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Research Article

Effects of K444T, N460K, F490S, L452R, and T478R Mutations on the Solubility, Allergenicity, and Immunogenicity of SARS-CoV-2-based Spike Protein Vaccines

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ABSTRACT

Background and Aim: The spike glycoprotein is a prime focal point for vaccine development due to its possession of numerous T-cell and B-cell epitopes. In this study, we investigated the effects of some important mutations (K444T, N460K, F490S, L452R, and T478R) on the immunogenicity of the spike protein in the Omicron variant. Additionally, we forecasted the effects of these mutations on the spike protein's solubility, allergenicity, and immunogenicity.

Methods: In this research, we obtained 100 SARS-CoV-2 spike sequences from two databases, namely the global initiative on sharing all influenza data (GISAID) EpiCoV and NCBI. We conducted a comparative analysis between the wild-type spike protein (Wuhan accession number: NC_045512.2) and the mutated spike proteins. The analysis focused on solubility, allergenicity, and immunogenicity. It was carried out using various bioinformatics servers, such as Dynamut, ToxinPred, SoluProt, Allertop, IEDB, and Vaxigen, as well as tools, like Mega XI and Pymol II.V.II visualizer.

Results: According to the prediction of the IEDB server, the K444T mutation is likely to decrease the humoral immune response. In addition, spike proteins in wild types and mutants do not have allergenic properties, and these proteins are soluble and can be expressed in *Escherichia coli*.

Conclusion: Vaccines formulated using spike protein design are effective. These findings indicate the potential for developing pan-coronavirus vaccines that offer protection not only against SARS-CoV-2 but also against a range of other coronaviruses in the future.

Keywords: COVID-19, Structural proteins, Bioinformatics analysis, Vaccine

Introduction

SARS-CoV-2 led to severe socio-economic burden and mourning of many families. This virus caused various pandemics in the world [1, 2]. SARS-CoV-2 is an enveloped single-stranded RNA virus that has spread rapidly in the last few years and caused a pandemic in the world. SARS-CoV-2 is the seventh coronavirus that has caused disease in humans. Its genome encodes four structural proteins, including spike, envelope, nucleocapsid, and membrane [3, 4].

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The initial stage of a viral infection involves the attachment of viral particles to receptors on the surface of host cells. Therefore, the ability of a cell or tissue to be susceptible to a virus is largely determined by the recognition of its receptors. In the case of coronaviruses, the spike glycoprotein plays a key role in mediating the entry process. This protein undergoes cleavage by host proteases, resulting in the formation of two subunits, S1 and S2. The conformational changes of these subunits are responsible for the recognition of the receptor and the fusion of the virus with the host cell membrane, respectively [5, 6].

The S1 subunit has a receptor-binding domain (RBD) domain that recognizes the receptor, and thus, it is considered a suitable candidate for the SARS-CoV-2 vaccine. Several strategies are employed in the development of vaccines against SARS-CoV-2, including the use of recombinant vaccines, inactivated viruses, live attenuated viruses, viral vector-based vaccines, and genomic vaccines [7-9]. The evolutionary characteristics of SARS-CoV-2 result in the emergence of novel strains that exhibit various mutations [10]. These mutations can lead to alterations in the virus behavior, such as increased infectivity, higher virulence, evasion of neutralizing antibodies, and reduced effectiveness of available diagnostic tests [11-14].

Also, due to the antigenic changes in this virus, it is always important to try to prevent this disease [4, 8, 15]. Vaccine companies worldwide have always prioritized the advancement of effective diagnostics, prognostic tools, and preventive interventions by placing significant emphasis on the design and production of multi-epitope and universal vaccines [16-19].

SARS-CoV-2 quickly is adapted to its host after spillover, while displaying minimal observable adaptation during the initial stages of the pandemic [8].

SARS-CoV-2 evolves rapidly on time scales of months or years, producing new variants to adapt to the host, but this has led to problems in the development of effective vaccines and antiviral drugs [7, 20-22].

The SARS-CoV-2 strains have experienced several non-synonymous mutations, specifically in the spike protein. Of these mutations, Omicron displayed the greatest number. These strains exhibited notable traits, including alterations in transmissibility and antigenicity. Currently, the World Health Organization (WHO) and national public health agencies have identified five SARS-CoV-2 variations as variants of concern (VOCs) due to significant changes in their ability to spread or resist the immune system. This underscores the crucial need for continuous monitoring [20, 23, 24].

In this study, we investigated the effects of some important mutations (K444T, N460K, F490S, L452R, and T478R) on the immunogenicity of the spike protein in the Omicron variant. We also examined the effects of mutations on spike protein in terms of solubility, allergenicity, and immunogenicity.

Materials and Methods

Retrieval sequences

In this study, we obtained 100 complete genome sequences of the Omicron variant from the GISAID EpiCoV database.

Multiple sequence alignment

The sequences were aligned and compared to the Wuhan reference (NC_045512.2) using Mega software, version XI and the Clustal W algorithm. The nucleotide sequences of the spike protein were converted into protein sequences [25].

Spike PDB protein construction (homology modeling)

The Swiss-model server was used for spike PDB protein construction [26].

Structure validation

SAVES server as a complete package was used to validate or evaluate the overall quality of the structure of the generated PDB protein [27].

Flexibility and stability analysis

The DynaMut server was employed to assess the influence of mutations on the stability and flexibility of protein structure [28].

Immunogenicity analysis

The effect of mutations on the immunogenicity of spike proteins was evaluated in comparison to the original spike protein by utilizing the immune epitope database (IEDB). This online resource is a comprehensive database specifically designed for analyzing antigens that target B cells and T cells. Furthermore, Disco Tope and Antigen Sequence Properties tools were employed

to predict both non-continuous and linear B cell epitopes, respectively [29, 30].

Solubility analysis

The production of numerous therapeutically and industrially valuable proteins is impeded by inadequate solubility. Traditional experimental approaches to enhance solubility have low success rates and may compromise biological activity. By relying solely on sequence information, computational techniques can forecast protein expressibility and solubility in *Escherichia coli*. Such predictions could help prioritize highly soluble proteins and reduce the expense of experimental studies [31].

Allergenicity analysis

An allergy occurs when the body's immune system overreacts to a substance that is usually harmless, causing symptoms, such as skin rashes, swelling of mucous membranes, sneezing, or wheezing. In this study, a method called auto cross-covariance (ACC) transformation was used to convert protein sequences into uniform vectors of equal length. This technique has been used in quantitative structure-activity relationship (QSAR) investigations of peptides with different lengths. The ACC transformation helps to analyze characteristics, such as amino acid hydrophobicity, molecular size, the potential for forming helices, the relative occurrence of amino acids, and the propensity for forming β-strands [32].

Toxicity analysis

Proteins and peptides exhibit great potential as therapeutic agents for various diseases. Nonetheless, toxicity poses a significant challenge to protein- and peptide-based therapies. This module enables users to create all feasible single mutant analogs of their peptides and forecast the analog's potential toxicity [33].

Results

Spike protein PDB validation

We carefully chose the optimal spike PDB structure using Swiss-model server criteria. These criteria include assessing Molprobity indices, Ramachandran plot, qualitative model energy analysis (QMEAN)Z-score, GMQE, and QMEANDisCo local model evaluation for both wild and mutant spike proteins. The ERRAT validation showed that the overall quality factor of these proteins was above 90. Additionally, the Ramachandran plot analysis conducted by PROCHECK indicated that 95.5% of the residues in all structure spike models were in the most favored regions, 4.4% were in additional allowed regions, 0.1% in generously allowed regions, and 0% in disallowed regions (Figure 1).

Immunogenicity analysis

The impact of mutations on spike protein immunogenicity, potential antibody-mediated detection escape, and the ability to detect and deliver to T cells, particularly

Table 1. Predicting the effect of mutations on Spike protein immunogenicity

Mutation	Disco Tope	ASP	MHC-I Immunogenicity	Major Histocompatibility Complex (MHC) Class II
K444T	W: -5.4	W: 0.59	W: 0.38	W: 1.10
	M: -6.09	M: 0.55	M: 0.633	M: 1.10
N460K	W: -5.10	W: 0.57	W: 0.38	W: 0.26
	M: -4.86	M: 0.59	M: 0.18	M: 0.22
F490S	W: -12.75	W: 0.55	W: 0.38	W: 1.8
	M: -10.93	M: 0.56	M: 0.11	M: 1.2
L452R	W: -13.95	W: 0.50	W: 0.38	W: 2.4
	M: -11.15	M: 0.55	M: 0.44	M: 2.1
T478R	W: -8.92	W: 0.55	W: 0.38	W: 1.8
	M: -7.33	M: 0.58	M: 0.39	M: 1.6

Abbreviations: W: Wild type; M: Mutant type; ASP: Antigen sequence properties.

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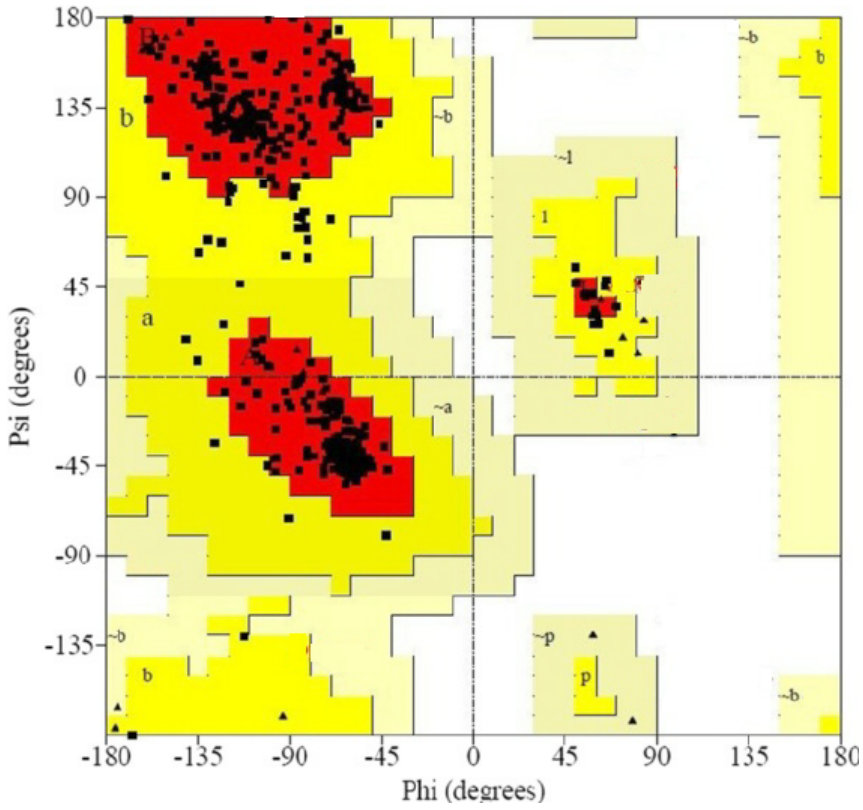

Figure 1. An example of a Ramachandran plot showing spike proteins

The majority of residues (95.5%) were found in the highly preferred regions, with an additional 4.4% in permissible regions, a mere 0.1% in moderately permissible regions, and none in prohibited regions.

helper and cytotoxic T cells, is highlighted in Table 1. The scores in the table correspond to the level of immunogenicity, with higher scores indicating greater immunogenicity. K444T mutation in both linear and spatial forms of the spike protein is likely to result in a reduced humoral immune response. Additionally, the N460K and F490S mutations may reduce the detection of the spike protein by molecules that provide recognition through MHC-I. However, the N460K mutation may also decrease the supply of the spike protein by MHC-II molecules to helper T cells.

Predicting the effect of mutations on the antigenicity using the Vaxigen server

The investigation using the Vaxigen server showed that creating the K444T, L452R, and N460K mutations for the peptide predicted by the IEDB server did not reduce the level of antigenicity and probably did not reduce the response rate of the vaccines designed for this region. It requires experimental studies, but for the mutations T478R and F490S for the peptide predicted by the IEDB server, the amount of antigenicity is reduced and the response rate of the vaccines designed for this region is decreased (Figure 2).

Predicting the effect of mutations on the toxicity using the ToxinPred server

The peptides in question before and after the mutation event did not have toxic properties (Figure 3).

Predicting the effect of mutations on the solubility using the SoluProt server

These peptides were soluble and could be expressed in *E. coli* (Figure 4).

Predicting the effect of mutations on the allergenicity using the AllerTop 2 server

According to the prediction of the server, spike protein in naive and mutant states did not have allergenic properties (Figure 4).

Predicting the effect of mutations on the stability and flexibility using the DynaMut server

Figure 2. Prediction of antigenicity by spike mutations K444T, L452R, N460K, T478R, and F490S

Mutations in the virus-binding regions, such as K444T, T478R, L452R, F490S, and N460K, destabilize the spike protein to enhance its flexibility, leading to changes in conformation and binding strength with ligands. This can decrease the binding affinity of monoclonal- or polyclonal-neutralizing antibodies, allowing the virus to evade the humoral immune system. Additionally, these mutations can affect processing and supply by MHCI/II molecules, reducing recognition by cellular immunity (TH/CTL), which may disturb various immune pathways involving TH cells and antibodies. The K444T mutation, for example, reduces the number and type of intermolecular interactions, as illustrated in Figure 5. In the wild-type K444, there are seven bonds, including four strong hydrogen bonds and three weak polar bonds. However, the K444T mutation reduces the frequency of bonds to five, and strong hydrogen bonds disappear.

Discussion

The spike protein is used due to its immunogenicity in all approved COVID-19 vaccines. However, VOCs contain spike protein mutations that enable them to evade immune responses induced by infection and vaccination, which can lead to reinfection [34-36]. Thus, extensive research is being conducted to develop new vaccines. Studying the epitopes of SARS-CoV-2 plays a vital role in vaccine development because it enables the identification of targets that can induce broadly neutralizing antibody responses and dominant T-cell epitopes. This knowledge would provide potential candidates for the future generation of COVID-19 vaccines [37]. Surveillance of asymptomatic individuals is important in controlling the spread of COVID-19 since asymptomatic cases are challenging to detect and pose a risk of

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Query Peptides

Peptide ID	Peptide Sequence	SVM Score	Prediction	Hydrophobicity	Steric hindrance	Sidebulk	Hydropathicity	Amphipathicity	Hydrophilicity	Net Hydrogen	Charge	pI	Mol wt
	NLDSKVGGNYNYLYRLFRKSNLKPFE	-1.53	Non-Toxin	-0.27	0.65	0.65	-0.95	0.66	0.07	1.12	3.00	9.71	3136.95
	LDSTVGGNYNYRYRLFRKSKLKPFE	-1.48	Non-Toxin	-0.33	0.65	0.65	-1.06	0.79	0.23	1.20	4.00	10.00	3052.85
	RDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT	-0.82	Non-Toxin	-0.09	0.63	0.63	-0.43	0.25	-0.32	0.74	-2.00	4.14	3891.77
	RDISTEIYQAGSRPCNGVEGFNCYSPLQSYGFQPT	-0.78	Non-Toxin	-0.16	0.63	0.63	-0.64	0.32	-0.14	0.86	-1.00	4.68	3886.75

1/1 50

Your job id is 55340

Figure 3. Prediction of toxic/non-toxic target peptides

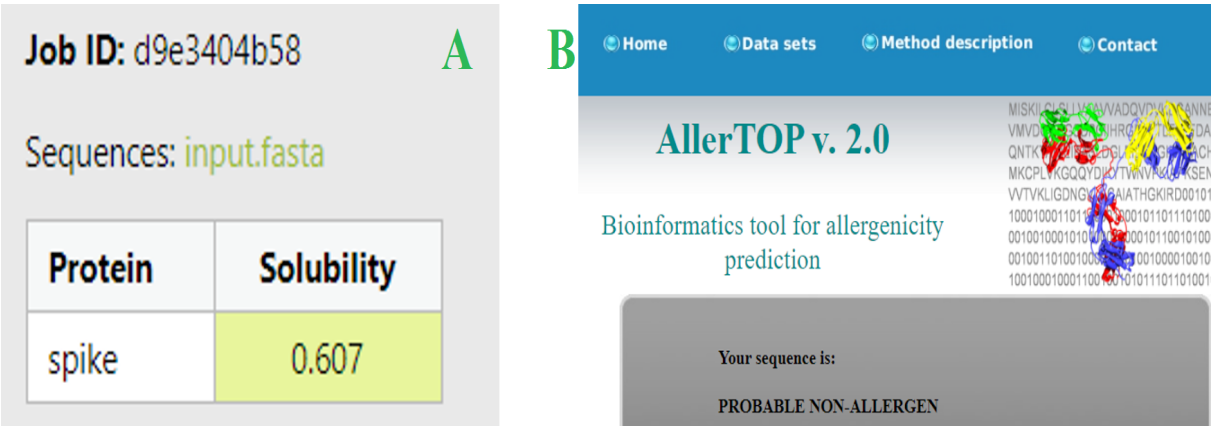

Figure 4. A) Prediction of the expression of solubility target peptides in *E. coli*, B) Predicting the effect of mutations on the allergenicity of spike protein

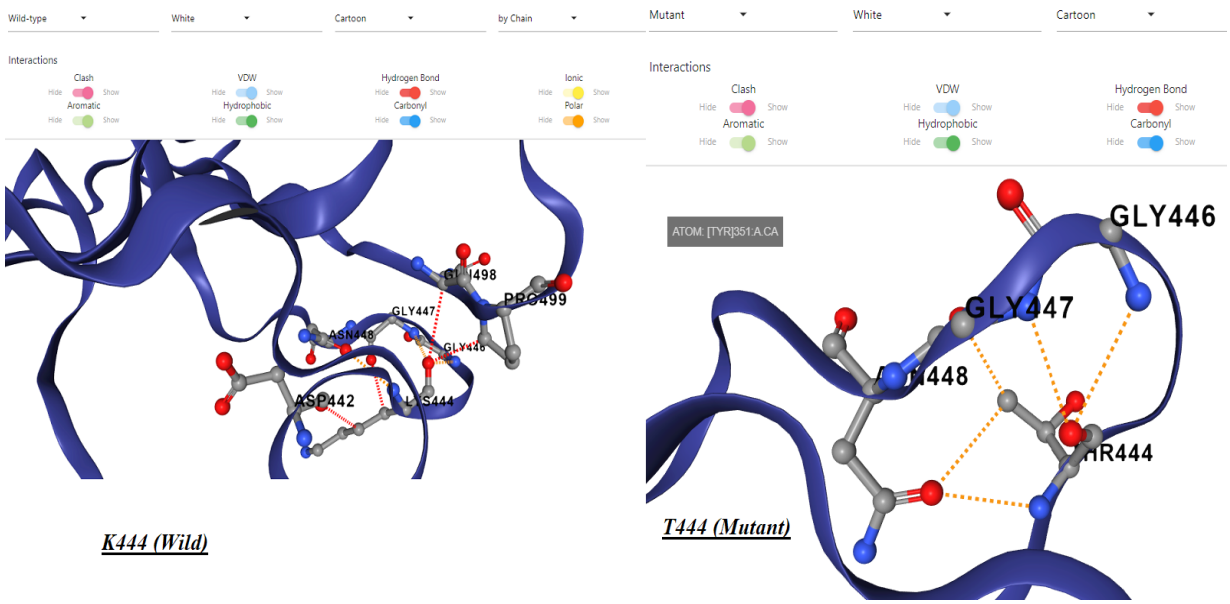

Figure 5. Predicting the effect of K444T mutation on the stability and flexibility of spike protein

transmission [38]. IgA antibodies against SARS-CoV-2 mediate the primary neutralizing humoral response [39]. Contreras et al. demonstrated the usefulness of the peptide sequence 455-LFRKSN (460) LKPFERD-467 in detecting asymptomatic carriers, which could be utilized to create an ELISA kit and screen patients [40].

The mutations N460K, L452R, and T478R involve the replacement of positively charged amino acids (lysine and arginine) with non-polar amino acids, resulting in a slightly more positive isoelectric pH (PI) (changing from 6.24 to 6.42). This alteration may affect the interaction between the ligand and the receptor, the processing of the spike protein in the proteasome complex, the cleavage site modification by host enzymes or proteases, the supply of the protein to immune cells, and the virus's ability to evade the immune system [41]. A comprehensive understanding of the structure, properties, and mutations of the spike protein is crucial for comprehending its significance in virus infection and for developing treatments and vaccines. Since vaccines have the potential to mitigate the disease and enhance population immunity, their safety, efficacy, and cost-effectiveness must be ensured. This information can be utilized to enhance vaccine efficacy [42].

Proteins are dynamic molecules, and their molecular motions are essential to their function. However, the computational simulation of protein dynamics can be costly, leading most structure-based approaches to rely on static structures to assess the impact of mutations on protein structure and function [43]. To tackle this challenge, we employed the DynaMut server. This server leverages two well-established techniques for investigating protein dynamics. It involves sampling different conformations and analyzing how mutations influence protein dynamics and stability by measuring changes in vibrational entropy [28]. Intermolecular interactions and ligand-to-receptor interactions, such as ACE2-mAb and CD147, are influenced by bond type, bond length, and the number of bonds [44]. Mutations in the virus-binding region, such as K444T, T478R, L452R, F490S, and N460K, destabilize the spike protein to enhance its flexibility, leading to changes in conformation and binding strength with ligands. This can decrease the binding affinity of monoclonal- or polyclonal-neutralizing antibodies, allowing the virus to evade the humoral immune system. Additionally, these mutations can affect processing and supply by MHCI/II molecules, reducing recognition by cellular immunity (TH/CTL), which may disturb various immune pathways involving TH cells and antibodies [45]. The K444T mutation, for example, reduces the number and type of intermolecular interactions.

It is crucial for vaccine and kit immunological structures to produce neutralizing antibodies while avoiding the creation of non-neutralizing antibodies that can cause cross-reactions and worsen the disease [46]. Identifying protective epitopes in SARS-CoV-2 proteins, including the RBD, is necessary for vaccine development. However, the RBD's limited immunogenicity, likely due to its small size and polymeric structure, poses a challenge for vaccine design [47].

To conquer this challenge, it is advisable to include various versions of the RBD in the vaccine components. This will allow better management of not only SARS-CoV-2 but also other beta coronaviruses, like MERS-CoV and SARS-CoV [48] To overcome this challenge, it is recommended to incorporate different versions of the (RBD) in the components of the vaccine.

Conclusion

The findings of this study suggest that vaccines targeting the spike protein are effective. It indicates that the development of pan-coronavirus vaccines, capable of protecting against not only SARS-CoV-2 but also other coronaviruses, is possible. While neutralizing antibody epitopes have received more attention, T-cell epitopes could offer new strategies to combat SARS-CoV-2 infection. T cells can recognize various antigens of SARS-CoV-2 beyond the spike protein, which reduces the potential for immune evasion by viral VOCs that primarily mutate the spike protein. Therefore, boosting T-cell responses through vaccination could provide adequate protection, even in the face of significant antibody evasion by VOCs. Further investigations should include laboratory and animal studies. It is crucial to explore alternative platforms for COVID-19 vaccines and assess the efficacy of convalescent serum. By utilizing the dominant epitopes of the SARS-CoV-2 protein spike, immunogenic structures could be widely employed in diagnostic kits, treatment, prevention, and vaccine production. Furthermore, a pan-coronavirus vaccine that covers all coronaviruses could be developed through a quantum vaccinomics approach, combining protective epitopes. With great effort, information sharing, and scientific support, we have contributed to the WHO slogan for an impressive reduction in morbidity and mortality rate of new variants of SARS-CoV-2 infection.

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Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research

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Authors' contributions

Conceptualization, ensure the accuracy of additional data: Bahman Aghcheli and Asyeh Yolmeh; Study design, supervision, designing graphical content, review, and editing: Mehdi Yolmeh; Creation of the workflow, coding, data analysis and writing the manuscript: Bahman Aghcheli and Mehdi Yolmeh; Data visualization: Asyeh Yolmeh; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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References

[1] Levin AT, Owusu-Boaitey N, Pugh S, Fosdick BK, Zwi AB, Malani A, et al. Assessing the burden of COVID-19 in developing countries: Systematic review, meta-analysis and public policy implications. BMJ Glob Health. 2022; 7(5):e008477. [DOI:10.1136/bmjgh-2022-008477] [PMID]

[2] COVID-19 National Preparedness Collaborators. Pandemic preparedness and COVID-19: An exploratory analysis of infection and fatality rates, and contextual factors associated with preparedness in 177 countries, from Jan 1, 2020, to Sept 30, 2021. Lancet. 2022; 399(10334):1489-512.[DOI:10.1016/S0140-6736(22)00172-6] [PMID]

[3] Aghcheli B, Tahamtan A, Razavi Nikoo H, Bazi Z, Kalani MR, Moradi A. Evaluation of mutations in SARS-CoV-2 N and S genes on the proteins stability, immunogenicity, and pathogenicity in Iranian patients from Golestan province. Int J Pediatr. 2022; 10(8):16486-97. [DOI:10.22038/ijp.2022.64880.4906]

[4] Polatoğlu I, Oncu-Oner T, Dalman I, Ozdogan S. COVID-19 in early 2023: Structure, replication mechanism, variants of SARS-CoV-2, diagnostic tests, and vaccine & drug development studies. MedComm (2020). 2023; 4(2):e228. [DOI:10.1002/mco2.228] [PMID]

[5] Pronker MF, Creutznacher R, Drulyte I, Hulswit RJG, Li Z, van Kuppeveld FJM, et al. Sialoglycan binding triggers spike opening in a human coronavirus. bioRxiv. Preprint. 2023; 1-29. [DOI:10.1101/2023.04.20.536837]

[6] Costello SM, Shoemaker SR, Hobbs HT, Nguyen AW, Hsieh CL, Maynard JA, et al. The SARS-CoV-2 spike reversibly samples an open-trimer conformation exposing novel epitopes. Nat Struct Mol Biol. 2022; 29(3):229-38. [DOI:10.1038/s41594-022-00735-5] [PMID]

[7] Markov PV, Ghafari M, Beer M, Lythgoe K, Simmonds P, Stilianakis NI, et al. The evolution of SARS-CoV-2. Nat Rev Microbiol. 2023; 21(6):361-79. [DOI:10.1038/s41579-023-00878-2] [PMID]

[8] Carabelli AM, Peacock TP, Thorne LG, Harvey WT, Hughes J; COVID-19 Genomics UK Consortium, et al. SARS-CoV-2 variant biology: Immune escape, transmission and fitness. Nat Rev Microbiol. 2023; 21(3):162-77. [DOI:10.1038/s41579-022-00841-7] [PMID]

[9] Umitaibatin R, Harisna AH, Jauhar MM, Syaifie PH, Arda AG, Nugroho DW, et al. Immunoinformatics study: Multi-Epitope based vaccine design from SARS-CoV-2 spike glycoprotein. Vaccines (Basel). 2023; 11(2):399. [DOI:10.3390/vaccines11020399] [PMID]

[10] Naz S, Aroosh A, Caner A, Şahar EA, Toz S, Ozbel Y, et al. Immunoinformatics approach to design a multi-epitope vaccine against Cutaneous Leishmaniasis. Vaccines (Basel). 2023; 11(2):339. [DOI:10.3390/vaccines11020339] [PMID]

[11] Sahu LK, Singh K. Cross-variant proof predictive vaccine design based on SARS-CoV-2 spike protein using immunoinformatics approach. Beni Suef Univ J Basic Appl Sci. 2023; 12(1):5. [DOI:10.1186/s43088-023-00341-4] [PMID]

[12] Evolution of SARS-CoV-2 Variants: Implications on immune escape, vaccination, therapeutic and diagnostic strategies. Viruses. 2023; 15(4):944. [DOI:10.3390/v15040944] [PMID]

[13] Thakur S, Sasi S, Pillai SG, Nag A, Shukla D, Singhal R, et al. SARS-CoV-2 mutations and their impact on diagnostics, therapeutics and vaccines. Front Med (Lausanne). 2022; 9:815389. [DOI:10.3389/fmed.2022.815389] [PMID]

[14] Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol. 2021; 19(7):409-24. [DOI:10.1038/s41579-021-00573-0] [PMID]

[15] Han W, Chen N, Xu X, Sahil A, Zhou J, Li Z, et al. Predicting the antigenic evolution of SARS-COV-2 with deep learning. Nat Commun. 2023; 14(1):3478. [DOI:10.1038/s41467-023-39199-6] [PMID]

[16] Khamjan NA, Lohani M, Khan MF, Khan S, Algaissi A. Immunoinformatics strategy to develop a novel universal multiple epitope-based COVID-19 vaccine. Vaccines (Basel). 2023; 11(6):1090. [DOI:10.3390/vaccines11061090] [PMID]

[17] Qin J, Jeon JH, Xu J, Langston LK, Marasini R, Mou S, et al. Design and preclinical evaluation of a universal SARS-CoV-2 mRNA vaccine. Front Immunol. 2023; 14:1126392. [DOI:10.3389/fimmu.2023.1126392] [PMID]

[18] Ojha R, Singh S, Gupta N, Kumar K, Padhi AK, Prajapati VK. Multi-pathogen based chimeric vaccine to fight against COVID-19 and concomitant coinfections. Biotechnol Lett. 2023; 45(7):779-97. [DOI:10.1007/s10529-023-03380-0] [PMID]

[19] Moustafa RI, Faraag AHI, El-Shenawy R, Agwa MM, El-sayed H. Harnessing immunoinformatics for developing a multiple-epitope peptide-based vaccination approach against SARS-CoV-2 spike protein. Saudi J Biol Sci. 2023; 30(6):103661. [DOI:10.1016/j.sjbs.2023.103661] [PMID]

[20] Islam MA, Shahi S, Marzan AA, Amin MR, Hasan MN, Hoque MN, et al. Variant-specific deleterious mutations in the SARS-CoV-2 genome reveal immune responses and potentials for prophylactic vaccine development. Front Pharmacol. 2023; 14:1090717. [DOI:10.3389/fphar.2023.1090717] [PMID]

[21] Gili R, Burioni R. SARS-CoV-2 before and after Omicron: Two different viruses and two different diseases? J Transl Med. 2023; 21(1):251. [DOI:10.1186/s12967-023-04095-6] [PMID]

[22] Arduini A, Laprise F, Liang C. SARS-CoV-2 ORF8: A rapidly evolving immune and viral modulator in COVID-19. Viruses. 2023; 15(4):871. [DOI:10.3390/v15040871] [PMID]

[23] Alquraan L, Alzoubi KH, Rababa'h SY. Mutations of SARS-CoV-2 and their impact on disease diagnosis and severity. Inform Med Unlocked. 2023; 39:101256. [DOI:10.1016/j.imu.2023.101256] [PMID]

[24] Focosi D, Quiroga R, McConnell S, Johnson MC, Casadevall A. Convergent evolution in SARS-CoV-2 spike creates a variant soup from which new COVID-19 waves emerge. Int J Mol Sci. 2023; 24(3):2264. [DOI:10.3390/ijms24032264] [PMID]

[25] Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol. 2021; 38(7):3022-7. [DOI:10.1093/molbev/msab120] [PMID]

[26] Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res. 2018; 46(W1):W296-303. [DOI:10.1093/nar/gky427] [PMID]

[27] Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Crystallogr. 1993; 26:283-91. [DOI:10.1107/S0021889892009944]

[28] Rodrigues CH, Pires DE, Ascher DB. DynaMut: Predicting the impact of mutations on protein conformation, flexibility and stability. Nucleic Acids Res. 2018; 46(W1):W350-5. [DOI:10.1093/nar/gky300] [PMID]

[29] Fleri W, Paul S, Dhanda SK, Mahajan S, Xu X, Peters B, et al. The immune epitope database and analysis resource in epitope discovery and synthetic vaccine design. Front Immunol. 2017; 8:278. [DOI:10.3389/fimmu.2017.00278] [PMID]

[30] Doytchinova IA, Flower DR. VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics. 2007; 8:4. [DOI:10.1186/1471-2105-8-4] [PMID]

[31] Hon J, Marusiak M, Martinek T, Kunka A, Zendulka J, Bednar D, et al. SoluProt: Prediction of soluble protein expression in Escherichia coli. Bioinformatics. 2021; 37(1):23-8. [DOI:10.1093/bioinformatics/btaa1102] [PMID]

[32] Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v.2--a server for in silico prediction of allergens. J Mol Model. 2014; 20(6):2278. [DOI:10.1007/s00894-014-2278-5] [PMID]

[33] Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R; Open Source Drug Discovery Consortium, et al. In silico approach for predicting toxicity of peptides and proteins. PLoS One. 2013; 8(9):e73957. [DOI:10.1371/journal.pone.0073957] [PMID]

[34] COVID-19 Forecasting Team. Past SARS-CoV-2 infection protection against re-infection: A systematic review and meta-analysis. Lancet. 2023; 401(10379):833-42. [DOI:10.1016/S0140-6736(22)02465-5] [PMID]

[35] Tan ST, Kwan AT, Rodríguez-Barraquer I, Singer BJ, Park HJ, Lewnard JA, et al. Infectiousness of SARS-CoV-2 breakthrough infections and reinfections during the Omicron wave. Nat Med. 2023; 29(2):358-65. [DOI:10.1038/s41591-022-02138-x] [PMID]

[36] Vicentini M, Venturelli F, Mancuso P, Bisaccia E, Zerbini A, Massari M, et al. Risk of SARS-CoV-2 reinfection by vaccination status, predominant variant and time from prior infection: A cohort study, Reggio Emilia province, Italy, February 2020 to February 2022. Euro Surveill. 2023; 28(13):2200494. [DOI:10.2807/1560-7917.ES.2023.28.13.2200494] [PMID]

[37] Rouet R, Henry JY, Johansen MD, Sobti M, Balachandran H, Langley DB, et al. Broadly neutralizing SARS-CoV-2 antibodies through epitope-based selection from convalescent patients. Nat Commun. 2023; 14(1):687. [DOI:10.1038/s41467-023-36295-5] [PMID]

[38] Park SW, Dushoff J, Grenfell BT, Weitz JS. Intermediate levels of asymptomatic transmission can lead to the highest epidemic fatalities. PNAS Nexus. 2023; 2(4):pgad106. [DOI:10.1093/pnasnexus/pgad106] [PMID]

[39] Sterlin D, Mathian A, Miyara M, Mohr A, Anna F, Claër L, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci Transl Med. 2021; 13(577):eabd2223. [DOI:10.1126/scitranslmed.abd2223] [PMID]

[40] Contreras M, Vicente J, Cerón JJ, Martinez Subiela S, Urra JM, Rodríguez-Del-Río FJ, et al. Antibody isotype epitope mapping of SARS-CoV-2 Spike RBD protein: Targets for COVID-19 symptomatology and disease control. Eur J Immunol. 2023; 53(4):e2250206. [DOI:10.1002/eji.202250206] [PMID]

[41] Yerukala Sathipati S, Shukla SK, Ho SY. Tracking the amino acid changes of spike proteins across diverse host species of severe acute respiratory syndrome coronavirus 2. iScience. 2022; 25(1):103560. [DOI:10.1016/j.isci.2021.103560] [PMID]

[42] Olukitibi TA, Ao Z, Warner B, Unat R, Kobasa D, Yao X. Significance of conserved regions in Coronavirus spike protein for developing a novel vaccine against SARS-CoV-2 infection. Vaccines (Basel). 2023; 11(3):545. [DOI:10.3390/vaccines11030545] [PMID]

[43] Yang LQ, Sang P, Tao Y, Fu YX, Zhang KQ, Xie YH, et al. Protein dynamics and motions in relation to their functions: Several case studies and the underlying mechanisms. J Biomol Struct Dyn. 2014; 32(3):372-93. [DOI:10.1080/07391102.2013.770372] [PMID]

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[44] Barthe M, Hertereau L, Lamghari N, Osman-Ponchet H, Braud VM. Receptors and cofactors that contribute to SARS-CoV-2 entry: Can skin be an alternative route of entry? Int J Mol Sci. 2023; 24(7):6253. [DOI:10.3390/ijms24076253] [PMID]

[45] Zhao Z, Zhou J, Tian M, Huang M, Liu S, Xie Y, et al. Omicron SARS-CoV-2 mutations stabilize spike up-RBD conformation and lead to a non-RBM-binding monoclonal antibody escape. Nat Commun. 2022; 13(1):4958. [DOI:10.1038/s41467-022-32665-7] [PMID]

[46] Lee WS, Wheatley AK, Kent SJ, DeKosky BJ. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. Nat Microbiol. 2020; 5(10):1185-91. [DOI:10.1038/s41564-020-00789-5] [PMID]

[47] Dormeshkin D, Katsin M, Stegantseva M, Golenchenko S, Shapira M, Dubovik S, et al. Design and immunogenicity of SARS-CoV-2 DNA vaccine encoding RBD-PVXCP fusion protein. Vaccines (Basel). 2023; 11(6):1014. [DOI:10.3390/vaccines11061014] [PMID]

[48] Hou XC, Xu HF, Liu Y, Sun P, Ding LW, Yue JJ, et al. A Vaccine with multiple receptor-binding domain subunit mutations induces broad-spectrum immune response against SARS-CoV-2 variants of concern. Vaccines (Basel). 2022; 10(10):1653. [DOI:10.3390/vaccines10101653] [PMID]