Hindawi BioMed Research International Volume 2020, Article ID 2683286, 12 pages https://doi.org/10.1155/2020/2683286



Research Article

Design of a Multiepitope-Based Peptide Vaccine against the E Protein of Human COVID-19: An Immunoinformatics Approach

Miyssa I. Abdelmageed,¹ Abdelrahman H. Abdelmoneim ,² Mujahed I. Mustafa ,³ Nafisa M. Elfadol,⁴ Naseem S. Murshed,⁵ Shaza W. Shantier,⁶ and Abdelrafie M. Makhawi³

Correspondence should be addressed to Mujahed I. Mustafa; mujahedibrahim44@gmail.com

Received 29 February 2020; Accepted 20 April 2020; Published 11 May 2020

Academic Editor: Bilal Alatas

Copyright © 2020 Miyssa I. Abdelmageed et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. A new endemic disease has spread across Wuhan City, China, in December 2019. Within few weeks, the World Health Organization (WHO) announced a novel coronavirus designated as coronavirus disease 2019 (COVID-19). In late January 2020, WHO declared the outbreak of a "public-health emergency of international concern" due to the rapid and increasing spread of the disease worldwide. Currently, there is no vaccine or approved treatment for this emerging infection; thus, the objective of this study is to design a multiepitope peptide vaccine against COVID-19 using an immunoinformatics approach. Method. Several techniques facilitating the combination of the immunoinformatics approach and comparative genomic approach were used in order to determine the potential peptides for designing the T-cell epitope-based peptide vaccine using the envelope protein of 2019-nCoV as a target. Results. Extensive mutations, insertion, and deletion were discovered with comparative sequencing in the COVID-19 strain. Additionally, ten peptides binding to MHC class I and MHC class II were found to be promising candidates for vaccine design with adequate world population coverage of 88.5% and 99.99%, respectively. Conclusion. The T-cell epitope-based peptide vaccine was designed for COVID-19 using the envelope protein as an immunogenic target. Nevertheless, the proposed vaccine rapidly needs to be validated clinically in order to ensure its safety and immunogenic profile to help stop this epidemic before it leads to devastating global outbreaks.

1. Introduction

Coronaviruses (CoV) are a large family of zoonotic viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). In the last decades, six strains of coronaviruses were identified; however, in December 2019, a new strain has spread across Wuhan City, China [1, 2]. It was designated as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO) [3]. In late January 2020, WHO declared the outbreak a global pandemic with cases in more

than 45 countries where the COVID-19 was spreading fast outside China, most significantly in South Korea, Italy, and Iran with over 2,924 deaths and 85,212 cases confirmed while 39,537 recovered on 29 February 2020, 06:05 AM (GMT).

COVID-19 is a positive-sense single-stranded RNA virus (+ssRNA). Its RNA sequence is approximately 30,000 bases in length [4]. It belongs to the subgenus *Sarbecovirus* and genus *Betacoronavirus* within the family *Coronaviridae*. The corona envelope (E) protein is a small, integral membrane protein involved in several aspects of the virus' life cycle, such as pathogenesis, envelope formation, assembly, and budding, alongside with its interactions with both other

¹Faculty of Pharmacy, University of Khartoum, Khartoum, Sudan

²Faculty of Medicine, Alneelain University, Khartoum, Sudan

³Department of Biotechnology, University of Bahri, Khartoum, Sudan

⁴Department of Molecular Biology, National University Biomedical Research Institute, National University, Khartoum, Sudan

⁵Department of Microbiology, International University of Africa, Khartoum, Sudan

 $^{^6}$ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, Khartoum, Sudan

CoV proteins (M, N, and S) and host cell proteins (release of infectious particles after budding) [5–9].

The infected person is characterized with fever, upper or lower respiratory tract symptoms, diarrhea, lymphopenia, thrombocytopenia, and increased C-reactive protein and lactate dehydrogenase levels or combination of all these within 3-6 days after exposure. Further molecular diagnosis can be made by real-time PCR for genes encoding the internal RNA-dependent RNA polymerase and Spike's receptor binding domain, which can be confirmed by Sanger sequencing and full genome analysis by NGS, multiplex nucleic acid amplification, and microarray-based assays [10–14].

A phylogenetic tree of the mutation history of a family of viruses is possible to reconstruct with a sufficient number of sequenced genomes. The phylogenetic analysis indicates that COVID-19 likely originated from bats [15]. It also showed that it is highly related with at most seven mutations relative to a common ancestor [16].

The sequence of COVID-19 RBD, together with its RBM that contacts receptor angiotensin-converting enzyme 2 (ACE2), was found similar to that of SARS coronavirus. In January 2020, a group of scientists demonstrated that ACE2 could act as the receptor for COVID-19 [17–21]. However, COVID-19 differs from other previous strains in having several critical residues at the 2019-nCoV receptor-binding motif (particularly Gln493) which provide advantageous interactions with human ACE2 [15]. This difference in affinity possibly explains why the novel coronavirus is more contagious than other viruses.

At present, there is no vaccine or approved treatment for humans, but Chinese traditional medicines, such as ShuFengJieDu capsules and Lianhuaqingwen capsules, could be possible treatments for COVID-19. However, there are no clinical trials approving the safety and efficacy for these drugs [22].

The main concept within all the immunizations is the ability of the vaccine to initiate an immune response in a faster mode than the pathogen itself. Although traditional vaccines, which depend on biochemical trials, induced potent neutralizing and protective responses in the immunized animals, they can be costly, allergenic, and time-consuming and require in vitro culture of pathogenic viruses leading to serious concern of safety [23, 24]. Thus, the need for safe and efficacious vaccines is highly recommended.

Peptide-based vaccines do not need in vitro culture making them biologically safe, and their selectivity allows accurate activation of immune responses [25, 26]. The core mechanism of the peptide vaccines is built on the chemical method to synthesize the recognized B-cell and T-cell epitopes that are immunodominant and can induce specific immune responses. A B-cell epitope of a target molecule can be linked with a T-cell epitope to make it immunogenic. The T-cell epitopes are short peptide fragments (8-20 amino acids), whereas the B-cell epitopes can be proteins [27, 28]. Therefore, in this study, we aimed to design a peptide-based vaccine to predict epitopes from the corona envelope (E) protein using immunoinformatics analysis [29–34]. Rapid further studies are recommended to prove the effi-

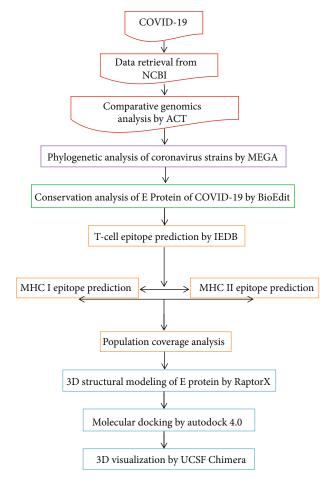


FIGURE 1: Descriptive workflow for the epitope-based peptide vaccine prediction.

ciency of the predicted epitopes as a peptide vaccine against this emerging infection.

2. Materials and Methods

The workflow summarizing the procedures for the epitopebased peptide vaccine prediction is shown in Figure 1.

- 2.1. Data Retrieval. Full GenBank files of the complete genomes and annotation of COVID-19 (NC_04551), SARS-CoV (FJ211859), MESA-CoV (NC_019843), HCoV-HKU1 (AY884001), HCoV-OC43 (KF923903), HCoV-NL63 (NC_005831), and HCoV-229E (KY983587) were retrieved from the National Center for Biotechnology Information (NCBI), while the FASTA format of the envelope (E) protein (YP_009724392.1), spike (S) protein (YP_009724390.1), nucleocapsid (N) protein (YP_009724397.2), and membrane (M) protein (YP_009724393.1) of 2019-nCoV and the envelope (E) protein of two Chinese and two American sequences (YP009724392.1, QHQ71975.1, QHO60596.1, and QHN73797.1) were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/).
- 2.2. The Artemis Comparison Tool (ACT). ACT is an in silico analysis software for visualization of comparisons between complete genome sequences and associated annotations

[35]. It is also applied to identify regions of similarity, rearrangements, and insertions at any level from base pair differences to the whole genome (https://www.sanger.ac.uk/science/tools/artemis-comparison-tool-act).

- 2.3. VaxiJen Server. It is the first server for alignment-independent prediction of protective antigens. It allows antigen classification solely based on the physicochemical properties of proteins without recourse to sequence alignment. It predicts the probability of the antigenicity of one or multiple proteins based on auto cross covariance (ACC) transformation of protein sequence. Structural CoV-2019 proteins (N, S, E, and M) were analyzed by VaxiJen with threshold of 0.4 [36] (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html).
- 2.4. BioEdit. It is a software package proposed to stream a distinct program that can run nearly any sequence operation as well as a few basic alignment investigations. The sequences of the E protein retrieved from UniProt were run in BioEdit to determine the conserved sites through ClustalW in the application settings [37].
- 2.5. The Molecular Evolutionary Genetics Analysis (MEGA). MEGA (version 10.1.6) is software for the comparative analysis of molecular sequences. It is used for pairwise and multiple sequence alignment alongside construction and analysis of phylogenetic trees and evolutionary relationships. The gap penalty was 15 for opening and 6.66 for extending the gap for both pairwise and multiple sequence alignment. Bootstrapping of 300 was used in construction of the maximum like hood phylogenetic tree [38, 39] (https://www.megasoftware.net).
- 2.6. Prediction of T-Cell Epitopes. IEDB tools were used to predict the conserved sequences (10-mer sequence) from HLA class I and class II T-cell epitopes by using an Artificial Neural Network (ANN) approach [40–42]. The Artificial Neural Network (ANN) version 2.2 was chosen as the prediction method as it depends on the median inhibitory concentration (IC50) [40, 43–45]. For the binding analysis, all the alleles were carefully chosen, and the length was set at 10 before prediction was done. Analysis of epitopes binding to the MHC class I and II molecules was assessed by the IEDB MHC prediction server at http://tools.iedb.org/mhci/ and http://tools.iedb.org/mhcii/, respectively. All conserved immunodominant peptides binding to the MHC I and II molecules at scores equal or less than 100 median inhibitory concentrations (IC50) and 1000, respectively, were selected for further analysis while epitopes with IC50 greater than 100 were eliminated [46].
- 2.7. Population Coverage Analysis. Population coverage for each epitope was carefully determined by the IEDB population coverage calculation tool. Due to the diverse binding sites of epitopes with different HLA alleles, the most promising epitope candidates were calculated for population coverage against the population of the whole world, China, and Europe to get and ensure a universal vaccine [47, 48] (http://tools.iedb.org/population/).

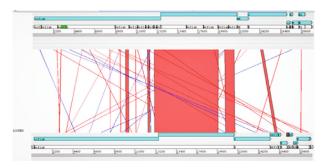


FIGURE 2: Artemis analysis of the envelope protein displaying 3 windows. The upper window represents the HCoV-HKU1 reference sequence, and its genes are highlighted in blue starting from orflab gene and ending with N gene. The middle window describes the similarities and the difference between the two genomes. Red lines indicate a match between genes from the two genomes; blue lines indicate inversion which represents the same sequences in the two genomes, but they are organized in the opposite direction. The lower window represents COVID-19 and its genes starting from orflab gene and ending with N gene.

Table 1: VaxiJen overall prediction of probable COVID-19 antigen.

Protein	Result	VaxiJen prediction
Protein E	0.6025	Probable antigen
Protein M	0.5102	Probable antigen
Protein S	0.4646	Probable antigen
Protein N	0.5059	Probable antigen

2.8. Tertiary Structure (3D) Modeling. The reference sequence of the E protein that has been retrieved from GenBank was used as an input in RaptorX to predict the 3D structure of the E protein [49, 50]; the visualization of the obtained 3D protein structure was performed in UCSF Chimera (version1.8) [51].

2.9. In Silico Molecular Docking

2.9.1. Ligand Preparation. In order to estimate the binding affinities between the epitopes and the molecular structure of MHC I and MHC II, in silico molecular docking was used. Sequences of proposed epitopes were selected from the COVID-19 reference sequence using UCSF Chimera 1.10 and saved as a PDB file. The obtained files were then optimized and energy minimized. The HLA-A*02:01 was selected as the macromolecule for docking. Its crystal structure (4UQ3) was downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), which was in a complex with an azobenzene-containing peptide [52].

All water molecules and heteroatoms in the retrieved target file 4UQ3 were then removed. The target structure was further optimized and energy minimized using Swiss PDB Viewer V.4.1.0 software [53].

2.9.2. Molecular Docking. Molecular docking was performed using AutoDock 4.0 software, based on the Lamarckian genetic algorithm, which combines energy evaluation through grids of affinity potential to find the suitable binding



FIGURE 3: Sequence alignment of the envelope protein of COVID-19 using BioEdit software (total conservation through the 4 strains: 2 from China and 2 from the USA).

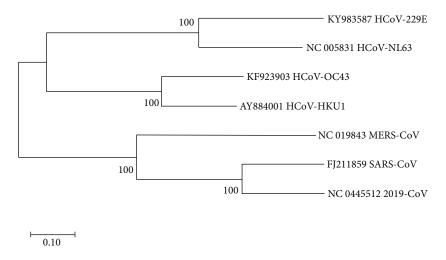


FIGURE 4: Maximum likelihood phylogenetic tree which describes the evolutionary relationship between the seven strains of coronavirus.

Table 2: The most promising MHC class I-related peptides in the envelope protein-based vaccine of COVID-19 along with the predicted coverage of the world, China, Europe, and East Asia.

Peptide	Alleles	Coverage	Combined coverage of 10 peptides
YVYSRVKNL	HLA-C*14:02, HLA-C*12:03, HLA-C*07:01, HLA-C*03:03, HLA-C*06:02	50.02%	World: 88.5%
SLVKPSFYV	HLA-A*02:06, HLA-A*02:01, HLA-A*68:02	42.53%	China: 78.17%
SVLLFLAFV	HLA-A*02:06, HLA-A*68:02, HLA-A*02:01	42.53%	Europe: 92.94%
FLAFVVFLL	HLA-A*02:01, HLA-A*02:06	40.60%	East Asia: 80.78%
VLLFLAFVV	HLA-A*02:01	39.08%	
RLCAYCCNI	HLA-A*02:01	39.08%	
FVSEETGTL	HLA-C*03:03, HLA-C*12:03, HLA-A*02:06, HLA-A*68:02, HLA-B*35:01	28.22%	
LTALRLCAY	HLA-A*01:01, HLA-A*30:02, HLA-B*15:01	26.34%	
LVKPSFYVY	HLA-B*15:01, HLA-A*29:02, HLA-A*30:02, HLA-B*35:01	21.72%	
NIVNVSLVK	HLA-A*68:01, HLA-A*11:01	20.88%	

position for a ligand on a given protein [54, 55] Polar hydrogen atoms were added to the protein targets, and Kollman united atomic charges were computed. The target's grid map was calculated and set to $60 \times 60 \times 60$ points with grid spacing of 0.375 Å. The grid box was then allocated properly in the target to include the active residue in the center. The genetic algorithm and its run were set to 100. The docking algorithms were set to default. Finally, results were retrieved as binding energies and poses that showed the lowest binding energies visualized using UCSF Chimera.

3. Results

- 3.1. The Artemis Comparison Tool. The reference sequence of the envelope protein was aligned with the HCoV-HKU1 reference protein using the Artemis Comparison Tool as illustrated in (Figure 2).
- 3.2. VaxiJen Server. The mutated proteins were tested for antigenicity using VaxiJen software, where the envelope protein was found as the best immunogenic target in Table 1.

 $T_{ABLE~3:} \ The \ most~promising~MHC~class~II-related~peptides~in~the~envelope~protein-based~vaccine~of~COVID-19~along~with~the~predicted~coverage~of~the~world,~China,~Europe,~and~East~Asia.$

Peptide sequence	Alleles	World coverage	Coverage/10 peptides
KPSFYVYSRVKNLNS	HLA-DPA1*01:03, HLA-DPB1*02:01, HLA-DPB1*03:01, HLA-DPB1*04:01, HLA-DPA1*02:01, HLA-DPB1*05:01, HLA-DPA1*03:01, HLA-DPB1*04:02, HLA-DPB1*06:01, HLA-DPB1*14:01, HLA-DPB1*01:01, HLA-DQA1*05:01, HLA-DQB1*04:02, HLA-DQA1*05:01, HLA-DQB1*05:01, HLA-DQA1*06:01, HLA-DQA1*01:02, HLA-DQB1*05:01, HLA-DQA1*02:01, HLA-DRB1*01:01, HLA-DRB1*07:01, HLA-DRB1*08:01, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB4*01:03, HLA-DRB1*04:01, HLA-DRB1*10:01, HLA-DRB1*04:05, HLA-DRB1*13:01, HLA-DRB1*08:02, HLA-DRB1*16:02, HLA-DRB1*15:01, HLA-DRB3*03:01, HLA-DRB5*01:01, HLA-DRB3*02:02, HLA-DRB1*04:04, HLA-DRB1*13:02	99.93%	World: 99.99%
VKPSFYVYSRVKNLN	HLA-DPA1*01:03, HLA-DPB1*02:01, HLA-DPB1*04:01, HLA-DPB1*03:01, HLA-DPB1*02:01, HLA-DPB1*05:01, HLA-DPB1*03:01, HLA-DPB1*04:02, HLA-DPB1*06:01, HLA-DPB1*01:01, HLA-DPB1*05:01, HLA-DPB1*06:01, HLA-DPB1*06:01, HLA-DQA1*05:01, HLA-DQB1*05:01, HLA-DQA1*02:01, HLA-DQB1*07:01, HLA-DRB1*08:01, HLA-DRB1*01:01, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB4*01:03, HLA-DRB1*15:01, HLA-DRB1*13:01, HLA-DRB3*03:01, HLA-DRB1*10:01, HLA-DRB1*16:02, HLA-DRB1*08:02, HLA-DRB1*04:05, HLA-DRB1*04:01, HLA-DRB1*13:02, HLA-DRB3*02:02, HLA-DRB1*04:01, HLA-DRB1*10:01, HLA-DRB1*04:01, HLA-DRB1*10:01, HLA-DRB1*04:01, HLA-DRB1*10:01, HLA-DRB1*04:01, HLA-DRB1*10:01, HLA-DRB1*04:01, HLA-DRB1*10:01, HLA-DRB1*04:01,	99.92%	China: 99.96%
LVKPSFYVYSRVKNL	HLA-DPA1*01:03, HLA-DPB1*02:01, HLA-DPB1*04:01, HLA-DPA1*02:01, HLA-DPB1*05:01, HLA-DPB1*06:01, HLA-DPA1*03:01, HLA-DPB1*04:02, HLA-DPA1*02:01, HLA-DPB1*01:01, HLA-DQA1*06:01, HLA-DQB1*04:02, HLA-DQA1*05:01, HLA-DQA1*02:01, HLA-DQA1*01:04, HLA-DQB1*05:03, HLA-DQA1*01:02, HLA-DQB1*05:01, HLA-DRB1*07:01, HLA-DRB1*08:01, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*01:03, HLA-DRB3*03:01, HLA-DRB1*01:01, HLA-DRB1*15:01, HLA-DRB1*16:02, HLA-DRB1*13:01, HLA-DRB1*10:01, HLA-DRB1*08:02, HLA-DRB5*01:01, HLA-DRB1*13:02, HLA-DRB1*04:05, HLA-DRB3*02:02, HLA-DRB1*04:01	99.90%	Europe: 100.0%
PSFYVYSRVKNLNSS	HLA-DPA1*01:03, HLA-DPB1*02:01, HLA-DPB1*03:01, HLA-DPB1*04:01, HLA-DPB1*04:02, HLA-DPB1*02:01, HLA-DPB1*05:01, HLA-DPB1*06:01, HLA-DPA1*05:01, HLA-DPB1*06:01, HLA-DQA1*05:01, HLA-DQB1*04:02, HLA-DQA1*01:02, HLA-DQB1*05:01, HLA-DQA1*06:01, HLA-DRB1*01:01, HLA-DRB1*08:01, HLA-DRB1*04:01, HLA-DRB1*11:01, HLA-DRB1*09:01, HLA-DRB1*07:01, HLA-DRB4*01:03, HLA-DRB1*04:05, HLA-DRB1*10:01, HLA-DRB1*13:01, HLA-DRB1*08:02, HLA-DRB1*16:02, HLA-DRB1*15:01, HLA-DRB3*03:01, HLA-DRB3*02:02, HLA-DRB1*13:02	99.86%	East Asia:99.91%
NIVNVSLVKPSFYVY	HLA-DPA1*01:03, HLA-DPB1*02:01, HLA-DPB1*04:01, HLA-DPB1*06:01, HLA-DPA1*02:01, HLA-DPB1*01:01, HLA-DQA1*01:02, HLA-DQB1*05:01, HLA-DQA1*05:01, HLA-DQB1*04:02, HLA-DQA1*02:01, HLA-DQB1*03:01, HLA-DQB1*03:03, HLA-DQB1*03:03, HLA-DQB1*04:02, HLA-DRB1*12:01, HLA-DRB1*01:01, HLA-DRB5*01:01, HLA-DRB3*03:01, HLA-DRB1*13:01, HLA-DRB1*07:01, HLA-DRB1*15:01, HLA-DRB4*01:03, HLA-DRB1*04:04, HLA-DRB1*08:02, HLA-DRB1*09:01, HLA-DRB1*13:02, HLA-DRB1*11:01, HLA-DRB1*04:05, HLA-DRB1*10:01	99.77%	

Table 3: Continued.

6

Peptide sequence	Alleles	World coverage	Coverage/10 peptides
LLVTLAILTALRLCA	HLA-DPA1*01:03, HLA-DPB1*02:01, HLA-DPB1*06:01, HLA-DPA1*03:01, HLA-DPB1*04:02, HLA-DQA1*01:02, HLA-DQB1*05:01, HLA-DQA1*02:01, HLA-DQB1*03:01, HLA-DQB1*03:03, HLA-DQA1*05:01, HLA-DQB1*04:02, HLA-DQA1*06:01, HLA-DQA1*01:03, HLA-DQB1*06:03, HLA-DRB4*01:03, HLA-DRB1*13:01, HLA-DRB1*04:04, HLA-DRB5*01:01, HLA-DRB3*03:01, HLA-DRB1*10:01, HLA-DRB1*07:01, HLA-DRB1*11:01, HLA-DRB1*15:01, HLA-DRB1*12:01, HLA-DRB1*11:01, HLA-DRB1*08:01, HLA-DRB1*12:01, HLA-DRB1*03:01, HLA-DRB1*10:01, HLA-DRB1*10:01, HLA-DRB1*10:01, HLA-DRB1*10:01, HLA-DRB1*10:01, HLA-DRB1*10:01, HLA-DRB1*10:01, HLA-DRB1*10:01, HLA-DRB1*10:02, HLA-DRB1*08:02	99.72%	
SFYVYSRVKNLNSSR	HLA-DPA1*01:03, HLA-DPB1*03:01, HLA-DPB1*02:01, HLA-DPA1*03:01, HLA-DPB1*04:02, HLA-DPA1*02:01, HLA-DPB1*05:01, HLA-DPB1*06:01, HLA-DQA1*05:01, HLA-DQB1*05:01, HLA-DQB1*04:02, HLA-DQB1*05:01, HLA-DRB1*04:01, HLA-DRB1*01:01, HLA-DRB1*11:01, HLA-DRB1*08:01, HLA-DRB1*07:01, HLA-DRB1*09:01, HLA-DRB4*01:03, HLA-DRB1*10:01, HLA-DRB1*13:01, HLA-DRB1*04:05, HLA-DRB1*08:02, HLA-DRB1*16:02, HLA-DRB3*02:02, HLA-DRB3*03:01, HLA-DRB1*04:04, HLA-DRB1*15:01, HLA-DRB5*01:01, HLA-DRB1*13:02	99.72%	
LVTLAILTALRLCAY	HLA-DPA1*01:03, HLA-DPB1*02:01, HLA-DPB1*06:01, HLA-DPA1*03:01, HLA-DPB1*04:02, HLA-DQA1*01:02, HLA-DQB1*05:01, HLA-DQA1*02:01, HLA-DQB1*03:01, HLA-DQB1*03:03, HLA-DQA1*05:01, HLA-DQB1*04:02, HLA-DQB1*06:02, HLA-DQA1*02:01, HLA-DQA1*06:01, HLA-DRB4*01:03, HLA-DRB1*01:01, HLA-DRB1*13:01, HLA-DRB1*04:04, HLA-DRB1*12:01, HLA-DRB1*10:01, HLA-DRB5*01:01, HLA-DRB1*15:01, HLA-DRB1*11:01, HLA-DRB3*03:01, HLA-DRB1*03:01, HLA-DRB1*08:01, HLA-DRB1*07:01, HLA-DRB1*08:01, HLA-DRB1*07:01, HLA-DRB1*08:02	99.69%	
VTLAILTALRLCAYC	HLA-DPA1*01:03, HLA-DPB1*06:01, HLA-DPA1*03:01, HLA-DPB1*04:02, HLA-DQA1*02:01, HLA-DQB1*03:01, HLA-DQA1*01:02, HLA-DQB1*05:01, HLA-DQB1*06:02, HLA-DQA1*05:01, HLA-DQB1*04:02, HLA-DQA1*05:01, HLA-DQB1*04:02, HLA-DQB1*01:01, HLA-DQA1*06:01, HLA-DQB1*04:02, HLA-DRB1*01:01, HLA-DRB4*01:03, HLA-DRB1*13:01, HLA-DRB1*04:04, HLA-DRB1*12:01, HLA-DRB1*10:01, HLA-DRB5*01:01, HLA-DRB1*15:01, HLA-DRB1*11:01, HLA-DRB1*03:01, HLA-DRB3*03:01, HLA-DRB1*08:01, HLA-DRB1*07:01, HLA-DRB4*01:01, HLA-DRB1*04:02	99.56%	
CNIVNVSLVKPSFYV	HLA-DPA1*01:03, HLA-DPB1*06:01, HLA-DPB1*04:02, HLA-DQA1*01:02, HLA-DQB1*05:01, HLA-DQA1*05:01, HLA-DQB1*05:01, HLA-DQB1*05:01, HLA-DQB1*03:01, HLA-DQB1*03:03, HLA-DQA1*01:03, HLA-DQB1*06:03, HLA-DRB3*03:01, HLA-DRB1*12:01, HLA-DRB5*01:01, HLA-DRB1*01:01, HLA-DRB1*07:01, HLA-DRB4*01:03, HLA-DRB1*13:01, HLA-DRB1*15:01, HLA-DRB1*08:02, HLA-DRB1*04:04, HLA-DRB1*09:01, HLA-DRB1*13:02, HLA-DRB1*11:01, HLA-DRB1*04:05, HLA-DRB4*01:01, HLA-DRB1*10:01	99.53%	

3.3. BioEdit. Sequence alignment of the COVID-19 envelope protein was done using BioEdit software which shows total conservation across four sequences which were retrieved from China and the USA (Figure 3).

3.4. The Molecular Evolutionary Genetics Analysis. To study the evolutionary relationship between all the seven strains of coronavirus, a multiple sequence alignment (MSA) was performed using ClustalW by MEGA software. This alignment

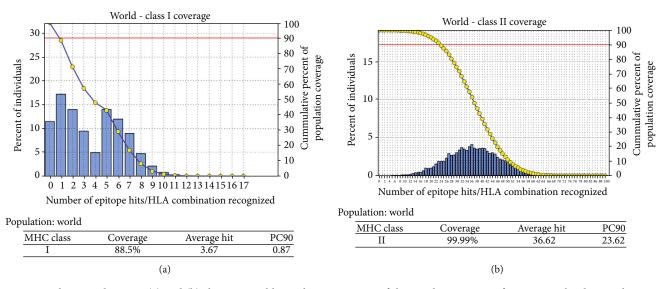


FIGURE 5: Schematic diagrams (a) and (b) showing world population coverage of the envelope protein of COVID-19 binding to the MHC class I and MHC class II molecules, respectively.

was used to construct the maximum likelihood phylogenetic tree as seen in Figure 4.

3.5. Prediction of T-Cell Epitopes and Population Coverage. The IEDB website was used to analyze the 2019-nCoV envelope protein for T-cell-related peptides. Results show ten MHC class I- and II-associated peptides with high population coverage (Tables 2 and 3; Figure 5). The most promising peptides were visualized using UCSF Chimera software (Figures 6(a) and 6(b)).

4. Discussion

Designing a novel vaccine is very crucial to defend against the rapid endless global burden of diseases [56–59]. In the last few decades, biotechnology has advanced rapidly, alongside with the understanding of immunology which assisted the rise of new approaches towards rational vaccine design [60]. Peptide-based vaccines are designed to elicit immunity particular pathogens by selectively stimulating antigenspecific B- and T-cells [25]. Applying the advanced bioinformatics tools and databases, various peptide-based vaccines could be designed where the peptides act as ligands [61–63]. This approach has been used frequently in Saint Louis encephalitis virus [64], dengue virus [65], and Chikungunya virus [66] proposing promising peptides for designing vaccines.

The COVID-19 is an RNA virus which tends to mutate more commonly than the DNA viruses [67]. These mutations lie on the surface of the protein, which makes COVID-19 more superior than other previous strains by inducing its sustainability leaving the immune system in a blind spot [68].

In our present work, different peptides were proposed for designing a vaccine against COVID-19 (Figure 1). In the beginning, the whole genome of COVID-19 was analyzed by a comparative genomic approach to determine the potential antigenic target [69]. The Artemis Comparison Tool (ACT) was used to analyze human coronavirus (HCoV-HKU1) reference sequence vs. Wuhan-Hu-1 COVID-19. Results obtained (Figure 2) revealed extensive mutations among the tested genomes. New genes (*ORF8* and *ORF6*) were found inserted in COVID-19 which were absent in HCoV-HKU1 that might be acquired by the horizontal gene transmission [70]. The high rate of mutation between the two genomes was observed in the region from 20,000 bp to the end of the sequence. This region encodes the four major structural proteins in coronavirus which are the envelope (E) protein, nucleocapsid (N) protein, membrane (M) protein, and spike (S) protein, all of which are required to produce a structurally complete virus [71, 72].

These conserved antigenic sites were revealed in previous studies through sequence alignment between MERS-CoV and bat coronavirus [73] and analyzed in SARS-CoV [74].

The four proteins were then analyzed by VaxiJen software to test the probability of antigenic proteins. Protein E was found to be the most antigenic gene with the highest probability as shown in Table 1. A literature survey confirmed this result in which protein E was investigated in Severe Acute Respiratory Syndrome (SARS) in 2003 and, more recently, Middle-East Respiratory Syndrome (MERS) [71]. Furthermore, the conservation of this protein against the seven strains was tested and confirmed through the use of the BioEdit package tool (Figure 3).

Phylogenetic analysis is a very powerful tool for determining the evolutionary relationship between strains. Multiple sequence alignment (MSA) was performed using ClustalW for the seven strains of coronavirus, which are COVID-19 (NC_04551), SARS-CoV (FJ211859), MESA-CoV (NC_019843), HCoV-HKU1 (AY884001), HCoV-OC43 (KF923903), HCoV-NL63 (NC_005831), and HCoV-229E (KY983587). The maximum likelihood phylogenetic

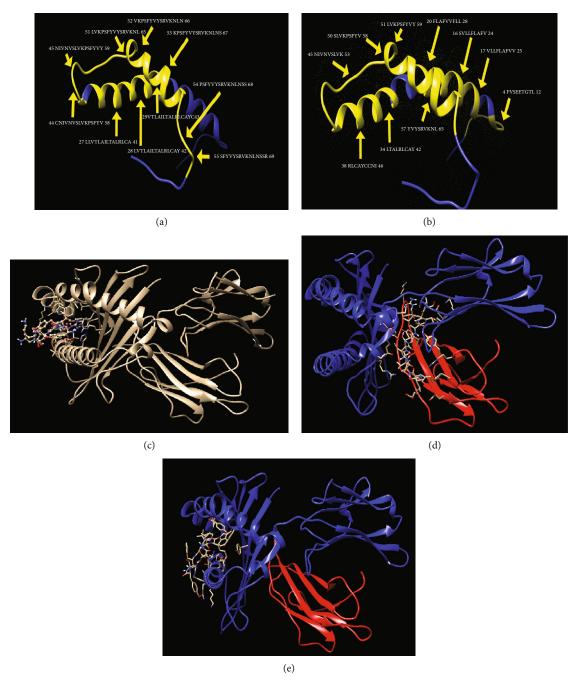


FIGURE 6: 3D structures visualized by UCSF Chimera: (a) and (b) show the most promising peptides in the envelope protein of COVID-19 (yellow colored) binding to MHC class I and MHC class II, respectively, while (c), (d), and (e) show the molecular docking of the YVYSRVKNL, LAILTALRL, and SLVKPSFYV peptides of coronavirus docked in HLA-A*02:01, respectively.

tree revealed that COVID-19 is found in the same clade of SARS-CoV; thus, the two strains are highly related to each other (Figure 4).

The immune response of T-cells is considered a long-lasting response compared to B-cells, where the antigen can easily escape the antibody memory response [75]. Vaccines that effectively generate cell-mediated responses are needed to provide protection against the invading pathogen. Moreover, the CD8+ and CD4+ T-cell responses play a major role

in antiviral immunity [76]. Thus, designing a vaccine against T-cells is much more important.

Choosing protein E as the antigenic site, the binding affinity to MHC molecules was then evaluated. The protein reference sequence was submitted to the IEDB MHC predication tool. 21 peptides were found to bind MHC class I with different affinities (Table 1), from which ten peptides were selected for vaccine design based on the number of alleles and world population percentage (Table 2; Figure 5).

Analysis in the IEDB MHC II binding prediction tool resulted in prediction of 61 peptides (Table 2), from which ten peptides were selected for vaccine design based on the number of alleles and world population percentage (Table 3; Figure 5). Unfortunately, IEDB did not give any result for B-cell epitopes; this might be due to the length of the COVID-19 (75 amino acids).

It is well known that peptides recognized with a high number of HLA molecules are potentially inducing immune response. Based on the aforementioned results and taking into consideration the high binding affinity to both MHC class I and II, conservancy, and population coverage, three peptides are strongly proposed to formulate a new vaccine against COVID-19.

These findings were further confirmed by the results obtained for the molecular docking of the proposed peptides and HLA-A*02:01. The formed complex between the MHC molecule and the three peptides (YVYSRVKNL, SLVKPSFYV, and LAILTALRL) has shown peptide amino- and carboxyltermini forming one and three hydrogen bonds, respectively, at the two ends of a binding groove with MHC residues with the least binding energy -13.2 kcal/mol, -11 kcal/mol, and -11.3 kcal/mol, respectively (Figures 6(c)-6(e)).

Although both flu and anti-HIV drugs are used currently in China for treatment of COVID-19, chloroquine phosphate, an old drug for treatment of malaria, has recently been found to have apparent efficacy and acceptable safety against COVID-19 [77, 78]; nevertheless, more studies are required to standardize these therapies. In addition, there has been some success in the development of mouse models of MERS-CoV and SARS-CoV infection, and candidate vaccines where the envelope (E) protein is mutated or deleted have been described [79–85]. To the best of our knowledge, this is the first study to identify certain peptides in the envelope (E) protein as candidates for COVID-19. Accordingly, these epitopes were strongly recommended as promising epitope vaccine candidates against T-cells.

5. Conclusion

Extensive mutations, insertion, and deletion were discovered in the COVID-19 strain using the comparative sequencing. In addition, a number of the MHC class I- and II-related peptides were found to be promising candidates. Among which, the peptides YVYSRVKNL, SLVKPSFYV, and LAILTALRL show high potentiality for vaccine design with adequate world population coverage. The T-cell epitope-based peptide vaccine was designed for COVID-19 using the envelope protein as an immunogenic target; nevertheless, the proposed vaccine rapidly needs to be validated clinically ensuring its safety and immunogenic profile to help stop this epidemic before it leads to devastating global outbreaks.

Abbreviations

WHO: World Health Organization

CoV: Coronaviruses

MERS-CoV: Middle East Respiratory Syndrome SARS-CoV: Severe Acute Respiratory Syndrome

COVID-19: Novel coronavirus

HCoV-HKU1: Human coronavirus HKU1 HCoV-OC43: Human coronavirus OC43 HCoV-NL63: Human coronavirus NL63 HCoV-229E: Human coronavirus 229E

ACE2: Angiotensin-converting enzyme 2

RBM: Receptor-binding motif
RBD: Receptor-binding domain

vs.: Versus

ACT: Artemis Comparison Tool ACC: Auto cross covariance

MEGA: Molecular Evolutionary Genetics Analysis

ANN: Artificial Neural Network
IEDB: Immune Epitope Database
IC50: Median inhibitory concentrations
MHC I: Major histocompatibility complex class I
MHC II: Major histocompatibility complex class II

PDB: Protein database

MSA: Multiple sequence alignment.

Data Availability

All data underlying the results are available as part of the article, and no additional source data are required.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

The contributions of the authors involved in this study are as follows: MIA: conceptualization, formal analysis, investigation, methodology, validation, visualization, and writing (original draft); AHA: formal analysis, investigation, and methodology; MIM: methodology, writing (original draft), and writing (review and editing); NME: formal analysis, methodology, and visualization; NSM: conceptualization, resources, and writing (review and editing); SWS: visualization, validation, and writing (review and editing); and AMM: data curation, conceptualization, project administration, supervision, and writing (review and editing). All authors have read and approved the final manuscript.

Acknowledgments

The authors acknowledge the Deanship of Scientific Research at University of Bahri for the supportive cooperation.

References

- [1] H. Lu, C. W. Stratton, and Y. W. Tang, "Outbreak of pneumonia of unknown etiology in Wuhan China: the mystery and the miracle," *Journal of Medical Virology*, vol. 92, no. 4, pp. 401-402, 2020.
- [2] D. S. Hui, E. I Azhar, T. A. Madani et al., "The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health — The latest 2019 novel coronavirus outbreak in Wuhan, China," *International Journal of Infectious Diseases*, vol. 91, pp. 264–266, 2020.

- [3] D. Benvenuto, M. Giovanetti, A. Ciccozzi, S. Spoto, S. Angeletti, and M. Ciccozzi, "The 2019-new coronavirus epidemic: evidence for virus evolution," *Journal of Medical Virology*, vol. 92, no. 4, pp. 455–459, 2020.
- [4] CoV2020, GISAID EpifluDB. Archived from the original on 12 January 2020, 2020.
- [5] P. Venkatagopalan, S. M. Daskalova, L. A. Lopez, K. A. Dolezal, and B. G. Hogue, "Coronavirus envelope (E) protein remains at the site of assembly," *Virology*, vol. 478, pp. 75–85, 2015.
- [6] J. L. Nieto-Torres, M. L. DeDiego, E. Álvarez et al., "Subcellular location and topology of severe acute respiratory syndrome coronavirus envelope protein," *Virology*, vol. 415, no. 2, pp. 69–82, 2011.
- [7] K. M. Curtis, B. Yount, and R. S. Baric, "Heterologous gene expression from transmissible gastroenteritis virus replicon particles," *Journal of Virology*, vol. 76, no. 3, pp. 1422–1434, 2002.
- [8] J. Ortego, D. Escors, H. Laude, and L. Enjuanes, "Generation of a replication-competent, propagation-deficient virus vector based on the transmissible gastroenteritis coronavirus genome," *Journal of Virology*, vol. 76, no. 22, pp. 11518– 11529, 2002.
- [9] T. R. Ruch and C. E. Machamer, "A single polar residue and distinct membrane topologies impact the function of the infectious bronchitis coronavirus E protein," *PLoS Pathogens*, vol. 8, no. 5, article e1002674, 2012.
- [10] J. F.-W. Chan, S. Yuan, K.-H. Kok et al., "A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster," *Lancet*, vol. 395, no. 10223, pp. 514–523, 2020.
- [11] N. Zhang, L. Wang, X. Deng et al., "Recent advances in the detection of respiratory virus infection in humans," *Journal* of *Medical Virology*, vol. 92, no. 4, pp. 408–417, 2020.
- [12] J. B. Mahony, A. Petrich, and M. Smieja, "Molecular diagnosis of respiratory virus infections," *Critical reviews in clinical lab*oratory sciences, vol. 48, no. 5-6, pp. 217–249, 2011.
- [13] V. M. Corman, O. Landt, M. Kaiser et al., "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR," Eurosurveillance, vol. 25, no. 3, 2020.
- [14] Y. Chen, Q. Liu, and D. Guo, "Emerging coronaviruses: genome structure, replication, and pathogenesis," *Journal of Medical Virology*, vol. 92, no. 4, pp. 418–423, 2020.
- [15] Y. Wan, J. Shang, R. Graham, R. S. Baric, and F. Li, "Receptor recognition by the Novel Coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS Coronavirus," *Journal of Virology*, vol. 94, no. 7, 2020.
- [16] T. Bedford and R. Neher, Genomic Epidemiology of Novel Coronavirus (nCoV) Using Data Generated by Fudan University, China CDC, Chinese Academy of Medical Sciences, Chinese Academy of Sciences and the Thai National Institute of Health Shared via GISAID, 2020.
- [17] M. Letko and V. Munster, Functional assessment of cell entry and receptor usage for lineage B β-coronaviruses, including 2019-nCoV, bioRxiv, 2020.
- [18] M. Letko, A. Marzi, and V. Munster, "Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses," *Nature Microbiology*, vol. 5, no. 4, pp. 562–569, 2020.
- [19] H. M. El Sahly, "Genomic characterization of the 2019 novel coronavirus," New England Journal of Medicine, Massachusetts, USA, 2020.

- [20] L. E. Gralinski and V. D. Menachery, "Return of the Coronavirus: 2019-nCoV," *Viruses*, vol. 12, no. 2, p. 135, 2020.
- [21] R. Lu, X. Zhao, J. Li et al., "Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding," *Lancet*, vol. 395, no. 10224, pp. 565–574, 2020.
- [22] H. Lu, "Drug treatment options for the 2019-new coronavirus (2019-nCoV)," *BioScience Trends*, vol. 14, no. 1, pp. 69–71, 2020.
- [23] Y. T. Lo, T. W. Pai, W. K. Wu, and H. T. Chang, "Prediction of conformational epitopes with the use of a knowledge-based energy function and geometrically related neighboring residue characteristics," *BMC Bioinformatics*, vol. 14, no. S4, Supplement 4, p. S3, 2013.
- [24] W. Li, M. Joshi, S. Singhania, K. Ramsey, and A. Murthy, "Peptide vaccine: progress and challenges," *Vaccines*, vol. 2, no. 3, pp. 515–536, 2014.
- [25] A. W. Purcell, J. McCluskey, and J. Rossjohn, "More than one reason to rethink the use of peptides in vaccine design," *Nature Reviews Drug Discovery*, vol. 6, no. 5, pp. 404–414, 2007.
- [26] N. L. Dudek, P. Perlmutter, M. I. Aguilar, N. P. Croft, and A. W. Purcell, "Epitope discovery and their use in peptide based vaccines," *Current Pharmaceutical Design*, vol. 16, no. 28, pp. 3149–3157, 2010.
- [27] S. Dermime, D. E. Gilham, D. M. Shaw et al., "Vaccine and antibody-directed T cell tumour immunotherapy," *Biochimica et Biophysica Acta (BBA) Reviews on Cancer*, vol. 1704, no. 1, pp. 11–35, 2004.
- [28] R. H. Meloen, J. P. M. Langeveld, W. M. M. Schaaper, and J. W. Slootstra, "Synthetic peptide vaccines: unexpected fulfillment of discarded hope?," *Biologicals*, vol. 29, no. 3-4, pp. 233–236, 2001.
- [29] V. Brusic and N. Petrovsky, "Immunoinformatics and its relevance to understanding human immune disease," Expert Review of Clinical Immunology, vol. 1, no. 1, pp. 145–157, 2014.
- [30] N. Tomar and R. K. De, "Immunoinformatics: a brief review," Methods in Molecular Biology, vol. 1184, pp. 23–55, 2014.
- [31] S. Khalili, A. Jahangiri, H. Borna, K. Ahmadi Zanoos, and J. Amani, "Computational vaccinology and epitope vaccine design by immunoinformatics," *Acta Microbiologica et Immunologica Hungarica*, vol. 61, no. 3, pp. 285–307, 2014.
- [32] N. Tomar and R. K. De, "Immunoinformatics: an integrated scenario," *Immunology*, vol. 131, no. 2, pp. 153–168, 2010.
- [33] N. R. Hegde, S. Gauthami, H. M. Sampath Kumar, and J. Bayry, "The use of databases, data mining and immunoinformatics in vaccinology: where are we?," *Expert Opinion on Drug Discovery*, vol. 13, pp. 117–130, 2017.
- [34] A. A. Bahrami, Z. Payandeh, S. Khalili, A. Zakeri, and M. Bandehpour, "Immunoinformatics:In SilicoApproaches and computational design of a multi-epitope, immunogenic protein," *International Reviews of Immunology*, vol. 38, no. 6, pp. 307–322, 2019.
- [35] T. J. Carver, K. M. Rutherford, M. Berriman, M. A. Rajandream, B. G. Barrell, and J. Parkhill, "ACT: the Artemis Comparison Tool," *Bioinformatics*, vol. 21, no. 16, pp. 3422-3423, 2005
- [36] I. A. Doytchinova and D. R. Flower, "VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines," *BMC Bioinformatics*, vol. 8, no. 1, p. 4, 2007.

[37] O. O. Oni, A. A. Owoade, and C. A. O. Adeyefa, "Design and evaluation of primer pairs for efficient detection of avian rotavirus," *Tropical Animal Health and Production*, vol. 50, no. 2, pp. 267–273, 2018.

- [38] G. Stecher, K. Tamura, and S. Kumar, "Molecular Evolutionary Genetics Analysis (MEGA) for macOS," *Molecular Biology* and Evolution, vol. 37, no. 4, pp. 1237–1239, 2020.
- [39] K. Tamura, J. Dudley, M. Nei, and S. Kumar, "MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0," *Molecular Biology and Evolution*, vol. 24, no. 8, pp. 1596–1599, 2007.
- [40] M. Nielsen, C. Lundegaard, P. Worning et al., "Reliable prediction of T-cell epitopes using neural networks with novel sequence representations," *Protein Science*, vol. 12, no. 5, pp. 1007–1017, 2003.
- [41] C. Lundegaard, K. Lamberth, M. Harndahl, S. Buus, O. Lund, and M. Nielsen, "NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11," *Nucleic Acids Research*, vol. 36, suppl_2, pp. W509–W512, 2008.
- [42] M. Andreatta and M. Nielsen, "Gapped sequence alignment using artificial neural networks: application to the MHC class I system," *Bioinformatics*, vol. 32, no. 4, pp. 511–517, 2016.
- [43] S. Buus, S. L. Lauemoller, P. Worning et al., "Sensitive quantitative predictions of peptide-MHC binding by a 'Query by Committee' artificial neural network approach," *Tissue Antigens*, vol. 62, no. 5, pp. 378–384, 2003.
- [44] C. Lundegaard, O. Lund, and M. Nielsen, "Accurate approximation method for prediction of class I MHC affinities for peptides of length 8, 10 and 11 using prediction tools trained on 9mers," *Bioinformatics*, vol. 24, no. 11, pp. 1397-1398, 2008.
- [45] C. Lundegaard, M. Nielsen, and O. Lund, "The validity of predicted T-cell epitopes," *Trends in Biotechnology*, vol. 24, no. 12, pp. 537-538, 2006.
- [46] S. Southwood, J. Sidney, A. Kondo et al., "Several common HLA-DR types share largely overlapping peptide binding repertoires," *The Journal of Immunology*, vol. 160, no. 7, pp. 3363– 3373, 1998.
- [47] A. Patronov and I. Doytchinova, "T-cell epitope vaccine design by immunoinformatics," *Open Biology*, vol. 3, no. 1, article 120139, 2013.
- [48] H. H. Bui, J. Sidney, K. Dinh, S. Southwood, M. J. Newman, and A. Sette, "Predicting population coverage of T-cell epitope-based diagnostics and vaccines," *BMC Bioinformatics*, vol. 7, no. 1, p. 153, 2006.
- [49] D. A. Benson, M. Cavanaugh, K. Clark et al., "GenBank," Nucleic Acids Research, vol. 45, no. D1, pp. D37–d42, 2017.
- [50] S. Wang, W. Li, S. Liu, and J. Xu, "RaptorX-Property: a web server for protein structure property prediction," *Nucleic Acids Research*, vol. 44, no. W1, pp. W430–W435, 2016.
- [51] E. F. Pettersen, T. D. Goddard, C. C. Huang et al., "UCSF Chimera–a visualization system for exploratory research and analysis," *Journal of Computational Chemistry*, vol. 25, no. 13, pp. 1605–1612, 2004.
- [52] J. A. Choo, S. Y. Thong, J. Yap et al., "Bioorthogonal cleavage and exchange of major histocompatibility complex ligands by employing azobenzene-containing peptides," *Angewandte Chemie (International ed in English).*, vol. 53, no. 49, pp. 13390–13394, 2014.

[53] N. Guex and M. C. Peitsch, "SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling," *Electrophoresis*, vol. 18, no. 15, pp. 2714–2723, 1997.

- [54] G. M. Morris, R. Huey, W. Lindstrom et al., "AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility," *Journal of Computational Chemistry*, vol. 30, no. 16, pp. 2785–2791, 2009.
- [55] G. M. Morris, D. S. Goodsell, R. S. Halliday et al., "Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function," *Journal of Computational Chemistry*, vol. 19, no. 14, pp. 1639–1662, 1998.
- [56] S. J. Marshall, "Developing countries face double burden of disease," *Bulletin of the World Health Organization*, vol. 82, p. 556, 2004.
- [57] A. S. De Groot and R. Rappuoli, "Genome-derived vaccines," Expert Review of Vaccines, vol. 3, pp. 59–76, 2014.
- [58] B. Korber, M. LaBute, and K. Yusim, "Immunoinformatics comes of age," *PLoS Computational Biology*, vol. 2, no. 6, p. e71, 2006.
- [59] A. S. Fauci, "Emerging and re-emerging infectious diseases: influenza as a prototype of the host-pathogen balancing act," *Cell*, vol. 124, no. 4, pp. 665–670, 2006.
- [60] S. Chaudhury, E. H. Duncan, T. Atre et al., "Combining immunoprofiling with machine learning to assess the effects of adjuvant formulation on human vaccine-induced immunity," *Human Vaccines & Immunotherapeutics*, vol. 16, no. 2, pp. 400–411, 2020.
- [61] S. S. Usmani, R. Kumar, S. Bhalla, V. Kumar, and G. P. S. Raghava, "In silico tools and databases for designing peptidebased vaccine and drugs," *Therapeutic Proteins and Peptides*, vol. 112, pp. 221–263, 2018.
- [62] S. K. Dhanda, S. S. Usmani, P. Agrawal, G. Nagpal, A. Gautam, and G. P. S. Raghava, "Novel in silico tools for designing peptide-based subunit vaccines and immunotherapeutics," *Briefings in Bioinformatics*, vol. 18, no. 3, pp. 467–478, 2017.
- [63] Y. Khazaei-Poul, S. Farhadi, S. Ghani, S. A. Ahmadizad, and J. Ranjbari, "Monocyclic peptides: types, synthesis and applications," *Current Pharmaceutical Biotechnology*, vol. 21, 2020.
- [64] M. A. Hasan, M. Hossain, and M. J. Alam, "A computational assay to design an epitope-based peptide vaccine against Saint Louis encephalitis virus," *Bioinformatics and Biology Insights*, vol. 7, pp. 347–355, 2013.
- [65] S. Chakraborty, R. Chakravorty, M. Ahmed et al., "A computational approach for identification of epitopes in dengue virus envelope protein: a step towards designing a universal dengue vaccine targeting endemic regions," *In Silico Biology*, vol. 10, no. 5,6, pp. 235–246, 2010.
- [66] M. T. U. Qamar, A. Bari, M. M. Adeel et al., "Peptide vaccine against Chikungunya virus: immuno-informatics combined with molecular docking approach," *Journal of Translational Medicine*, vol. 16, no. 1, p. 298, 2018.
- [67] S. S. Twiddy, E. C. Holmes, and A. Rambaut, "Inferring the rate and time-scale of dengue virus evolution," *Molecular Biology and Evolution*, vol. 20, no. 1, pp. 122–129, 2003.
- [68] J. M. Christie, H. Chapel, R. W. Chapman, and W. M. Rosenberg, "Immune selection and genetic sequence variation in core and envelope regions of hepatitis C virus," *Hepatology*, vol. 30, no. 4, pp. 1037–1044, 1999.
- [69] R. C. Hardison, "Comparative genomics," PLoS Biology, vol. 1, no. 2, p. E58, 2003.

[70] C. Gilbert, A. Chateigner, L. Ernenwein et al., "Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons," *Nature Communications*, vol. 5, no. 1, p. 3348, 2014.

- [71] D. Schoeman and B. C. Fielding, "Coronavirus envelope protein: current knowledge," *Virology Journal*, vol. 16, no. 1, p. 69, 2019.
- [72] E. Mortola and P. Roy, "Efficient assembly and release of SARS coronavirus-like particles by a heterologous expression system," *FEBS Letters*, vol. 576, no. 1-2, pp. 174–178, 2004.
- [73] R. Sharmin and A. B. Islam, "Conserved antigenic sites between MERS-CoV and Bat-coronavirus are revealed through sequence analysis," Source Code for Biology and Medicine, vol. 11, no. 1, p. 3, 2016.
- [74] J. Xu, J. Hu, J. Wang et al., "Genome organization of the SARS-CoV," Genomics Proteomics Bioinformatics, vol. 1, no. 3, pp. 226–235, 2003.
- [75] M. Black, A. Trent, M. Tirrell, and C. Olive, "Advances in the design and delivery of peptide subunit vaccines with a focus on toll-like receptor agonists," *Expert Review of Vaccines*, vol. 9, no. 2, pp. 157–173, 2010.
- [76] D. Sesardic, "Synthetic peptide vaccines," *Journal of Medical Microbiology*, vol. 39, no. 4, pp. 241-242, 1993.
- [77] J. Gao, Z. Tian, and X. Yang, "Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies," *Bioscience trends*, vol. 14, no. 1, pp. 72-73, 2020.
- [78] R. Arya, A. Das, V. Prashar, and M. Kumar, *Potential inhibitors against papain-like protease of novel coronavirus* (COVID-19) from FDA approved drugs, 2020.
- [79] J. W. Westerbeck and C. E. Machamer, "A Coronavirus E Protein Is Present in Two Distinct Pools with Different Effects on Assembly and the Secretory Pathway," *Journal of Virology*, vol. 89, no. 18, pp. 9313–9323, 2015.
- [80] M. L. Dediego, L. Pewe, E. Alvarez, M. T. Rejas, S. Perlman, and L. Enjuanes, "Pathogenicity of severe acute respiratory coronavirus deletion mutants in hACE-2 transgenic mice," *Virology*, vol. 376, no. 2, pp. 379–389, 2008.
- [81] J. Netland, M. L. DeDiego, J. Zhao et al., "Immunization with an attenuated severe acute respiratory syndrome coronavirus deleted in E protein protects against lethal respiratory disease," *Virology*, vol. 399, no. 1, pp. 120–128, 2010.
- [82] P. B. McCray Jr., L. Pewe, C. Wohlford-Lenane et al., "Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus," *Journal of Virology*, vol. 81, no. 2, pp. 813–821, 2007.
- [83] J. A. Regla-Nava, J. L. Nieto-Torres, J. M. Jimenez-Guardeño et al., "Severe acute respiratory syndrome coronaviruses with mutations in the E protein are attenuated and promising vaccine candidates," *Journal of Virology*, vol. 89, no. 7, pp. 3870–3887, 2015.
- [84] J. Zhao, K. Li, C. Wohlford-Lenane et al., "Rapid generation of a mouse model for Middle East respiratory syndrome," *Pro*ceedings of the National Academy of Sciences, vol. 111, no. 13, pp. 4970–4975, 2014.
- [85] X. Guo, Y. Deng, H. Chen et al., "Systemic and mucosal immunity in mice elicited by a single immunization with human adenovirus type 5 or 41 vector-based vaccines carrying the spike protein of Middle East respiratory syndrome coronavirus," *Immunology*, vol. 145, no. 4, pp. 476–484, 2015.