA-I and HLA-II* presenting these selective peptides were also predicted (Table1). Totally, 17 peptides were predicted for HLA-I peptides and 9 peptides were found for HLA-II. As indicated in Table1, the different conserved sequences (15-mer sequence) were attached to different sub-classes of *HLA-I* and *HLA- II*. The predicted proteasome target site of the E protein has been presented in Figure1.

Table 1. The HLA-I and HLA-II related peptides in E protein of SARS-CoV-2 based on predicted percentile IC50 <100

Peptide Sequence	HLA-I	Peptide Sequence	HLA-II
			HLA-DRB1*12:01
	HLA-B*15:01		HLA-DRB4*01:03
LTALRLCAY	HLA-B*15:25	LAILTALRLCAYCCN	HLA-DRB1*01:01
	HLA-B*35:01		HLA-DRB1*13:01
			HLA-DPA1*01:03/DPB1*06:01
			HLA-DQA1*01:02/DQB1*05:01
FLAFVVFLL	HLA-A*02:01		HLA-DRB1*04:04
	HLA-A*02:06	FLLVTLAILTALRLC	HLA-DRB4*01:03
	HLA-A*68:02		HLA-DRB1*01:01
			HLA-DRB1*13:01
TLAILTALR	HLA-A*68:01		HLA-DRB5*01:01
	HLA-A*33:03		HLA-DRB1*10:01
	HLA-A*33:01		HLA-DRB3*03:01
	HLA-A*31:01		HLA-DPA1*01:03/DPB1*06:01
			HLA-DQA1*01:02/DQB1*05:01
VLLFLAFVV	HLA-A*02:01		HLA-DQA1*02:01/DQB1*03:03
	HLA-A*02:06		HLA-DQA1*02:01/DQB1*03:01
	HLA-A*02:06		
SVLLFLAFV	HLA-A*02:01	LAFVVFLLVTLAILT	HLA-DRB3*03:01
	HLA-A*68:02		HLA-DPA1*01:03/DPB1*06:01
			HLA-DQA1*01:02/DQB1*05:01
	HLA-A*02:01		HLA-DRB3*03:01
FLLVTLAIL	HLA-A*02:06	SEETGTLIVNSVLLF	HLA-DRB1*13:02
			HLA-DQA1*02:01/DQB1*03:01
			HLA-DQA1*01:02/DQB1*05:01
NSVLLFLAF	HLA-B*15:25	SVLLFLAFVVFLLVT	HLA-DPA1*01:03/DPB1*06:01
	HLA-B*35:01		HLA-DPA1*01:03/DPB1*02:01
FVVFLLVTL	HLA-A*02:06	LVKPSFYVYSRVKNL	HLA-DRB1*08:01
	HLA-A*68:02		HLA-DRB1*07:01
	HLA-A*02:01		HLA-DRB1*09:01

Table 1. The HLA-I and HLA-II related peptides in E protein of SARS-CoV-2 based on predicted percentile IC50 <100

FVSEETGTL	HLA-C*03:02 HLA-C*12:03 HLA-C*03:02 HLA-C*03:04 HLA-C*03:03 HLA-C*03:04 HLA-C*03:03 HLA-C*12:03 HLA-C*03:02 HLA-C*12:02 HLA-C*02:02 HLA-C*02:09 HLA-C*16:01		HLA-DRB1*15:01 HLA-DRB1*11:01 HLA-DRB4*01:03 HLA-DRB3*03:01 HLA-DRB1*01:01 HLA-DRB1*07:01 HLA-DRB1*09:01 HLA-DRB1*15:01 HLA-DRB1*08:01 HLA-DRB4*01:03 HLA-DRB1*13:01 HLA-DRB3*03:01 HLA-DRB1*01:01 HLA-DQA1*06:01/DQB1*04:02
VTLAILTAL	HLA-A*02:06 HLA-C*16:01 HLA-C*03:04 HLA-C*03:03 HLA-C*03:02 HLA-C*12:03	TLIVNSVLLFLAFVV	HLA-DRB3*03:01 HLA-DRB1*13:02 HLA-DRB3*03:01 HLA-DRB1*13:02 HLA-DPA1*01:03/DPB1*02:01 HLA-DPA1*01:03/DPB1*06:01 HLA-DQA1*01:02/DQB1*05:01
LAILTALRL	HLA-C*03:04 HLA-C*03:03 HLA-C*03:02 HLA-C*12:03		
LVKPSFYVY	HLA-B*15:25 HLA-B*15:01 HLA-B*15:02 HLA-A*30:02 HLA-A*29:02 HLA-B*35:01		HLA-DRB1*04:01 HLA-DRB1*08:01 HLA-DRB1*08:02 HLA-DRB1*04:05 HLA-DRB1*09:01 HLA-DRB1*11:01
LVKPSFYVY	HLA-B*15:25 HLA-B*15:01 HLA-B*15:02 HLA-A*30:02 HLA-A*29:02 HLA-B*35:01	FYVYSRVKNLNSSRV	HLA-DRB1*01:01 HLA-DRB1*07:01 HLA-DRB1*13:02 HLA-DRB3*02:02 HLA-DRB1*16:02
SSRVPDLLV	HLA-A*30:01 HLA-B*15:25 HLA-B*35:01		HLA-DRB1*04:04HLA-DRB1*10:01HLA-DQA1*01:02/DQB1*05:01
SEETGTLIV	HLA-B*40:01 HLA-B*40:02		
VSLVKPSFY	HLA-A*30:02 HLA-B*15:25		
VSEETGTLI	HLA-C*05:01		

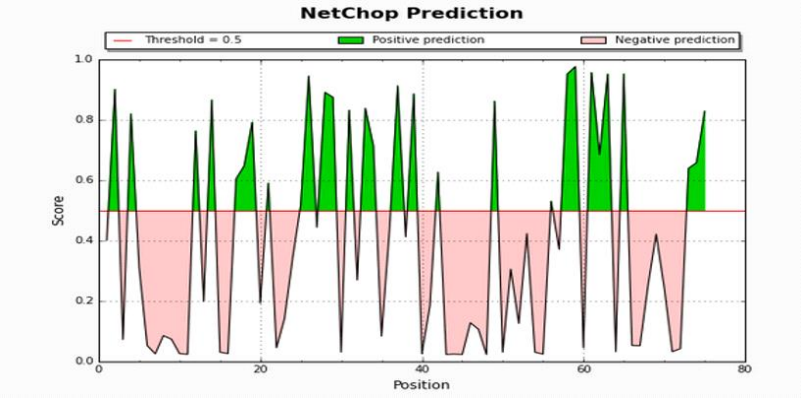
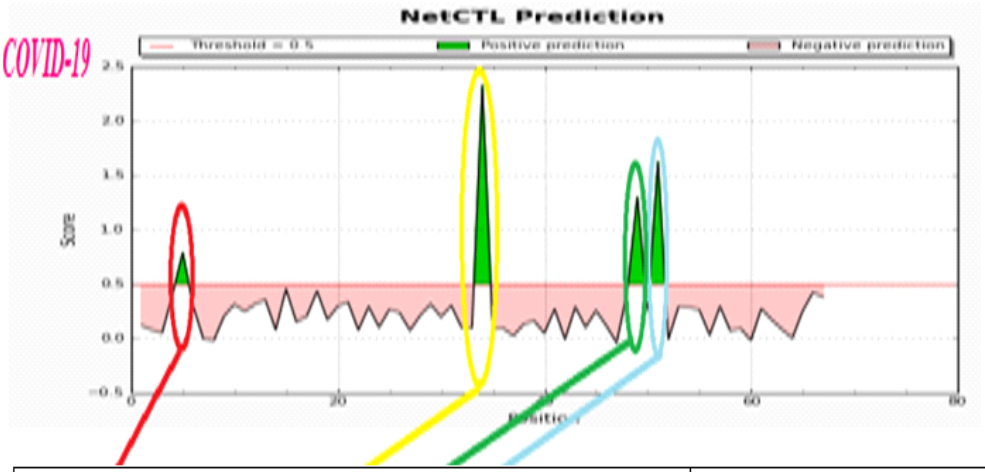


Figure 1. NetChop predicts the residues, which are targets for cleavage by the human proteasome. The positive predictions are displayed in green, while the predictions below the threshold value are in red.

Following epitope prediction, four T cell epitopes were selected from the SARS-CoV-2 E protein. These included: 1) "*VSEETGTLI", 2) "LTALRLCAY", 3)"VSLVKPSFY", and 4) "LVKPSFYVY"*. The corresponding positions have been shown in Figure 2. All of these predicted epitopes have a high affinity for binding to *HLA* molecules (Score > 0.5). It has been found that epitopes "*VSEETGTLI* "and "*LTALRLCAY"* had high immunogenicity than the peptides "*LVKPSFYVY"* and "*VSLVKPSFY"* (Table 2).



Peptide sequence	Predicted MHC binding affinity	Rescale binding affinity	C-terminal cleavage affinity	TAP transport efficiency	Prediction score
VSEETGTLI	*0.1122*	*0.7620*	*0.9211*	*0.4910*	*0.9247*
LTALRLCAY	*0.3125*	*2.1226*	*0.6321*	*2.9220*	*2.3636*
VSLVKPSFY	*0.1672*	*1.1358*	*0.6687*	*3.0430*	*1.3882*
LVKPSFYVY	*0.1947*	*1.3223*	*0.9991*	*3.0630*	*1.6254*

Figure 2. NetCTL predicts CTL epitopes in protein sequences, which is the main epitope in designing vaccine based on E-protein sequence because of their essential role in the stimulation of CTL through HLA-I.

Table 2. NetCTL software analysis

Peptide	Length	Score
VSEETGTLI	9-mer	0.22923
LTALRLCAY	9-mer	0.01886
LVKPSFYVY	9-mer	-0.11106
VSLVKPSFY	9-mer	-0.25372

Prediction results are sorted by descending score values. This table shows that four selective epitopes extracted with NetCTL software have higher immunogenicity scores and indicate a greater probability of eliciting an immune response and were finally chosen as targets for vaccination.

Then, selective peptides were ranked based on binding to various sub-classes of *HLA-II* using *NN-align* (As illustrated in Table 3). The results of the analysis demonstrated that some epitopes in the E protein of SARS-CoV-2 had a relatively higher immunogenicity score than the other ones, some of them are "*SEETGTLIVNSVLLF", "TLIVNSVLLFLAFVV", "LAFVVFLLVTLAILT", "LAILTALRLCAYCCN"*, and "*SVLLFLAFVVFLLVT"*. Following the antibody-epitope prediction, we found that a region containing amino acid residue 11-65 has a high antigenicity in E protein of SARS-CoV-2 (Figure 3a). The hydrophilic residues are located on the initial part of this region and the last 30 amino acids of the protein sequences (Figure 3b). Additionally, the results of more analysis represented that "*FVSEET"* and "*PSFYVYSRVKNLNSSRVP"* sequences of SARS-CoV-2 have a higher probability to be more accessible as the selective antigens (Figure 3c), the sequences related to high antigenicity, hydrophilicity, and surface accessibility have been located in the C-terminal of SARS-CoV-2 E-protein (As shown in figure 3). According to the location of immunogen epitopes stimulating B and T lymphocytes, the 3D structure of the SARS-CoV-2n E-protein was designed, as shown in Figure 4.

Table 3. Immunogenicity score of different top epitopes of SARS-CoV-2 E protein. These scores have been calculated based on binding to determinant HLAs.

Peptide	Start	End	Immunogenicity Score	HLA-DRB1:03:01	HLA-DRB1:07:01	HLA-DRB1:15:01	HLA-DRB3:01:01	HLA-DRB3:02:02	HLA-DRB4:01:01	HLA-DRB5:01:01
SEETGTLIVNSVLLF	6	20	93.3656	5.7	8.3	21	14	13	51	66
TLIVNSVLLFLAFVV	11	25	89.262	5.7	8.7	0.53	21	19	30	23
SVLLFLAFVVFLLVT	16	30	84.595	17	4.4	0.53	24	83	6.8	25
LAFVVFLLVTLAILT	21	35	86.8259	9.3	3.3	2.5	24	71	19	14
FLLVTLAILTALRLC	26	40	76.2224	19	1.5	4.2	49	37	23	7
LAILTALRLCAYCCN	31	45	86.3331	9.6	6.2	6.6	48	51	34	15
LVKPSFYVYSRVKNL	51	65	63.0479	21	2.8	17	23	8.6	67	11
FYVYSRVKNLNSSRV	56	70	64.5749	21	2.9	15	38	2.2	43	13

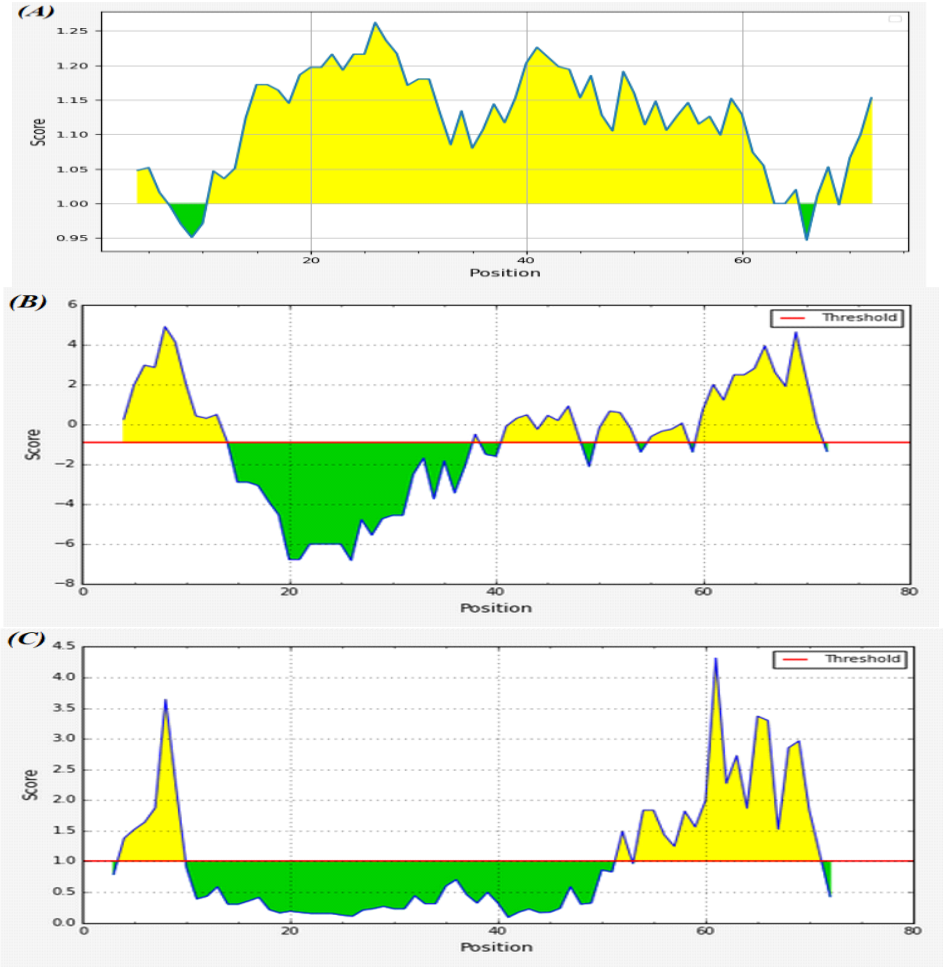


Figure 3. These curves are related to data analysis with the Antibody Epitope Prediction server. The result of Kolaskar and Tongaonkar server demonstrates that a high antigenicity scale of residues with scores above the threshold (default value is 1.000) is predicted to be part of the E protein sequences of SARS-CoV-2 and colored in yellow on the graph (where Y-axes depicts residue scores and X-axes residue positions in the sequence) (3a). Besides, The results of analyzed data with Parker Hydrophilicity Prediction show the hydrophilic residues of E protein of SARS-CoV-2, which is colored in yellow on the graph (3b). Also, analyzed data with Emini server predicts the residues with a higher score of surface accessibility of E protein sequences of SARS-CoV-2 above the threshold (default value is 1.000) (3c).

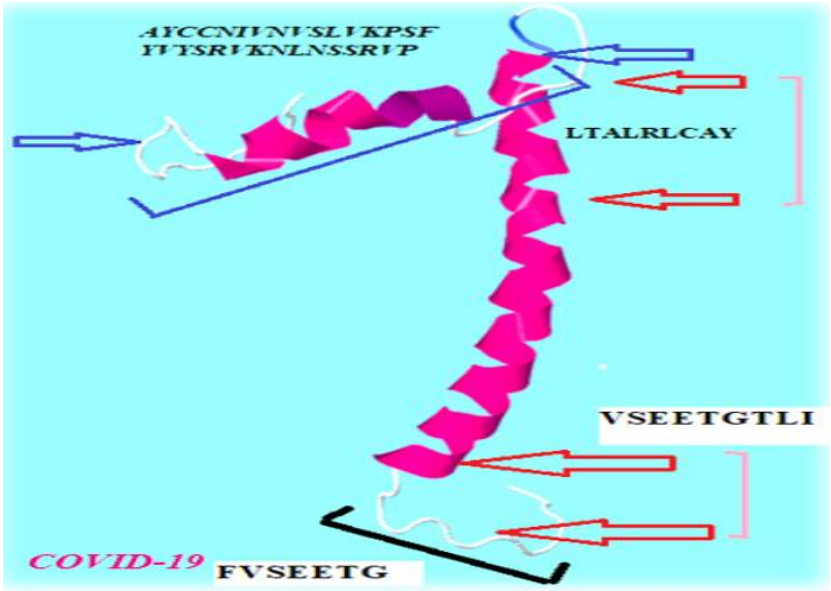


Figure 4. The tertiary structure (3D) modeling of SARS-CoV-2 E-protein was designed based on their structural characteristics using RaptorX software. In tertiary structure (3D) modeling, the immuno-dominant sites for B and CTL cells have been colored blue and red, respectively.

Discussion

The present study aimed to compare the antigenic potential of N, M, S and E proteins of the COVID-19. A high rate of mutations was detected at the end of COVID-19 genome, which encodes the major structural proteins in SARS-CoV-2 including envelope (E) protein, nucleocapsid (N) protein, membrane (M) protein, and spike (S) protein (21, 22). Our finding showed that SARS-CoV-2 E-protein showed a higher antigenicity value than the corresponding values for N, M, and S proteins. In previous studies, these conserved antigenic sites were revealed through sequence alignment between MERS-CoV and Bat-coronavirus (23) and analyzed in SARS-CoV (24). Since these conserved genes are crucial for the survival of the virus, and substantial mutations in these genes might be caused more potential to adapt with new conditions and antibiotic resistance by microorganisms, we aimed to find conserved coding genes in the E protein of viruses to the vaccine design. Besides, the results of study by Abdelmageed M *et al*. revealed that SARS-CoV-2 protein E is a gene with the highest probability antigenicity (25).

Given, the fact that the Cellular immunity is an *HLA*-restricted response depending on several factors including proteasomal cleavage, MHC binding affinity, and TAP transport efficiency are crucial factors in stimulating immunity against viruses. Here, we found 26 peptides binding to *HLA-I* and *HLA-II* as candidates for a vaccine designed based on the maximum affinity for *HLA* binding. We furthermore evaluated the proteasomal cleavage sites, *HLA* binding affinity, C-terminal cleavage, TAP transport efficiency via *NetCTL* and *NetChop* software, and final reported scores determine the immunogenicity level related to each peptide. Based on our findings in this part, four top immunogen epitopes binding *HLA-I* were selected for the SARS-CoV-2 E protein, including *VSEETGTLI*, *LTALRLCAY*, *VSLVKPSFY*, and *LVKPSFYVY*. Then, antigen potency of all selected epitopes was confirmed via *IEDB* tools, and ultimately two epitopes of E protein, including *VSEETGTLI* and *LTALRLCAY*, were introduced as immunogenic epitopes for SARS-CoV-2. Overall, given the selected immunogen epitopes of E protein, some *HLA-I* alleles such as *HLA-C*05:01*, *HLA-B*15:01*, *HLA-B*15:02*, *HLA-B*15:25*, *HLA-A*29:02*, *HLA-A*30:02*, *HLA-B*35:01* are considered the highlighted *HLA-I* molecule in virus infections with SARS-CoV-2.

On the other hand, a similar analysis was performed for detecting the immunomodulatory epitopes binding *HLA-II*. Our findings predicted various peptides binding *HLA-II* and a list of certain alleles in *HLA-II*. Moreover, the immunogenicity score of all of the elected

epitopes was computed based on the potency of the interaction with different *HLA-II* alleles (*HLA-DRB1*03:01, HLA-DRB1*07:01, HLA-DRB1*15:01, HLA-DRB3*01:01, HLA-DRB3*02:02, HLADRB4*01:01, HLADRB5*01:01*). Finally, a list of epitopes related to COVID-19 E protein was scoring as the selective immunomodulatory sequences. Accordingly, the results of the analysis also showed that some epitopes in the SARS-CoV-2 E protein (*SEETGTLIVNSVLLF, TLIVNSVLLFLAFVV, LAFVVFLLVTLAILT, LAILTALRLCAYCCN*, and *SVLLFLAFVVFLLVT)* have a relatively higher immunogenicity score than the other ones.

Our analysis showed that certain sequences of SARS-CoV-2 E protein were dominant for B cell responses and that those regions were well conserved in terms of sequence with SARS-CoV-2 E protein. On the other hand, the initial sequences of the SARS-CoV-2 E protein contain hydrophilic epitopes. Our analysis predicted that the residues with a higher score of surface accessibility of the E protein sequences of SARS-CoV-2 are located in the elementary and middle parts of the E protein sequence. Altogether, the initial and middle parts of the E protein sequence have a relatively higher potential for stimulating immune responses.

Conclusions

Altogether, despite the length of the polypeptide chain of E protein is so shorter compared with the other structural proteins (75 amino acids), this protein can notably motivate the adaptive immune system and B cells. It has also been found that different selective peptides can act as promising epitopes in this way.

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