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Immunoinformatics Prediction of Peptide-Based Vaccine against MERS-Corona-viruses

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Abstract

MERS-Corona-viruses cause massive and pandemic outbreaks of respiratory infection in several regions of continents' and revealed a global epidemic trend. However, no effective antiviral drug or vaccine has been developed to treat coronavirus. Aim of the study to detect epitopes can be as vaccine. A total of 28 outer spike glycoprotein (s) sequences of Corona-viruses were retrieved from the National Center for Biotechnology Information database (NCBI) on them, several tests were conducted using Immune Epitope Analysis Database (IEDB) to detect the highly conserved immunogenic epitopes of B and T cells from which all possible epitopes that can be used as a therapeutic peptide vaccine to be selected. Several conserved cytotoxic T-lymphocyte epitopes, linear and conformational B cell epitopes were predicted for Corona-viruses spike glycoprotein and their antigenicity was calculated. Among B-cell epitopes 106–SQDVKQ-111 is antigenic and in the case of T cell epitopes, 279-FQFATLPVY-287 and 786-FSFGVTQEY-794 and 69-ITYQGLFPY-77 and, 924- AQYVAGYKV-938, 1271-ALNESYIDL-1285, and 1300-AGLVALALC-1314 are extremely antigenic promising for vaccination against Corona-viruses They demonstrated population coverage against the whole world **91.81%**.The study led to the discovery of various epitopes, conserved among various strains belonging to different countries. The potential antigenic epitopes can be successfully utilized in designing novel vaccines for combating and eradication of MERS Corona-viruses disease.

Keywords: Immunoinformatics, Vaccination, MERS Coronaviruses, Peptide.
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Introduction:

The rapid emergence and dissemination of infectious diseases have taken a heavy toll on humans since the beginning of the twenty-first century. One of the most familiar examples was the outbreak of severe acute respiratory syndrome (SARS) in the winter of 2002 and 2003, 2019 caused by a novel coronavirus (SARS-CoV) novel coronavirus disease (COVID-19) have increased not only in Wuhan, Hubei Province but also China and the world[1-3]. Since it was discovered, coronavirus was considered relatively harmless to humans until the outbreaks of SARS and MERS and COVID-19 in 2003 and 2012, 2019 respectively. SARS-CoV is a new type of coronavirus identified after the discovery of SARS-CoV, belongs to the Beta coronavirus lineage C (3, SARS-CoV that causes severe acute respiratory disease with a high fatality rate [4-6].

MERS-Coronavirus is approved and long – acquainted virus system that can be classified into four categories depend on their genome sequence:

Alpha coronavirus, Beta coronavirus, Gamma coronavirus, and Delta coronavirus . Coronavirus is a class of enveloped RNA virus with a 27–3l kb long single-stranded positive-sense genome. The genome includes two large replicase open reading frames, ORF1a and ORF1b, encoding two viral replicase polyproteins. The region downstream of ORF1 contains at least 10 small ORFs, encoding the spike protein (S), a small envelope protein (E), membrane protein (M), nucleocapsid protein (N), and the assumed nonstructural proteins [7].

MERS -CoV belongs to the genus Betacoronavirus in the Family Coronaviridae, as SARS-CoV does. However, they do not use the same host cell receptor for infection [8]. Complete genome sequencing indicated that this new virus is the first lineage C Beta coronavirus species known to infect humans [9] MERS -CoVs are positive-strand RNA viruses. The virion includes a nucleocapsid (N) core surrounded by an envelope containing three membrane proteins: spike (S), membrane, and envelope. The S protein of MERS -CoV, a 1353 - amino-acid type I membrane glycoprotein, is known to be responsible for receptor binding [9], membrane fusion [10], and the induction of neutralizing antibodies [11]. Although the S protein of MERS -CoV shares little amino-acid identity with that of other CoVs (< 30%), it shares common structural features with the S proteins of other CoVs [12]. Its two components are S1, which contains the receptor-binding domain (RBD) [13]. Moreover, a combination of computed tomography imaging, whole-genome sequencing, and electron microscopy computed tomography (CT) and real-time reverse-transcriptase-polymerase chain reaction (rRT-PCR) and Serological assays were initially used to screen and identify SARS-CoV- 1 & 2 [14-17]. However, no effective antiviral drug or vaccine has been developed to treat MERS-Coronavirus. Traditional vaccines use completely killed viruses or weakened viruses to stimulate the immune response and create protective immunity. Nevertheless, sometimes you want to target immunity to specific parts of the virus and not to others. Alternatively, you may want to generate immunity against a protein that is not naturally immunogenic. We utilize computational protein design to place antigenic loops on the surfaces of other proteins, stabilizing them and making them polyvalent for a better immune response. Aim of the study to detect epitopes can be a vaccine.

Materials and Methods

Immunogenic Part for MERS Virus:

Protein Sequence Retrieval

The twenty-nine strains of MERS Coronaviruses spike glycoprotein were retrieved from NCBI in April 2019 (https://www.ncbi.nlm.nih.gov/protein/term = Nipah + virus + G + glycoprotein). The retrieved strains were from different parts of the world. The retrieved strains and their accession numbers were depicted in **figure**1.

Determination of MERS Coronaviruses spike glycoprotein MERS-Conserved Regions:

The retrieved sequences of MERS-Coronaviruses spike glycoprotein strains were aligned to obtain conserved regions using multiple sequence alignment (MSA). Sequences were aligned with

the aid of ClustalW as implemented in the BioEdit program, version 7.2.5. Then epitopes prediction and analysis of each protein were done using different tools of immune epitope database IEDP software (http://www.iedb.org) (18).

Epitopes Prediction:

To detect the candidate epitopes from MERS - Coronaviruses spike glycoprotein, for B and T cells, several analysis prediction tools from Immune Epitope Database (IEDB) (http://www. iedb .org/) were used(18).

B-cell Epitope Prediction:

B cell epitope is the portion of an immunogenic which interacts with B lymphocytes. B-lymphocytes upon exposure differentiated into plasma cells and memory cells. Thus B cell epitopes are shown to being accessible and antigenic. Accordingly, the classical propensity scale methods and hidden Markov model programmed software from IEDB analysis resource were used for the following aspects:

Prediction of Linear B-cell Epitopes:

Bepipred from immune epitope database (http://toolsiedb.ofg/bcell/) was used as a linear B-cell epitopes prediction from the conserved region of MERS Coronaviruses spike glycoprotein with a default threshold value of 0.5 (19).

Prediction of Surface Accessibility:

Emini surface accessibility prediction tool of the immune epitope database (IEDB) was used (http://tools.immuneepitope.org/tools/bcell/iedbT he surface accessible epitopes were predicted from the conserved region of MERS spike glycoprotein with the default threshold value is it 1.000 (19).

Prediction of Epitopes Antigenicity:

The kolaskar and tongaonker antigenicity method was used to determine the antigenic sites with a default threshold value of 1.034 (http://tools.immuneepitope.org/bcell/) [20].

T-cell Epitopes Prediction:

MHC Class I Binding Predictions:

Analysis of peptide binding to MHC class I molecules was assessed by the IEDB MHC-I prediction tool at (http://tools.iedb.org/mhci/n). MHC-I peptide complex presentation to T-lymphocytes underwent several steps. For instance, the attachment of cleaved peptides to MHC-1 molecules was predicted by an Artificial Neural Network (ANN) [20]. Also, all of the epitope's lengths were set as 9 amino acids. Besides, all the conserved epitopes that bind to alleles at a score equal to or less than 100 half-maximal inhibitory concentrations (IC50) were selected for further analysis [21].

MHC Class II Binding Predictions:

Analysis of peptide binding to MHC class II molecules was assessed by the IEDB MHC II prediction tool at (http://tools.immuneepitope. org/mhcii/) [23-24]. For MHC-II binding prediction, human allele references set were used. MHC class II groove can bind peptides with different lengths. Therefore, for the analysis, the NN-align as prediction method from the IEDB MHC-II prediction tool was used. It allows for identification of the MHC class II binding core and epitopes binding affinity All conserved

epitopes that bind to many alleles at score equal or less than 500 half-maximal inhibitory concentrations (IC50) were selected for further analysis.

Population Coverage Calculation:

For the calculation of the population coverage for all potential MHC-I and II epitopes bindings, the IEDB tools (http://tools.iedb.org/ tools/ population/ iedb_input) was used. The MERS virus spike glycoprotein was assessed for population coverage against the whole world and North Africa with selected MHC-I and MHC-II interacted alleles [25].

Homology Modeling:

Raptor X protein structure prediction server was used for creating the 3D structure of virus spike glycoprotein (http://raptorx.uchicago.edu/StructurePrediction/predict/) [26]. The reference sequence [NP_112027.1] was used as an input and Chimera 1.8 was used as a tool to visualize the selected epitopes belonging to B cell and T cell (MHC-I and MHC-II). Homology modeling was used for visualization of the surface accessibility of the B lymphocytes predicted candidate epitopes as well as for visualization of all predicted T cell epitopes in the structural level [27].

Results

Multiple sequence alignment of the Retrieved Strains

All retrieved sequences of MERS Coronaviruses spike glycoprotein strains were aligned to obtain conserved regions using multiple sequence alignment (MSA) with accession number which revealed a few discrepancies in amino acid A instead of V and S in position 26 and 226 respectively Figure1

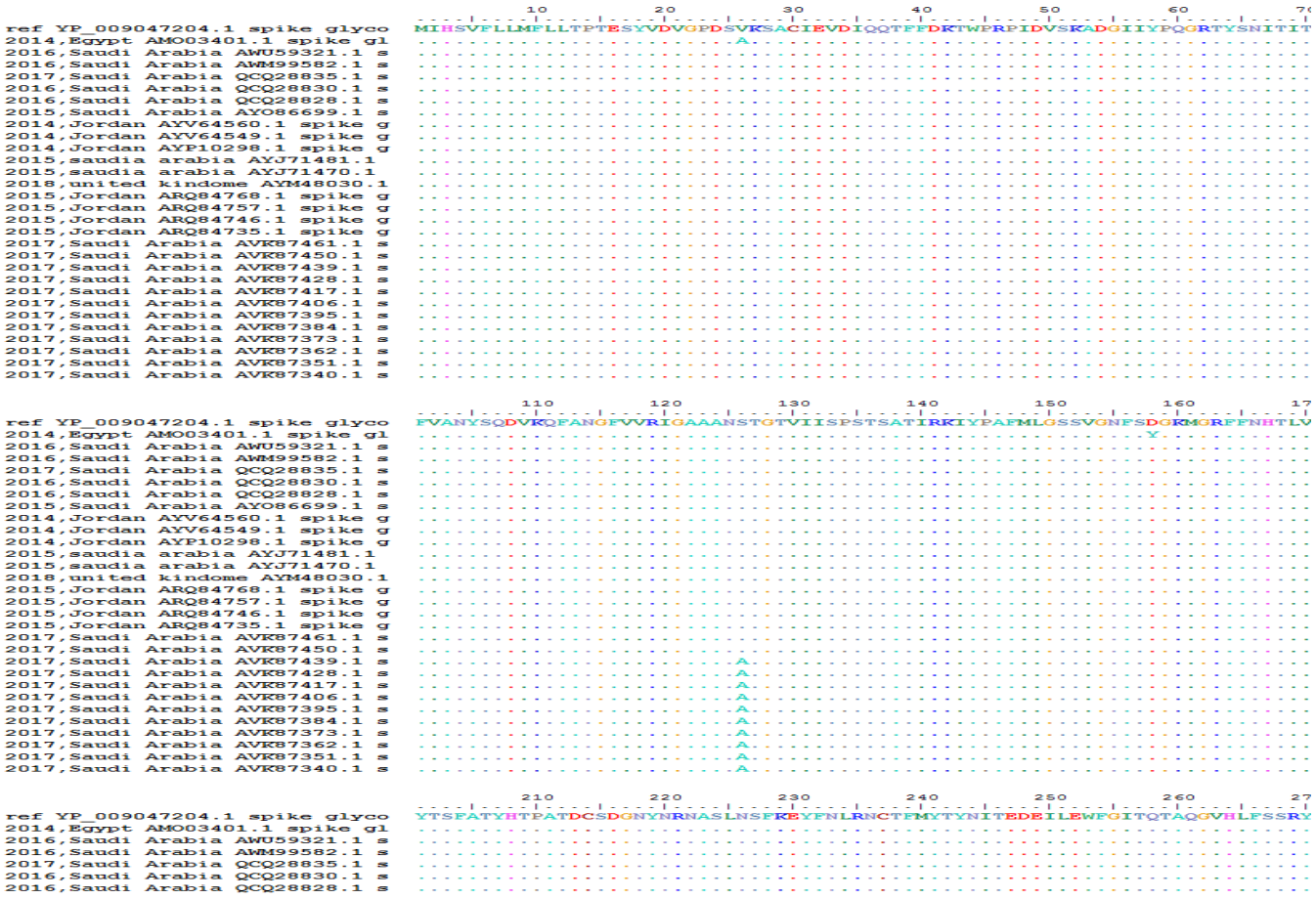

Figure 1 Sequence alignment showed that some regions were mutated region, and dots show the conservancy between different retrieved sequences

Prediction of B-cell Epitope

The reference sequence of MERS-Coronavirus glycoprotein was subjected to Bepipred linear epitope, Emini surface accessibility, Kolaskar, and Tongaonkar antigenicity methods in IEDB to predict the likelihood of specific regions in the protein that bind to B cell receptor, being in the surface and immunogenic respectively. The thresholds of Bepipred linear epitope, Emini surface accessibility, and Kolaskar and Tongaonkar antigenicity were shown in Figures 2 & 3 and Table 1. For Bepipred linear epitope prediction method, the average binding score of viral protein to B cell was predicted as a linear epitope and Emini surface accessibility provided epitopes that were potentially predicted on the surface bypassing the default threshold 1.000. Kolaskar and Tongaonkar antigenicity provided epitopes that gave a score above the default threshold 1.045. The epitope predicted by these different tools against B cell were provided in Table 1. Accordingly, one conserved epitope was successfully predicted to elicit the B cell lymphocytes since they were conserved among all retrieved strains, got higher score values in Emini surface accessibility, and Kolaskar and

Tongaonkar antigenicity prediction methods. These epitopes were **106- SQDVKQ -111**. The three-dimension structural (3D) level of this epitope was shown in Figure 4.

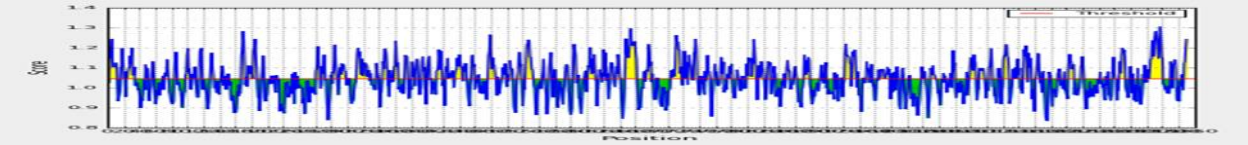

Figure 2 surface accessibility analyses using the Emini surface accessibility scale

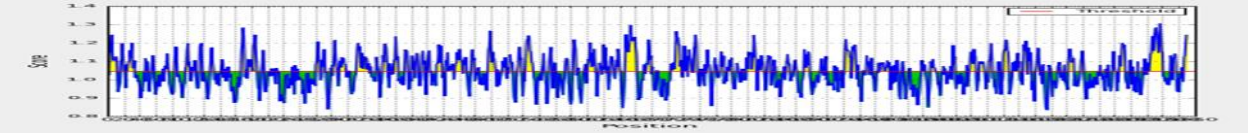

Figure 3 Kolaskar and Tongaonker antigenicity

Table 1: **B-cell Epitopes Prediction, the Position of Peptides is According to the Position of Amino Acids in the Spike Glycoprotein of the** MERS- **Corona Viruses.**

No.	Start	End	Peptide	Length	a.emini score	b.koloskare score
6	**106**	**111**	**SQDVKQ**	**6**	**2.651**	**1.037**

a: default threshold value 1.000

b: default threshold value 1.034

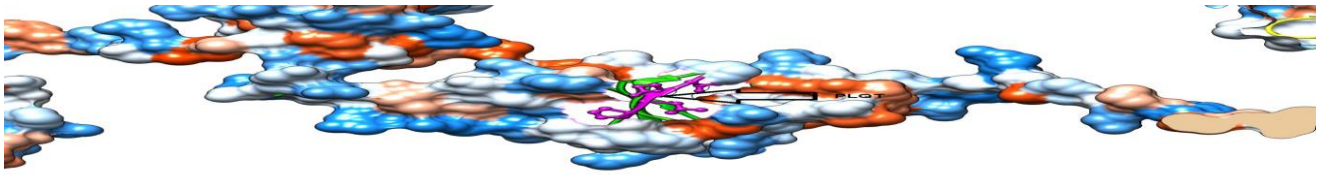

Figure 4. ***Position of Proposed Conserved B Cell Epitopes in Structural Level of spike Glycoprotein of*** MERS ***-Coronavirus*.**

T lymphocytes Epitopes Binding Prediction:

MHC-I Binding Predictions:

The reference structural protein (spike glycoprotein) was analyzed using the IEDB MHC-1 binding prediction tool to predict T cell epitopes interacting with different types of MHC-I alleles. Conserved peptides were predicted to interact with different MHC-1 alleles. The peptide **279-FQFATLPVY-287** and **786-FSFGVTQEY-794** and **69-ITYQGLFPY-77** also interacted with two alleles as shown in **Table 2**. These three epitopes and their positions in the structural level of spike glycoprotein were shown in **Figure 5**.

Table 2: List of Top Epitopes that had Binding Affinity with MHC-I alleles. The position of peptides is according to the position of amino acids in spike glycoprotein of the MERS **-Coronavirus.**

Allele	Start	End	Peptide
HLA-A*02:06, HLA-A*29:02, HLA-A*30:02, HLA-B*15:01, HLA-B*18:01, HLA-B*15:02, HLA-B*35:01, HLA-C*12:03	***279***	***287***	***FQFATLPVY***
HLA-A*29:02, HLA-A*26:01, HLA-A*30:02, HLA-A*68:01, HLA-B*15:01, HLA-B*35:01, HLA-B*46:01, HLA-B*58:01, HLA-C*12:03	**786**	**794**	**FSFGVTQEY**
HLA-A*11:01, HLA-A*29:02, HLA-A*30:02, HLA-A*32:01, HLA-B*15:01, HLA-B*35:01, HLA-B*58:01, HLA-C*12:0	**69**	**77**	**ITYQGLFPY**

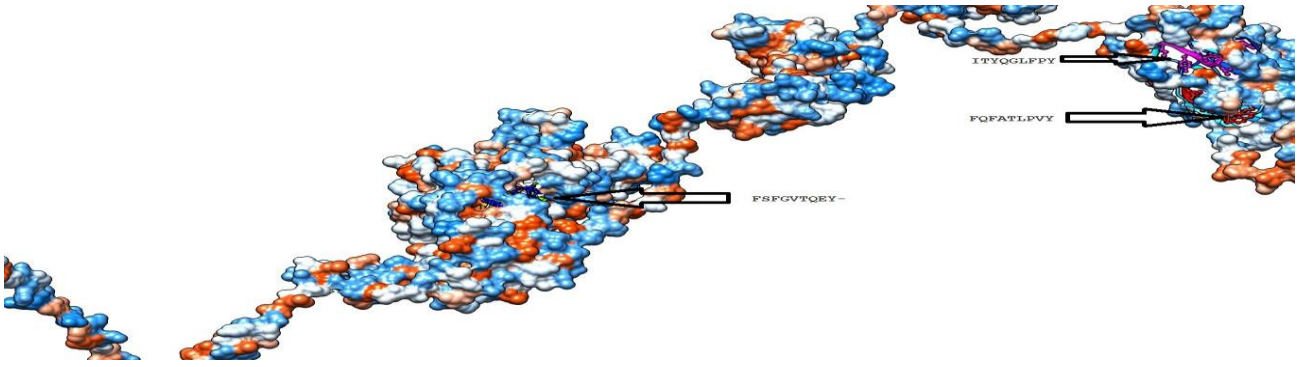

Figure 5: Position of Proposed Conserved T Cell Epitopes that Interact with MHC-I in Structural level of spike glycoprotein of MERS **-Corona Virus**

Table 3: List of Top Epitopes that had Binding Affinity with MHC-II alleles.

Allele	Start	End	Peptide
HLA-DQA1*05:01/DQB1*03:01	**923**	**937**	**AQYVAGYKV**
HLA-DRB1*01:01	**924**	**938**	
HLA-DRB1*07:01	**922**	**936**	
	926	**940**	
	925	**939**	
	920	**934**	
	921	**936**	
HLA-DPA1*03:01/DPB1*04:02	**1271**	**1285**	**ALNESYIDL**
HLA-DRB1*01:01	**1269**	**1283**	
HLA-DRB4*01:01	**1270**	**1284**	
	1272	**1286**	
	1268	**1282**	
HLA-DQA1*01:02/DQB1*06:02	**1299**	**1313**	**AGLVALALC**
HLA-DQA1*03:01/DQB1*03:02 HLA-	**1300**	**1314**	
DQA1*04:01/DQB1*04:02	**1301**	**1315**	
	1298	**1312**	
	1302	**1316**	
	1303	**1317**	
	1304	**1318**	

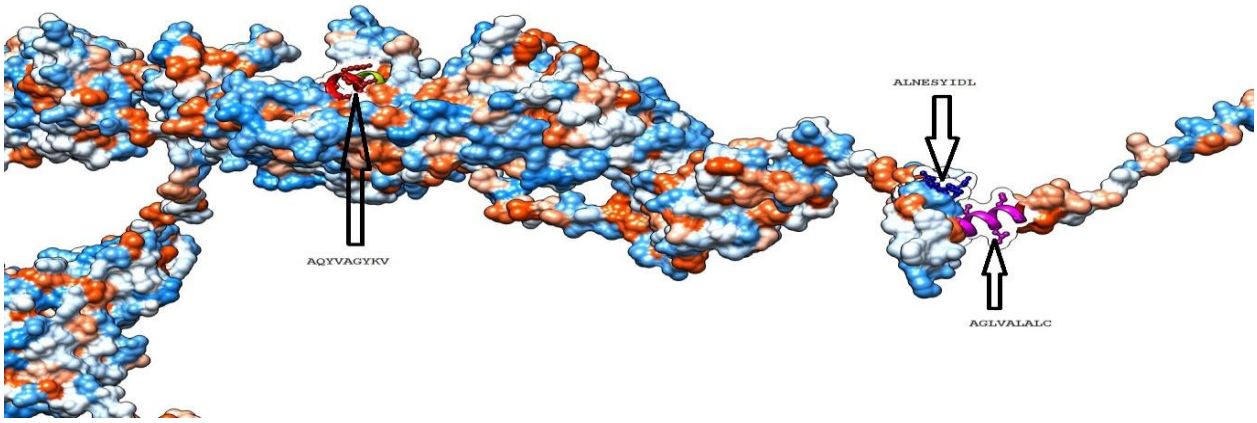

Figure 6: Position of proposed conserved T cell epitopes that interact with MHC-II

Population Coverage

Population coverage was performed for predicted T-cell epitopes and their respective MHC-I and MHC-II alleles preliminarily. We selected only three epitopes that interacted with most frequent MHC-I alleles **279-FQFATLPVY-287 and 786-FSFGVTQEY-794** and **69-ITYQGLFPY-77**. They demonstrated population coverage against the whole world **60.81%** Three epitopes, **924-AQYVAGYKV-938**, **1271-ALNESYIDL-1285** and**1300-AGLVALALC-1314**, demonstrated population coverage against the whole world 65.76% against MHC-II. Interestingly the epitope **924-AQYVAGYKV -938** was shown to interact with both MHC-I and MHC-II alleles. The overall epitope sets for the predicted epitopes against MHC-I and MHC-II alleles were **91.18%** as shown in Figure 6. We selected only nine countries of the MENA region while predicting population coverage, which are North Africa, Iran Israel, Jordan, Lebanon, Oman, Saudi Arabia, Turkey, and the United Arab of Emirate. In North Africa, population coverage was 74.33%, Iran 78.30%, Israel 69.89%, Jordan 66.94%, Lebanon 68.38%, Oman 55.47%, Saudi Arabia 84.68%, Turkey 79.73% and United Arab of Emirate 2.19%. The highest population coverage 84.68%, was seen for Saudi Arabia and the lowest at 2.19%.was seen for the United Arab of Emirate.

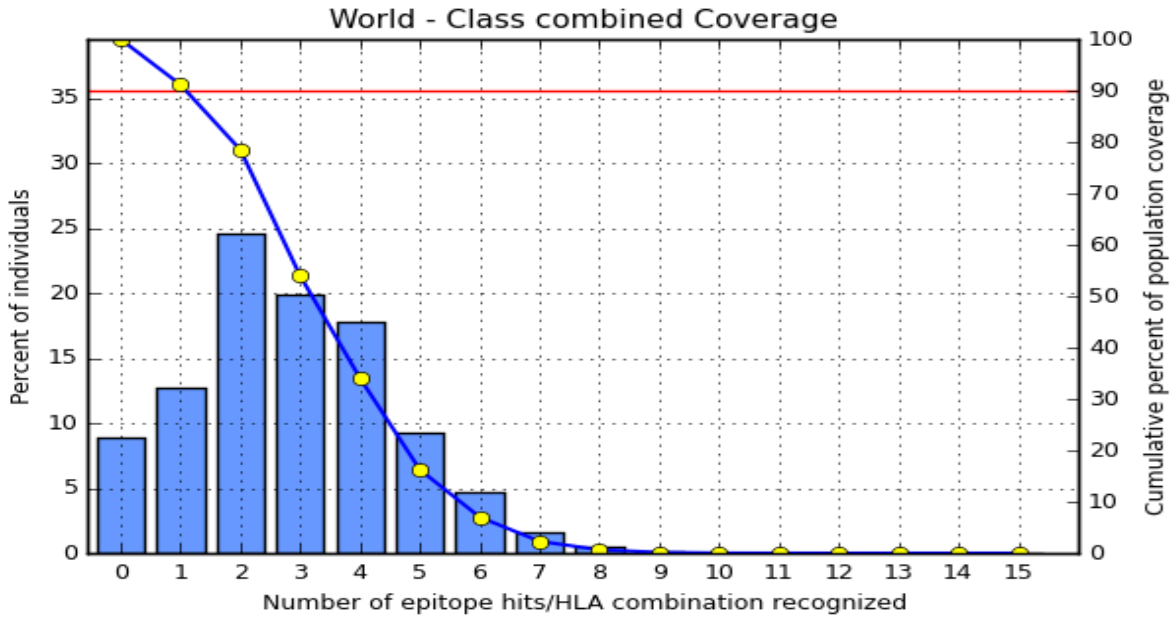

Figure 6 Combined population coverage of MHC class I and class II for proposed peptides from Selected Spike glycoprotein of MERS **-Coronaviruses.**

Discussion

Researchers to predict possible antigenic epitopes from MERS -Corona-virus proteins (especially Spike glycoprotein) for peptide-based vaccine development have instantly utilized advancements of immuno-informatics tools. *In-silico* adducts aided over *in vitro* experimental techniques and tools in vaccine design individually in terms of cost as well as time. Moreover, MERS -COV is being a positive-strand RNA virus is more vulnerable to mutation due to a lack of proofreading activity of RNA polymerase. However, for reliable vaccine candidates, a highly conserved epitope will ensure effective and long-lasting immunity. Therefore, this study aims to identify potential epitopes that can induce cellular and humoral immune reactions and act as a candidate for vaccine development. Therefore, we used immune- informatics tools to identify epitopes for multiple peptide vaccine for Spike glycoprotein. One epitope from the only conserved region was found to interact with Spike glycoprotein, It was found that the most satisfactory peptide is 6 amino acid **106 – SQDVKQ-111** B cell epitope from 106 to 111 with antigenicity score of 1.037 and 2.651 Score for Emini surface accessibility and chosen as a proposed peptide that can activate B cell to produce

antibodies against the virus or it had a domain that can neutralize antibodies [28]. While three Epitopes from structural Spike glycoprotein interacted with MHC class I HLA alleles. T cell immune response is essential for longer-lasting

responses [28]. The proposed T cell peptide **279-FQFATLPVY-287 and 786-FSFGVTQEY-794** and **69-ITYQGLFPY-77** with potential population coverage of **60.81%** this peptide considered as a candidate for vaccine production. Three epitopes, **924- AQYVAGYKV-938**, **1271-ALNESYIDL-1285**, and **1300-AGLVALALC-1314**, demonstrated population coverage against the whole world 65.76% against MHC-II. Interestingly the epitope **924-AQYVAGYKV -938** was shown to interact with both MHC-I and MHC-II alleles. The following proposed peptides are recommended for multiple peptides vaccine designs against Coronavirus **106–SQDVKQ-111**, **279-FQFATLPVY-287, and 786-FSFGVTQEY -794** and **69-ITYQGLFPY-77, and 924-AQYVAGYKV-938**, **1271-ALNESYIDL-1285**, and **1300-AGLVALALC-1314**. This vaccine will ensure good population coverage **91.18%** and fewer side effects that can be seen with the live-attenuated vaccine with T cell response against the vaccine. The peptides found in the present study may prove more immunogenic as compared to the earlier reported peptides [29]. Predicted peptides might show the physicochemical instability, to overcome this limitation, several structural as well as physical modification strategies are available to enhance the poor physicochemical stability of peptides. These strategies are including peptidomimetic approach, prodrug approach, analog formations, hydrophobic ion pairing, conjugation with fatty acids, and use of substitute methods of drug administration. Researchers have been working to gather data linked to Corona-virus to understand its biology, transmission, and pathophysiology to eliminate the disease. Shortly, we anticipate that predicted epitopes have therapeutic potential with an outstanding scope. Our immune-informatics examinations have proposed a strong T cell epitope along with a B cell epitope that will efficiently support the development of potent peptide-based vaccines to deal with the MERS -Corona-virus challenge. In recent studies, novel antigenic epitopes of some essential and vital proteins revealed that could victoriously elicit the response of the immune system, therefore, becoming great peptide vaccines targets and protecting the host from virus attack. Therefore, the current research was conducted to predict antigenic epitopes of spike glycoprotein of MERS -Corona-virus. We carried out sequence, structure, and conservation analysis as well as homology modeling of spike glycoprotein of Corona-virus. These epitopes were capable to induce a particular immunologic response. Hopefully, that few of the antigenic epitopes suggested and screened in this work might present a preliminary set of peptides for future vaccine development against MERS -Corona-virus for control and prevention of this devastating epidemic. Limitation occurs where there is no alleles of Sudanese have not been screened. There are only 29 sequences of Spike glycoproteins available in the database; more sequences are needed to increase the significance of the result. This vaccine will ensure a good population. Coverage and fewer side effects that can be seen with life attenuated vaccine.

Conclusion and Recommendations

The efficacy and safety of predicted epitopes by this computational analysis are needed to evaluate animal model studies, to confirm whether they can induce a protective immune response or not. More sequences are needed to increase the significance of the result. The following proposed peptides are recommended for multiple peptides vaccine designs against MERS **106–SQDVKQ-111**, **279-FQFATLPVY-287, and**, **1300-AGLVALALC-1314**. This vaccine will ensure a good population. Coverage and fewer side effects that can be seen with life attenuated vaccine.

Recommendations: Using Animal model studies, to confirm whether they can induce a protective immune response or not.

Declaration: The views expressed in the submitted article are the author's own and not an official position of the institution or funder.

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