Scientific Inquiry and Review (SIR)
Volume 10 Issue 2, 2026
ISSN(P): 2521-2427, ISSN(E): 2521-2435
Homepage: https://journals.umt.edu.pk/index.php/SIR

Title:	**Evaluation of Immune Evasive and Infectivity Potential of Viral Molecular Evolution of SARS-CoV-2 Emerging Variants**
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Academic Editor	Imran Tipu
DOI:	https://doi.org/10.32350/sir.102.03
History:	Received: February 17, 2026, Revised: April 14, 2026, Accepted: May 25, 2026, Published: June 22, 2026
Citation:	Saddique N, Khan IN, Ahmad J, Muhammad H, Rashid MI. Evaluation of immune evasive and infectivity potential of viral molecular evolution of SARS-CoV-2 emerging variants. *Sci Inq Rev*. 2026;10(2): 47–69. https://doi.org/10.32350/sir.102.03
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Conflict of Interest:	Author(s) declared no conflict of interest

A publication of
The School of Science
University of Management and Technology, Lahore, Pakistan

Evaluation of Immune Evasive and Infectivity Potential of Viral Molecular Evolution of SARS-CoV-2 Emerging Variants

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ABSTRACT

In December 2019, the world was shocked by a new and highly infectious pathogen named SARS-CoV-2 which caused a very high number of cases and deaths, worldwide. Several vaccines were introduced to tackle the virus but complications arose when its different variants were identified. World Health Organization (WHO) has classified them as "Variants of Concern" and "Variants of Interest". VOCs include Alpha (B.1.17), Beta (B.1.351), Gamma (P.1), Delta (B.1.617), and Omicron (BA.1, BA.2, BA.4 and BA.5). The primary focus of this study was the mutations reported in the spike protein of these VOCs and their role in immune evasion and infectivity potential of the virus. This study employed an *in silico* approach to provide the detailed structure of the spike protein of all variants. The immune threshold of the VOCs and Wuhan-ref was compared. Further, differences in the interaction of the spike glycoprotein of the variants and Wuhan-ref with the *h*ACE2 receptor were analyzed to show variations in the spike protein of different variants. The findings indicate that as the number of mutations increased in the emerging variants, so did their infectivity potential. Furthermore, when different epitopes were examined for immune evasion, it was found that molecular evolution has made them more immune evasive but less severe than the Wuhan-ref.

Keywords: hACE2, immune evasion, infectivity potential, SARS-CoV-2, spike protein, Variants of Concern (VOCs),

Highlights

- *In silico* analysis of SARS-CoV-2 variants suggests their potential for immune evasion.
- Mutations in the S-protein may alter hACE2 receptor binding and viral entry.

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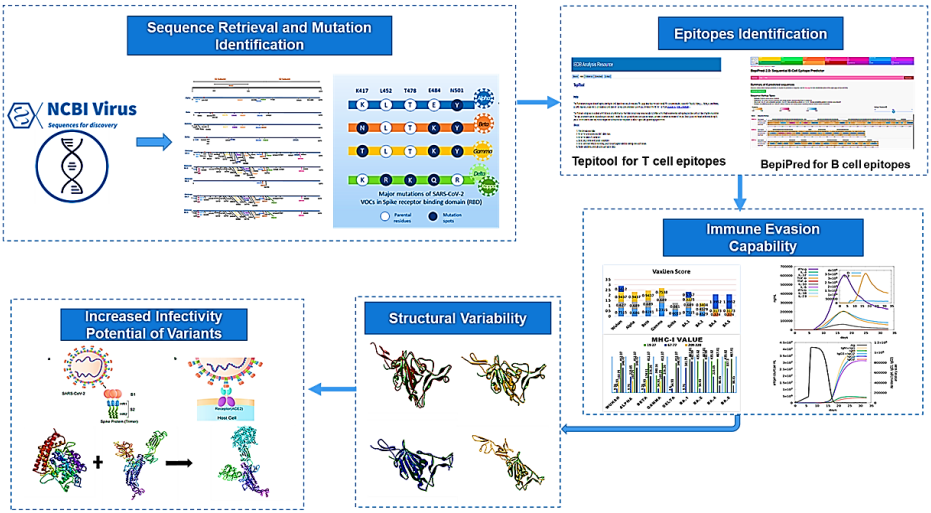
- Insertions and deletions can reshape the 3D conformation of the RBD.

GRAPHICAL ABSTRACT



1. INTRODUCTION

A wave of fear was created when an unknown pathogen (later named SARS-CoV-2) was identified in Wuhan, China back in December 2019 [1, 2]. Since then, this new pathogen has spread worldwide and infected countless number of people, resulting in a significant number of deaths [3]. By the end of January 2020, this infection was declared as an international public health emergency by the World Health Organization (WHO) [4].

The SARS-CoV-2, a single-stranded positive-sense RNA, has a genome comprising 29,903 bp with 50 different Open Reading Frames (ORFs). The viral genome carries the information for multiple structural, non-structural, and accessory proteins, involved in various functions enabling the success of the viral infection [5, 6]. The two ORFs (ORF1a & ORF1ab) comprise the first two-thirds of the viral genome which produce polyproteins (PP1 & PP1ab). The larger polyprotein PP1ab, containing about 16 non-structural proteins (Nsp16), is involved in a variety of roles in biological processes vital to the virus, including replication, replication error correction, translation, inhibition of host proteins, immune response blocking, and RNA stabilization [6]. Structural proteins (i.e., spike (S), membrane (M), nucleocapsid (N), and envelope (E)), which specify the viral structure, are

encoded in the final one-third of the RNA [5,6]. The spike protein or S-protein of the virus is an important protein that participates in the emergence of the variants. The S-protein comprises two subunits S1 and S2, where S1 is responsible for binding to the host ACE 2 receptor and helps in virus entry into the host cell, while S2 contains the required elements for viral and cellular membrane fusion [7-9]. Various mutations observed in the S-protein cause conformational changes that increase the pathogenicity of the virus [10].

As per the WHO classification, the viral variants are grouped into 3 categories. These include the "Variants of Concern" (VOC), the "Variants of Interest" (VOI), and the "Variants under Monitoring" (VUM). The VOC are defined as the variants with increased transmissibility, a significant decrease in neutralization by antibodies produced during vaccination or previous infection, more severe disease, diagnostic failure, and reduced effectiveness of treatments. The first VOC reported was B.1.1.7 (Alpha), which originated in the UK but rapidly became the dominant strain by January 2021. This was the first variant with a higher transmissibility rate [11]. The Beta (B.1.351) and Gamma (P.1) variants were first identified in the United Kingdom, Brazil, and South Africa and later spread throughout the world, according to the WHO.

The most prominent of all the variants is B.1.617 (Delta) which emerged in India. This lineage includes three main subtypes: B.1.617.2, B.1.617.3, and B.1.617.1. The range of mutations for this lineage is highly diverse and includes the N-terminal domain and Receptor Binding Domain (RBD) regions of the SARS-CoV-2 spike protein. It is believed that these changes increase the immune evasion potential of the Delta variant [12]. In late November 2021, a new highly immune evasive variant emerged from South Africa. WHO labelled it as Omicron, while its PANGO lineage is BA.1 [13-15]. Omicron has 32 new mutations, 15 of them are present in the RBD region of the S-protein of the virus [16]. Omicron is becoming the dominant variant as its new sub-lineages are emerging which are more immune evasive and added to the VOC list of WHO. These sub-variants include BA.2, BA.4, and BA.5.

All VOCs reported so far have almost the same range of mutations with few exceptions, including the N501Y in Alpha, K417N in Beta, Gamma, and Delta, and D614G in most VOCs. Studies show that the D614G mutation increases the binding affinity to the ACE2 receptor [17]. Further,

infectivity [17,18] and transmissibility in the human population are enhanced [19]. Several sub-lineages of the Omicron variant have emerged, including BA.2.75 and BA.2.75.2, which exhibit increased virulence and transmissibility. In October 2022, the WHO began monitoring a new Omicron variant called XBB, which is a recombinant of BA.2.10.1 and BA.2.75, along with its sub-lineages [20]. The BA.5/BA.2 lineage and its descendants are currently widespread and recombined versions of BA.2 (e.g. XBB variant) are prevalent in South and Southeast Asian countries. These variants have varying characteristics due to specific mutations. On November 20, 2022, the WHO designated BA.5, BA.2.75, BA.4.6, XBB, and BA.2.3.20 as Omicron sub-variants of interest under close monitoring (VUM) [21].

The high rate of the emergence of variants is believed to be linked to error-prone viral replication and the escape from immune selective pressures. The point of concern is that these mutants are resistant to the antibodies produced by COVID-19 patients [22-24], as well as those who have been vaccinated [25]. This research aims to investigate the mutational differences in VOCs and their impact on virulence, antigenicity, and transmission. The overall workflow of this study is provided below in Figure 1.

2. MATERIALS AND METHODS

2.1. Sequence Retrieval

The first step involves retrieving protein sequences of the S-protein for Wuhan-ref and all other variants. These protein sequences were retrieved from the NCBI Virus database (ncbi.nlm.nih.gov/labs/virus/) [26]. The accession numbers for each sequence used for retrieval are given below in Table 1.

2.2. Epitopes and Antigenic Potential Prediction

The immune evasion potential was predicted by identifying B-Cell and T-Cell (MHC Class I and II) epitopes through different online servers. BepiPred v2.0 [27] was used to identify B-Cell epitopes and the antigenic potential for these epitopes was predicted using 'VaxiJen' v2.0 [28]. A threshold of 0.5 to calculate the VaxiJen score was set. MHC Class I and II epitopes were identified using TepiTool available at the Immune Epitope Database (https://www.iedb.org/) [29]. TepiTool predicted MHC Class-I and II epitopes for the selected proteins using IC50 value <500nM. C-

IMMSIM was used to predict the score of the immune response, levels of IgG, IgM, and cytokines [30].

Table 1. Variants and their Accession Numbers

Sr. No.	Variants	Accession Numbers
1.	Wuhan-Ref	YP_009724390
2.	Alpha	QWP89177.1
3.	Beta	QWP89165.1
4.	Gamma	QVE55277.1
5.	Delta	QWM92219.1
6.	Omicron BA.1	UFO69279.1
7.	Omicron BA.2	UHV00336.1
8.	Omicron BA.3	URN53606.1
9.	Omicron BA.4	UOZ79885.1
10.	Omicron BA.5	UOZ63337.1

2.3. Post-translational Modifications

Post-Translation Modifications (PTM) are important as they affect the functioning of the protein in many ways. Among the PTMs, only two types of glycosylation (O-Glycosylation and N-Glycosylation), acetylation, and phosphorylation were studied using different tools. For glycosylation, online servers DictyOGlyc-1.1 [31] and NetNGlyc-1.0 [32] were used. NetPhos-3.1 server predicts threonine, tyrosine, or serine phosphorylation sites [33,34].

2.4. 3D Structure Building and Optimization

The structures of the RBD regions of the S-protein of Wuhan-ref and all its variants were built using the SWISS-MODEL server, an online web server used for building and predicting protein structures [35]. The structures obtained were then evaluated utilizing the 'Structure Validation Server' (https://saves.mbi.ucla.edu/). This server consists of PROCHECK, used for the accuracy of stereochemical parameters including G factor, conformational angles, and bond length.

The structure of Human ACE2 (PDB ID: 6M0J, chain A) was accessed through the PDB server (https://www.rcsb.org/) [36], which was downloaded in PDB format. The energy of the structures was minimized using the Modrefiner server.

2.5. Protein-Protein Docking and Superimposition of Structures

The structures of S-protein of all VOCs were docked with human ACE2 receptor 6M0J: chain A. Cluspro 2.0 (https://cluspro.bu.edu/login.php), a docking web server, was used for this step [37]. The energy profiles were evaluated using electrostatic and Van der Waals interactions (vdW), based on the binding interactions, by applying the equation given below:

E= 0.40Erep + -0.10Eatt + 600Eelec + 1.00EDARS

For each protein, lower energy models were selected and subjected to docking analysis with the RBD region of the S-protein. For visualization, Biovia Discovery Studio 2021 was used for the obtained models [38].

YASARA visualization software and YASARA MUSTANG (Multiple Structural Alignments) method were used to superimpose S-protein structures for the analysis of the difference in structures between Wuhan-ref and all VOCs [39].

3. RESULTS

3.1. Retrieval of Sequences and Identification of Mutations

The retrieved sequences for all variants were analyzed to identify the mutations using alignment. The mutations in each variant were identified and listed in ***Table S1.***

3.2. Immune Evasion Capability of the Variants

BepiPred predicted B-cell epitopes for the S-protein of all the variants and Wuhan-ref. The antigenic potential of the B-cell epitopes was then predicted using VaxiJen scores, with a threshold of 0.5. Most epitopes have VaxiJen scores greater than 0.5, indicating their antigenic potential. TepiTool predicted Class I and II MHC epitopes for selected proteins using IC50 value <500nM [40], resulting in the prediction of over 1000 epitopes. Epitope regions predicted by TepiTool were selected to cover the mutations and compared to Wuhan-ref. Table 2 shows the VaxiJen score of selected B cell epitopes (Five Main Epitopes (13-37, 65-80, 204-219, 436-465 and 469-496) were taken as reference for all the epitopes), while Table 3 and 4 show IC50 values of selected MHC-I and II epitopes. Graphical representations for these values are presented in Figures 2a, 2b, and 2c.

Table 2. B-Cell Epitopes of All the Variants Along with Their Vaxijen Score

Variants	13-37	65-80	204-219	436-465	469-496
Wuhan	0.7515		0.627	0.9437	0.5612
Alpha	0.686		0.689	0.9437	
Beta	0.8281		0.689	0.9437	
Gamma	1.2315		0.689	0.7538	
Delta	0.6013		0.661		
BA.1	0.7515		0.689	0.3225	0.5757
BA.2	0.4529		0.4578	0.3404	
BA.4		0.374		0.3173	1.3952
BA.5		0.374		0.3173	1.3952

Table 3. MHC I Epitopes of the Variants and Their IC50 Values

Variants	19-27	67-77	209-220	433-441	478-489
Wuhan	9.76	54.93	160.25	412.37	346.47
Alpha	9.76	223.05	160.25	412.37	346.47
Beta	9.76	54.93	174.51	412.37	226.26
Gamma	240.16	54.93	160.25	412.37	226.26
Delta	2.6	54.93		412.37	346.47
BA.1	9.76	341.75		467.41	430.99
BA.2	435.62	54.93		467.41	430.99
BA.4	435.62	223.05		467.41	98.45
BA.5	435.62	337.5		467.41	98.45

Table 4. MHC II Epitopes of the Variants and Their IC50 Values

Variants	13-37	65-80	204-219	436-465	469-496
Wuhan	0.7515		0.627	0.9437	0.5612
Alpha	0.686		0.689	0.9437	
Beta	0.8281		0.689	0.9437	
Gamma	1.2315		0.689	0.7538	
Delta	0.6013		0.661		
BA.1	0.7515		0.689	0.3225	0.5757
BA.2	0.4529		0.4578	0.3404	
BA.4		0.374		0.3173	1.3952
BA.5		0.374		0.3173	1.3952

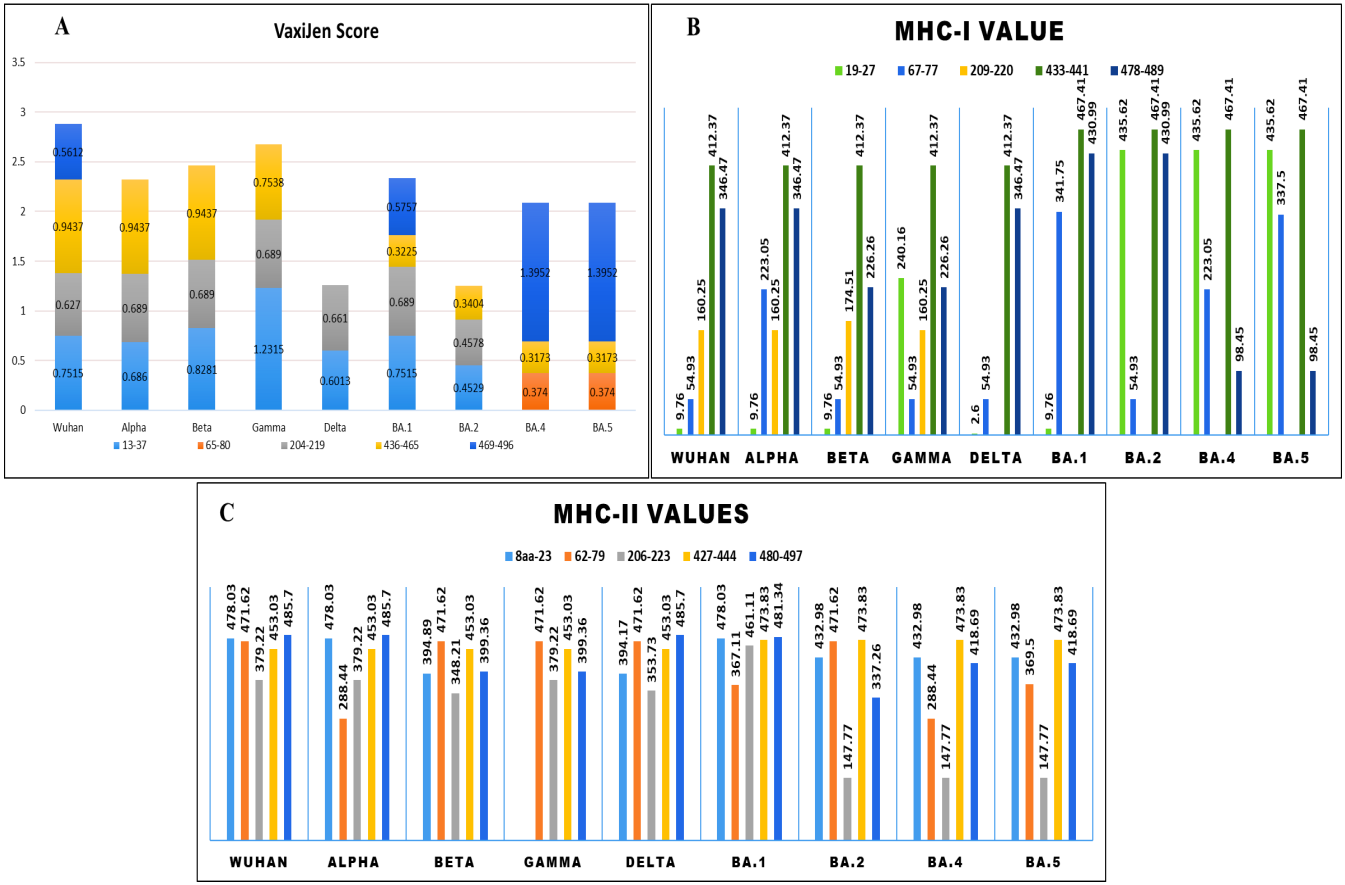


Figure 2. Immunogenic Values of Mutant Epitopes. **A.** VaxiJen Scores for the B-Cell Epitopes of All Variants. **B.** IC50 Values of MHC-I Epitopes of All Variants. **C.** IC50 Values of MHC-II epitopes of All Variants.

In Figure 2a if a score is greater than the threshold of 0.5, it indicates that the epitopes are likely to be antigens. While, the scores below the threshold indicate that the variants are potentially able to evade immune mechanisms. In 2b a higher IC50 value indicates that a greater concentration of epitope is needed to induce an immune response. The graph shows that the variants have a higher IC50 value, indicating their immune evasion capability. In 2c a higher IC50 value indicates that a greater concentration of epitope is needed to induce an immune response. The graph shows that the variants have a higher IC50 value, indicating their immune evasion capability.

3.3. Immune Response using C-IMMSIM

C-IMMSIM was used to measure the levels of immune response, including IgM, IgG, and cytokines after viral infection was induced for all S-proteins. The levels of IgM and IgG were calculated after the induction of viral load. It was found that the levels of IgG and IgM on the 15th day after the induction of the viral load were lower for Wuhan-ref and higher for Omicron and its sub-variants.

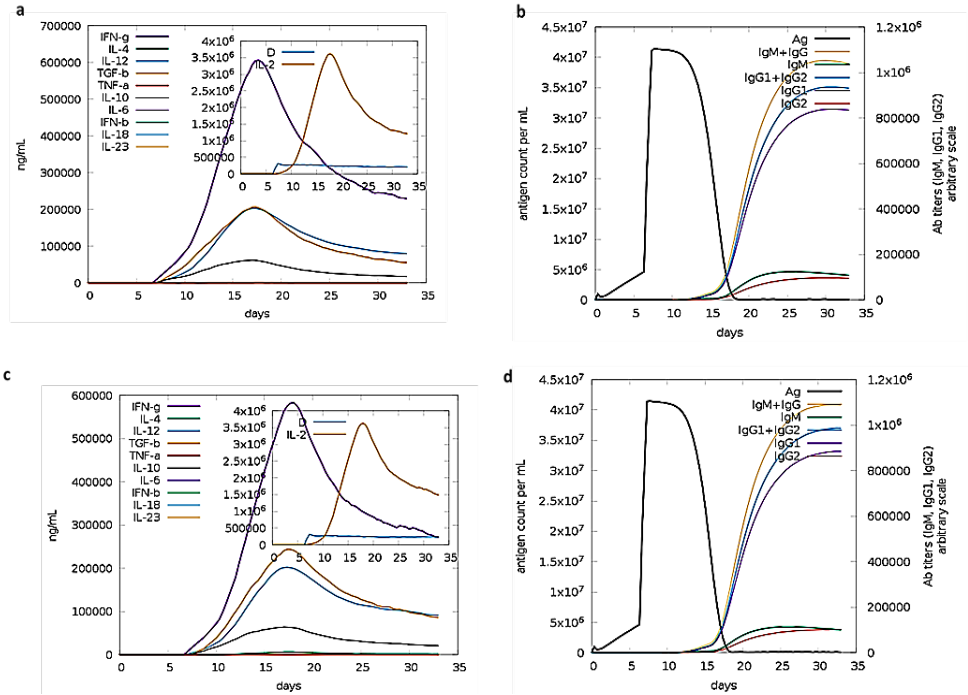


Figure 3. Simulated Immune Responses

Figure3a shows the level of cytokines after the induction of viral load of Wuhan-ref. Figure 2b shows the levels of IgG and IgM after the induction of viral load of Wuhan-ref. Figure 2c shows the levels of cytokines that are released after the induction of the viral load of the BA.1 variant. Figure 2d shows the levels of IgG and IgM after the induction of the viral load of the BA.1 variant.

Comparing the values of all variants and Wuhan-ref, it was observed that the levels of cytokines, preferably IFN-γ, were lower for some variants. Specifically, the levels were comparatively lower for Omicron than for Wuhan-ref [Figure 3]. Omicron variants are less severe as compared to other variants (including the Wuhan-ref) and the obtained values could explain the less severe symptoms of Omicron. Although the difference is not significant, it still highlights many important aspects, such as the severity and transmissibility of the emerging variants. Severity is an important factor for diseases that spread widely and the severity of COVID-19 may be linked to cytokine levels produced during the infection. So, we hypothesize that lower levels of IFN-γ for Omicron might be the reason for it being less severe than other variants. Whereas, higher levels of IFN-γ lead to a higher expression of hACE2 receptors, increasing viral load as well as the severity of the disease.

3.4. Overall Influence of Post-Translational Modifications (PTMs)

Post-Translational Modifications (PTMs) are important as they affect the functioning of the protein. The mutations identified were also assessed for PTMs in order to identify whether they have some influence on the pathogenesis and evasion capability of the virus. Two types of glycosylation (O-Glycosylation and N-Glycosylation), acetylation, and phosphorylation were studied using different tools. The overall result is compiled in Table S2 which shows that O-Glycosylation is present in the variants. Glycosylation contributes to viral amplification including viral receptor binding inside the cell and helps viruses connect to the receptors and infect immune cells. So, its presence may contribute to increased virulence and immune evasion in the variants. Hence, the results indicate that these PTMs in Alpha, Delta and BA.1 may contribute towards their increased virulence.

3.5. Structure Building

The structures for all the variants and Wuhan-Ref S-protein were obtained using homology modeling and various parameters were checked

to ensure quality structures. The selected structures were based on their QMEAN values and the models were refined before being used for further analysis. ***Table S3*** provides information about the structure validation values of the Ramachandran plot obtained from PROCHECK and for structure sequence compatibility through VERIFY 3D, all of which confirmed that the structures were of high quality.

3.6. Effect of Mutations on Spike-hACE2 Interaction

To observe the effects of mutations in the RBD regions of the variants, a superimposition between variants and Wuhan-ref was carried out and the RMSD values were noted. The RMSD differences for all the variants were significant, with 0.997Å for Alpha, 1.034 Å for Beta, 0.811 Å for Gamma, 1.416 Å for Delta, 1.021 Å for BA.1, 1.081 Å for BA.2, 0.873 Å for BA.4, and 1.156 Å for BA.5, indicating structural variation [Figure 4].

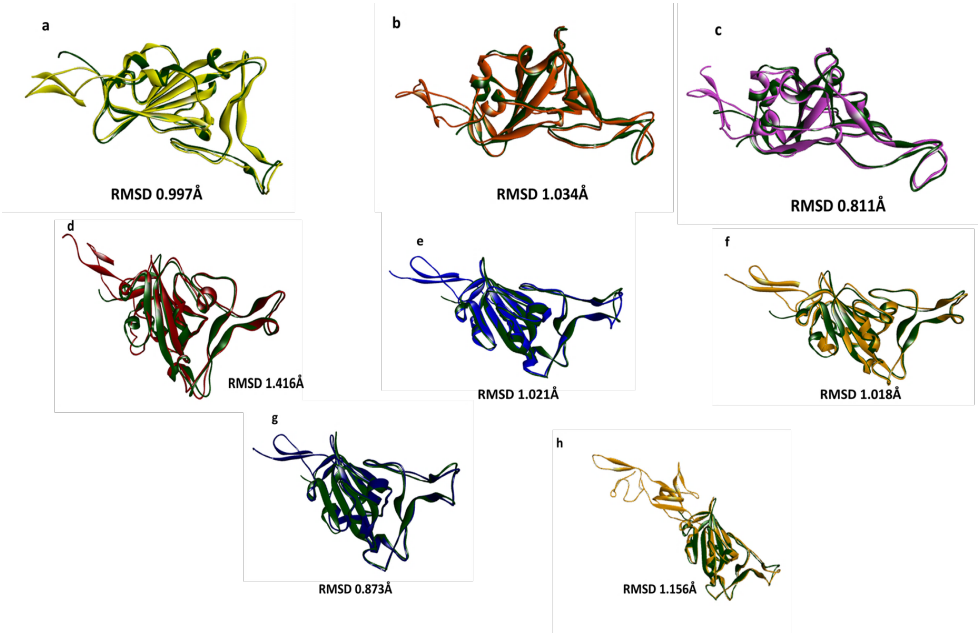


Figure 4. Superimposed Structures of Wuhan-ref (green) and **a)** Alpha (yellow) **b)** Beta (orange) **c)** Gamma (purple) **d)** Delta (red) **e)** BA.1 (light blue) **f)** BA.2 **g)** BA.4 **h)** BA.5

The predicted structure of S-protein for all variants and Wuhan-ref were then docked with the hACE2 and the results were analyzed with Biovia Discovery Visualization studio. It was observed that the mutated regions of

the variants formed a stronger bond with hACE2 than the wild-type S-protein. In comparison to Wuhan-ref, which formed only nine hydrogen bonds, Omicron BA.1 and BA.2 were found to have a higher number of interactions with 15 and 14 interactions, respectively. These interactions involved various amino acids, such as ASN414, ASN474, LYS475, ARG490, SER493, and TYR498 [Figure 5].

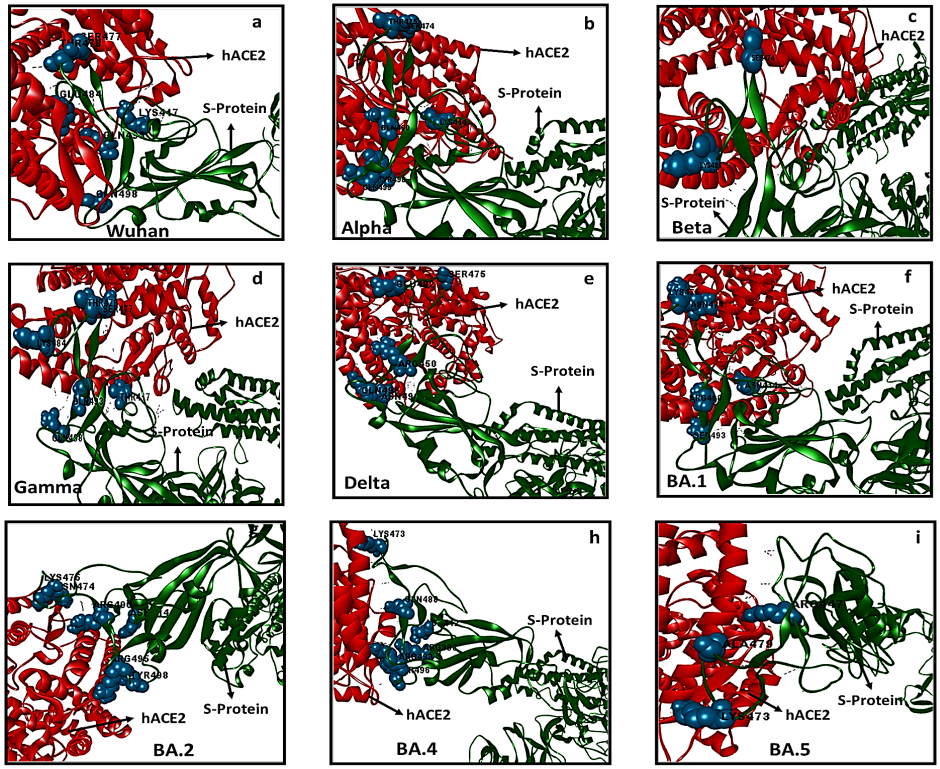


Figure 5. Depiction of 3D Docked Structures of **a)** Wuhan-ref with hACE2 **b)** Alpha with hACE2 **c)** Beta with hACE2 **d)** Gamma with hACE2 **e)** Delta with hACE2 **f)** BA.1 with hACE2 **g)** BA.2 with hACE2 **h)** BA.4 with hACE2 **i)** BA.5 with hACE2

4. DISCUSSION

SARS-CoV-2 emerged in Wuhan, a city in the Hubei province of China, back in December 2019 and spread to the whole world by May 2020 [2]. Despite ongoing efforts to control it, the virus continues to produce new variants, likely due to its error-prone replication and immune evasion

mechanisms. Mutations in the S-protein of these variants have been reported to cause conformational changes, contributing to viral pathogenicity and infection severity [10]. This study focused on the effects of mutations on the immune evasive, severity, and infectivity potential of different variants.

TepiTool and BepiPred were employed to study the immune evasion potential of SARS-CoV-2 variants. The analysis showed that the variants are immune evasive, as evidenced by their higher IC50 values for T-cell epitopes and lower VaxiJen scores for B-cell epitopes. We specifically targeted the NTD regions and found that they have higher IC50 values for MHC-I and MHC-II, which indicates their capability of immune evasion. Previous studies by Andreano, E., et al. and McCarthy, K.R., et al. also demonstrated that mutations in the NTD region of the S-protein play a crucial role in immune escape, especially the deleted residues of the regions [41,42].

In addition to exploring immune evasion, the C-IMMSIM server was used to examine the severity of different variants. The virus can either cause asymptomatic infections or could result in varying symptoms and severity, potentially affecting multiple organs (nervous system, cardiovascular system, gastrointestinal system, and most importantly, the respiratory system). Factors contributing to clinical presentation include host-specific factors, including age, gender, underlying health conditions, vaccination, and previous infection history [43,44]. The characteristics and mutations in the virus can impact the severity of the disease. Variants with different point mutations have been reported to impact disease severity and clinical presentation [45]. Viral evolution across the globe in diverse geographical regions over time has resulted in diverse clinical presentations and severity. This strongly suggests the potential role of host immunity not only acting as the selective pressure for viral evolution but also dictating the manifestation of the infection.

When a new variant emerges after a large segment of the population has developed immunity through vaccination or previous infection, it may appear less severe not necessarily because of the mutations in the virus but due to host immunity protecting against the disease severity. The mutations in the virus can also affect how the disease presents by changing the tissues it tends to infect or by increasing its virulence [46].

For example, variants preferentially infecting upper respiratory tract may cause predominantly mild mucosal symptoms, while the variants targeting alveoli are more likely to cause pneumonia and ARDS. Consistent with this distinction, sore throat was prominent during the Omicron wave, whereas loss of smell was more characteristic of the Delta variant [45]. Omicron may employ a distinct host adoption strategy as compared with delta and earlier variants. Alternatively, pre-existing host immunity may cause limited lower respiratory tract involvement and reduced incidence of pneumonia.

Some variants may have a preference for the respiratory system, leading to symptoms like cough and sore throat, while others may target the gastrointestinal tract, causing symptoms such as nausea and diarrhea. Spike mutations in the RBD and NTD can confer partial escape from neutralizing antibodies and humoral immunity, potentially increasing reinfections and breakthrough infections as observed with Omicron [47]. Although these mutations are less likely to evade cellular immunity, the virus can modulate MHC-I expression, potentially impairing T-cell responses [48]. Viral mutations may also affect host interferon responses and innate immunity [49]. Thus, such mechanisms may enhance immune evasion and influence the clinical phenotype and severity of infection in populations with pre-existing immunity.

The findings suggest that IFN-γ levels in Omicron and its sub-variants have lower values and are less severe. We hypothesize that the Omicron variant of SARS-CoV-2 causes milder symptoms than other variants because it triggers lower levels of cytokines, especially IFN-γ, after the virus infects cells [50]. IFN-γ has numerous functions, from stimulating macrophages to enhancing the levels of antigen-presenting cells and immunoglobins [51], and activating signaling pathways like JAK/STAT. IFN-γ, along with other chemokines and cytokines, helps to regulate the activation of STAT1 through serine phosphorylation [52]. When it comes to diseases that spread widely, such as SARS-CoV-2, severity becomes an important factor. In early COVID-19 cases, high levels of cytokines were associated with more severe symptoms, as they promoted inflammation and reduced the production of IFN-γ by CD4+ T cells.

The S-protein plays a crucial role in the entry of the virus into host cells. Further, its evolution into different variants is linked to stronger interaction with the human ACE2 receptor. This study examined the impact of

mutations in the S-protein on its interaction with hACE2 using ClusPro 2.0. The results indicated that its binding affinity with hACE2 increases as the number of mutations in variants increases. For example, the Alpha variant with N501Y mutation forms 11 hydrogen bonds, with one specifically at position 501. This mutation was found in interactions with hACE2 receptors more frequently than others. The highest number of hydrogen bonds were observed in Omicron BA.1 and BA.2, with 15 and 14 bonds, respectively. Specifically, in BA.1, 4 bonds were observed at Asn477 and 3 bonds at Lys478. While, in BA.2, 3 bonds were observed at Asn477 and 2 at Lys478, respectively.

Previous studies showed that certain mutations facilitate the Spike-hACE2 binding capability. Liu, H., et al reported that mutant RBD, having tyrosine (Y) at position 501, showed a 10-fold increase in binding affinity towards the hACE2 receptor due to additional hydrogen bonds [53]. Another study showed that the mutant N501Y increased binding affinity, while D614G decreased it [54]. N439K, S477N, and T478K also showed a higher binding affinity towards the hACE2 receptor. This was concluded through a molecular docking study, which showed extra salt bridges, hydrogen bonds, and non-bond contacts between the mutant S-protein and hACE2 receptor. Thus, giving a reason for higher binding affinity [55]. The increased binding affinity of these variants was attributed to deviations from the Wuhan-ref RBD region. Furthermore, prior research demonstrated a substantial correlation between RBD stability and affinity, with mutations that boost structural stability and rigidity, coinciding with increases in binding affinity [55].

4.1. Conclusion

This study applied an *in silico* approach to predict the structural characteristics of the S-protein of the emerging SARS-CoV-2 variants and assessed the potential impact on severity, immune evasion, and interaction with the hACE2 receptor. The analyses indicated that mutations within the S-protein are associated with altered T-cell and B-cell epitope characteristics (higher predicted T-cell epitope IC50 values and lower VaxiJen antigenicity scores), suggesting a potential reduction in immune recognition and an immune-evasive tendency. In addition, the predicted reduction in levels of IFN-γ observed for some variants may indicate altered immune responses; however, these computational predictions should not be interpreted as direct evidence of reduced disease severity or altered clinical

outcomes. Structural analyses further suggested that insertions and deletions in certain variants may influence the 3D conformation of the RBD. The observed differences in predicted RBD–hACE2 interactions and structural deviation from the Wuhan reference sequence suggest that these mutations may influence receptor-binding properties and could potentially contribute to altered viral entry. However, an increased predicted receptor interaction does not by itself establish increased infectivity or transmissibility. Overall, the findings provide computational evidence for potential differences in immune recognition and hACE2-binding characteristics among emerging SARS-CoV-2 variants and generate hypotheses regarding mechanisms that may contribute to their biological behavior. Since this study was exclusively based on *in silico* analyses, the predicted effects on immune evasion, receptor binding, viral infectivity, transmissibility, and disease severity should be interpreted cautiously and require validation through experimental, epidemiological, and clinical studies.

Author Contribution

Nayyer Saddique: data curation, methodology, investigation, writing - original draft, visualization. **Ishaq N. Khan:** supervision. **Jalal Ahmed:** methodology. **Hafsah Muhammad:** supervision. **Muhammad Ibrahim Rashid:** conceptualization, supervision, writing - review & editing.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request

Funding Details

No funding has been received for this research.

Generative AI Disclosure Statement

The authors did not use any type of generative artificial intelligence software for this research except for the graphical abstract.

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