Multi Epitopes Potential on Surface SARS-CoV-2 Protein as a Covid-19 Vaccine Candidate

by Khoirul Anam

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RESEARCH ARTICLE

Multi Epitopes Potential on Surface SARS-CoV-2 Protein as a Covid-19 Vaccine Candidate

Khoirul Anam[1,2]*, Bobi Prabowo[3,4], Meike Tiya Kusuma[3], Yuliati[3], Sri Winarsih[5], Tri Yudani Marcahing Raras[6], Sumarno Reto Prawiro[5]

[1]Doctoral Program in Medical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.
[2]Study Program of Medical Laboratory Technology, Institute of Health and Science Technology, Wiyata Husada Samarinda, Indonesia.
[3]Master Program in Biomedical Science, Faculty of Medicine, Universitas Brawijaya. Malang Indonesia.
[4]Department of Emergency Medicine, Dr. Iskak General Hospital, Tulungagung, Indonesia.
[5]Department of Clinical Microbiology, Faculty of Medicine, Universitas Brawijaya. Malang Indonesia
[6]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Brawijaya Malang. Indonesia.
*Corresponding Author E-mail: **sanambwi@yahoo.co.id**

ABSTRACT:
Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the etiology of an outbreak Covid-19. SARS-CoV-2 has a structural part consisting of spike glycoprotein, nucleoprotein N, membrane M and envelopes small membrane pentamer E. Immunoinformatic approach epitope analysis is developed to identify both weak and robust epitopes. Our study aims to identify several epitopes present in the spike glycoprotein, envelope, and membrane protein from the SARCoV-2 surface, with the help of insilico approach that highly potential as vaccine candidates. Analysis of antigeninicity was performed with the Kolaskar and Tongaonkar Antigenicity software. Epitope Mapping was analyzed using Linear Epitope Prediction Bepired. The structure of proteins with epitope regions was visualized by software Pyrex and PyMOL. Conserve analysis was performed using bio edit software. HLA mimicry was analyzed through HLAPred software. Molecular docking between the epitope with HLA I and HLA II was validated by Chimera and PyMOL software. The toxicity test for candidate vaccine peptides was carried out using ToxinPred software. Our study found seven potential epitope candidates as vaccine candidates. The seven epitopes were derived from spike proteins (5 epitopes), envelope proteins (1 epitope), and membrane proteins (1 epitope). All epitope codes are conserved and are not the same as HLA in Humans. The docking test results show a value with low affinity so that a strong bond can provide a high immune response. Toxicity tests show that all epitopes are non-toxic and safe to use as vaccine ingredients. Seven peptides from the spike, envelope, membrane protein that showed potential as vaccine candidates against Covid-19.

KEYWORDS: Immunoinformatic, Surface protein, Epitope, Covid-19, Vaccine candidate.

INTRODUCTION:

Covid-19 pandemic is outbreak diseases caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This virus is positive single-stranded RNA virus and enveloped.

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Based on phylogenetic tree analysis, SARS-CoV and SARS-CoV-2 are a subgenus of Sarbecovirus within the genus of Betacoronavirus[1,2]. The SARS-CoV-2 has six genome encoding spike glycoprotein trimer S, nucleoprotein N, membrane protein M and envelopes small membrane protein pentamer E[3,4].

The conserved region in designing vaccines is a viable strategy to develop vaccine efficacy against variable pathogens that affect adaptive immune responses. Multiple sequence alignment (MSA) was analyzed for

recognition of conserved regions in viral structural proteins. All the sequences of SARS CoV-2 spike protein are highly conserved. Thus, a vaccine based on its sequence will be highly likely to have broad-spectrum immunological implications[5,6].

Immunoinformatic epitope analysis approaches are developed to identify both weak and strong epitopes[4]. Modern technology can be used for the production of protein synthesis containing epitopes. The development of an epitope-based peptide vaccine candidate is a new step for an effective SarsCov 2 vaccine[7,8]. The immunoinformatic approach aims to predict antibody production and a long-lasting immunological response[3].

Several proteins were analyzed to screen only useful epitopes based on criteria to find the most suitable and authentic epitopes, for further testing in a wet laboratory[9,10]. This study aims to identify several epitopes on the glycoprotein spike, envelope, and membrane proteins from the surface of SARCoV-2, which can be recommended as vaccine candidates.

MATERIAL AND METHODS:

The method used in this study is bioinformatic based using the approach of Antigenicity, Epitope Mapping, Multi Sequence Alignment, HLA-I and HLA-II Prediction, Toxicity Prediction, Tertiary Structure Prediction, and Molecular docking.

Analysis of the identified antigenic protein was carried out using the approach in silico bioinformatics with the Kolaskar and Tongaonkar Antigenicity software from the Epitope Immune Database and Analysis Resource (http://www.iedb.org) with values threshold (threshold value) 1.0[7]. Epitope Mapping using Linear Epitope Prediction Bepired with a threshold value (entry) 0.35 of IEDB. The structure of proteins with epitope regions visualized by software Pyrex and PyMOL[11,12].

Conservation analysis was carried out using bio edit software. Our protein sequences were obtained from NCBI or UniProt data. BioEdit was used to determine conserved sites via ClustalW[13].

HLA mimicry was analyzed through HLAPred software (http://crdd.osdd.net/raghava/hlapred/). Human HLA mimicry analysis to see the similarities between epitope and peptide sequences in humans. This method identifies and predicts the HLA binding region of the antigen sequence for the prediction of HLA binding based on the HLA classification performed on Indonesian HLA[14].

Antigen visualization was visualized using Molecular Evolutionary Genetics Analysis (MEGA), followed with models the 3D structure using SWISS-MODEL (https://swissmodel.expasy.org). The results of 3D models were displayed in the PyMol and Modloop software.

Molecular docking was performed between the epitope with HLA-I and HLA-II. Chimera, PyMOL, Plus PLIP software were used to visualize the interaction between candidate epitope wih HLA-I (B*44:03) dan HLA-II (DRB-1*07:01). Binding affinity values were scored by Autodock Vina[15].

The toxicity test for vaccine candidate peptides was carried out using ToxinPred software (http://crdd.osdd.net/raghava/toxinpred/). The ToxinPred web server is utilized for determining the toxicity scoring of epitopes[6].

RESULT AND DISCUSSION:

The complete amino acid sequences of spike, envelop and membrane protein of SARS-CoV-2 were downloaded in FASTA format from National Centre for Biotechnological Information (NCBI) database. The potential epitopes were predicted by performin, as shown in Fig 1 and Table 1.

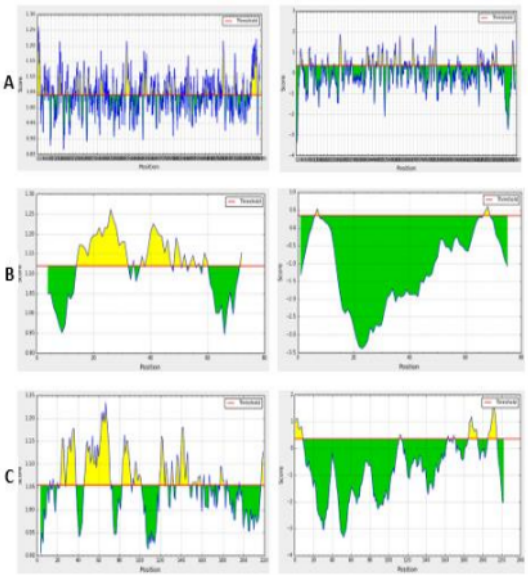

Fig 1. The results of antigenicity analysis and epitope mapping using Kolaskar & Tongaonkar Antigenicity and Bepipred Linear Epitope Prediction software to obtain potential epitopes. A) analysis of the antigenicity and epitope mapping of the Sars-Cov2 spike glycoprotein; B) antigenicity analysis and epitope mapping of the envelope Sars-Cov2; C) analysis of the antigenicity and epitope mapping of the Sars-Cov2 membrane.

Table 1. Candidate Epitope for multiepitope Covid-19 vaccine design results from antigenicity analysis and epitope mapping.

S. No	Sequences Peptide (Epitope)	Location	Length	Description
1	FLVLLPLVSSQCVNL	4-18	15	Spike glycoprotein
2	ILPDPSKPSKRS	805-816	12	
3	YQPYRVVVLSFELLHAPATVCGP	505-527	23	
4	YGFQPTNGVGYQ	495-506	12	
5	YFPLQSYGF	489-497	9	
6	VNSVLLFLAFVVFLLVTLASS	14-32	21	Envelope protein
7	LYIIKLIFLWLLWPVTLACFVLAAVY	47-71	26	Membrane protein

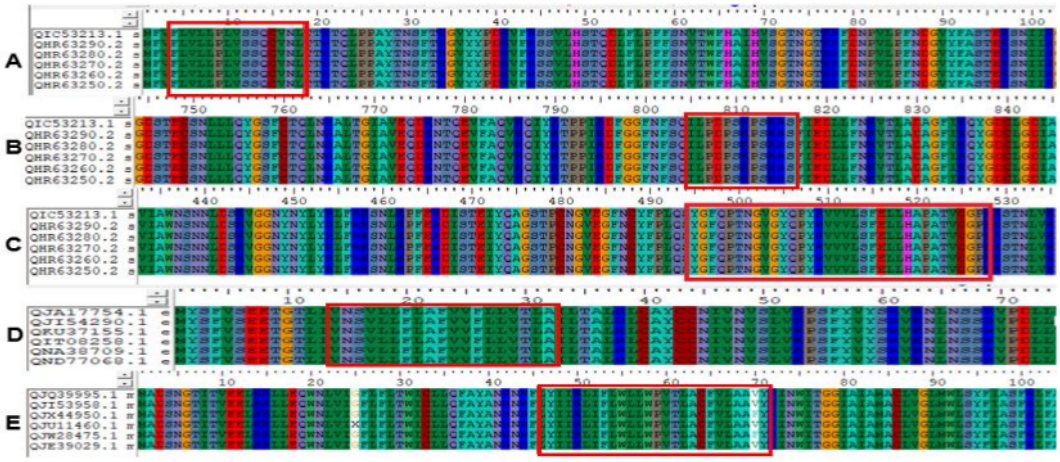

Fig 2. Multi Sequence Alignment (MSA) epitope 1-7 using Bioedit software. A) analysis of conserve epitope 1; B) analysis of conserve epitope 2; C) analysis of conserve epitope 3-5; D) analysis of conserve epitope 6; E) analysis of conserve epitope 7.

The epitope candidate from the surface SARS-CoV-2 protein was subjected to conserve analysis using the bioedit software to identify–whether the amino acid sequence that was the epitope's target was an area that underwent mutation (Fig 2).

The results from antigenicity analysis and epitope mapping were analyzed using human HLA mimicry analysis to reveal the similarity between peptide sequences and peptides in humans (Table 2).

Table 2 Analysis of Mimicry HLA was analyzed by web server.

Epitope	Sequence	Hits Against Genome	Comments
1	FLVLLPLVS	No Identical Sequence Found	The predicted binder can be recommended as a potential vaccine candidate because it has nothing in common with humans (eukaryotic organisms)
2	ILPDPSKPS	No Identical Sequence Found	
3	YQPYRVVVL	No Identical Sequence Found	
4	YGFQPTNGV	No Identical Sequence Found	
5	YFPLQSYGF	No Identical Sequence Found	
6	FLAFVVFLL FVVFLLVTL LFLAFVVFL LLFLAFVVF	No Identical Sequence Found	
7	FLWLLWPVT	No Identical Sequence Found	

The protein structures with epitope regions were visualized using the ModLoop design on the SARSCoV-2 protein surface (Fig 3).

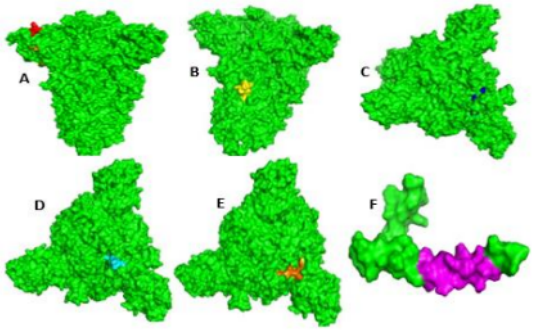

Fig 3. Visualization of the multiepitope on the protein of SARSCoV-2 (ModLoop designed missing residue). A) Visualization of Epitope 1(red color) by 6ZGI; B) Visualization of Epitope 2 (yellow color) by PDB 6ZGI; C) Visualization of Epitope 3 (blue color) by PDB 6ZGI; D) Visualization of Epitope 4 (cyan color) by PDB 6ZGI; E) Visualization of Epitope 5 (orange color) by PDB 6ZGI; F) Visualization of Epitope 6 by PDB 2MM4 (magenta color). Visualization of epitope 7, like the visualized structure of *Mycobacterium tuberculosis* [13], has a similarity value of 42,3% of membrane glycoprotein SARSCoV-2.

Molecular docking of epitope with HLA-I (HLA-B*44:03: PDB 3KPN). Binding affinity values were scored by Autodock Vina [12]. Visualization HLA

B*44:03 with candidate epitope was analyzed by Chimera and PyMOL software, and Plus PLIP web server to predict interaction preview between HLA and epitope (Fig 4 and Table 3).

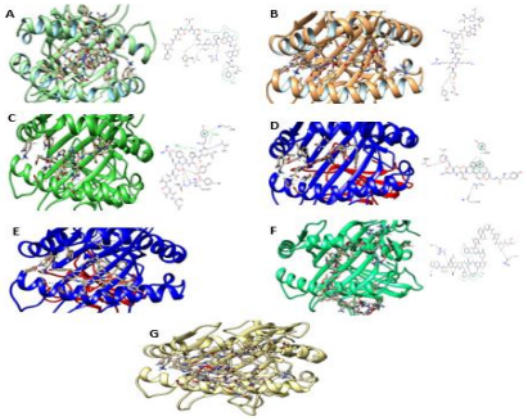

Fig 4. The molecular interaction between HLA-B*44:03 and epitope 1-7. The interaction was analyzed by Plus PLIP software. Figure A-G shows epitope 1-7

Table 3. Autodock Vina scored the binding affinity score molecular interaction between HLA-B*44:03 with candidate epitope values.

Epitope	Binding Affinity Score (kcal/mol)	RMSD u.b
1	-8.7	2.004
2	-7.4	5.198
3	-7.4	12.013
4	-11.0	2.441
5	-10.4	10.708
6	-8.9	17.764
7	-5.8	10.50

Molecular docking of epitope with HLA II (PDB HLA DRB-1*04:01: 6BIJ). Autodock Vina scored binding affinity values, Visualization HLA DRB-1*07:01 with candidate epitope was analyzed by PyMOL, and interaction preview between HLA and epitope was described by Plus PLIP (Fig 5 and Table 4).

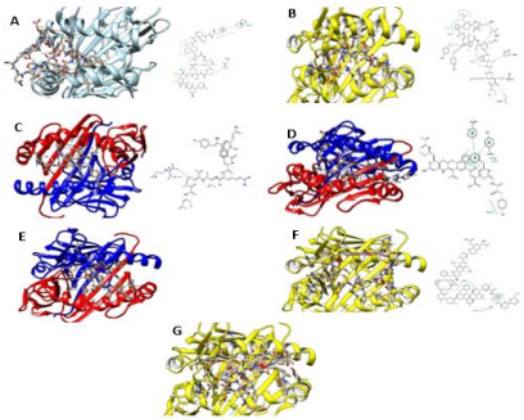

Fig 5. The molecular interaction between HLA DRB-1*04:01 and epitope 1-7. PLIP analysed interaction. Figure A-G shows visualization of epitopes 1-7

Table 4. Autodock Vina scored the binding affinity score molecular interaction between HLA DRB-1*04:01 with candidate epitope values.

Epitope	Binding Affinity Score (kcal/mol)	RMSD u.b
1	-7.4	11.678
2	-7.0	11.757
3	-8.5	2.610
4	-10.4	3.149
5	-11.2	11.847
6	-9.6	2.184
7	-6.5	13.40

Epitope candidates were analyzed using the prediction of toxicity to see the toxicity of the candidate epitope (Table 5).

Table 5. The results of prediction of toxicity properties of several epitopes using the ToxinPred software.

Epitope	Peptide Sequences	Prediction score	Comment	Hydrophilicity	Mol wt
1	FLVLLPLVSSQCVNL	-1.01	Non-Toxin	-1.07	1645.27
2	ILPDPSKPSKRS	-0.88	Non-Toxin	0.78	1324.70
3	YQPYRVVVL	-1.20	Non-Toxin	-0.86	1136.49
4	YGFQPTNGV	-1.37	Non-Toxin	-0.70	982.19
5	YFPLQSYGF	-0.49	Non-Toxin	-1.21	1121.38
6	VNSVLLFLAFVVFLLVTLASS	-1.45	Non-Toxin	-1.24	2253.09
7	LYIIKLIFLWLLWPVTLACFVLAAVY	-1.34	Non-Toxin	-1.49	3083.33

Researcher initiated to study peptide based-vaccine as a safe alternative. We have successfully identified several epitopes derived from spike, envelope and membrane protein using immunoinfomatic tools. Analysis of potential epitope identification was carried out by antigenicity and epitope mapping tests on B cells using Kolaskar and Tongaonkar Antigenicity and Linear Epitope Prediction Bepired software (http://www.iedb.org). B-cells are a significant component of humoral immunity as long as the adaptive immune response produces antibodies that recognize antigens. The results showed that seven potential epitopes showed potential as vaccine candidates. This study's results are in line with previous studies that predict the epitope of potential spike and envelope protein [16,17], although we did not use linker to generate multiple epitopes.

Peptides from protein sequences that are predicted as epitope regions need to be ensured that these sequences

are conserved areas that do not undergo mutation. The analysis was performed using bio edit software, and it was found that the seven-candidate epitopes from this study after alignment did not experience mutations in these sequences. These results indicate that the epitope candidate from the spike, envelope, and membrane proteins can be recommended as candidate materials for the Covid-19 vaccine. The recombinant RBD protein S consisting of multiple epitopes which cause high antibody titers that be used as a neutralizer against SARS-CoV-2[18]. Envelope protein is an important virulence factor[19]. In addition, it has been reported that the transmembrane domain of M protein containing epitope groups of T cells was able to induce cellular immune response[20]. In addition, M protein is highly conserved in several different species[21,22].

With a help of Human HLA mimicry analysis, the similarities between epitope and peptide sequences in humans can be identified. The similarity in molecular structure between class II HLA genes and exogenous antigens can reflect autoimmunity through the concept of molecular mimicry. The similarity in molecular structure between class II HLA genes and exogenous antigens can cause autoimmunity[23,24]. The mimicry analysis results show that all epitope candidates did not show any similarity to human HLA, so the candidate epitopes in our study can be considered safe from autoimmunity. Some of the peptides on the SARSCoV-2 spike, which is the epitope, were analyzed for their similarity to the *Homo sapiens* body receptor's cell surface. The results show that some of these epitopes have nothing in common with the cell surface receptors of the *Homo sapiens* body[5,25].

ModLoop is an online web tools for the automatic protein structure modelling (http://salilab.org/bioinformatics_resources.shtml)[21]. The spike protein epitope used modeling of the 6ZGI protein (epitope 1= red color, epitope 2=yellow color, epitope 3=blue color, epitope 4=cyan color, epitope 5=orange color). Visualization of the SARS CoV2 spike protein using PDB: 6ZGI has the same value as 96.7%[26,27]. Visualization of epitope six by PDB 2MM4 (magenta color). PDB: 2MM4 represents the coronavirus protein envelope / SARS-CoV-2 envelope[28], has a similarity value of 91.4% with the SARS-CoV-2 envelope. Visualization of epitope seven, like the visualized structure of *Mycobacterium tuberculosis*[29], has a similarity value of 42,3% of membrane glycoprotein SARSCoV-2.

Protein Plus and PLIP web server described visualization of docking analysis results between epitope and HLA I was analyzed by Chimera and PyMOL and interaction preview between HLA and epitope. Visualization of the docking analysis results between the epitopes and HLA II was also analyzed using Chimeras and PyMOL. Protein Plus and PLIP web server described an interaction preview between HLA and epitope. The results of binding affinity score molecular interaction between HLA with candidate epitope values were scored by Autodock Vina[30]. The affinity binding between HLA I and the epitope is as follows: -8.7; -7.4; -7.4; -11.0; -10.4; -8.9; -5.8 for epitope 1, 2, 3, 4, 5, 6, 7 subsequently. Meanwhile, the binding affinity between HLA II and the epitope is as follows: -7.4; -7.0; -8.5; -10.4; -11.2; -9.6; -6.5. The docking simulation results between peptide and HLA can be recommended for use as vaccine candidates due to low energy affinity score. The lowest binding energy as the result of molecular docking, the higher probability the formation of molecular complex[31]. Hydrogen bonding and hydrophobicity will affect the molecular bond energy score[32]. The immune response of B cells is influenced by the stability of the molecular complex[33].

The peptide candidate we obtained is a material that is safe to use as a vaccine candidate. Vaccine candidate materials are declared safe and meet the requirements for vaccine candidates if they are not toxins. The results of the toxicity analysis showed that the 7 peptides we obtained were not toxic.

CONCLUSION:

Our finding has identified high potential seven epitopes as vaccine candidate Sars-Cov-2. However, the proposed epitope requires trials through *in vitro* and *in vivo* studies.

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CONFLICT OF INTEREST:

The authors confirm that this article content has no conflicts

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