wjpmr, 2021,7(3), 215 – 225.

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

IDENTIFICATION OF B CELL EPITOPES ON SPIKE PROTEIN (SURFACE GLYCOPROTEIN) OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

Nithiyanandan Saravanan[1], Prashanth Rajendiran[1], Mageshbabu Ramamurthy[1*], Balaji Nandagopal[1], Swarnalatha Purushotham[2]

[1]Sri Sakthi Amma Institute of Biomedical Research, Sri Narayani Hospital and Research Centre, Sripuram, Vellore - 632055, Tamil Nadu, India.

[2]Department of Information Security, School of Computer Science and Engineering, VIT University, Vellore-632014, Tamil Nadu, India.

***Corresponding Author: Mageshbabu Ramamurthy**
Sri Sakthi Amma Institute of Biomedical Research, Sri Narayani Hospital and Research Centre, Sripuram, Vellore - 632055, Tamil Nadu, India. **DOI: https://doi.org/10.17605/OSF.IO/ZRSBX**

Article Received on 05/01/2021 **Article Revised on 25/01/2021** **Article Accepted on 15/02/2021**

ABSTRACT

The emergence and rapid spread of corona virus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the case fatality rate is estimated to range from 2 to 3%. Prevention entails home isolation of suspected cases and those with mild illnesses and strict infection control measures at hospitals that include contact and droplet precautions. At this time, there are no specific vaccines or treatments for COVID-19. However, there are many ongoing clinical trials evaluating potential treatments. So far, the real-time PCR is the gold standard clinical diagnosis method of COVID-19 is nucleic acid detection in the nasal and throat swab sampling or other respiratory tract samplings. The novel Corona virus disease virions are surrounded by a lipid bilayer from which spike protein trimers. The Spike proteins are known to play major role in corona virus pathogenesis, and antibodies against linear epitopes are protective against COVID-19 infection so the spike protein peptides are most sensitive peptides for serodiagnosis. In this Corona virus Spike protein's (Indian strains) consensus sequence was used in BepiPred 2.0, ABCpred, and IEDB analysis resource for the B cell epitope prediction. Based on the bioinformatics program results one epitope of 16mer **HTPINLVRDLPQGFSA** was recognized in the highly conserved regions of spike protein with 81% surface exposed residues. The identified candidate peptide could be used for screening antibody response in patients who have had COVID-19. This study could help us to use the predicted peptide as an immunogenic for the development of diagnostics and vaccines against COVID-19.

KEYWORDS: Corona virus, SARS-CoV-2, COVID-19, Spike protein, epitope

INTRODUCTION

Coronaviruses are enveloped, positive-sense, single stranded RNA viruses belonging to the family of beta corona virus.[1] The novel Corona virus disease (n-COVID 19) pandemic first case was identified in Wuhan, China [2] and reported to World Health Organization (WHO) on 31st December 2019 by the time virus has spread around the world.[3] On January 7, 2020, the 2019 novel Corona virus disease (**n- COVID 19**) was officially renamed as Severe Acute Respiratory Syndrome Corona Virus 2 **(SARSCoV-2)** and the disease was subsequently termed as corona virus disease **(COVID-19)** by WHO.[4, 5] Corona viruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N). These outer membrane proteins play major roles in the entry of the virus into host cells and subsequent division of viral particles. Immune responses including antibody production against the outer membrane proteins are necessary to limit the spread of the infection.[6]

Fever, cough, Shortness of breath or difficulty in breathing, tiredness is the Common symptoms that have been specifically linked to COVID-19.[7] Other symptoms also reported which includes myalgia, chills, sore throat, runny nose, headache, chest pain and loss of smell.[8] Globally people infected with COVID-19 primarily causes respiratory illness ranging from mild disease to severe disease and death.[9] Some people infected with the virus never develop symptoms and recover without requiring special treatment.[10] Older people and those with chronic illness like cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop serious illness.[11]

The COVID-19 virus spreads primarily through the droplets of saliva[12] or discharge from the nose when an infected person coughs or sneezes, so it's important to wear protective masks.[13] The possible mode of transmission for COVID-19, include contact, droplet, airborne, fecal-oral, blood borne, mother-to-child, and animal-to-human transmission.[14, 15]

The best way to prevent and slow down transmission is to be well informed about the COVID-19 virus, the disease it causes and how it spreads. Protect yourself and others from infection by washing your hands or using an alcohol based sanitizers frequently and not touching your face.[16, 17] At this time, there are no specific vaccines or treatments for COVID-19.[18] However, there are many ongoing clinical trials evaluating potential treatments.[19, 20]

So far, the real-time PCR is the gold standard clinical diagnosis method of COVID-19 is nucleic acid detection in the nasal and throat swab sampling or other respiratory tract samplings.[21] ELISA and IFA were suitable for clinical application because of costs, time-to-results, relative simplicity and specificity. Currently these assays are only used for the diagnosis of asymptomatic patient samples not for the conformational test like RT-PCR.[22] Peptide based ELISA has been widely used for the sero-diagnosis of viral infections. It provides the simple, rapid, sensitive, reliable diagnostic tool with reducing cost and time consumption.[23]

Comparisons of the genetic sequences of corona virus have shown similarities to bat corona viruses.[24] IgM is the first immunoglobulin to be produced in response to an antigen and will be primarily detectable during the early onset of the disease.[25] Corona virus enter into host cells through the transmembrane spike (S) glycoprotein[26] that forms homotrimers protruding from the viral surface. Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry.[27] In this study we aim to target the spike protein of corona virus (Indian strains) for the candidate B-cell peptide selection using Bioinformatics approach. The Spike proteins are known to play major role in corona virus pathogenesis, and antibodies against linear epitopes are protective against COVID-19 infection[28, 29], so the spike protein peptides are most sensitive peptides for serodiagnosis.

METHODOLOGY

Corona virus Spike protein's (Indian strains) available full-length amino acid sequences (n=119) were retrieved from the NCBI resources. A consensus amino acid sequence was aligned from these 119 spike protein sequences using CLC sequence viewer 8.0 program.[30] The study methodology outline is shown in **Figure 1**. The homology of consensus amino acid (1273aa) sequence was checked with the complete amino acid sequence (29,800aa) of corona virus 2 (COVID19) using BLASTp (protein-protein) analysis which showed 100% similarity.

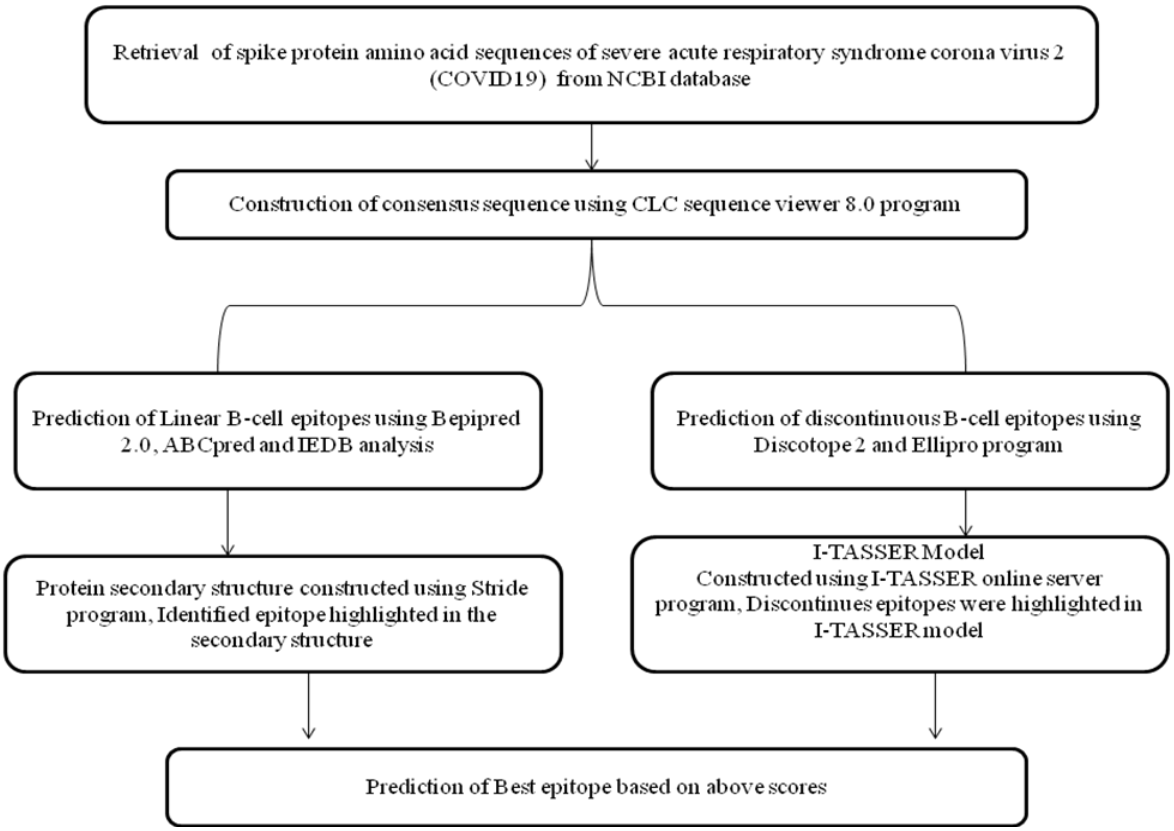


Figure 1: Flow chart of the Study.

Linear B-cell epitopes were predicted using three different programs which includes BepiPred 2.0,[31] http://www.cbs.dtu.dk/services/BepiPred/, ABCpred,[32] http://crdd.osdd.net/raghava/abcpred/ and IEDB analysis resource,[33] http://tools.iedb.org/bcell/.

The consensus sequence of spike protein was used to predict the epitopes in all the three programs BepiPred 2.0, ABCpred and IEDB analysis resource. CLC Sequence viewer program was applied to analyze the conserved regions across all available COVID19 Indian strain. The epitopes that were predicted by all the three programs matched with CLC consensus sequences.

The I-TASSER online server platform,[34] https://zhanglab.ccmb.med.umich.edu/ITASSER/ was used to model the 3D protein structure for spike protein consensus sequence. The epitope regions were labeled using The PyMOL[35] (Molecular visualization System ver 2.4.0 Edu Schrödinger, Limited Liability Company, New York). The modeled 3D structure of spike protein was verified by using Ramachandran plot was modeled by RAMPAGE program[36], http://mordred.bioc.cam.ac.uk/~rapper/rampage.php.The Ramachandran plot analysis shows the statistical distribution of the combinations of the backbone torsional angles ϕ and ψ of amino acid residues in the modeled protein structure for possible confirmation of the peptide region. Percentages of residues in various regions like favored region, allowed region, and outlier region results were analyzed by the RAMPAGE program. The stability of the protein considered by higher percentage of residues pin the allowed region. STRIDE web interface[37] was used for the derivation of protein secondary structure from 3D coordinates of spike protein modeled in this study. This program utilizes both hydrogen bond energy and main chain dihedral angles (phi and psi), to derive secondary structures for structurally known proteins. The α-helix, β-strand, coil, and turns were considered as described in **figure 2**. The BepiPred2.0 program was used to calculate the surface accessibility (buried/exposed) of the selected epitopes.

The prediction of discontinuous epitopes was done by using, Discotope 2 program[38] (http://www.cbs.dtu.dk/services/DiscoTope/) and Ellipro program[39] (http://tools.iedb.org/ellipro/) with default prediction parameters. The 3D structure generated by I-TASSER was used for the discontinuous epitope prediction. The final scores calculation was done by combining the propensity scores (probability of the residue in the interface/probability of the residue on the surface) of residues in spatial proximity and the contact numbers (surface accessibility).[40] The latter, ElliPro program correlate each identified epitope with a score, defined as protrusion index (PI) (the extent to which a residue protrudes from the surface of a protein) averaged over epitope residues.

RESULTS

Linear B-cell peptide prediction

The B-cell epitopes was predicted from one consensus amino acid sequence using the three different programs (Bepipred 2.0, ABCpred and IEDB analysis resource) and analyzed for conservation across the 119 individual sequences. BepiPred 2.0 program recognized 16 peptide epitopes **(Table.1)** with varying lengths.[15-40] Among them, one epitope of 16mer **HTPINLVRDLPQGFSA** was recognized in the highly conserved regions of spike protein with 81% surface exposed residues.

Table 1: List of B-cell epitopes identified by BEPIPRED 2.0 program for the selection of Candidate peptide ELISA.

Position	PEPTIDE	Length	Exposed	Exposed %
786-800	KQIYKTPPIKDFGGF	15	9	60
207-222	**HTPINLVRDLPQGFSA**	**16**	**13**	**81**
694-709	**AYTMSLGAENSVAYSN**	**16**	**12**	**75**
828-843	LADAGFIKQYGDCLGD	16	9	56
628-644	QLTPTWRVYSTGSNVFQ	17	8	47
673-691	**SYQTQTNSPRRARSVASQS**	**19**	**14**	**74**
1252-1270	SCCKFDEDDSEPVLKGVKL	19	12	63
338-356	FGEVFNATRFASVYAWNRK	19	11	58
516-535	ELLHAPATVCGPKKSTNLVK	20	13	65
141-163	LGVYYHKNNKSWMESEFRVYSSA	23	13	56
14-36	QCVNLTTRTQLPPAYTNSFTRGV	23	12	52
59-81	FSNVTWFHAIHVSGTNGTKRFDN	23	11	48
455-477	LFRKSNLKPFERDISTEIYQAGS	23	11	48
402-424	IRGDEVRQIAPGQTGKIADYNYK	23	7	30
371-395	SASFSTFKCYGVSPTKLNDLCFTNV	25	12	48
1133-1172	VNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGI	40	25	62

Epitopes with at least 70% of exposed residues are shown in bold faces.

The peptide epitopes generated by IEDB program recognized 34 predicted epitopes out of which 16 epitopes (15-40mer) were within the suitable size range **(Table. 2)**. Among them two epitopes (16mer) were within 75% surface exposed residues: **KHTPINLVRDLPQGFS** (position 206-221), YTMSLGAENSVAYSNN (position 695-710) and one epitope(19mer) with 79% surface exposed residues: **ASYQTQTNSPRRARSVASQ** (position 672-690).

Table 2: List of B-cell epitopes identified by IEDB analysis resource program for the selection of Candidate peptide ELISA.

Position	PEPTIDE	Length	Exposed	Exposed %
786-800	KQIYKTPPIKDFGGF	15	9	60
828-842	LADAGFIKQYGDCLG	15	8	53
206-221	**KHTPINLVRDLPQGFS**	**16**	**12**	**75**
617-632	CTEVPVAIHADQLTPT	16	3	19
695-710	**YTMSLGAENSVAYSNN**	**16**	**12**	**75**
1252-1267	SCCKFDEDDSEPVLKG	16	11	69
138-154	DPFLGVYYHKNNKSWME	17	10	59
304-322	KSFTVEKGIYQTSNFRVQP	19	10	52
672-690	**ASYQTQTNSPRRARSVASQ**	**19**	**15**	**79**
516-536	ELLHAPATVCGPKKSTNLVKN	21	14	66
59-81	FSNVTWFHAIHVSGTNGTKRFDN	23	11	48
404-426	GDEVRQIAPGQTGKIADYNYKLP	23	6	26
13-37	SQCVNLTTRTQLPPAYTNSFTRGVY	25	12	48
369-393	YNSASFSTFKCYGVSPTKLNDLCFT	25	11	44
329-363	FPNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	35	17	48
1133-1172	VNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGI	40	25	62

Epitopes with at least 70% of exposed residues are shown in bold faces.

ABCPred program generated a total of 99 peptides (20mer) of different ranks **(Table.3),** of which one peptide epitope **VTLADAGFIKQYGDCLGDIA** was at position 826 scored first(score = 0.90) with 60% surface-exposed residues, and was highly conserved across the sequences analyzed. Among the epitopes identified, the 20 mer: **KHTPINLVRDLPQGFSALEP** (score = 0.88, position 206) was100% conserved across the sequences. Other epitopes generated from all the above programs had variability at multiple sites and excluded from consideration for conferring immunogenic status and not shown in respective tables.

Table 3: List of B-cell epitopes identified by ABCpred program for the selection of Candidate peptide ELISA.

Rank	Sequence 20Mer	Position	Score
1	VTLADAGFIKQYGDCLGDIA	826-845	0.9
1	IGAEHVNNSYECDIPIGAGI	651-670	0.9
1	TNVYADSFVIRGDEVRQIAP	393-412	0.9
1	VYYHKNNKSWMESEFRVYSS	143-162	0.9
2	IHVSGTNGTKRFDNPVLPFN	68-87	0.89
2	GVNCTEVPVAIHADQLTPTW	614-633	0.89
2	SYLTPGDSSSGWTAGAAAYY	247-266	0.89
3	**KHTPINLVRDLPQGFSALEP**	206-225	0.88
3	VVIKVCEFQFCNDPFLGVYY	126-145	0.88
Sequence 18Mer			
1	VYSSANNCTFEYVSQPFL	159-176	0.93
2	YKLPDDFTGCVIAWNSNN	423-440	0.91
3	EMIAQYTSALLAGTITSG	868-885	0.9
3	NFRVQPTESIVRFPNITN	317-334	0.9
Sequence 16Mer			
1	AGTITSGWTFGAGAAL	879-894	0.97
2	GVSVITPGTNTSNQVA	594-609	0.95
2	GWTAGAAAYYVGYLQP	257-272	0.95
2	PQIITTDNTFVSGNCD	1112-1127	0.95
3	HRSYLTPGDSSSGWTA	245-260	0.92
3	QKEIDRLNEVAKNLNE	1180-1195	0.92

Epitopes from 1 to 3 ranks only shown in table(ABCpred based on score) others were excluded.

The protein secondary structure of spike protein identified from the modeled 3D structure revealed to possess helices (23.1%), coils (46.2%) and strands (30.7%). The selected peptide **HTPINLVRDLPQGFSA** highlighted had high (81%) exposed residues. The selected epitopes are shown in **Figure 2(A)**. The Ramachandran plot analysis identified from Rampage server showed the model generated by I-TASSER (**Figure.3**) was acceptable compared with other programs (Phyre2 and Swiss Model). The number of residues in favored, allowed, and outlier regions were 73.9%, 18.3%, and 7.8%, respectively.

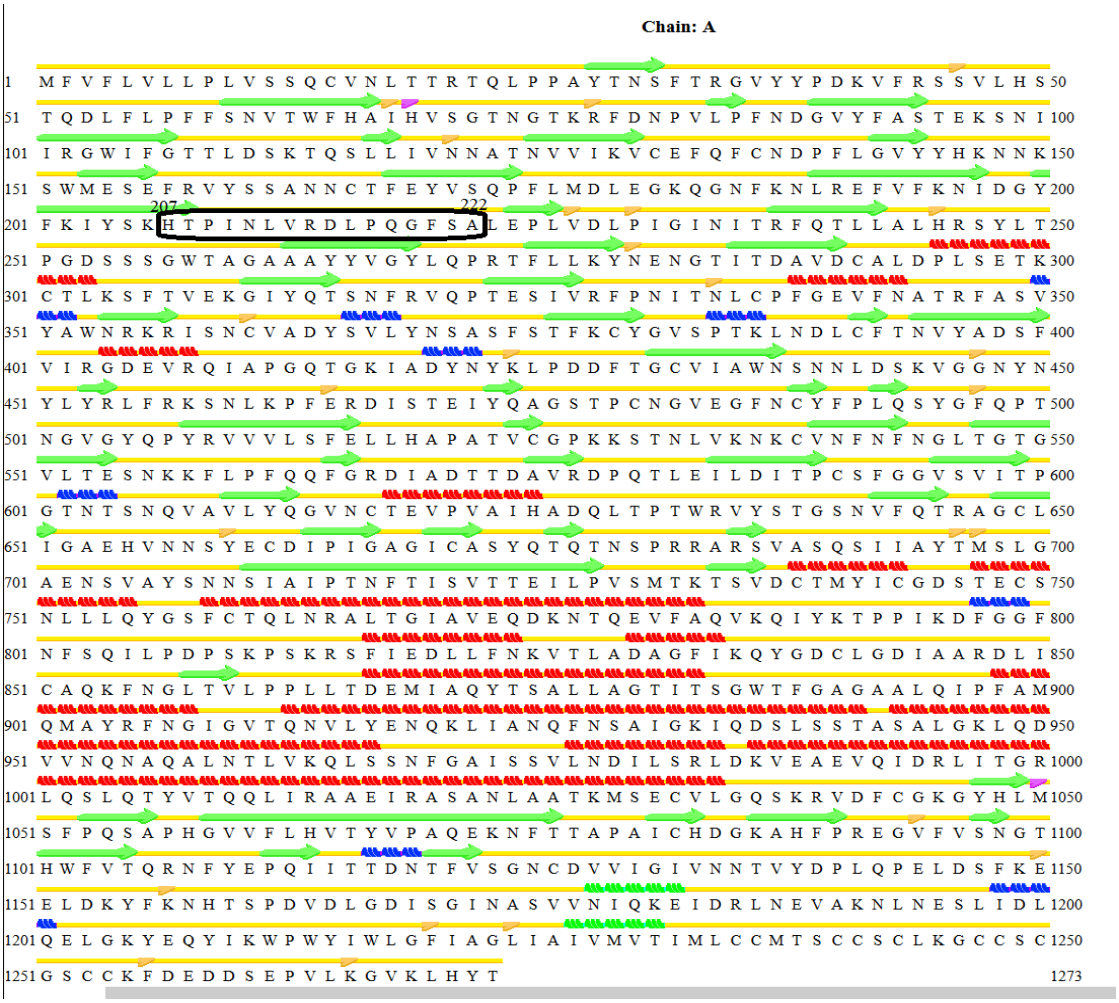
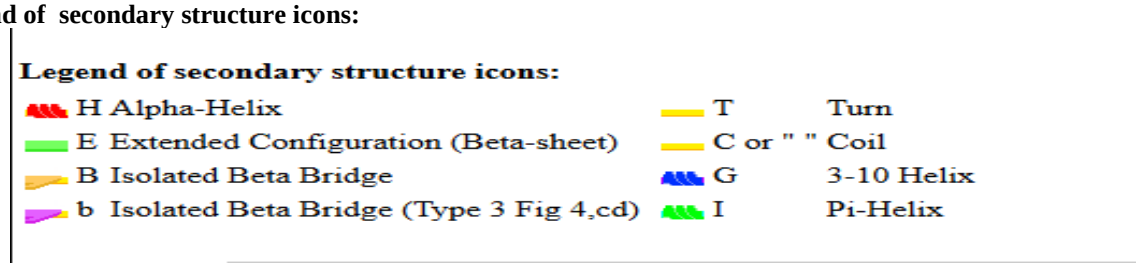


Figure 2: Structure of Spike protein of severe acute respiratory syndrome corona virus 2 (COVID19) Indian strains. Secondary protein structure indicating helices, beta strands, coils and turns. Identified B-cell epitope highlighted in box.

Legend of secondary structure icons:



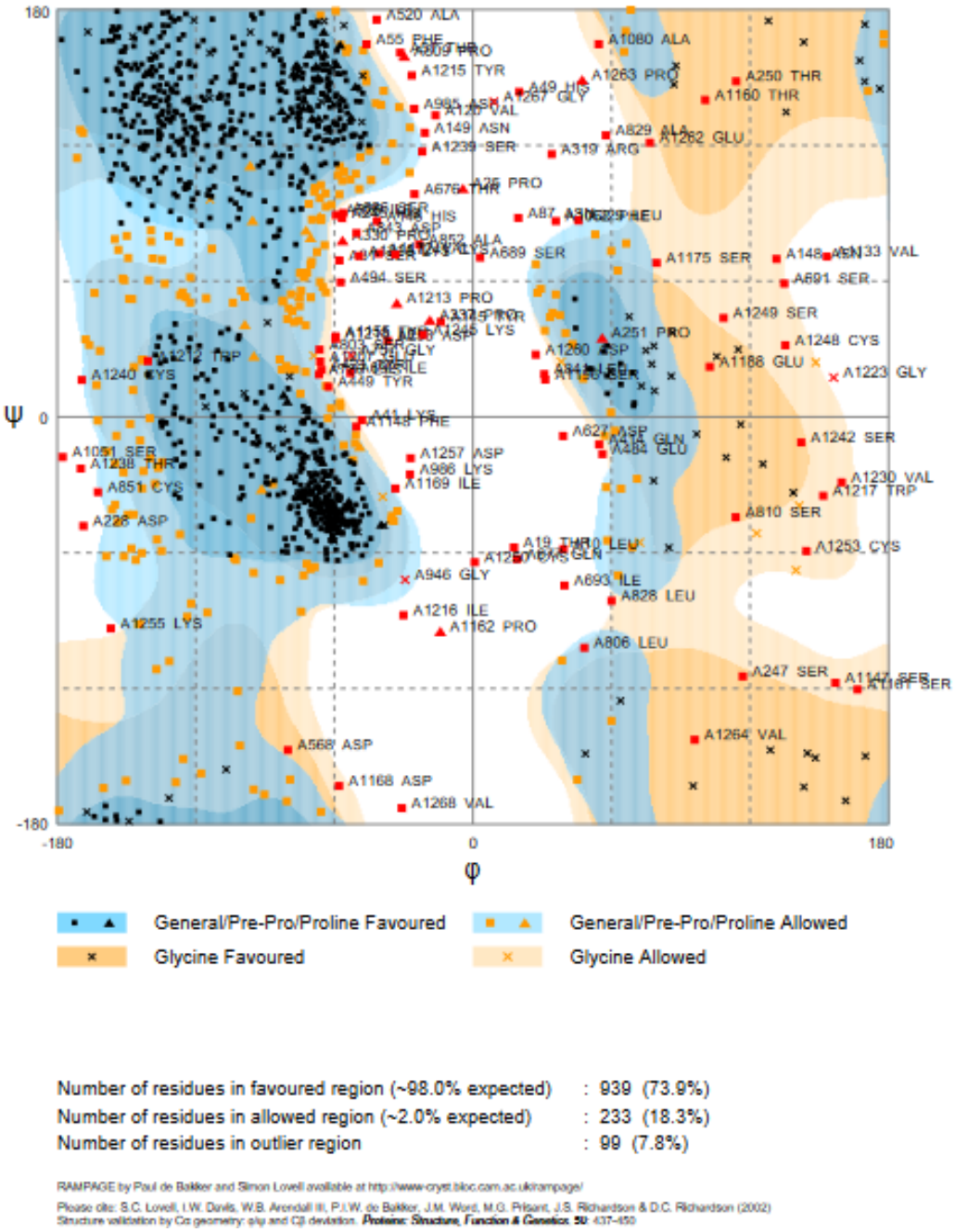


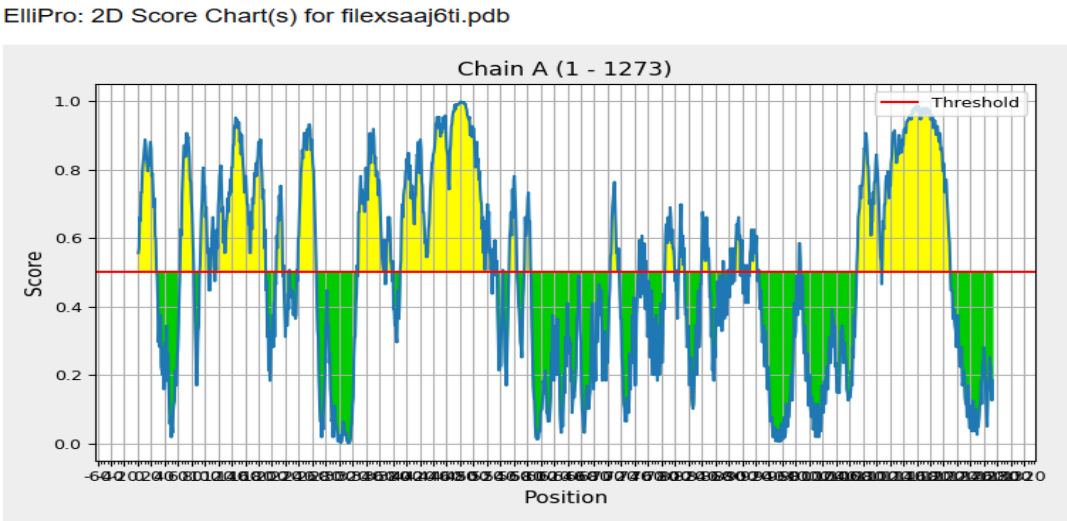
Figure 3: Molecular characterization of Spike protein of severe acute respiratory syndrome corona virus 2 (COVID19) Indian strains using RAMPAGE program with residues scores.

Discontinuous B - Cell Peptide Prediction

Discontinuous epitopes were predicted by Ellipro and Discotope 2 program using the modeled 3D protein structure of spike protein in protein data bank (PDB) format. The 2D score chart showed discontinuous epitopes of 147 residues and a score of 0.791 as predicted by Ellipro prediction program.

The residues that are above the threshold score are considered as potential epitopes and indicated in yellow **(Figure 4.A)**. Here, the protein's structure is approximated by a number of ellipsoids. The ellipsoid with PI=0.75 would include within 75% of the protein residues with 25% of the protein residues being outside of the ellipsoid. The discontinuous epitope as predicted by Discotope 2 program is highlighted in the modeled protein structure **(Figure 4.B)**. The identified residues position are 73; 146 to 151;180;182-185; 211; 250;255; 416; 439 to 440;444 to 447; 449; 458 to 460;476 to 477; 494;496 to 505; 556;558;560;686;703 to 704;706 to 707;716;793;808 to 812; 914;917;1071;1073 to 1074;1099 to 1100; 1139 to 1185;1188;1190 to 1192;1194 to 1208.

(A)



(B)

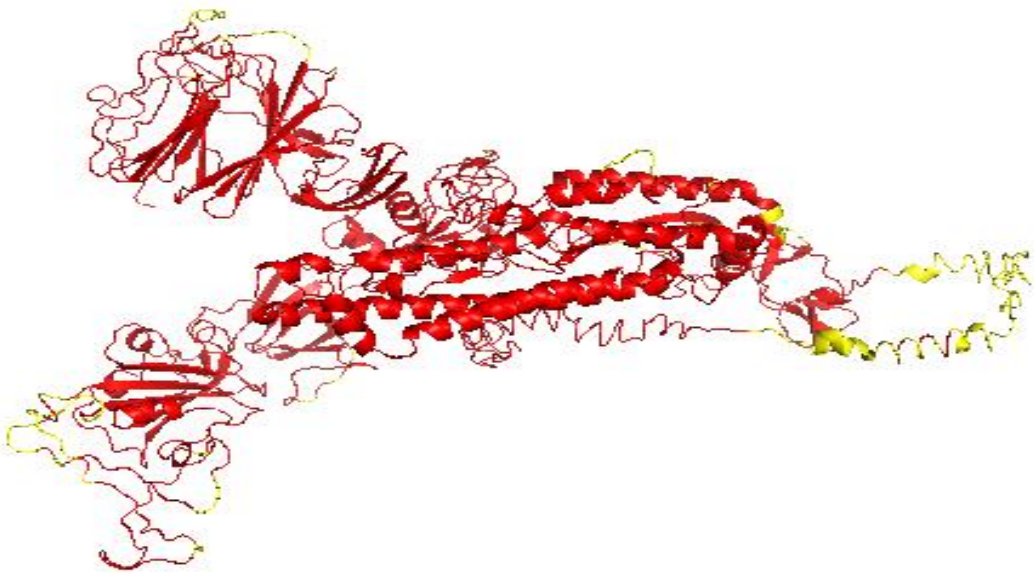


Figure 4: Prediction of discontinuous epitopes by Ellipro program and Discotope 2 program. A, The discontinuous epitopes predicted by Ellipro program. Figure 4 (B), The discontinuous epitopes predicted by discotope 2 program. The residues that are above threshold score as consider as potential epitopes and are indicated in yellow.

DISCUSSION

Corona virus, the causative pathogen for severe acute respiratory syndrome, According to the situation report (reported by 04 September 2020) of WHO, in worldwide, 28 million confirmed cases were reported, of which 0.89 million cases were with deaths. (https://covid19.who.int/)[41]

In this study we used BepiPred-2.0 a web server for B-cell epitopes prediction from SARS-COV-2 spike protein sequences. BepiPred-2.0 is based on a random forest algorithm trained on epitopes interpreted from antibody-antigen protein structures.[42] ABCpred server is to predict Linear B cell epitope regions in an antigen sequence, using artificial neural network. This server will assist in locating epitope regions that are useful in selecting synthetic vaccine candidates, disease diagnosis and also in allergy research.[43] The Immune Epitope Database (IEDB) captures experiments that identify and characterize epitopes and epitope specific immune receptors along with various other details such as host organism, immune exposures, and induced immune responses.[44]

A recent study has also predicted B cell epitopes for COVID-19 that may be presented by a population from the Asia-Pacific region.[45, 46] Again, there are multiple differences to our work. Firstly, the authors focused on B cell epitope prediction using only IEDB server only and

the other computational tools were not analyzed. In our study we used multiple bioinformatics tools and compared our results with other bioinformatics programs based on the exposed residues, conserved regions and prediction ranks.

In our present study, we attempted to find out various B-cell epitopes against SARS-COV-2, using the Bioinformatics, as quick identification of B-cell epitopes for peptide based in-house ELISA development. Reliable B-cell epitope prediction bioinformatics tools are important in therapeutic antibody development and vaccine design.[46] The spike glycoprotein was analyzed for B-cell epitope identification in the IEDB server, and 34 linear B-cell epitopes were identified as a result. Subsequently, the sequence was also analyzed in BepiPred 2.0 and ABCPred servers for the identification of the linear B-cell epitope. Fortunately, we found 1 epitope of spike protein that can be possibly used for candidate peptide ELISA.[47] All the observations of our present work depict the effectiveness of selected epitopes within the spike glycoprotein of SARS-COV-2. These epitopes can be used to make an immunogenic epitopic peptide ELISA against SARS-COV-2.

Discontinuous B-cell epitope prediction methods require the 3-D structure of the antigen as input. Ellipro and Discotope 2 programs were used to predict Discontinuous epitopes using the modeled 3D protein structure of spike protein in protein data bank (PDB) format.[48] The major portions of amino acid residues were comparable between the two programs. Ellipro server results represent the protein structure as an ellipsoid and calculate protrusion indexes for protein residues outside of the ellipsoid.[49] Discotope 2 utilizes calculation of surface accessibility and a novel epitope prediction amino acid score. The final scores are calculated by combining the prediction scores of residues in spatial proximity and the surface accessibility.[50]

B-cell epitope prediction is important for vaccine design, development of diagnostic reagents, and interpretation of the antigen-antibody interactions on a molecular level.[51] Localizing epitopes by experimental methods is expensive in terms of time, cost, and effort; therefore, computational methods feature for its low cost and high speed was employed to predict B-cell epitopes. In these years, lots of computational methods have been proposed for epitope prediction. These methods predict epitopes either by antigen structure or by mapping mimotopes to the original antigen surface.[52]

The B-cell peptide identified in this study possibly valuable for the development of immunodiagnostic tools towards detection of IgM antibodies in ELISA or point of care test for diagnosis. Substantially the peptides that are at least of 15 to 20mers are flexible for the development of specific Enzyme Immunoassays (EIA) based immunodiagnostic tools.[53]

CONCLUSION

In this study, we report the potential B-cell peptide recognized by bioinformatics approach. Peptide synthesis would be less expensive and today easy to synthesize. Peptide candidates may be used to raise very specific antibodies for research or diagnostics. Peptide-based sensitive and rapid diagnostic kits are considered a better alternative to the conventional serological tests, including whole antigenic protein.[54] The peptide based immunodiagnostics would be more stable and relatively safe. The B-cell peptide epitopes could be explored as an antigen for a micro plate ELISA or point of care test. The peptide is located on the Spike protein with 81% surface exposed residues. An advantage of the epitope reported in this study is match with all the three programs with highest exposed residues and percentile score. The identified candidate peptide could be used for screening antibody response in patients who have had COVID-19. This study could help us to use the predicted peptide as an immunogenic for the development of diagnostics and vaccines against COVID-19.

REFERENCES

1. Keni R, Alexander A, Nayak PG, Mudgal J, Nandakumar K: COVID-19: Emergence, Spread, Possible Treatments, and Global Burden. Front Public Health. 2020 May 28; 8: 216.
2. Rothan HA, Byrareddy SN: The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020 May; 109:102433.
3. Tommaso Lupia , Silvia Scabini , Simone Mornese Pinna , Giovanni Di Perri , Francesco Giuseppe De Rosa , Silvia Corcione: 2019 novel coronavirus (2019-nCoV) outbreak: A new challenge. J Glob Antimicrob Resist. 2020 Jun; 21: 22-27.
4. Anthony R. Fehr and Stanley Perlman: Corona viruses: An Overview of Their Replication and Pathogenesis. Corona viruses Methods in Molecular Biology. Methods Mol Biol.2015; 1282: 1-23.
5. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X: Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020; 63: 457–460.
6. Ursula J Buchholz [1] , Alexander Bukreyev, Lijuan Yang, Elaine W Lamirande, Brian R Murphy, Kanta Subbarao, Peter L Collins: Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci U S A, 2004 Jun 29; 101(26): 9804-9.
7. Sasmita Poudel Adhikari, Sha Meng, Yu-Ju Wu, Yu-Ping Mao , Rui-Xue Ye, Qing-Zhi Wang, Chang Sun , Sean Sylvia, Scott Rozelle, Hein Raat, Huan Zhou: Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. Infect Dis Poverty, 2020 Mar 17; 9(1): 29.

8. Catherine R. Jutzeler, Lucie Bourguignon, Caroline V. Weis, Bobo Tong, Cyrus Wong, Bastian Rieck, Hans Pargger, Sarah Tschudin-Sutter, Adrian Egli, Karsten Borgwardt, Matthias Walter: Co morbidities, clinical signs and symptoms, laboratory findings, imaging features, treatment strategies, and outcomes in adult and pediatric patients with COVID-19: A systematic review and meta-analysis. Travel Med Infect Dis. 2020 September-October; 37: 101825.
9. Yang Y, Peng F, Wang R, Yange M, Guan K, Jiang T, Xu G, Sun J, Chang C: The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. J Autoimmun. 2020 May; 109: 102434.
10. Gulati A, Pomeranz C, Qamar Z, Thomas S, Frisch D, George G, Summer R, DeSimone J, Sundaram B: A Comprehensive Review of Manifestations of Novel Coronaviruses in the Context of Deadly COVID-19 Global Pandemic. Am J Med Sci. 2020 Jul; 360(1): 5-34.
11. 11. Mahumud RA, Kamara JK, Renzaho AMN: The epidemiological burden and overall distribution of chronic comorbidities in coronavirus disease-2019 among 202,005 infected patients: evidence from a systematic review and meta-analysis. Infection. 2020 Aug 19: 1-21.
12. Li Y, Ren B, Peng X, Hu T, Li J, Gong T, Tang B, Xu X, Zhou X: Saliva is a non-negligible factor in the spread of COVID-19. Mol Oral Microbiol. 2020 Aug; 35(4): 141-145.
13. Tirupathi R, Bharathidasan K, Palabindala V, Salim SA, Al-Tawfiq JA: Comprehensive review of mask utility and challenges during the COVID-19 pandemic. Infez Med. 2020 Jun 1; 28(suppl 1):57-63.
14. Park SE: Epidemiology, virology, and clinical features of severe acute respiratory syndrome - coronavirus-2 (SARS-CoV-2; Coronavirus Disease-19). Clin Exp Pediatr. 2020 Apr; 63(4):119-124.
15. Amirian ES: Potential fecal transmission of SARS-CoV-2: Current evidence and implications for public health. Int J Infect Dis. 2020 Jun; 95: 363-370.
16. Mayar Allam, Shuangyi Cai, Shambavi Ganesh, Mythreye Venkatesan, Saurabh Doodhwala, Zexing Song, Thomas Hu, Aditi Kumar, Jeremy Heit, COVID-19 Study Group, Ahmet F Coskun: COVID-19 Diagnostics, Tools, and Prevention. Diagnostics (Basel) 2020 Jun; 10(6): 409.
17. Zhao S, Chen H: Modeling the epidemic dynamics and control of COVID-19 outbreak in China. Quant Biol. 2020 Mar 11:1-9.
18. Ahn DG, Shin HJ, Kim MH, Lee S, Kim HS, Myoung J, Kim BT, Kim SJ: Current Status of Epidemiology, Diagnosis, Therapeutics, and Vaccines for Novel Coronavirus Disease 2019 (COVID-19). J Microbiol Biotechnol. 2020 Mar 28; 30(3): 313-324.
19. Bimonte S, Crispo A, Amore A, Celentano E, Cuomo A, Cascella M: Potential Antiviral Drugs for SARS-Cov-2 Treatment: Preclinical Findings and Ongoing Clinical Research. In Vivo. 2020 Jun; 34(3 Suppl): 1597-1602.
20. Abrams-Downey A, Saabiye J, Vidaurrazaga M: Investigational Therapies for the Treatment of COVID-19: Updates from Ongoing Clinical Trials. Eur Urol Focus. 2020 Sep 15; 6(5):1028-1031.
21. Severino Jefferson Ribeiro da Silva, Caroline Targino Alves da Silva, Klarissa Miranda Guarines, Renata Pessôa Germano Mendes, Keith Pardee, Alain Kohl, Lindomar Pena: Clinical and Laboratory Diagnosis of SARS-CoV-2, the Virus Causing COVID-19. ACS Infect Dis. 2020 Sep 11;6(9):2319-2336.
22. Linda J Carter, Linda V Garner, Jeffrey W Smoot, Yingzhu Li, Qiongqiong Zhou, Catherine J Saveson, Janet M Sasso, Anne C Gregg, Divya J Soares, Tiffany R Beskid, Susan R Jervey, Cynthia Liu: Assay Techniques and Test Development for COVID-19 Diagnosis. ACS Cent Sci. 2020 May 27; 6(5): 591-605.
23. Melissa E. Dubois, Erika Hammarlund, Mark K. Slifka: Optimization of Peptide-Based ELISA for Serological Diagnostics: A Retrospective Study of Human Monkeypox Infection. Vector Borne Zoonotic Dis. 2012 May; 12(5): 400–409.
24. Yosra A. Helmy, Mohamed Fawzy, Ahmed Elaswad, Ahmed Sobieh, Scott P. Kenney, Awad A. Shehata: The COVID-19 Pandemic: A Comprehensive Review of Taxonomy, Genetics, Epidemiology, Diagnosis, Treatment, and Control. J Clin Med. 2020 Apr; 9(4): 1225.
25. Rachael Racine, Gary M Winslow: IgM in microbial infections: taken for granted?. Immunol Lett . 2009 Aug 15; 125(2): 79-85.
26. M Alejandra Tortorici, David Veesler: Structural insights into coronavirus entry. Adv Virus Res, 2019; 105: 93-116.
27. Offringa A, Montijn R, Singh S, Paul M, Pinto YM, Pinto-Sietsma SJ: The mechanistic overview of SARS-CoV-2 using angiotensin-converting enzyme 2 to enter the cell for replication: possible treatment options related to the renin-angiotensin system. Eur Heart J Cardiovasc Pharmacother. 2020 Sep 1;6(5): 317-325.
28. Yashpal Singh Malik, Shubhankar Sircar, Sudipta Bhat, Khan Sharun, Kuldeep Dhama, Maryam Dadar, Ruchi Tiwari, Wanpen Chaicumpa: Emerging novel coronavirus (2019-nCoV)—current scenario, evolutionary perspective based on genome analysis and recent developments. Vet Q. 2020; 40(1): 68–76.
29. Fang Li: Structure, Function, and Evolution of Coronavirus Spike Proteins, Annu Rev Virol. available in PMC 2017 Jun 5. Annu Rev Virol. 2016 Sep 29; 3(1): 237–261.
30. Sudeep Roy, Nidhi Maheshwari, Rashi Chauhan, Naresh Kumar Sen, Ashok Sharma: Structure prediction and functional characterization of

secondary metabolite proteins of *Ocimum*. Bioinformation. 2011; 6(8): 315–319.
31. Hong-Zhi Chen, Ling-Li Tang, Xin-Ling Yu, Jie Zhou, Yun-Feng Chang, Xiang Wu: Bioinformatics analysis of epitope-based vaccine design against the novel SARS-CoV-2. Infect Dis Poverty. 2020; 9: 88.
32. Xiangyu Wang, Qi Sun, Zhonghua Ye, Ying Hua, Na Shao, Yanli Du, Qiwei Zhang, Chengsong Wan: Computational approach for predicting the conserved B-cell epitopes of hemagglutinin H7 subtype influenza virus. Exp Ther Med. 2016 Oct; 12(4): 2439–2446.
33. Ward Fleri, Sinu Paul, Sandeep Kumar Dhanda, Swapnil Mahajan, Xiaojun Xu, Bjoern Peters, Alessandro Sette: The Immune Epitope Database and Analysis Resource in Epitope Discovery and Synthetic Vaccine Design. Front Immunol. 2017; 8: 278.
34. Jianyi Yang, Yang Zhang: Protein Structure and Function Prediction Using I-TASSER. Curr Protoc Bioinformatics. 2015; 52: 5.8.1–5.815.
35. Martina L. Jones, Fiona S. Legge, Kebaneilwe Lebani, Stephen M. Mahler, Paul R. Young, Daniel Watterson, Herbert R. Treutlein, Jun Zeng: Computational Identification of Antibody Epitopes on the Dengue Virus NS1 Protein. Molecules. 2017 Apr; 22(4): 607.
36. Al-Refaei MA, Makki RM, Ali HM: Structure prediction of transferrin receptor protein 1 (TfR1) by homology modelling, docking, and molecular dynamics simulation studies. Heliyon. 2020 Jan 29; 6(1).
37. Matthias Heinig and Dmitrij Frishman: STRIDE: a web server for secondary structure assignment from known atomic coordinates of proteins. Nucleic Acids Res. 2004 Jul 1; 32.
38. Vahid Bayrami, Mehrnaz Keyhanfar, Hassan Mohabatkar, Manijeh Mahdavi, Violaine Moreau: In silico prediction of B cell epitopes of the extracellular domain of insulin-like growth factor-1 receptor. Mol Biol Res Commun. 2016 Dec; 5(4): 201–214.
39. Julia Ponomarenko, Huynh-Hoa Bui, Wei Li, Nicholas Fusseder, Philip E Bourne, Alessandro Sette, Bjoern Peters: ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinformatics. 2008; 9: 514.
40. Jens Vindahl Kringelum[1], Claus Lundegaard, Ole Lund, Morten Nielsen: Reliable B cell epitope predictions: impacts of method development and improved benchmarking. PLoS Comput Biol, 2012; 8(12).
41. https://covid19.who.int/, WHO Corona virus Disease (COVID-19) Dashboard.
42. Martin Closter Jespersen, Bjoern Peters, Morten Nielsen, and Paolo Marcatili: BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res. 2017 Jul 3; 45.
43. Sudipto Saha, G P S Raghava: Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins. 2006 Oct 1; 65(1): 40-8.
44. Jens Vindahl Kringelum, Claus Lundegaard, Ole Lund, Morten Nielsen: Reliable B cell epitope predictions: impacts of method development and improved benchmarking. PLoS Comput Biol. 2012; 8(12): e1002829.
45. Manojit Bhattacharya, Ashish R Sharma, Prasanta Patra, Pratik Ghosh, Garima Sharma, Bidhan C Patra, Sang-Soo Lee, Chiranjib Chakraborty. Development of epitope-based peptide vaccine against novel coronavirus 2019 (SARS-COV-2): Immunoinformatics approach. J Med Virol, 2020 Jun; 92(6): 618-631.
46. Pritam Kumar Panda, Murugan Natarajan Arul, Paritosh Patel, Suresh K. Verma, Wei Luo, Horst-Günter Rubahn, Yogendra Kumar Mishra, Mrutyunjay Suar,and Rajeev Ahuja: Structure-based drug designing and immunoinformatics approach for SARS-CoV-2. Sci Adv, 2020 Jul 10; 6(28)
47. Naz A, Shahid F, Butt TT, Awan FM, Ali A, Malik A: Designing Multi-Epitope Vaccines to Combat Emerging Coronavirus Disease 2019 (COVID-19) by Employing Immuno-Informatics Approach. Front Immunol. 2020 Jul 10; 11: 1663.
48. Sankar S, Saravanan N, Rajendiran P, Ramamurthy M, Nandagopal B, Sridharan G: Identification of B- and T-cell epitopes on HtrA protein of Orientia tsutsugamushi. J Cell Biochem. 2019 Apr; 120(4): 5869-5879.
49. Matteo Castelli, Francesca Cappelletti, Roberta Antonia Diotti, Giuseppe Sautto, Elena Criscuolo, Matteo Dal Peraro, Nicola Clementi: Peptide-based vaccinology: experimental and computational approaches to target hypervariable viruses through the fine characterization of protective epitopes recognized by monoclonal antibodies and the identification of T-cell-activating peptides. Clin Dev Immunol, 2013; 2013: 521231.
50. Pernille Haste Andersen, Morten Nielsen, and Ole Lund, Prediction of residues in discontinuous B-cell epitopes using protein 3D structures, Protein Sci. 2006 Nov; 15(11): 2558–2567.
51. Kalaiselvan S, Sankar S, Ramamurthy M, Ghosh AR, Nandagopal B, Sridharan G: Prediction of B Cell Epitopes Among Hantavirus Strains Causing Hemorragic Fever With Renal Syndrome. J Cell Biochem. 2017 May; 118(5): 1182-1188.
52. Nimrod G, Fischman S, Austin M, Herman A, Keyes F, Leiderman O, Hargreaves D, Strajbl M, Breed J, Klompus S, Minton K, Spooner J, Buchanan A, Vaughan TJ, Ofran Y: Computational Design of Epitope-Specific Functional Antibodies. Cell Rep. 2018 Nov 20; 25(8): 2121-2131.
53. Silvia Rodriguez, Patricia Wilkins, and Pierre Dorny: Immunological and molecular diagnosis of cysticercosis. Pathog Glob Health. 2012 Sep; 106(5): 286–298.

54. Zhao S, Chen H: Modeling the epidemic dynamics and control of COVID-19 outbreak in China. Quant Biol. 2020 Mar 11:1-9.