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Immunoinformatics approaches to explore B and T cell epitope-based vaccine designing for SARS-CoV-2 Virus

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Abstract: SARS-CoV-2, a new world coronavirus belonging to class *Nidovirales* of *Coronaviridae* family causes COVID-19 infection which is the leading cause of death worldwide. Currently there are no approved drugs and vaccines available for the prevention of COVID-19 infection, although couples of immunizations are being tested in clinical trials. However, the present efforts are focused on computational vaccination technique for evaluating candidates to design multi-epitope-based vaccine against pathogenic mechanism of novel SARS-COV-2. Based on recent published evidence, we recognized spike glycoprotein and envelope small membrane protein are the potential targets to combat the pathogenic mechanism of SARS-CoV-2. Similarly, in the present study we identified epitope of both B and T cell associated with these proteins. Extremely antigenic, conserve, immunogenic and nontoxic epitope of B and T cell of Spike protein are WPWYVWLGFI, SRVKNLNSSEGVPDLLV whereas the CWCARPTCIK and YCCNIVNVSL are associated with envelope small membrane protein were selected as potential candidate for vaccine designing. These epitopes show virtuous interaction with HLAA0201 during molecular docking analysis. Under simulation protocol the predicted vaccine candidates show stability. Collectively, this work provides novel potential candidates for epitope-based vaccine designing against COVID-19 infection.

Keywords: SARS-CoV-2, vaccine, immunoinformatics, multi-epitope, COVID-19

INTRODUCTION

Coronaviruses are positive non-segment RNA viruses in both the Coronaviridae and Nidoviral families that are widely spread among humans and other mammals (Malik *et al.,* 2020). Although usually mild in human coronavirus infections, epidemics of the two beta coronaviruses, extreme acute respiratory coronavirus syndrome (SARS-CoV) and Middle East respiratory coronavirus syndrome (MERS-CoV) have caused an addition of more than 10,000 mutual cases over the past two years, with decease rates of 10% for SARS-CoV and 37% for MERS-CoV respectively. The coronavirus already identified can only be the tip of the iceberg, with many more novel and extreme zoonotic cases to be discovered (Huang *et al.,* 2020). The recent 2019-20 epidemic of coronavirus disease (COVID-19) resulted in 366896 deaths, with 5,80,7149 cases reported worldwide, and the World Health Organization (WHO) proclaimed COVID-19 a public health emergency (Guan, Ni *et al*. 2020). SARS-CoV-2 is spherical, has a diameter of around 125nm and genome (~30kb) contains at least six open reading frames, which encode 16 non-structural protein and nearly 3'end of genome ORFs, encodes four main structural proteins recognized as spike protein (S). The glycoprotein spike (S) binds to the angiotensin translating enzyme 2 (ACE2) of the cellular receptor and is responsible for inducing viral infection (Chen *et al.,* 2020). Significant coronaviruses extracted from bats and other animals are assumed to have a viral gene pool in wild organisms. Coronavirus can cross the fence directly and contaminate people with extreme mortality (Qiang *et al.,* 2020). With the advances in computational biology the drug discovery process and vaccine production are now accelerated, and traditional approaches have been outstripped. Many studies related to the production of insilico-immuno informatics-based epitope B and T-cells have been published (Damfo *et al.,* 2017; Arnon & Tamar, 2003; Hughes *et al.,* 2011; Yong *et al.,* 2019).

In the present efforts an attempt is made to strategy an epitope-based peptide vaccine in contradiction of the spike (A0A1B3Q5W5 9BETC) and Envelope small membrane (P59637 VEMP CVHSA) proteins. The antigenicity, allergen city and physiochemical properties of B- and T-cell epitopes were also measured. Additionally, we employed the molecular docking strategy, thermodynamics stability profiling, agent-based modelling and *insilico* expression approaches to authenticate the constancy, appearance and immune retort

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reaction triggered by the ultimate vaccine. The interaction of a multi-epitope vaccine construct designed through an integrated modelling approach may trigger innate and specific adaptive immunity by activating TLR signalling pathways and may produce a promising immune response against SARS-CoV-2.

MATERIALS AND METHODS

Proteome dataset

The FASTA formatted sequence of whole proteome dataset of coronavirus were retrieve from NCBI databases. After processing the spike (A0A1B3Q5W5 9BETC) and envelop small membrane protein (P59637 VEMP CVHSA) of SARS-COV-2 were selected for vaccine designing.

Viral strain selection

The ExPASy Bioinformatics tool Portal database Viral Region was used for the 26660-genome selection, host, transmission, ailment, genus, families and proteomes of Coronavirus and its associated data. The primary coronavirus sequences were contained in the UniProt Database (Apweiler *et al.,* 2004).

Identification of antigenic score of proteins

To expose the uppermost antigenic protein, the FASTA format ~~s~~equence of amino acid spike protein (A0A1B3Q5W5 9BETC) and Envelope small membrane protein (P59637 VEMP CVHSA) of SARS-CoV-2 virus were succumbed to the VaxiJen v2.0 server (http://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html) on behalf of antigenicity prediction (Doytchinova and Darren, 2007). This method directed to the selection of the extremely antigenic protein for further assessment.

Identification of T cell epitope and MHC1 binding alleles

NetCTL 1.2 was employed to envisage the T cell epitopes from the protein sequences (Larsen *et al.,* 2007). To assume the MHC-I links, a method of the Immune Epitope Database was used. The Stable Matrix Base Process (SMM) (Peters & Alessandro, 2005) was used to assess the estimates of peptide binds to MHC-I at half-maximal inhibitory concentration (IC50). For binding, each allele was selected, in the duration of 9.0. An online database was utilized to investigate proteasome cleavage, TAP transport and MHC-I for designated epitopes (Tenzer *et al.,* 2005).

Identification of B cell epitopes

B-cell epitope identification is the dynamic phase for epitope-based peptide vaccine strategy. Subsequently, the B-cell epitopes were recognized from the maximum antigenic protein using the available BepiPred-2.0 (http:/www.cbs. dtu.dk/services/BepiPred/) (Karplus & Schulz, 1985). IEDB tools have been used to identify B cell antigenicity containing Kolaskar and Tongaonkar antigenicity (Thevenet *et al.,* 2012), Schulz flexibility and Karplus analysis (Berman *et al.,* 2002), Accessibility Emini- surface analysis (Kolaskar and Prasad, 1990), and Chou and Fasman prediction analysis (Emini *et al.,* 1985) with 0.2 to 0.3 threshold value.

Identification of Allegenicity score for protein

To create the vaccine, Aller Hunter system (Muh *et al.,* 2009) was used to estimate the allergenicity of the potential epitope. The Aller Hunter predicts highly specific allergens and in fact, non-allergens. This allows Aller Hunter an invaluable method for detecting allergens for cross-reactivity (Liao & WIilliam, 2003).

Molecular docking approach

To apply molecular docking technique, PEPFOLD server (Larsen *et al.,* 2006) was subjected to the T and B cell epitope for 3D structural transformation and for the assessment of the interaction among the peptide and human HLA-A0201 we used online web server Galaxy PEPP-DOCK sever (Chou and Gerald, 1978). It calculates separating scores, surface fix coordinating scores, and depiction of atomic shape on behalf of molecular docking. The server splits both the epitopes and HLA-A0201 into minor squares in contract with the surface shape. These minor squares bear a resemblance to exclusive shapes, which can differentiate among puzzle fragments visually. One more algorithm perform superimposition of these minor squares after identification of the squares. The refined complexes provided by Galaxy PEPPDOCK tools are based on numerous aspects including atomic contact energy, partial electrostatics and vdW.

Protein structure simulation

By using the CABS Flex software, we calculate the RMSF value of all amino acids frame works. RMSF is calculated to permit for command-line contact and to evaluate the simulation procedure. CABS-flex is prepared with landscapes including modelling of multimeric and large-size protein schemes, contact map visualizations, exploration of similarities to the reference structure and configurable modelling procedure to calculate the RMSF of all the frameworks (Kuriat *et al.,* 2018).

Insilico vaccine expression and codon optimization

To accomplish extreme expression in E. coli cellular apparatus, Jcat explored the GC content and CAI scores for the vaccine sequence to confirm extreme appearance. Jcat was employed for reverse translation, codon optimization, listed prokaryotic ribosome and restriction binding locations, and choices for rho-independent termination. The reverse translated sequence was applied to restriction sites of Xhol and Ndel. The ultimate vaccine cloned and inserted into plasmid pET-32a(+) via the snap-gene program (Grote *et al.,* 2005). Flow chart demonstrating the inclusive strategy of epitope-based vaccine plan against COVID19 is illustrated in fig. 1.

RESULTS

Extraction of protein sequences profile

The amino acid sequences of the chosen proteins spike (A0A1B3Q5W5 9BETC) and Envelope small membrane protein (P59637 VEMP CVHSA) are obtained from UniProtKB database. Spike protein and envelope small membrane proteins play vital role against SARS-COV-2, these two proteins template available in whole countries population.

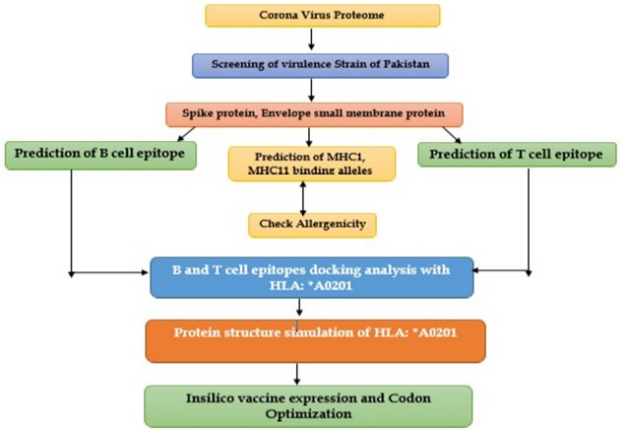

Fig. 1: The overall methodology flow of prediction of peptides against COVID-19 via T and B cell epitope prediction

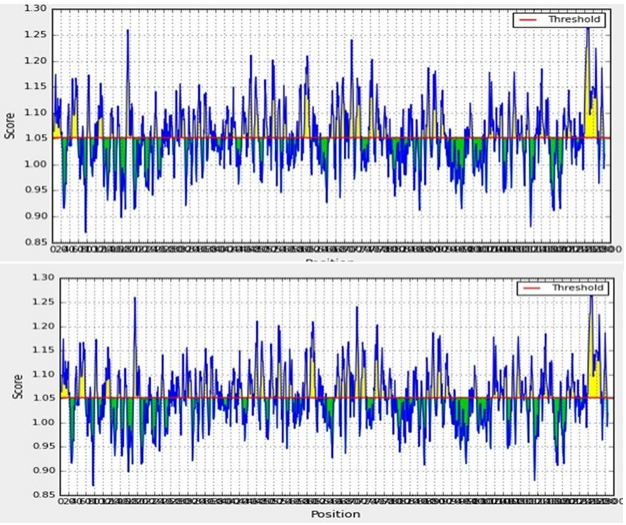

Fig. 2: Antigenicity prediction of Spike and Envelope protein via Kolashkar and Tongaonkar graph**.**

Predication of antigenic score

Vaxijen2.0 web server was employed to determine the antigenicity of each protein. The outcomes reveal that all protein owns antigenic characteristics. Scores showed by mentioned server includes 0.48 for spike (A0A1B3Q5W5 9BETC) and 0.6 for Envelope small membrane protein (P59637 VEMP CVHSA) respectively.

Identification of T cell epitopes

The two effective T cell epitopes from both selected protein sequences such as WPWYVWLGFI and SRVKNLNSSEGVPDLLV with a specificity score of 0.4837 and 0.6025, a sensitivity score of 0.80 is predicted via the NetCTL server. The NetCTL server shows the identification of MHC ligand were 47 for the number of peptide is 1248 for spike protein and identification of MHC ligand were 3 for a number of the peptide is 67 for envelope protein shown in (fig. 2 and table 1) indicates the MHC-I alleles on behalf of which the epitopes disclosed greater affinity that is IC50 < 200 nM. Between the two T cell epitopes, WPWYVWLGFI and SRVKNLNSSEGVPDLLV, interrelated with a maximum of the MHCI alleles, comprising HLA:*H2Db, HLA: *A0201, HLA: *A0206, HLA: *B3501, HLA:* DRB0101, HLA:*IAb, HLA:*IAs and TAP with developed affinity values in between 0.75-1.0.

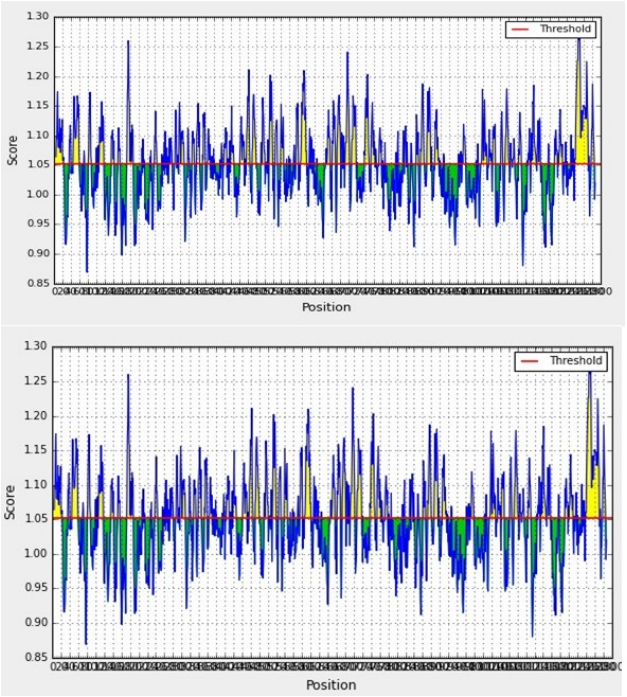

Fig. 3: Antigenicity prediction of Spike and Envelope protein by Emini surface accessibility graph

Predication of allergenicity score for protein

The sequence-based allergenicity prediction method precisely determined with the use of Aller Hunter method was 0.02, 0.46 with a sensitivity of 74.4%, 72.3% and a specificity value of 13.1%.

Prediction of B cell epitope on different factor

The Kolaskar and Tongaonkar antigenicity evaluation approach tested the antigenicity of the B-cell epitope based on the physiochemical characteristics of the amino acids, using various techniques to determine possible B-cell epitopes. The total value obtained for antigenicity

was 1.013. In (table 2 and fig. 3), eleven epitopes described an ability for expressing the B cell response.

The probable beta turn region predicted through Chou and Fasman that is 14-23 (CWCARPTCIK) and 42-51 (YCCNIVNVSL) regions. Through Kolaskar and Tongaonkar antigenicity probable predict the B-cell epitopes that is CWCARPTCIK, YCCNIVNVSL. For a probable epitope, a B-cell needs to be completely accessible to the surface. Hence the prediction of Emini surface accessibility was created. The regions with residues of 14-23 and 42-51 amino acids were more usable. Fig. 3 illustrates the prediction residues. Consequently, the antigenicity prediction system Karplus and Schulz were used, the area of 20-26 and 59-65 amino acid residues were shown as the most suitable region. The Bipred approach calculates the probability of triggering the desired immune responses including B-cell epitopes with peptide-sequence from 14-23 and 42-51 amino acids. (table 3 and fig. 4) display these domains.

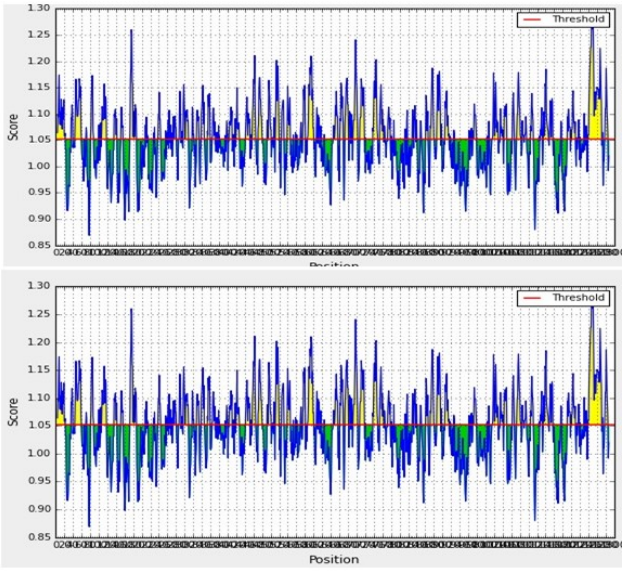

Fig. 4: Antigenic graph B cell epitope of Spike and Envelope protein through Bepipred linear epitope prediction

Molecular docking analysis of B and T-cell epitope

Based on interactions with a maximum number of alleles, the T-cell epitope WPWYVWLGFI, SRVKNLNSSEGVPDLLV and B-cell epitope CWCARPTCIK, YCCNIVNVSL were selected based on the specific amino acid residues. The T and B-cell epitopes WPWYVWLGFI, SRVKNLNSSEGVPDLLV and CWCARPTCIK, YCCNIVNSL were docked with human HLA:*A0201. The outcomes reveal that the epitopes have been fitted into the alpha-helix alloy slot properly that indicates its stability and efficacy as a probable SARSCoV-2 vaccine. The docked complexes are displayed in fig. 5 & 6. Based on all the computational analysis, it is recommended that the selected epitopes can be a suitable accessible result to cure infections of the SARS-COV-2 virus.

Protein structure simulation

The 'Fluctuation plot' tab stipulates an interactive 2D plot representing residue-wise fluctuations noted during the simulation. Fluctuations are intended as RMSF later than global superposition. For multi chain proteins a distinct plot is created for individual chain. They can be presented by choosing it within the 'Chains' panel. The human leukocyte antigen HLA Complexes with the designated receptors (1A1M, 5TXS) were exposed to MD simulations to compute the residual constancy and fluctuations. Root mean square deviation (RMSD) is assessed to enumerate all system's constancy while Root mean square fluctuation (RMSF) was estimated as represented in fig. 7 on behalf of residual fluctuation. Number of simulations cycle for all the systems were recognized, that described variable RMSF including 0.5 nm (1A1M), 0.5 nm (5TXS). Residual fluctuation RMSF was detected in the acceptable range except a few larger fluctuating residues.

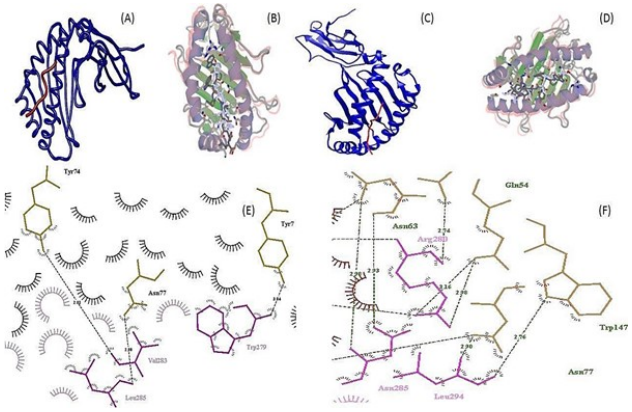

Fig. 5: Proposed T cell epitopes of Spike and Envelope small protein docking analysis results of HLA:*A0201. (A), (C) shows the model similarity structure, light color indicates the peptide and protein template. (B), (D) shows the template of peptide and protein, the blue color represents the protein and blue color represents the peptide. (E) Shows the docking interaction analysis of protein with T cell epitope WPWYVWLGFI. (F) Shows the docking interaction analysis of protein with T cell epitope SRVKNLNSSEGVPDLLV, all interacting residues bond length are greater than 2.5.

In silico vaccine appearance and codon optimization

In this, Jcat, was used to calculate the expression level of the multi-epitopes vaccine within Escherichia coli (K12 strain). An overall 1050 nucleotides were utilized as input data. Codon optimization was achieved and Codon Adaptation Index (CAI) was predicted that is 0.95 along with GC contents of 72%. These outcomes suggest that the final multiple-epitope vaccine production in E coli (K-12 strain) showed virtuous expression. For better

Table 1: The two predicted T-cell epitopes, along with their target amino group, predicted IC50 value and interacting alleles.

Position	Start	End	Peptides	Length
1	21	36	CIKPTETWGTPSFTGV	16
2	38	45	YVPHNTTY	8
3	61	68	YMAHSGQT	8
4	80	80	N	1
5	83	83	V	1
6	85	85	P	1
7	87	88	TP	2
8	97	103	NVTPGVY	7
9	127	135	DNPNRTCQE	9
1	14	32	VNSVLLFLAFVVFLLVTLA	19
2	39	47	LCAYCCNIV	9

Table 2: B-cell epitopes based on Kolaskar and Tongaonkar antigenicity analysis

Epitope	Predict Target Amino Group	Predicted Ic50 Value	Interacting Alleles
WPWYVWLGFI	PWYVWLGFI,PWYVWLGFI, WPWYVWLGF	235.50, 849.18,177.42	A0201, H2db, B3501
SRVKNLNSSEG VPDLLV	SRVKNLNSS, RVKNLNSSE, SEGVPDLLV, NLNSSEGVP, LNSSEGVPD, NSSEGVPDL, SSEGVPDLL, KNLNSSEGV	13.18, 20.18, 48.31, 57.68, 124.17,124.45, 178.65, 306.2	A0206, Drb0101

Table 3: Selected B cell epitope prediction by Bepipred linear way.

NO.	START	END	Peptide
62	1272	1279	YEEHEDVY
61	1156	1164	PAVDTSSFN
60	1131	1132	IS
59	1100	1104	TGYAP
58	1082	1084	QPT
57	1068	1071	QMAP
1	6	9	SEET
2	57	71	Yvysrvknlnssrvp

expression 35% to 70% of GC content is necessary. Addition of 5′and 3′end restriction sites (NdeI and XhoI) and cloning of the augmented nucleotide sequence in the pET32a (+) vector was achieved. The inclusive construction of this vaccine is given in fig. 8 in consort with the vector sites and restriction sites.

DISCUSSION

Bioinformatics use *in-Silico* approaches to identified the location or specific gene, prediction of transcript of a particular genes and protein structure and location inside the cell or disease associated with abnormal structure of that protein involves devising the effective drug designing strategy relative to a particular protein involved in a disease pathway (Ahmad and Qadus *et al*. 2020) therefore, we used Immunoinformatics techniques in current research to confirm the vaccine protein's stability, efficacy, and high-level expression in the host E coli. In comparison, the use of an agent-based model estimated the correct reaction when the vaccine sequence was injected. The vaccine is safe, non-allergenic and antigenic and can successfully regulate the corona virus. More experimental trials are needed to test the vaccine's efficiency. Developing new vaccines in a shorter timeframe is very necessary to deal with the increasingly growing diseases (Marshall S, 2004; Purcell *et al.,* 2007). Since sequence-based research is progressing, there is already lots of information available on the genomes and proteomes of several viruses. As a result, the vaccines based on peptides that be produced with the aid of several bioinformatics apparatuses. Although the concept of

epitope-based vaccine strategy is being considered, there is still no work on epitope-based vaccine design for SARS-COV-2. In this research, an attempt has been applied to improve the *Insilico* epitope-based vaccine designing against SARS-CoV2. Usually, the vaccines development are based on B-cell immunity. However, immunization has been energized considering the T cell epitope, as the host can produce a vigorous immune response to the infected cell by the CD8 + T cell (Shrestha *et al.,* 2004). With time, every foreign molecule that gets away from the memory retort of an antibody because of antigenic drift; however, the mediated immune responses from T cell regularly offer long-lasting immunity. There are some criteria which must be follow to develop an epitope-based vaccine designing, and in our study to designing the vaccine against SRAS-COV-2 all the criteria match that's why our strategy prove to be successful method. Basically, Allegenicity is one of the most severe impediments for the manufacture of vaccines. Still, however, most immune activating vaccines are screened for adverse allergic reactions, first T help type 2 cells (Th2) and immunoglobulin E (IgE) activate. Aller Hunter value is a probability of a cross-receptive allergen being a given set. However, the limit for measuring allergic cross reactivity is approximately above 0.08. There the possible epitope's level of allergenicity was 0.06, which resulted in it being non-allergenic. Both these expected findings *in-silico* method was used to focused on sequence analysis of immune databases. Therefore, it is proposed that the epitope suggested well induce in a vigorous immune response as an in vivo peptide immunization. (Khan *et al.,* 2014). The use of antibiotics is not only costly to contain infectious diseases but also precariously contributes to the continuous development of resistant microbes.

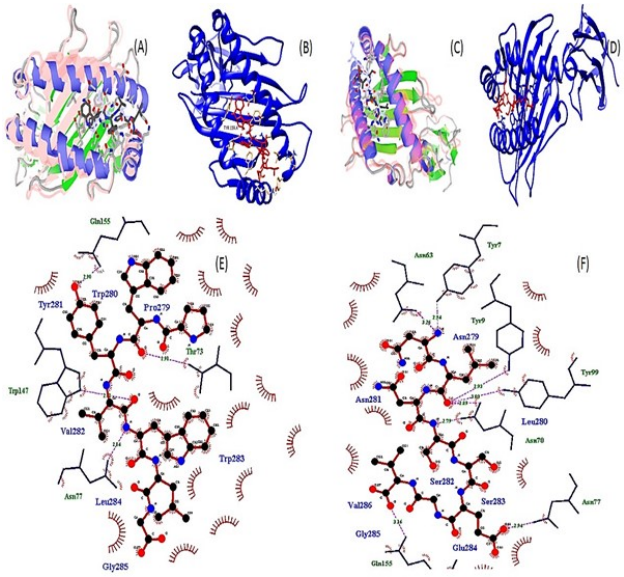

Fig. 6: Proposed B cell epitopes of Spike and Envelope small protein docking analysis results of HLA:*A0201. (A), (C) shows the model similarity structure, light colour indicates the peptide and protein template. (B), (D) shows the template of peptide and protein, the blue colour represents the protein and blue colour represents the peptide. (E) Shows the docking interaction analysis of protein with T cell epitope CWCARPTCIK. (F) Shows the docking interaction analysis of n with T cell epitope YCCNIVNVSL, all interacting residues bond length are greater than 2.5

Instead, vaccines can be introduced successfully to avoid diseases in a large population. Conversely, currently cutting-edge technology has facilitated the development of "vaccines subunit," that make up the inimitable pathogenic protein sequences of the bacteria and are entirely equipped successful immune response activation. Because of the existence of inclusive evidence on the microbes' genomes and proteomes developing new and competent subunit vaccines is practically possible. As traditional methods to the production of vaccines are currently obsolete because of their little efficiency, extreme expenditure and time outlays.

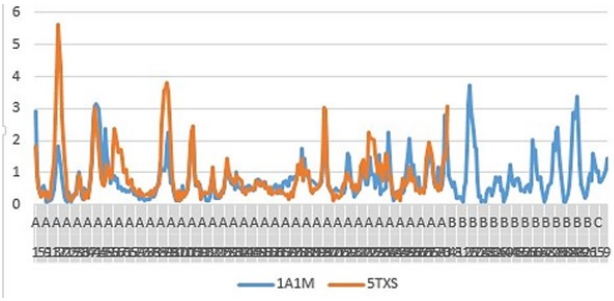

Fig. 7: RMSFs for all the systems are provided for residual stability and fluctuations. Simulations were performed in five nanoseconds. The RMSF panel is provided in the Proper panel

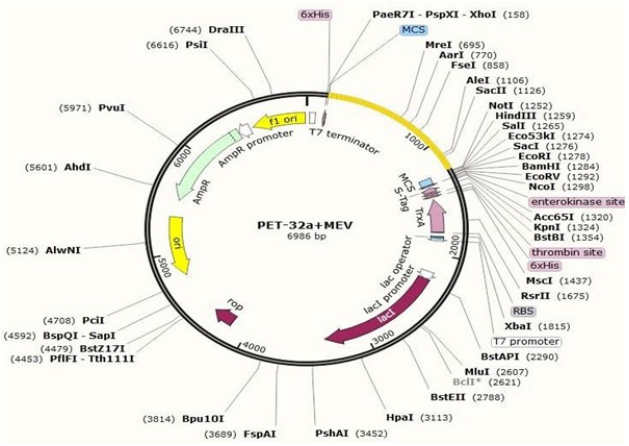

Fig. 8: In-silico vector structure pET32a (+) build including vaccine along with vector sites and restriction sites. The list of vaccines is displayed in dark grey colour.

Vaccine design via the immunoinformatics approach is moderately steady, innocuous, inexpensive, precise and extra effective. Using the cutting edge immune-

informatics technique the proteome of the targeted spike and shell classify for targeting the sequences to design the SARS-COV-2 subunit vaccine. As already mentioned, spike and envelope protein are the most recognized toxic factors in SARS-COV-2 virus. An inclusive analysis was performed consuming online servers to determine the immunogenic efficacy of these proteins and online resources were used to produce an effective subunit vaccine template. Also, the epitopes of the B-lymphocytes and T-lymphocytes were investigated using the sequences of selected proteins. Jcat software was introduced for maximized codon expression and optimization, and E. coli strain K12 was employed. The content of GC and CAI determined via Jcat suggested sophisticated expression while the validation of the program reported maximum solubility of the anticipated vaccine with the obligatory expression in E. coli. Finally, disulfide bonds have been accustomed to ensuring vaccine protection.

CONCLUSION

Present research stipulate valuable insights for the designing of B and T cell based potential Vaccine candidates against the highly emerging COVID-19 infection via the immunoinformatics as well as vaccinomics approaches. To achieve high level of outcomes, molecular docking assessment, thermodynamics stability profiling, agent-based simulation and *in-silico* expression were performed. Based on these approaches, the mapping of T-cell and B-cell epitopes in the spike and envelope small membrane protein were accomplished and putative SARS-COV-2 vaccine candidate were selected. Additionally, the identified epitopes possessed T-cell and B-cell selectivity, maximum conservancy, non-allergenicity, nontoxicity, higher resident's coverage and display extremely good contact with HLA: *A0201 by virtuous affinity. Nevertheless, these outcomes will provide primary data for further *in vitro* and *in vivo* analysis to design vaccine against COVID-19 infection after proper clinical trials.

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