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ORIGINAL ARTICLE

Prediction of B- and T-cell epitopes using *in-silico* approaches: a solution to the development of recombinant vaccines against COVID-19

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ABSTRACT

BACKGROUND: The novel Coronavirus (2019-nCoV, SARS-CoV-2, or COVID-19) is a recent type of RNA virus. The disease caused by the coronavirus, called COVID-19, was first identified in Wuhan, China. COVID-19 is a new virus and is related to families of viruses such as acute respiratory syndrome and other common colds. Symptoms include fever, cough and shortness of breath. In more severe cases, the infection can lead to pneumonia, kidney failure and in some cases the disease will be fatal. There is currently no known treatment for the virus. However, *in-silico* approaches help produce efficient novel vaccine in a short time and at low cost compared with previous methods.

METHODS: In this study, immunoinformatics tools was used to predict MHC-I, MHC-II, CTL and B-cell epitopes of spike protein and envelope protein of SARS-CoV-2 that could be appropriate to trigger immune system response. We identified potential epitopes against SARS-CoV-2 that could potentially stimulate both T-cell and B-cell immune system response with increased effective potential due to the presence of MHC-I, MHC-II, CTL and B-cell epitopes. Immunoformatics tools were used to physicochemical property analysis of the protein sequences and detect highly antigenic, non-toxin, non-allergen and highly immunogenic MHC-I, MHC-II, CTL and B-cell epitopes of the spike protein and envelope protein of the 2019-nCoV was performed in ProtParam, ANTIGENpro, ToxinPred, AlgPred, IEDB, RANKPEP, CTLpred and BepiPred servers. The safety and stability of epitopes were analyzed for the binding potential with the Major histocompatibility complex (MHC) alleles using FireDock and Patch dock tools.

RESULTS: Based on the results, the best epitopes were selected. Selected epitopes may be used as target candidates in recombinant vaccines to combat COVID-19 virus.

CONCLUSIONS: These findings are likely to help develop the recombinant vaccine against COVID-19 disease.

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KEY WORDS: Severe acute respiratory syndrome; Coronavirus; COVID-19; Molecular docking simulation.

Can immunoinformatics help researchers fight with novel Coronavirus? Extensive applications of trustworthy statistical formalization in immunoinformatics researches are essentially to use for faster promotion and use of medical services in medicine. With the emergence of this knowledge, researchers are predicting pathogenic peptides among thousands of peptide sequences using computational approaches.[1-3]

The microorganism called the corona virus is a main class of RNA viruses. This virus is an enveloped, positive-sense, ssRNA virus and its structural protein molecules consist of nucleocapsid (N), spike (S), envelope (E), membrane (M) and have crown-like stumps on their wall surface.[4-6]

Coronaviruses cause cold-like illness in living beings, and some of these viruses damage the respiratory system. So far, seven human coronaviruses have been discovered, including: HCoV-229E, HCoV-OC43, HCoV-NL63,

HCoV-HKU1, SARS-CoV (severe acute respiratory-Coronaviruses), MERS-CoV (Middle East respiratory syndrome-Coronaviruses), SARS-CoV-2.[7-9]

In December 2019, SARS-COV-2 in Wuhan, China, was identified as a pneumonia cluster.[10, 11] The 2019-nCoV genome is approximately 70% similar to SARS-CoV.[12] Characteristics of four structural proteins related to SARS-CoV-2 include: The spike is involved in the viral fusion and attachment with host cell to help viral arrival into the cell. The envelope has a significant pattern in pathogenesis and assembly of the virion and it's an integral membrane protein. The nucleocapsid protein plays a dynamic monitoring role in virus replication as well as transcription. The function of the membrane protein is in the structure of novel coronavirus.[13-15]

Symptoms novel coronavirus that causes COVID-19, usually a few days after exposure to the virus starts. Based on research, symptoms may include: fever (≥38 °C), dry cough, respiratory disorder, fatigue, muscle pain and diarrhea.[16, 17] As of April 11th, 2020, COVID-19 has affected more than 1,610,909 people and killed more than 99,690 in 213 countries, number is expected to increase in the coming days. According to the World Health Organization declared this prevalence a public health emergency of universal distress.[18]

Unfortunately, there is no treatment for this disease, including vaccines and drug.[19] Thus, in this critical situation, the necessity for designing drug and multi-epitope vaccine appropriate versus the 2019-nCoV infection was growing.[20]

Designing an epitope-based novel vaccine using bioinformatics and immunoinformatics tools helps to achieve the recombinant vaccine required in the least time, low cost and high safety.[21, 22]

Several studies related to Coronavirus propose a protective role of both B-cell and T-cell immune responses system. On the other, an appropriate recombinant vaccine may contain T-cell and B-cell epitopes, with combination of which recombinant vaccine is capable to either induce special cellular or humoral immune response against infectious diseases efficiently.[23-25]

In this research, we applied immunoinformatics tools to recognize CTL, MHC-I, MHC-II and B-cell peptides based on the envelope protein and spike protein of 2019-nCoV. The HLA alleles were calculated for MHC-I and MHC-II epitopes and the Toxicity, allergenicity and antigenicity of all the epitopes were evaluated. Physiochemical characteristics were also checked for exploring the safety, antigenicity and stability of the recognized epitopes.

Materials and methods

Data retrieval of viral protein sequences

The FASTA format of spike glycoprotein (GenBank: QIC53213.1), envelope protein (GenBank: QIA98556.1) of SARS-CoV-2 were obtained from the National Center of Biotechnology Information (NCBI).[26]

Physicochemical property analysis of the protein sequences

The antigen sequences were analyzed by ProtParam server (https://web.expasy.org/protparam/) to determine their various physicochemical characteristics.[27] This server compute molecular weight, theoretical pI, EI (extinction coefficient), instability index II, GRAVY (grand average hydropathy) and AI (aliphatic index).

Prediction of MHC-I epitopes

The MHC-I epitope predicted by using online server IEDB. IEDB at: http://tools.iedb.org/mhci/ is a first server that used stabilized matrix method (SMM) artificial neural network (ANN) and average relative binding (ARB). SMM and ARB methods model using position-specific scoring matrices (PSSM) to predict binding specificity of an MHC-I molecule. The ANN method predicts model-binding specificity by using neural networks with various sequence encoding outlines.[28]

Prediction of MHC-II epitopes

The MHC-II epitope predicted by using online server RANKPEP. RANKPEP (http://imed.med.ucm.es/Tools/rankpep.html) is the second tool for MHC-II epitope prediction. RANKPEP server that uses PSSMs for the prediction of epitope-MHC-II binding as a foundation for CD4 T-cell peptide identification.[29]

Prediction of CTL epitopes

Cytotoxic T lymphocyte (CTL) epitopes are possible candidates for final epitopes for several infections. CTL epitope prediction performed by CTLpred server. CTLpred server (http://crdd.osdd.net/raghava/ctlpred/index.html) is based on machine learning techniques such as ANN and support vector machine (SVM) and quantitative matrix (QM).[30]

Prediction of B-cell epitopes

The B-cell epitope prediction server based on immunoinformatics data including BepiPred have been used. The BepiPred server (http://www.cbs.dtu.dk/services/

BepiPred/) has been used for prediction of linear B-cell epitopes using propensity scale method and hidden Markov model.[31]

Antigenicity prediction of the selected epitopes

To predict antigenicity of antigenic protein sequences was using a web server called ANTIGENpro (http://scratch.proteomics.ics.uci.edu). The server mentioned is a pathogen-independent predictor. It is an alignment-free and sequence-based with the accuracy of 82%.[32]

Toxicity and allergenicity prediction of the epitopes

The prediction of toxicity selected epitopes was performed applying ToxinPred server (http://crdd.osdd.net/raghava/toxinpred/). ToxinPred is a bioinformatic tool, which is developed to design and predict non-toxic and toxic epitopes. The essential dataset used in this tool comprised of 1805 toxic protein sequences. This server using SVM based method, upkeep total the parameters default.[33]

The prediction of allergenicity selected epitopes was performed using AlgPred at http://www.imtech.res.in/raghava/algpred. This server utilizes dataset of 700 non-allergens and 578 allergens for prediction of allergenic antigens and mapping of IgE peptides.[34]

Generation of the 3D structures of the selected epitopes

The three-dimensional structure of the selected peptides was generated applying PEP-FOLD3 server (http://bioserv.rpbs.univparis-diderot.fr/services/PEP-FOLD3/).[35]

Molecular docking of the selected epitopes

Designed construct obtained from PEP-FOLD were used for docking with HLA-A*02:01 allele (PDB ID: 5F9J) and HLA-DRB1*15:01 (PDB ID: 5V4M) molecule attained from Protein Data Bank (PDB). Docking experiments performed using PatchDock (https://bioinfo3d.cs.tau.ac.il/PatchDock/patchdock.html). This server is an algorithm for protein-protein docking.[36] The inputs are two macromolecules of each type: DNA, peptides and proteins. The output is table of potential collection organized by form complementary criteria. Protein-protein docking was performed applying similar method of we formerly published research. At the end, a root mean square deviation (RMSD) score is used to the verified model solutions. afterwards, the redundant solutions are discarded according to analyzing the root mean square deviation score. Higher score solutions are considered as higher ranked solutions by this tool.

In order to cheque the refinement of docking results, we used FireDock (http://bioinfo3d.cs.tau.ac.il/FireDock/php.php).[37]

Ethical approval

The present study was approved by The Ethics Committee of Lorestan University of Medical Sciences (IR.LUMS.REC.1398.293). This article does not contain any studies with human participants or animals performed by any of the authors.

Results

Data retrieval of viral protein sequences

Two antigens sequences: spike glycoprotein (GenBank: QIC53213.1) and envelope protein (GenBank: QIA98556.1) retrieved from the NCBI in fasta format.

Physicochemical property analysis of the protein sequences

The physicochemical parameters evaluation was conducted for spike protein and envelope protein. The physicochemical characteristics of the two proteins such as GRAVY, theoretical pI, aliphatic index, half-life, instability index, amino acid composition and molecular weight are shown in Table I.

Prediction of B-cell and T-cell epitopes and their antigenicity, toxicity and allergenicity determination

The HLA-A* 02:01 was selected for MHC class-I, and HLA-DRB1*15:01 was selected for MHC class-II were adjusted as β and α chains based on data calculated from AFND (allele frequency net) for Iranian people (http://www.allelefrequencies.net/hla6006a.asp).

TABLE I.—*The physicochemical characteristics analysis of the selected proteins.*

Protein	N. of amino acids	Molecular weight	Theoretical pI	Ext. coefficient (in M-1 cm-1)	Estimated half-life (in mammalian cell)	Instability index	Aliphatic index	Gravy
Spike glycoprotein	1273	141,178.47	6.24	148,960	30	33.01	84.67	-0.079
Envelope protein	75	8365.04	8.57	6085	30	38.68	144.00	1.128

TABLE II.—*MHC-I epitopes prediction and allergenicity, antigenicity and toxicity analysis of the epitopes of spike glycoprotein and envelope protein.*

Protein	Start	Epitope	Percentile rank	Antigenicity	Allergenicity	Toxicity
Spike glycoprotein	1095	FVSNGTHWFV	0.21	Non-antigen	Non-allergen	Non-toxin
	2	FVFLVLLPLV	0.28	Antigen	Non-allergen	Non-toxin
	269	YLQPRTFLL	0.3	Antigen	Allergen	Non-toxin
	1220	FIAGLIAIV	0.4	Non-antigen	Non-allergen	Non-toxin
	386	KLNDLCFTNV	0.42	Antigen	Non-allergen	Non-toxin
Envelope protein	20	FLAFVVFLLV	0.15	Antigen	Non-allergen	Non-toxin
	26	FLLVTLAIL	0.43	Antigen	Non-allergen	Non-toxin
	50	SLVKPSFYV	0.5	Antigen	Allergen	Non-toxin
	16	SVLLFLAFVVFLLV	0.73	Antigen	Non-allergen	Non-toxin

Low percentile rank = good binders.

TABLE III.—*MHC-II epitopes prediction and allergenicity, antigenicity and toxicity analysis of the epitopes of spike glycoprotein and envelope protein.*

Protein	Start	Epitope	Scores	Antigenicity	Allergenicity	Toxicity
Spike glycoprotein	351	YAWNRKRIS	26.728	Antigen	Non-allergen	Non-toxin
	145	YHKNNKSWM	24.739	Non-antigen	Non-allergen	Non-toxin
	350	VYAWNRKRI	24.285	Antigen	Allergen	Non-toxin
	1209	YIKWPWYIW	23.44	Antigen	Non-allergen	Non-toxin
Envelope protein	62	VKNLNSSRV	7.557	Antigen	Non-allergen	Non-toxin
	14	VNSVLLFLA	6.327	Non-antigen	Non-allergen	Non-toxin

Top percentile score = good binders.

TABLE IV.—*CTL epitopes prediction and allergenicity, antigenicity and toxicity analysis of the epitopes of spike glycoprotein and envelope protein.*

Protein	Start	Epitope	Scores	Antigenicity	Allergenicity	Toxicity
Spike glycoprotein	226	YVSQPFLMD	1.000	Non-antigen	Non-allergen	Non-toxin
	461	DEVRQIAPG	1.000	Antigen	Allergen	Non-toxin
	635	PQTLEILDI	1.000	Antigen	Non-allergen	Non-toxin
Envelope protein	18	INSEVEREA	1.000	Non-antigen	Non-allergen	Non-toxin
	106	VKPSFYVYS	1.000	Antigen	Allergen	Non-toxin
	3	GQIAENVEL	0.970	Antigen	Allergen	Non-toxin

Top percentile score = good binder.

TABLE V.—*B-cell epitopes prediction and allergenicity, antigenicity and toxicity analysis of the epitopes of spike glycoprotein and envelope protein.*

Protein	Start	Epitope	Scores	Antigenicity	Allergenicity	Toxicity
Spike glycoprotein	879	AGTITSGWTFGAGAAL	0.97	Non-antigen	Non-allergen	Non-toxin
	594	GVSVITPGTNTSNQVA	0.95	Antigen	Non-allergen	Non-toxin
	257	GWTAGAAAYYVGYLQP	0.95	Antigen	Non-allergen	Non-toxin
	1112	PQIITTDNTFVSGNCD	0.95	Non-antigen	Allergen	Non-toxin
Envelope protein	30	TLAILTALRLCAYCCN	0.85	Antigen	Non-allergen	Toxin
	48	NVSLVKPSFYVYSRVK	0.80	Antigen	Non-allergen	Non-toxin

Top percentile score = good binders.

The MHC-I, MHC-II, CTL and B-cell epitopes were determined for potential epitopes. The IEDB server were applied for identify top ranked MHC Class-I binding epitopes (Table II). The RANKPEP server were applied for identify top ranked MHC class-II binding epitopes (Table III). The CTLpred server were applied for top ranked CTL binding epitopes prediction (Table IV). The BepiPred server were applied for top ranked B-cell binding epitopes prediction

(Table V). The epitopes used in the next step were selected based on the epitope score, antigenicity, non-allergenic, non-toxic. Two MHC-I selected epitopes FVFLVLLPLV and KLNDLCFTNV, Two MHC-II selected epitopes YAWNRKRIS and YIKWPWYIW of spike glycoprotein and Three MHC-I FLAFVVFLLV, FLLVTLAIL and SVLLFLAFVVFLLV, One MHC-II epitope VKNLNSSRV were selected for envelope protein.

Generation of the 3D structures of the epitopes and molecular docking

Molecular docking was performed to determine, whether whole the selected epitopes had the capability to bind with the $CD4^+$ and $CD8^+$ T-cell proteins or not. For docking MHC class 1 and 2 epitopes of the HLA-A*02:01 allele (PDB ID: 5F9J) and HLA-DRB1*15:01 (PDB ID: 5V4M) were selected as the receptor. Amongst MHC-I and MHC-II epitopes of spike protein, FVFLVLLPLV and YAWNRKRIS demonstrate the outcome with the lowest and best global energy score. Amongst MHC-I and MHC-II epitopes of envelope protein, FLAFVVFLLV and VKNLNSSRV demonstrate the outcome with the lowest and best global energy score (Table VI, VII).

Discussion

The COVID-19 infection is a serious security threat to the all-world, so the need for an effective vaccine is urgent. Symptoms of COVID-19 disease consist of fever, lung infections, dry cough, muscle pain and shortness of breath. The World Health Organization says the incubation period of the infection lasts up to 14 days.[38, 39]

It is essential to identify effective epitopes of immunity. The spike protein is an essential component of SARS-CoV-2 that mediates attachment coronavirus entry events. This protein plays an important role in the life cycle of coronavirus.[40] The envelope protein is the smallest and most enigmatic of the coronavirus structural proteins. During the coronavirus replication cycle, the envelope protein is highly expressed in the infected host cell. Envelope protein plays an important role in the production and also maturation of coronavirus.[41] Bioinformatics tools is important in designing multi-epitope vaccines against 2019-CoV. Bioinformatics tools are an important method in analyzing and developing recombinant vaccines because they can improve the effectiveness of time and cost.[42, 43]

In this research, prediction of high-potential and high-safety epitopes for use in the design of novel vaccines, it used immunoinformatics tools to identify potential epitopes for the 2019-CoV.[44] To carry out the recognize potential epitopes, two candidate antigens (envelope protein and spike protein) of the 2019-CoV were identified and elected from the National Center for Biotechnology Information gene bank. Alone highly antigenic proteins sequences were elected for research since the potential epitopes can induce good immune system response.[45]

Because envelope protein and spike protein have been identified as antigenic proteins, they have been considered for potential epitopes.

Physicochemical parameters were performed for both novel coronavirus proteins, including envelope and spike protein. an effective antigenic protein should induce potent immunogenic responses and also should have admissible physicochemical characteristics. Therefore, the physicochemical parameters of the selected proteins were analyzed using immunoinformatics method. According to the physicochemical properties results (Table I), Our proteins can be used as suitable proteins for the selection of potential epitopes.

TABLE VI.—*Results of molecular docking analysis of the selected epitopes.*

Protein	Epitope	MHC allele	Global energy	Hydrogen bond energy
Spike glycoprotein	FVFLVLLPLV	MHC-I	-46.53	-1.24
	KLNDLCFTNV		-34.05	-2.77
Envelope protein	FLAFVVFLLV		-59.84	-1.27
	FLLVTLAIL		-31.35	-0.49
	SVLLFLAFVVFLLV		-30.41	-0.88

TABLE VII.—*Results of molecular docking analysis of the selected epitopes.*

Protein	Epitope	MHC allele	Global energy	Hydrogenbond energy
Spike glycoprotein	YAWNRKRIS	MHC-II	-20.45	-1.50
	YIKWPWYIW		-36.32	0.00
Envelope protein	VKNLNSSRV		-9.26	-1.56

Predictions of B-cell, MHC-I, MHC-II and CTL epitopes were performed. Several epitopes of B-cell, MHC-I, MHC-II and CTL of both antigenic proteins were considered by appropriate servers. Then, epitopes were selected based on high antigenicity, non-toxicity and non-allergenicity.

Molecular docking was performed to determine whether the selected epitopes are capable of binding to their MHC-I and MHC-II alleles. Therefore, amongst the final epitopes, FVFLVLLPLV, YAWNRKRIS, FLAFVVFLLV and VKNLNSSRV generated the good molecular docking scores.

Conclusions

The SARS-CoV-2 is spreading around the world. Countless countries from Asia to Africa are affected by the virus. No definitive treatment has been reported for COVID-19. In this research, the potential epitopes of two SARS-CoV-2 proteins (spike and envelope protein) were predicted using immunoinformatics tools. The epitopes selected in this paper should be tested in future studies for multi-epitope vaccines. in silico approaches will be useful for developing a safe and potential treatment, such as designing recombinant vaccines COVID-19 challenging.

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