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In-silico immunoinformatic analysis of SARS-CoV-2 virus for the development of putative vaccine construct

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ABSTRACT

COVID-19 (CoronaVirus disease 2019) is caused by the SARS-CoV-2 virus (severe acute respiratory syndrome coronavirus 2). SARS-CoV-2 virus is highly contagious and affects the human respiratory tract resulting in symptoms such as high fever, body ache, cough, dysfunctions of tastebuds and smelling sense of body. The objective of the present study involves immunoinformatic analysis to predict COVID-19 protein for vaccine construct based on the genomic information SARS-CoV-2 virus. At present, as per WHO estimates, around 133 COVID-19 novel vaccines under development. Three amino acid sequences of SARS-CoV-2 were retrieved from the NCBI database for the analysis of vaccine construct. This study involves computational and immunoinformatic methods. The Immunoinformatic tools used in the present study are NetCTL server, IFN epitope server, Toxin PRED, BCPred, CTL+HTL+ADJUVANTS+LINKERS, AlgPredserver, VaxiJenserver, ProtParam to predict vaccine construct. The secondary and tertiary structure prediction is done by PSIPRED, I-TASSER, Galaxy refine, proSA+ Ramachandran. Finally, docking of the vaccine constructs and ligand was done with the help of Cluspro 2.0. C-ImmSimm webserver to simulate the potential vaccine construct. The present study demonstrated three potential Vaccine constructs for the SARS-CoV-2 virus, which were docked with TLR8 (Toll-like receptor8). Interestingly from these, all constructs one having a high potential for the inhibition effect of the SARS-CoV-2 virus. Immunological simulation data shows significant elevated amount of memory B cell; also, the high response was seen in TH(Helper) and TC(cytotoxic) cell population from the vaccine construct proposed in the current study. Hence, these constructs are suitable vaccine candidates that might be useful in developing a novel vaccine.

1. Introduction

Coronavirus disease 19 (COVID-19) is caused by the SARS-CoV-2 virus responsible for pandemic across the world. The symptoms associated with COVID-19 disease are fever, body ache, high body temperature, cough, and breathlessness (Struyf et al., 2020). The severity of COVID-19 causes accumulation of mucous in the respiratory tract resulting in respiratory failure leading to the death of the patient (Wang, 2020). The primary risk associated with SARS-CoV-2 virus infection is that it is highly contagious and spread very fast from human to human contact (Sanche et al., 2020) and there are some studies which describe that virus could affect other animals too, some are pet animals (Salajegheh Tazerji, 2020). The prionness of SARS-CoV-2 through ACE2 receptor in chicken is very less, while horse shows strong interactions (Kumar et al., 2020). The genome of the SARS-CoV-2 virus consists of around 30 K base pairs. The 5’ end of the genome comprises more than two-thirds of the total genome. It code for ORF1 ab poly protein. It is the largest coding region made up of around 21 K base pairs. The remaining one-third of the genome comprises 3’ end. The 3’ end comprises gene encoding structural proteins, including surface proteins, envelope proteins, membrane proteins, and nucleocapsid proteins. Gene coding for envelope proteins is made up of 228 base pairs. The membrane protein gene is 669 nucleotides long. Nucleocapsid N is the biggest structural gene having 908 nucleotides (Khailany et al., 2020). The viral outer layer is formed by S-protein formed; then protein helps in the viral replication, genome construction, and host cellular response; envelope shape is determined by the M-protein and maturation of the SARS-CoV-2 virus is done by the E-protein (Astuti and Ysrafil, 2020). Tilocca, B., et al
describe the significance of predicting B cell epitopes and T cell epitopes as major proteins, which play important role in the immunogenicity towards SARS-CoV-2 envelop protein, membrane protein, spike protein and nucleocapsid protein (Tiloca et al., 2020).

Based on this genomic information, a new generation of vaccines can be developed to stimulate both humoral and cellular responses. The various factors associated with the successful development of vaccines include the production of long term T and B memory cells, immunity mediated by neutralizing antibodies, and the stable nature of antigens. Cytotoxic T lymphocyte (CTL) response plays a significant role in identifying the virus infected cells by recognizing specific epitopes of viral proteins. Epitopes are particular regions of proteins with short amino acid sequences that can impart direct and indirect immune responses (Ju et al., 2020; Braun, 2020).

The patients who recover from COVID-19 disease has an evoked T-cells immune response made by T-cell epitopes of SARS-CoV-2 spike protein, and most of these immunogenic epitopes were localized to the S protein of the virus (Asutti and Ysrifaril, 2020). The size of T-cell epitopes can vary from 8 to 20 amino acids, whereas the B-cell epitopes can be larger than T-cell epitopes (Dermine et al., 2004; Molten et al., 2001).

T cell produces cellular immunity, which is vital for the functioning of a vaccine. T lymphocytes are having crucial participation in cellular and antibody-mediated immunity. CD3+ T cells, CD4+ T cells, CD8+ T cells, natural killer cells, B cells, and T lymphocytes all identify the antigen, a peptide in nature (Xu et al., 2020). T helper cells response having inter connection with the production of neutralizing antibodies (Janice Oh et al., 2012). Earlier SARS-COV research demonstrated that acquired immune response against spike glycoprotein is defensive (Zhu et al., 2007). So, CD4+ T Cells are beneficial in the production and maturation of antibodies for SARS-CoV-2 (Ju et al., 2020). B cells are also a major component of the immune system against any viral infection and play an essential role in the generation of antibodies and provide immune protection. The immunological role of B cells in COVID-19 is still not much explored, so more studies could be conducted for the same (Creed, 2020).

Multi epitope peptide vaccine including T cell and B cell epitopes in a combined form that can generate a robust immune response. The significant advantages are easy manufacturing, physicochemical stability, and fewer adverse effects than conventional vaccines. This ideal characteristic makes peptide and epitope vaccine most preferable for vaccine construct (Patronov and Doytchinova, 2013). In the se multi-epitope peptide vaccine construction, prediction of B cell, cytolytic T cell, helper T cell epitopes are playing an important role (Chiarella, 2009).

The whole protein sequence is not responsible for stimulating the immunogenic response, whereas only antigenic epitopes are responsible for the same, and these epitopes are easy to predict (Zheng et al., 2017). Tiloca, B., et al studied the Mycobacterium avium Paratuberculosis proteins which causes paratuberculosis, finding of the epitope binding sites which further helps in the early detection and the vaccine construct for the disease (Tiloca et al., 2020). Sarkar, B., et al has taken four different proteins from SARS-CoV-2 virus, and made the three possible construct for the vaccines (Sarkar et al., 2020). In the Nucleocapsid protein, at three different places i.e. 229 to 268, 349 to 399 and 405 to 419, these all amino acids having B cell epitopes and MHC II binding epitopes (Dai et al., 2020). Humoral and cell mediated immunity generated against virus by using peptide based multi epitope vaccine (Rahman et al., 2020). In spike glycoprotein there are 13 epitopes in MHC-I and 3 epitopes in MHC-II. Docking interactions with TLR 5 demonstrated these possibilities (Bhattacharyya et al., 2020). Vaccine construct for these epitopes in spike glycoproteins are made with help of in-silico method (Bhattacharyya, 2020). Some phytochemicals like sarsasapogenin, ursolic acid, curcumin, ajmalicine, novobiocin, silymarin and aronatin, piperine, gingerol, rosmarinic acid, and alpha terpinyl acetate also helpful in inhibition of viral infection by acting on Non structural protein 15 (Kumar, 2021).

The objective of the present study involves in-silico peptide vaccine construct based on the genomic information of the SARS-CoV-2 virus. Peptide based vaccines are biologically safe as they don’t need in vitro culture, and selectivity activates immune responses. B-cell and T-cell epitopes that are immunodominant and can induce specific immune responses are constructed using immunoinformatic tools. The present study aimed to construct a peptide-based vaccine against SARS-CoV-2 virus using computational and immunoinformatic tools.

2. Material and methods

2.1. Retrieval of sequences

Three amino acid sequences of SARS-CoV-2 globally known as COVID-19 were retrieved from NCBI in FASTA format with accession number YP_009724390.1, YP_009724393.1 and YP_009724392.1.

2.2. CTL (cytotoxic T-cell) epitope prediction

NetCTL1.2 web server is used to decipher CTL epitopes in all protein sequences taken for study (Larsen et al., 2007). MHC-I binding peptide prediction, proteasomal C terminal cleavage, and the transportation efficiency Transporter Associated with antigen Processing (TAP) is the foremost essential part of this analysis. Proteasomal C terminal cleavage and MHC binding are predicted through artificial neural networks; on the other hand, the efficiency of TAP transporter is done through the weight matrix method. For finding the CTL epitopes, 0.75 was the adjusted threshold.

2.3. HTL (helper T-cell) epitope prediction

IEDB online server was used to predict epitopes for proteins of the SARS-CoV-2 virus (Zhu et al., 2007). Server predicts Five epitopes of 15-mer length for human alleles (HLA-DRB1*01:01, HLA-DRB1*01:02, HLA-DRB1*01:03, HLA-DRB1*01:04, HLA-DRB1*01:05). IC50 score is used for peptide affinity for receptors.

2.4. Toxicity prediction

ToxinPred online server is used to find out the toxicity of selected epitopes. Only non-toxic epitopes are taken further studies, physicochemical assets are the critical point in the toxicity prediction (Gupta et al., 2013).

2.5. B cell epitope prediction

B cell has a crucial job in making host antibodies. BCpred server is used to predict the B cell epitopes (EL-Manzalawy et al., 2008). This web portal consists of a machine learning kernel algorithm that is very helpful in finding B Cell epitopes. BCpred makes predictions based on SVM with the deployment of Amino acid pair antigenicity (Patronov and Doytchinova, 2013).

2.6. Preparation of multi-epitope vaccine constructs

From earlier results of CTL, HTL, and B cell epitopes, the multi-epitope vaccine sequence is made by using linkers. The different epitopes were linked together using AAY, GPGPG, and KK linkers. To improve the immunogenicity of the vaccines, three different adjuvants were chosen and retrieved from NCBI protein Database WP 003403353. 50S ribosomal protein L7/L12 Mycobacterium (Lee et al., 2014), AAQ97601.1 human beta-defensin-3 Mature peptide, partial synthetic sequence taken for study (Larsen et al., 2007). Sarkar, B., et al has taken four different proteins from SARS-CoV-2 virus, and made the three possible construct for the vaccines (Sarkar et al., 2020). In the Nucleocapsid protein, at three different places i.e. 229 to 268, 349 to 399 and 405 to 419, these all amino acids having B cell epitopes and MHC II binding epitopes (Dai et al., 2020). Humoral and cell mediated immunity generated against virus by using peptide based multi epitope vaccine (Rahman et al., 2020). In spike glycoprotein there are 13 epitopes in MHC-I and 3 epitopes in MHC-II. Docking interactions with TLR 5 demonstrated these possibilities (Bhattacharyya et al., 2020). Vaccine construct for these epitopes in spike glycoproteins are made with help of in-silico method (Bhattacharyya, 2020). Some phytochemicals like sarsasapogenin, ursolic acid, curcumin, ajmalicine, novobiocin, silymarin and aronatin, piperine, gingerol, rosmarinic acid, and alpha terpinyl acetate also helpful in inhibition of viral infection by acting on Non structural protein 15 (Kumar, 2021).

The objective of the present study involves in-silico peptide vaccine construct based on the genomic information of the SARS-CoV-2 virus. Peptide based vaccines are biologically safe as they don’t need in vitro culture, and selectivity activates immune responses. B-cell and T-cell epitopes that are immunodominant and can induce specific immune responses are constructed using immunoinformatic tools. The present study aimed to construct a peptide-based vaccine against SARS-CoV-2 virus using computational and immunoinformatic tools.
2.7. Allergenicity prediction of the vaccine

AlgPred server issued to predict the allergenicity of the vaccine construct (Saha and Raghava, 2006). The most accurate results of this server are because of its six various methods in prediction; 85% accuracy is obtained with a 0.4 threshold value.

2.8. Antigenicity prediction of the vaccine

VaxiJen server is used to decipher the antigenicity of the vaccine construct (Doytchinova and Flower, 2007). Results are predicted on the basis of physiochemical assets of amino acid sequence. The virus is set as the model organism with a threshold value of 0.4 to get optimum results.

### Table 1

Predicted CTL epitopes for surface glycoproteins. Finally, the selection is made w.r.t to c-terminal, TAPscores antigenicity and toxicity.

| Residue number | Peptide sequence | MHC Binding affinity | Rescale binding affinity | C terminal cleavage affinity | Transport affinity | Prediction score | Antigenic | Toxicity |
|----------------|------------------|----------------------|-------------------------|----------------------------|------------------|-----------------|-----------|----------|
| 865            | LTDEMAQY         | 0.7953               | 3.3768                  | 0.9723                     | 2.779            | 3.6616          | Non       |          |
| 258            | WTAGAAAYY        | 0.6735               | 2.8596                  | 0.7339                     | 2.863            | 3.1128          | Antigenic  | Non      |
| 604            | TSNQVAVLY        | 0.6559               | 2.7847                  | 0.944                      | 2.991            | 3.0758          | Antigenic  | Non      |
| 361            | GVADYSVLY        | 0.5348               | 2.2705                  | 0.9754                     | 3.18             | 2.5759          | Non       |          |
| 733            | KTSVDCMTMY       | 0.4908               | 2.084                   | 0.9649                     | 3.016            | 2.3795          | Antigenic  | toxin    |
| 746            | STECSNLLL        | 0.5136               | 2.1808                  | 0.8879                     | 0.703            | 2.3492          | Antigenic  | Non      |
| 652            | GAEJVNNSY        | 0.4042               | 1.7163                  | 0.9769                     | 2.663            | 1.996           | Non       |          |
| 196            | NIDGYFKIY        | 0.3921               | 1.6649                  | 0.9664                     | 3.015            | 1.9606          | Non       | antigenic |
| 160            | YSSANNCTF        | 0.3975               | 1.6878                  | 0.9032                     | 2.598            | 1.9531          | Non       | toxin    |
| 152            | WMESEFRVY        | 0.3902               | 1.6569                  | 0.7993                     | 2.929            | 1.9232          | Non       |          |
| 162            | SANNCTFEY        | 0.3737               | 1.5865                  | 0.9196                     | 2.99             | 1.8739          | Non       |          |
| 687            | VASQSIAY         | 0.3529               | 1.4986                  | 0.9656                     | 3.089            | 1.7978          | Non       | antigenic |
| 30             | NSFTRGVYY        | 0.3389               | 1.4389                  | 0.6421                     | 3.124            | 1.6915          | Non       |          |
| 136            | CNDPFLGVY        | 0.2613               | 1.1095                  | 0.69                        | 2.45             | 1.3355          | Non       | antigenic |
| 392            | FTNYADSF         | 0.2704               | 1.148                   | 0.38                       | 2.317            | 1.3208          | Non       | antigenic |
| 261            | GAAAYVGYG        | 0.2253               | 0.9568                  | 0.7608                     | 2.969            | 2.194           | Antigenic  | Non      |
| 357            | RICSCVAYD        | 0.2106               | 0.8941                  | 0.9292                     | 3.394            | 1.2032          | Non       | antigenic |
| 465            | ERDISTEIY        | 0.2097               | 0.8903                  | 0.9744                     | 2.646            | 1.1687          | Non       | antigenic |
| 285            | ITDAVDCAL        | 0.235                | 0.9979                  | 0.8708                     | 0.79             | 1.168           | Non       | antigenic |
| 1039           | RVDFCGGKY        | 0.2036               | 0.8644                  | 0.7618                     | 2.323            | 1.1403          | Non       | antigenic |
| 343            | NATRFSAVY        | 0.1955               | 0.83                     | 0.9342                     | 2.873            | 1.1138          | Non       | antigenic |
| 1237           | MTSCCSCCLK       | 0.226                | 0.9595                  | 0.7525                     | 0.479            | 1.0963          | Antigenic  | toxin    |
| 50             | STQDLFLPF        | 0.1974               | 0.8383                  | 0.553                      | 2.511            | 1.0468          | Antigenic  | Non      |
| 1096           | VSGTCHWFV        | 0.2012               | 0.8544                  | 0.6143                     | 0.218            | 0.9574          | Non       | antigenic |
| 880            | GTITSGWTF        | 0.1656               | 0.7031                  | 0.7489                     | 2.557            | 0.9433          | Non       | antigenic |
| 815            | RSFIEDLLF        | 0.1421               | 0.6035                  | 0.5938                     | 3.032            | 0.8441          | Non       | antigenic |
| 1264           | VLKGVKLHV        | 0.1262               | 0.5356                  | 0.9783                     | 2.859            | 0.8253          | Antigenic  | Non      |
| 748            | ECSNILLQY        | 0.1413               | 0.6                     | 0.5316                     | 2.747            | 0.8171          | Non       | antigenic |
| 370            | NSASFSSTFK       | 0.1671               | 0.7093                  | 0.5456                     | 0.507            | 0.8165          | Non       | antigenic |
| 372            | ASPSTFCKY        | 0.118                | 0.501                   | 0.9587                     | 3.275            | 0.8085          | Non       | antigenic |
| 628            | QLTFTWRVY        | 0.1189               | 0.5047                  | 0.9661                     | 2.782            | 0.7887          | Antigenic  | Non      |
| 296            | LSETKCTLK        | 0.1515               | 0.6432                  | 0.8919                     | 0.22             | 0.7879          | Antigenic  | Non      |
| 192            | FVFKNIDGY        | 0.1358               | 0.5767                  | 0.4093                     | 2.913            | 0.7837          | Non       | antigenic |
| 445            | VGGYNYYLY        | 0.1164               | 0.4941                  | 0.9518                     | 2.658            | 0.7698          | Non       | antigenic |
| 83             | VLPFNDGVY        | 0.113                | 0.4797                  | 0.9703                     | 2.846            | 0.7675          | Non       | antigenic |
| 1095           | FVSNGTHWF        | 0.1232               | 0.5231                  | 0.7203                     | 2.621            | 0.7622          | Non       | antigenic |
| 612            | YQDVNCTEV        | 0.1531               | 0.6501                  | 0.587                      | 0.242            | 0.7502          | Antigenic  | Non      |

2.7. Allergenicity prediction of the vaccine

AlgPred server issued to predict the allergenicity of the vaccine construct (Saha and Raghava, 2006). The most accurate results of this server are because of its six various methods in prediction; 85% accuracy is obtained with a 0.4 threshold value.

2.8. Antigenicity prediction of the vaccine

VaxiJen server issued to decipher the antigenicity of the vaccine construct (Doytchinova and Flower, 2007). Results are predicted on the basis of physiochemical assets of amino acid sequence. The virus is set as the model organism with a threshold value of 0.4 to get optimum results.

2.9. Secondary structure prediction

All physiochemical properties like nature of amino acids, half-life, PI, hydrophathy plot, the molecular weight of sequence, instability index, in vitro and in vivo half-life, and grand average of hydropathicity are calculated with the help of online web server ProtParam (Wilkins, 1999).
2.10. Tertiary structure prediction

I-TASSER (Iterative Threading Assembly Refinement) server was used to do homology modeling of the final constructs of vaccine peptides (Yang and Zhang, 2015). The 3D structure was generated from the FASTA format of amino acid sequence. This model is developed through multiple alignments and built simulated iterative structures.

2.11. Tertiary structure refinement and validation

Galaxy refine webserver was used in the refinement of the tertiary structure of the vaccine peptide Construct (Heo et al., 2013). Validation of the tertiary structure is done by pros A + Ramachandran. Ramachandran plot is also helping validate the 3D structure of a protein by plotting a graph that deciphers the presence of all nature and structure of the amino acid sequence. ProSA analyzed the 3D structure of the protein, whether it is showing relatedness to the native protein structure or having some mistakes in the structure (Wiederstein and Sippl, 2007).

2.12. Docking with TLRL (Toll-like receptor ligand)

TLR Docked with three vaccine constructs (A, B, C) using ClusPro protein-protein docking server. Balanced docking method was chosen using PIPER algorithm. PIPER algorithm represents the interaction energy between two proteins using an expression of the form \( E = w_1E_{\text{rep}} + w_2E_{\text{attr}} + w_3E_{\text{elec}} + w_4E_{\text{DARS}} \), where \( E_{\text{rep}} \) and \( E_{\text{attr}} \) denote the repulsive and attractive contributions to the van der Waals interaction energy, and \( E_{\text{elec}} \) is an electrostatic energy term. \( E_{\text{DARS}} \) is a pairwise interaction energy, and \( E_{\text{elec}} \) is an electrostatic energy term. EDARS is a pairwise state (DARS) approach. The coefficients \( w_1, w_2, w_3, \) and \( w_4 \) define the weights of the corresponding terms. The best-Docked Structures with Coefficient Weights and Cluster Scores were retrieved and taken for further reference.

2.13. Immune simulation

ClmmSim server was used to characterize the immunogenicity and immune response profile of the chimeric peptide. This server also conducts in-silico immune simulations (Doytchinova and Flower, 2007). A position-specific scoring matrix (PSSM) used by this server for the prediction of immune epitope and immune interactions are calculated by machine learning techniques.

3. Results

3.1. Collection of proteins sequences for B and T-cell epitopes prediction

COVID-19 proteins (surface, envelope, and the membrane) amino acid sequences were retrieved from NCBI. The amino acid sequences were used to predict the B and T cell epitopes for designing the multi-epitope sub unit vaccine.

3.2. Cytotoxic T lymphocytes (CTL) epitopes prediction

Prediction of CTL epitopes was made by using an online tool, NetCTL1.2. Total 37 CTL epitopes of length 9-mer were predicted. From37 epitopes by analyzing toxicity prediction and immunogenicity prediction, three epitopes were selected, which were non-toxic, antigenic (Table 1), and highly immunogenic (Table 2). For membrane protein, a total of eight CTL epitopes were predicted. Out of eight, only one epitope is selected on the basis of non-toxic, antigenic (Table 3) and highly immunogenic (Table 4). For envelope protein total of three

| Residue number | Peptide sequence | MHC Binding affinity | Rescale binding affinity | C terminal cleavage affinity | Transport affinity | Prediction score | Antigenic | Toxicity |
|----------------|------------------|----------------------|-------------------------|-----------------------------|-------------------|----------------|----------|---------|
| 182            | SSDNIALLV        | 0.6531               | 2.7729                  | 0.9682                      | 0.286             | 2.9325         | Non-antigen | TOXIN   |
| 140            | ATSRITLSY        | 0.5463               | 2.3195                  | 0.9375                      | 3.09              | 2.6146         | ANTIGEN   | NON – TOXIN |
| 165            | YSRIRIGNY        | 0.3214               | 1.3648                  | 0.9345                      | 3.148             | 1.6623         | NON-ANTIGEN | TOXIN |
| 8              | YANKRNFLY        | 0.3305               | 1.4031                  | 0.4099                      | 3.017             | 1.6155         | NON-ANTIGEN | TOXIN |
| 139            | VARTSRLS        | 0.2752               | 1.1684                  | 0.9679                      | 3.013             | 1.4642         | ANTIGEN   | NON – TOXIN |
| 56             | LVGLMWLSY        | 0.2694               | 1.144                   | 0.724                       | 2.897             | 1.3974         | ANTIGEN   | NON-TOXIN |
| 157            | AGDSGFAAY        | 0.1341               | 0.5695                  | 0.9652                      | 2.673             | 0.848          | ANTIGEN   | NON – TOXIN |
| 181            | SSDNIALLL       | 0.1487               | 0.6313                  | 0.9630                      | 1.098             | 0.8308         | NON-ANTIGEN | TOXIN |

weights of the corresponding terms. The best-Docked Structures with Coefficient Weights and Cluster Scores were retrieved and taken for further reference.

Table 5

Predicted T-cell epitopes for Envelope protein. Finally, selection is done w.r.t to c-terminal, TAP scores antigenicity and toxicity.

| Peptide   | Length | Scores |
|-----------|--------|--------|
| LVKPSFYVV | 9      | –0.11106 |
| VSLVPVFSY | 9      | –0.25372 |

Table 2

Immunogenicity results of the non-toxic + antigenic epitopes. The first three based on top scores were taken.

| Peptide     | Length | Scores |
|-------------|--------|--------|
| QLTPTWRY    | 9      | 0.31555 |
| WTAGAAGYY   | 9      | 0.15259 |
| CNDNPFGVY   | 9      | 0.15232 |
| VLPFNDG     | 7      | 0.12878 |
| GAAAYVGVY   | 9      | 0.09663 |
| ITDAVDCAL   | 9      | 0.08501 |
| YQGQVNTCEV  | 9      | 0.08295 |
| STQDLPFLP   | 9      | 0.06828 |
| GASHVNSY    | 9      | –0.00296 |
| TSNQAVLY    | 9      | –0.01327 |
| VGGNNYNLY   | 9      | –0.0148 |
| KTSVDCMTY   | 9      | –0.11115 |
| LSETCTKLN   | 9      | –0.16291 |
| VLEKGVHLY   | 9      | –0.18916 |
| STECSSLIL   | 9      | –0.20478 |
| MTSCRSCLKL  | 9      | –0.36816 |

Table 3

Immunogenicity results of the non-toxic + antigenic epitopes. Epitope the basis of top scores were taken.

| Peptide     | Length | Scores |
|-------------|--------|--------|
| AGDSGFAAY   | 9      | 0.03981 |
| LVGLMWLSY   | 9      | –0.06867 |
| ATSRITLSY   | 9      | –0.11604 |
| VARTSRLS    | 9      | –0.17295 |

Table 4

Immunogenicity results of the non-toxic + antigenic epitopes. Epitope the basis of top scores were taken.

| Peptide     | Length | Scores |
|-------------|--------|--------|
| LVKPSFYVV   | 9      | –0.11106 |
| VSLVPVFSY   | 9      | –0.25372 |

weights of the corresponding terms. The best-Docked Structures with Coefficient Weights and Cluster Scores were retrieved and taken for further reference.

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Predicted T-cell epitopes for Envelope protein. Finally, selection is done w.r.t to c-terminal, TAP scores antigenicity and toxicity.

| Peptide   | Length | Scores |
|-----------|--------|--------|
| LVKPSFYVV | 9      | –0.11106 |
| VSLVPVFSY | 9      | –0.25372 |
epitopes were predicted out of three; two were further selected for the experiment based on non-toxic, antigenic (Table 5) and Immunogenicity scores (Table 6). Epitopes with non-antigenic + non-toxic, toxin +

Table 6

| Residue number | Peptide sequence | MHC | Rescale binding affinity | C terminal cleavage affinity | Transport affinity | Prediction score | Antigenic | Toxicity |
|---------------|----------------|-----|--------------------------|-----------------------------|-------------------|-----------------|-----------|----------|
| 34            | LTALRLCAY       |     | 0.5594                   | 2.3751                      | 0.6272            | 2.933           | 2.6158    | Non-Antigen | Toxin    |
| 49            | VSLKPSFY        |     | 0.3533                   | 1.4999                      | 0.3714            | 3.186           | 1.7149    | Antigen   | Non-toxin |
| 51            | LVKPSFYVY       |     | 0.1343                   | 0.5702                      | 0.9767            | 3.119           | 0.8726    | Antigen   | Non-toxin |

Table 7

| Allele         | Start | End  | Length | Non Allergens peptide sequence | Percentile rank | Alegpred score | antigenic | toxicity | If n+/- |
|----------------|-------|------|--------|--------------------------------|-----------------|----------------|----------|----------|---------|
| HLA-DRB5*01:01 | 1     | 235  | 15     | ITRFQTLLAHRSYL                 | 0.26            | −0.41          | Non-Antigen | Toxic    |         |
| HLA-DRB5*01:01 | 1     | 234  | 15     | NITRQFTLLAHRSY                 | 0.32            | −0.45          | Non-Antigen | Toxic    |         |
| HLA-DRB5*01:01 | 1     | 233  | 15     | GINTRQFTLLAHR                  | 0.52            | −0.48          | Antigenic   | Non-Toxic | Ifn+    |
| HLA-DRB5*01:01 | 1     | 209  | 15     | PINLVRDLPGFSAL                 | 0.49            | −0.78          | Antigenic   | Non-Toxic |         |
| HLA-DRB5*01:01 | 1     | 64   | 14     | ATRFASYAVWRRK                  | 0.49            | −0.43          | Non-Antigen | Toxic    |         |
| HLA-DRB5*01:01 | 1     | 65   | 14     | TRFASYAVWRR                   | 0.52            | −0.55          | Non-Antigen | Toxic    |         |
| HLA-DRB3*01:01 | 1     | 207  | 15     | HTPINLVRDLPGFS                  | 0.51            | −0.98          | Non-Antigen | Toxic    |         |
| HLA-DRB3*01:01 | 1     | 210  | 15     | INLVRDLPGFSALE                 | 0.51            | −0.48          | Antigenic   | Non-Toxic |         |

Table 8

| Allele         | Start | End  | Length | Non Allergens peptide sequence | Percentile rank | Alegpred score | antigenic | toxicity | If n+/- |
|----------------|-------|------|--------|--------------------------------|-----------------|----------------|----------|----------|---------|
| HLA-DRB1*07:01 | 166   | 180  | 15     | KEITVATSRTLSYYK                  | 2.1             | Antigenic       | Non-Toxin |         |         |
| HLA-DRB3*01:01 | 175   | 189  | 15     | TLSYKLGASQVRAG                   | 2.1             | Antigenic       | Non-Toxin |         |         |
| HLA-DRB1*07:01 | 164   | 178  | 15     | LPKEITVATSRTLSY                  | 2.2             | Antigenic       | Non-Toxin |         |         |
| HLA-DRB1*07:01 | 88    | 102  | 15     | VGLMSYFHASFR                   | 3.4             | Antigenic       | Non-Toxin |         |         |
| HLA-DRB5*01:01 | 174   | 188  | 15     | RTLSYKLGASQVRQA                  | 6.4             | Antigenic       | Non-Toxin |         | Ifn+    |

Table 9

| Allele         | Start | End  | Length | Non Allergens peptide sequence | Percentile rank | Alegpred score | antigenic | toxicity | If n+/- |
|----------------|-------|------|--------|--------------------------------|-----------------|----------------|----------|----------|---------|
| HLA-DRB1*15:01 | 9     | 23   | 15     | TGTLIVNSVLFAL                   | 0.3354          | Non-allergen   | Antigenic | Non-Toxin | Ifn+    |
| HLA-DRB1*03:01 | 10    | 24   | 15     | GTLIVNSVLLFLAFV                 | 0.3383          | Non-allergen   | Antigenic | Non-Toxin | Ifn+    |
| Non Overlapping Sequences | GTLIVNSVLLFLAFV | | | | | | | | |

epitopes were predicted out of three; two were further selected for the experiment based on non-toxic, antigenic (Table 5) and Immunogenicity scores (Table 6). Epitopes with non-antigenic + non-toxic, toxin +

Table 10

| Epitope         | ANTIGENICITY | Score | TOXIN SCORE | TOXIN/ NON TOXIN |
|-----------------|--------------|-------|-------------|------------------|
| GSVITPCTNTSNQVA | Probable     | 0.4651| −1.58       | Non-Toxin        |
| GWTAGAAAAYYVGYQP| Probable     | 0.0621| −1.29       | Non-Toxin        |
| HRSYLTPGDSSSGWTA| Probable     | 0.6017| −0.7        | Non-Toxin        |
| TVEKGIYTQNTSNFRVQ| Probable     | 0.438 | −1.78       | Non-Toxin        |

3.3. Helper T cell epitope prediction

HTL epitopes for human alleles were predicted by the MHC II prediction module of IEDB. For surface protein HLA DRB5*01:01 with position 232–246, HLA DRB 3*01:01:029-223 with a length of 15-mer were selected on the basis of antigenic, non-toxic and non-overlapping epitopes (Table 7). In the case of membrane protein HLA DRB1*07:01 with position 88–102 and HLA DRB 5*01:01 with position 174–188 with 15-mer length based on Antigenicity and non-toxicity both were selected (Table 8). For envelope protein, HLA DRB1*15:01 start 9 ends with 23, and HLA DRB1*03:01 start 10 and end with 24 were predicted, based on parameters mentioned above, only HLA DRB1*03:01 selected for further investigation (Table 9). Antigenic + Non-allergenic + Non-Toxic +
3.4. B cell epitope prediction

BCPred online server was used to predict the b cell epitopes for three mentioned proteins for each protein non-toxicity and antigenicity parameter was used to qualify epitope for further analysis. For surface protein, two epitopes with the highest score of 0.6210 and 0.6017 were selected (Table 10). In the case of membrane protein, from nine epitopes, two were chosen further, with the highest score of 0.9132 and 0.9510. One epitope is discarded as it shows toxicity (Table 11). For envelope protein, from four epitopes, two were predicted as toxin, and two were predicted as non-toxin. Two non-toxin, having a score of 0.66 and 0.54, were selected (Table 12).

3.5. Construction of multi-subunit vaccine

Total 17 epitopes were selected from three protein shaving CTL, HTL and B-cell epitopes. For joining of adjuvant with CTL epitopes, EAAAK linker was used. AAY linker was used to connect each CTL epitope. GPQGPG linker was used to join CTL epitope with HTL epitope and to connect each HTL epitope. KK linker was used to join HTL with B cell epitope and to connect each B-cell epitope (Figs. 1a–1c). Allergenicity of vaccine construct was checked by ALgPred, and antigenicity was reviewed by Vaxijen Server (Table 13). All three vaccine constructs qualify the parameters of non-allergen, and antigenic three final vaccine constructs were created by using three different adjuvants (Table 13).

Physicochemical analysis of vaccine constructs:

The molecular weight of the three constructs ranges from 35 to 52 KDa. Construct three shows maximum weight. PI value indicates that three constructs are basic in nature. The instability index is a parameter that is used to calculate the stability inside the test tube. Instability index for the final constructs predicted less than 40, which is considered as stable in nature. GRAVY value for three constructs indicates that vaccine construct 1 has a hydrophobic score while vaccine construct 3 has a hydrophilic score suggesting its hydrophilic nature (Table 14).

Homology modelling and tertiary structure refinement:

For tertiary structure prediction, all the three constructs sequences were submitted to TASSER online web server. I-TASSER resulted in five models for each vaccine construct. Thereafter each model was submitted to Galaxy Refine server for model refinement of structure, which results back with total of 75 models for each I-TASSER vaccine constructs model. RMSD score, clash score and rama favoured best models for three constructs were selected for further analysis. For vaccine construct 1, model no. 2, having 88.0% rama favourable regions was selected (Table 15). Whether in the case of vaccine construct 2, model no. 5, having 83.4% rama favourable regions was selected (Table 16). For Vaccine construct 3, least poor rotamers, lowest clash scores and most rama favoured region model was selected (Table 17). Three vaccine constructs were used for final epitope prediction.

Table 11
B-cell epitope prediction of membrane protein. Top antigenic scores were taken for further investigations.

| Epitope       | ANTIGEN | Score | Score toxicity | Toxicity |
|---------------|---------|-------|----------------|----------|
| RSMWSFPETNILNV | ANTIGEN | 0.4451 | −1.2 | Non-Toxin |
| SFLFARTRSMWSFNP | ANTIGEN | 0.951 | −0.84 | Non-Toxin |
| RFYIKIFLIFLWLLWP | ANTIGEN | 0.4532 | −0.56 | Non-Toxin |
| GDSFAAYSYRIRIGNY | ANTIGEN | 0.898 | −1.19 | Non-Toxin |
| RCDKDLPREITVATS | ANTIGEN | 0.5606 | −1.1 | Non-Toxin |
| RINWITGIAIAMACL | ANTIGEN | 1.2392 | −0.71 | Non-Toxin |
| PKETVATSRLSYYK | ANTIGEN | 0.5935 | −1.26 | Non-Toxin |
| GIAAMACLVGLMWLS | ANTIGEN | 0.9132 | −0.93 | Non-Toxin |
| LVIGFLFTWICLLQF | ANTIGEN | 0.9967 | 0.15 | Toxin |

Table 12
B-cell epitope prediction of Envelope protein. Top antigenic scores were taken for further investigations.

| PEPTIDE       | ANTIGEN | Score | TOXIN SCORE | TOXIN/ NON TOXIN |
|---------------|---------|-------|-------------|-----------------|
| TLAILTALRLCAYCNN | ANTIGEN | 0.6628 | 0.54 | Toxin |
| NVSLVKKPSYYSRRVK | ANTIGEN | 0.7965 | −1.44 | Non-Toxin |
| YYVSRVKNLNSSRVPD | ANTIGEN | 0.5457 | −0.9 | Non-Toxin |
| LCAAYCNIVNVSLVVP | ANTIGEN | 0.7286 | 0.92 | Toxin |

Fig. 1a. Final Multi-epitope vaccine structure 1.
Secondary structures of three vaccine constructs: Secondary structures of vaccine construct peptides obtained from PSIPRED are shown in (Figs. 3a, 3b, 3c (see Supplementary Figures)). Every vaccine construct has alpha helix, beta-sheet, and beta-turn.

Tertiary structure validation:
PROSA server was used to verify the structures. Z-score determines the overall model quality and is represented in the plot. From the server, it was investigating that all the vaccine constructs show homology with X-ray structures (Figs. 4a, 4b, 4c (see Supplementary Figures)) (Figs. 5–8).

3.6. Comparative results of docking of three vaccine constructs

The TLR 8 protein structure was docked to three vaccine construct with the ClusPro server. Balanced docking method was chosen among available four methods: Balanced, Electrostatic-favored, Hyrdophobic-favored and VdW + Elec. Coefficient Weight were calculated by using algorithm $E = 0.40E_{rep} + 0.40E_{att} + 600E_{elec} + 1.00EDARS$. The best orientation PDB files with Coefficient Weights and Cluster Scores were retrieved and taken for protein-ligand interactions studies. The cluster score of all three docked complexes is given in Table 18. Interaction studies were done by Dimplot and Ligplot. Hydrogen bond interactions vary from 21 to 34, as shown in Tables 19–21. Vaccine Construct 2 with 21 Hydrogen Bonds showed least interaction with TLR8, whereas Vaccine Construct 3 interacted with 34 Hydrogen Bonds showed maximum interaction with TLR8. Interacting residues of TLR8 with all three Vaccine Construct ligand along with name and position are given in Tables 19–21. Based on the Coefficient Weights score of the docked complex obtained from ClusPro Protein-Protein Docking Server, along with Hydrogen Bonds and AAs interactions between TLR8 and vaccine construct predicted by Dimplot, Vaccine Construct 3 seems to be promising to fight against the COVID-19 pandemic.

Immunological simulations of final potential construct:
The TLR 8 protein structure was docked to three vaccine construct with the ClusPro server. Balanced docking method was chosen among available four methods: Balanced, Electrostatic-favored, Hyrdophobic –
favored and VdW + Elec. Coefficient Weights were calculated by using algorithm $E = 0.40 \text{Erep} + 0.40 \text{Eatt} + 600\text{Edi} + 1.00\text{Eelec}$. The best orientation PDB files with Coefficient Weights and Cluster Scores were retrieved and taken for protein-ligand interactions studies. The cluster score of all three docked Complexes is given in Table 18. Interaction studies were done by Dimplot and Ligplot + Hydrogen bond interactions vary from 21 to 34, as shown in Tables 19–21. Vaccine Construct 2 with 21 Hydrogen Bonds showed least interaction with TLR8, whereas Vaccine Construct 3 interacted with 34 Hydrogen Bonds showed maximum interaction with TLR 8. Interacting residues of TLR 8 with all three Vaccine Construct ligand along with name and position are given in Tables 19–21.

Based on the Coefficient Weights score of the docked complex obtained from ClusPro Protein–Protein Docking Server, along with Hydrogen Bonds and AAs interactions between TLR8 and vaccine construct predicted by Dimplot, Vaccine Construct 3 seems to be promising to fight against the COVID-19 pandemic.

### Table 13
Three Final multi-epitope vaccine construct sequences further predicted for allergen toxicity and antigenicity.

| Construct No. | Vaccine Sequence | Allergenic | Toxicity | Antigenicity |
|---------------|------------------|------------|----------|--------------|
| 1 | MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAPAGAAVEAEQSEFDVILEAAGDKKGVKVVR ETVSGLGLKEAKDLVPGPKPELVKVEAADEKAK LEAGATVTKVEAAAKQLPTWRRVYAAAYWTAGAAAYY AANCNIPGLVAYADGSAFAYAALYVKPSFYVAAAYV YLYVKKPSFYGPGPNITRFQTLALHRPGPGPNINLRVLD LPGQGFSALPGPGPILWLSYTAISFRPGPGPGTPSLYYK QAQSROQVAGPGPGPPGTLTVNSLVLFLALPKVPPPQK GWTTAGAAAYYVGGQPLKKHSLYTPDSSGWTAKNVSLKPS FYVYYRVSNNVHFLSVDKSFRLPARKTSMSWFNSF PKQGIAHAMACLVLGMWLS | NO | NO | YES |
| 2 | GIINTLQKVCRGVRGCAVLSCPLKQEEKEIKXCSTRGRKCCRRKKEAAAKQLTPTWRVYAVY WTAAGAAAYACNPDELGYYAAYADGSA AAYYLVKPSFYVAYAVSLLKPSFYGPGP GINTRQFTQLALHRPGPGPNINLRVLD LPGQGFSALPGPGPILWLSYTAISFRPGPGPGTPSLYYK QAQSROQVAGPGPGPPGTLTVNSLVLFLALPKVPPPQK GWTTAGAAAYYVGGQPLKKHSLYTPDSSGWTAKNVSLKPS FYVYYRVSNNVHFLSVDKSFRLPARKTSMSWFNSF PKQGIAHAMACLVLGMWLS | NO | NO | YES |
| 3 | MAENPNIDDLPAVLAAALGAAALATVDNL AINHLQAEETAAETRTRVEEERARLTKFQE DLPSQFOELRDFEYELKLAAEXGvYLAATN YNHELVEGAELQRLLQFQAFDASARAG YVQAOALQTEALGVTAQTVGAEAEAKLvl GEJPGKAEAGKQAQAHAAAPAKKAAPAAKAAK KVTQKEAANLQPTWP YAYWTTAGAAYAYAYYCNDFLGVYWAA YAGDSFGAAAYLКPSFYAYAAYVSLKPSFYGPGGPGPGPNINLRVLD LPGQGFSALPGPGPILWLSYTAISFRPGPGPGTPSLYYK QAQSROQVAGPGPGPPGTLTVNSLVLFLALPKVPPPQK GWTTAGAAAYYVGGQPLKKHSLYTPDSSGWTAKNVSLKPS FYVYYRVSNNVHFLSVDKSFRLPARKTSMSWFNSF PKQGIAHAMACLVLGMWLS | NO | NO | YES |

### Table 14
Physiochemical properties of three constructs:

| S.No | Vaccine Construct | Molecular weight (kDa) | Total Amino Acid | PI | GRAVY | Instability Index | Aliphatic Index |
|------|-------------------|------------------------|------------------|----|-------|-------------------|----------------|
| 1    | C1                | 44.033                 | 412              | 9.56 | 0.097 | 28.64             | 88.20          |
| 2    | C2                | 35.753                 | 327              | 10.13| 0.045 | 35.48             | 79.11          |
| 3    | C3                | 52.768                 | 487              | 9.89 | 0.215 | 36.05             | 81.54          |

### Table 15
Galaxy refine results showing different scores for vaccine construct Model 1.

| Model | GDT-HA | RMSD | MolProbity Clash score | Poor rotamers | Rama favored |
|-------|--------|------|------------------------|---------------|--------------|
| Initial | 1     | 0.9284 | 0.466 | 2.326 | 13.3 | 87.6 |
| MODEL 1 | 0.9302 | 0.468 | 2.342 | 14.3 | 1.3 | 88.7 |
| MODEL 2 | 0.9175 | 0.506 | 2.487 | 14.3 | 1.9 | 87.6 |
| MODEL 3 | 0.9272 | 0.472 | 2.307 | 14.9 | 0.6 | 86.6 |
| MODEL 4 | 0.9278 | 0.473 | 2.285 | 14.3 | 0.6 | 86.8 |

### Table 16
Galaxy refine results showing different scores for vaccine construct Model 2.

| Model | GDT-HA | RMSD | MolProbity Clash score | Poor rotamers | Rama favored |
|-------|--------|------|------------------------|---------------|--------------|
| Initial | 1     | 0.9205 | 0.487 | 2.673 | 25.9 | 1.2 | 81.2 |
| MODEL 1 | 0.9251 | 0.478 | 2.709 | 28.5 | 1.2 | 81.5 |
| MODEL 2 | 0.9159 | 0.505 | 2.883 | 28.9 | 2 | 81.5 |
| MODEL 3 | 0.9159 | 0.501 | 3.011 | 26.2 | 3 | 80.6 |
| MODEL 4 | 0.9235 | 0.483 | 2.81 | 25.9 | 2 | 83.4 |

3.7. Immunological simulations of final potential construct:

When antigen was initially exposed to the system, a very high level of IgM noticed. After the secondary and tertiary response, there was an
Fig. 2. 3D model structures of vaccine peptides predicted by I-TASSER.

Fig. 5. Most stable orientation of TLR8 Docked with three vaccines constructs (A, B, C). Docked Complexes were obtained from the ClusPro.

Fig. 6. H Bond interactions as plotted by Dimplot Between TLR8 and vaccine construct 1. Dashed lines Hydrogen bonds, arcs hydrophobic interactions.
increase in the concentration of IgM + IgG, Ig1 and Ig2 with the decrease in the concentration of antigen (Fig. 9A). Total B cell concentration from three exposures results in increasing while memory B cell concentration remains high for several months (Fig. 9B). Similarly, a high response was seen in TH (Helper) and TC (cytotoxic) cell population (Fig. 9C, 9D). Interferon – Gamma and II-2 concentration rose with exposure to antigen. They remained at a high level with exposure to the antigen, which indicates that it leads to an increase in TC, B cell and immunoglobins productions.

**Table 18**

Weighted Score of TLR8 and ligand (Vaccine Construct 1–3) obtained from the ClusPro.

| S.No. | Docked Complex         | Weighted Score | Center Energy | Lowest energy |
|-------|------------------------|----------------|---------------|---------------|
| 1.    | TLR8 + Vaccine Construct 1 | 999.3          | 1382.7        |               |
| 2.    | TLR8 + Vaccine Construct 2 | 837.0          | 1059.4        |               |
| 3.    | TLR8 + Vaccine Construct 3 | 1175.7         | 1478.0        |               |

**Fig. 7.** H Bond interactions as plotted by Dimplot Between TLR8 and vaccine construct 2. Dashed lines Hydrogen bonds, arcs hydrophobic interactions.

**Fig. 8.** H Bond interactions as plotted by Dimplot Between TLR8 and vaccine construct 3. Dashed lines Hydrogen bonds, arcs hydrophobic interactions.
In the present study, SARS-CoV-2 three proteins antigenic cines (Groot and Rappuoli, 2004; Korber et al., 2006; Purcell et al., 2007). In the present study, SARS-CoV-2 three proteins antigenic cines (Groot and Rappuoli, 2004; Korber et al., 2006; Purcell et al., 2007).

In-silico approach for designing and developing multi-epitope vaccine construct and its immune simulation to identify predicted immune responses. The in-silico approach is used in the present scenario because it saves a lot of time, does not require microbial culture and safe to develop effective new generation vaccine using modern computational and immunoinformatic tools (Scarselli et al., 2005). Recent studies have shown that advanced in-silico tools not only helping in the prediction of immune response but also paying the way for designing and developing new generation vaccines (Groot and Rappuoli, 2004; Korber et al., 2006; Purcell et al., 2007). In the present study, SARS-CoV-2, three proteins antigenic epitope prediction, the interaction of MHC alleles with epitopes have been identified for generation of multi-epitope vaccine constructs. Initially, these proteins were checked in the BlastP online program of NCBI to ensure that they don’t show any similarity with human proteome. Further B-cell and T cell epitopes were retrieved from online servers. B-cells are associated with the generation of humoral response by secreting antibodies from plasma cells and memory B cells, providing lifelong immunity (Silva et al., 2016).

The purpose of choosing B-cell epitopes because they will generate both cell-mediated and humoral response. B-cell epitopes are the group of amino acid sequence present on the cell surface identified by specific antibodies or BCR that generate a humoral or cell-mediated response. The generated antibodies will neutralize the virus, and cell-mediated immunity will kill infected cells. The idea of choosing T cell epitopes was because it was presented by APC, bound by MHC molecules to generated immune response. T cell identifies MHC class I and II in the cell surface. Class I MHC molecule generally present Peptide molecule between 8 and 10 amino acid length to be identified by Cytotoxic T cells having marker CD8+. Class II MHC molecule has a peptide length of 12to25 amino acid, identified by Helper T cell with marker CD4+. Ifa sufficient epitope is presented, the T cell may generate a significant adaptive immune response specific against the virus-specific pathogen. Because of this, all these epitopes from three proteins of SARS-CoV-2 were assembled to make three final constructs with the help of linkers and three different adjuvants. For vaccine construct I - non-allergenic score was 0.424, Non-toxic, and antigenicity score was 0.5292. The construct-II non-allergenic score was -1.0073, Non-toxic, the antigenic score was 0.59512. Construct-III Non-allergenic score was 1.029, Non-toxic, the antigenic score was 0.4861. All the three vaccines constructs show highly antigenic, non-toxic and non-allergen by passing the threshold cut-offs. Previous studies have shown that using immunoinformatic tools, epitopes were synthesized from the parasite proteome such as Leishmania braziliensis is and assessed in-vitro found to be immunogenic and elicit an immune response (Silva et al., 2016). Another study was shown to generate T cell response using T cell epitopes derived from Mycobacterium tuberculosis proteome using an immunoinformatic approach (Khan et al., 2014). Based on these studies, the present study attempted to design and develop the vaccine construct against SARS-CoV-2 virus using computational and immunoinformatic approach. Furthermore, the molecular weight of construct I, II, III was identified as 44.033 kDa, 35.753 kDa, 32.768 kDa that shows molecular weight with in acceptable values for construction of vaccine. All three constructs show basic in nature and Instability index values of three shows all are thermally

### Table 19
H Bond Interactions between TLR8 and ligand as Predicted by Dimplot (Vaccine Construct 1).

| No. | Hydrogen Bonds | Interacting AAs of TLR8 | Interacting AAs of ligand |
|-----|----------------|------------------------|--------------------------|
| 1   | 33             | ASN 262                | ARG 387                  |
|     |                | ALA 263                | ARG 381                  |
|     |                | TYR 348                | ARG 373                  |
|     |                | LYS 350                | ARG 235                  |
|     |                | ARG 541                | ASN 232                  |
|     |                | TYR 567                | LYS 396                  |
|     |                | ASN 595                | SER 391                  |
|     |                | TYR 597                | LEU 369                  |
|     |                | ARG 619                | SER 371                  |
|     |                | ARG 650                | GLU 33                   |
|     |                | ARG 696                | GLU 20                   |
|     |                | ARG 797                | THR 31                   |
|     |                | ASP 72                 | PRO 228                  |
|     |                | ASP 673                | TYR 147                  |
|     |                | ASN 99                 | ALA 146                  |
|     |                | PRO 264                | ASP 236                  |
|     |                | HIS 566                | HIS 223                  |
|     |                | HIS 593                | ASN 393                  |
|     |                | ASP 645                | SER 388                  |
|     |                | SER 647                | ARG 224                  |
|     |                | GLU 768                | LYS 395                  |

### Table 20
H Bond Interactions between TLR8 and ligand as Predicted by Dimplot (Vaccine Construct 2).

| No. | Hydrogen Bonds | Interacting AAs of TLR8 | Interacting AAs of ligand |
|-----|----------------|------------------------|--------------------------|
| 1   | 21             | ASN 262                | TYR 9                    |
|     |                | ARG 375                | ARG 12                   |
|     |                | SER 513                | THR 5                    |
|     |                | ASN 539                | LYS 8                    |
|     |                | ARG 541                | TYR 243                  |
|     |                | ASP 72                 | ARG 241                  |
|     |                | ASN 99                 | ARG 197                  |
|     |                | CYS 260                | SER 195                  |
|     |                | CYS 267                | GLN 196                  |
|     |                | GLU 427                | ARG 178                  |
|     |                | PHE 467                | VAL 198                  |
|     |                | TYR 468                | TYR 190                  |
|     |                | TYR 563                | ALA 175                  |
|     |                | SER 565                |                            |

### Table 21
H Bond Interactions between TLR8 and ligand as Predicted by Dimplot (Vaccine Construct 3).

| No. | Hydrogen Bonds | Interacting AAs of TLR8 | Interacting AAs of ligand |
|-----|----------------|------------------------|--------------------------|
| 1   | 34             | GLN 136                | GLY 364                  |
|     |                | TYR 182                | GLY 365                  |
|     |                | PHE 183                | GLY 362                  |
|     |                | ASN 184                | SER 331                  |
|     |                | LYS 185                | LEU 327                  |
|     |                | SER 212                | MET 328                  |
|     |                | ASN 262                | TYR 222                  |
|     |                | LYS 350                | ARG 338                  |
|     |                | SER 352                | TYR 234                  |
|     |                | GLN 380                | TYR 231                  |
|     |                | LYS 407                | TYR 224                  |
|     |                | SER 516                | PRO 361                  |
|     |                | ASN 539                | PRO 363                  |
|     |                | ARG 541                | GLN 356                  |
|     |                | ASN 156                | VAL 358                  |
|     |                | ASN 157                | ALA 359                  |
|     |                | ASN 180                | GLY 324                  |
|     |                | CYS 187                | ASP 249                  |
|     |                | CYS 260                | TYR 258                  |
|     |                | TYR 353                | PRO 262                  |
|     |                | GLU 427                | GLN 211                  |

### 4. Discussion

Vaccines are developed with the purpose to generate immune responses that protect human being from several viral diseases. Recently, epitope vaccines were developed by engineering CTL, HTL and B-cell epitopes. The advantages associated with epitope vaccines as compared to conventional vaccines are their non-toxicity, highly immunogenic and Non-allergen. The present study based on in-silico approach for designing and developing multi-epitope vaccine construct and its immune simulation to identify predicted immune responses. The in-silico approach is used in the present scenario because it saves a lot of time, does not require microbial culture and safe to develop effective new generation vaccine using modern computational and immunoinformatic tools (Scarselli et al., 2005). Recent studies have shown that advanced in-silico tools not only helping in the prediction of immune response but also paying the way for designing and developing new generation vaccines (Groot and Rappuoli, 2004; Korber et al., 2006; Purcell et al., 2007). In the present study, SARS-CoV-2, three proteins antigenic epitope prediction, the interaction of MHC alleles with epitopes have been identified for generation of multi-epitope vaccine constructs. Initially, these proteins were checked in the BlastP online program of
stable. Two and three-dimensional structures of three vaccines constructs were identified was using online servers, which were further preceded by structural validations.

Docking analysis was conducted to comprehend the immune response of TLR8 of three vaccine constructs. By comparing three vaccine constructs, construct III shows the lowest energy and is taken into further consideration for immune simulations. Immune simulation studies with repeated exposure of antigen show a significant increase in the immune response. Initially, there was a high level of IgM present; when exposed to Secondary and tertiary responses, there was also an increase in IgM and IgG. From three exposures, memory B cell remain high for several months in the case of T h and T c cells shows massive high response Interferon-gamma and IL-2 remains at a peak which shows there was an enormous production of Ig. Immune simulations of the final vaccine construct show better result in the modelled environment; hence by compilation of these new immunoinformatic approach in this study to the formation of potential, non-toxic, non-allergenic, antigenic with high immune response to control SARS-CoV-2.

5. Conclusion

In this study, an immunoinformatic approach employed to develop a multi-epitope vaccine construct against SARS-CoV-2. The different vaccine constructs are docked with TLR 8, showing good binding affinity. The predicted humoral and cell-mediated responses are also significant using simulation studies. Physicochemical structure analysis and immune simulation of vaccine construct are performed to check how vaccine constructs behave in cell environment and how much immune response is generated. Preferred vaccine construct will need in-vivo validations further to ensure its activity, stability and enhancement of immune response. This research will be assisted infection control by enhancing the immune response against SARS-CoV-2.

Author contributions

A.K. conceived the idea. A.S. & S.P. performed data retrieval, analysis and characterization. A.S., S.P. & A.P. Performed methodology, software, data curation and characterization under the guidance of A.K. The manuscript was written by A.K., A.S., S.P., A.P. & S.K. S.K. and A.K.

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Fig. 9. C-ImmSim demonstration of antigenic peptide through immune simulation. (A) Feedback of antigenic injections w.r.t. production of immunoglobulin (black vertical lines); specific subclasses are indicated as coloured peaks. (B) Concentration of B-cell population after three exposures. (C) Indicating concentration of TC cells. (D) Evolution of TH cells.
contributed to the analysis and discussion of the results. Review & editing done by A.K. & S.K.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imbio.2021.152134.

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