

# SARS-CoV-2 Mutations and Their Impacts on Virulence and Transmissibility of The Virus

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## ARTICLE INFO

### Article History:

Received: July 18, 2023

Accepted: January 04, 2024

ePublished: July 23, 2024

### Keywords:

SARS-CoV-2, COVID-19, Mutation, Variant of concern, Omicron

## Abstract

The emergence of SARS-CoV-2 in 2019 and its rapid global spread led to infrequent problems for human society. This vast spreading and high mortality rate were related mainly to the potential of this virus to develop mutated variants. Due to the emergence of hyper mutated strains escaping from vaccine-induced immune responses, the pandemic is still ongoing despite the discovery of various vaccines, and there is little chance that it will be eradicated in the near future. Therefore, it is crucial to monitor covid-19 mutations globally in order to develop new control measures. The SARS-CoV-2 mutation's emergence and molecular characteristics have been the major talking points of this article, with a focus on significant variants classified as variants of concern (VOC).

## Introduction

With the emergence of the novel coronavirus in 2019, the world has faced a new pandemic disease called COVID-19. Due to its resemblance to the coronavirus that causes severe acute respiratory syndrome (SARS-CoV), a new member of the Coronaviridae family has been named SARS-CoV-2 by the International Committee on Taxonomy of Viruses.<sup>1</sup> Similar to other coronaviruses, SARS-CoV-2 is an enveloped positive-sense virus. Fusion of the virus with the host membrane and penetration into the host cell is assisted by the envelope structure.<sup>2</sup> The virus enters the host cell by adhering the viral spike to the receptor of angiotensin-converting enzyme 2 (ACE-2) on the surface of the host cell membrane.<sup>3</sup>

The timeline of SARS-CoV-2 spreading worldwide, divided into parts by CDC. First part was "Late 2019" which the WHO country office in China has received reports of an unidentified cause of pneumonia in Wuhan, China, presenting symptoms such as breathlessness and fever. Second part was in "Early 2020" that the CDC shares information about the outbreak of the 2019 novel coronavirus (2019-nCoV) caused by the SARS CoV-2 virus on its website. By "Late 2020" the second wave of

COVID-19 accrued and in "Early 2021", some countries experienced a third wave of COVID-19 cases. These waves were often associated with the emergence of new variants of the virus, which were more transmissible and sometimes exhibited increased disease severity. As of 22 May 2023, statistics from the WHO website show that SARS-CoV-2 has infected more than 766 million individuals and caused more than 6.9 million fatalities globally.

This virus is distinguished from other coronaviruses by the occurrence of successive mutations, which lead to increased pathogenicity and disease transmission. The effectiveness of vaccines, treatment strategies, and diagnostic methods may be affected by the recent global emergence of novel SARS-CoV-2 mutated variants. In addition, some of these mutations have increased the virus spreading rate and could make it more contagious and deadly.<sup>4</sup> Therefore, it is important to continuously monitor viral mutations and their impact on pathogenicity, virulence, and transmission of the virus.

This article is dedicated to the investigation and follow-up of SARS-CoV-2 mutations and their spread worldwide. Also, effects of mutations on virus structure, immunogenicity, and transmission have been discussed.

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## Materials and Methods

### Structure of SARS-CoV2 spike antigen

The Spike protein contains S1 and S2 domains, in which the S1 region consists of RBD and NTD subdomains. These areas are immunogenic and essential for viral attachment and cell entry.<sup>5-7</sup> Most SARS-CoV-2 vaccines target this region of the S-glycoprotein. Many reported mutations are detected in glycoprotein S and affect the manner in which the virus attaches to host cells.<sup>8</sup> These mutations enhance the likelihood of infection or reinfection, decrease the potency of vaccination, and allow the virus to escape neutralizing antibodies.

### Nomenclature of mutations

SARS-CoV-2 has been classified using various nomenclature systems, including Pango, NextStrain, GISAID, and the Greek alphabet. The Greek and Pangolin letters were used in this manuscript for mentioning different variants. The Pangolin nomenclature, which stands for the Phylogenetic Assignment of Named Global Outbreak Lineages, was created to apply the dynamic naming of SARS-CoV-2 lineages.<sup>9,10</sup>

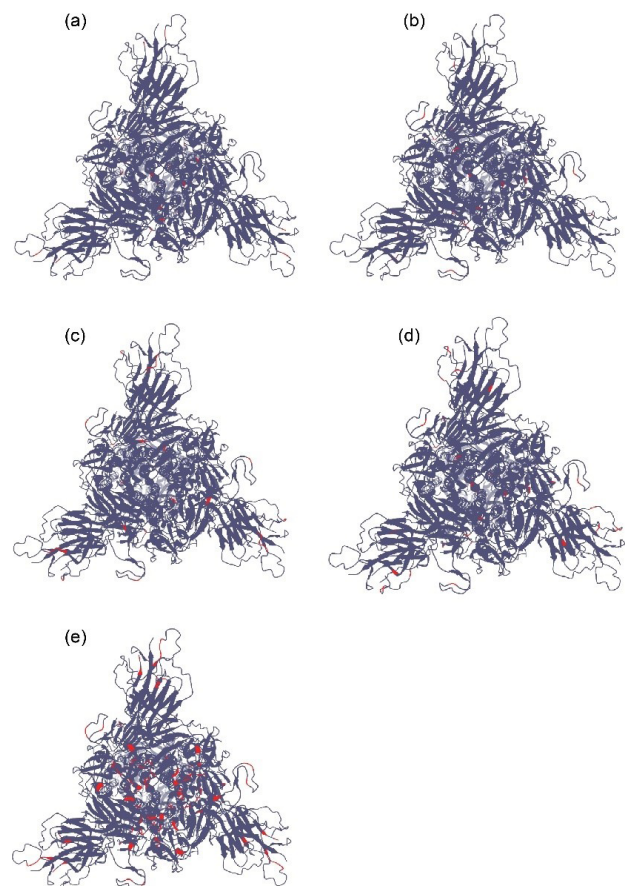
## Results and Discussion

### Origin, emergence, and dissemination of SARS-CoV-2 mutations

According to previous studies, shortly after the initiation of the pandemic, new strains emerged from the primary Wuhan-Hu-1 wild-type variant of SARS-CoV-2, each containing mutations that were correlated with the variant's death rate, transmissibility, immune evasion, and resistance to neutralizing antibodies.

The first important mutant was the *Alpha* (B.1.1.7) (Figure 1a) strain reported in the United Kingdom in December 2020. It then spread worldwide and became the dominant variant in most patients; the WHO classified this mutant as a variant of concern (VOC).<sup>11,12</sup> It has 23 mutations compared with the Wuhan-Hu-1 wild-type strain.<sup>13</sup> By December 2020, *Beta* (B.1.351) (Figure 1b) with 13 mutations had been documented in South Africa.<sup>13</sup> In October 2020, *Delta* (B.1.617.2) (Figure 1c), a new VOC, was reported in India. It is more contagious in comparison with the initial variant of SARS-CoV-2 and spreads very fast worldwide.<sup>13-16</sup> In January 2021, the National Institute of Infectious Diseases of Japan reported a new variant of SARS-CoV-2 isolated from passengers who had traveled to Brazil; thus, the WHO labeled *Gamma* (P.1) (Figure 1d). It contains 17 mutations and is considered VOC.<sup>13,17</sup>

In November 2021, a new variant was reported from South Africa, *Omicron* (B.1.1.529) (Figure 1e), and was considered a new VOC. This new variant has rapidly spread worldwide and has become the dominant variant in many countries. Omicron has approximately 37 mutations in its spike protein in comparison with the initial Wuhan-Hu-1. It is thought that this variant was not derived from former strains and may have a different origin.<sup>18</sup> The transmissibility rate of Omicron was much higher than



**Figure 1.** (a) A 3-dimensional model of the Alpha variant of SARS-CoV-2 (S1 face). Red regions indicate mutated parts in comparison with Wuhan-Hu-1. (b) A 3-dimensional model of the Beta variant of SARS-CoV-2 (S1 face). Red regions indicate mutated parts in comparison with Wuhan-Hu-1. (c) A 3-dimensional model of the Delta variant of SARS-CoV-2 (S1 face). Red regions indicate mutated parts in comparison with Wuhan-Hu-1. (d) A 3-dimensional model of the Gamma variant of SARS-CoV-2 (S1 face). Red regions indicate mutated parts in comparison with Wuhan-Hu-1. (e) A 3-dimensional model of the Omicron variant of SARS-CoV-2 (S1 face). Red regions indicate mutated parts in comparison with Wuhan-Hu-1.<sup>20</sup>

that of other variants, and a higher peak was observed in the infection diagrams for each country.<sup>19</sup>

### Types of mutations

In upper airway epithelial cells, the mutation N501Y enhanced the affinity of viral spike and replication. Based on the work of Liu et al, N501Y in the spike receptor-binding domain (RBD) is thought to lead to more contact with the human ACE2 receptor at K353, raising the receptor's affinity. Using a bio-layer interferometry system, they conducted binding tests utilizing recombinant spike RBD and human ACE2 proteins to verify this. The binding was enhanced by the N501Y substitution. These findings suggest that N501Y increases viral fitness for replication in the upper airway, leading to enhanced transmission via enhanced spike receptor interactions. These results are in line with previous work showing that spike residue 501 is crucial for ACE2 receptor binding.<sup>21</sup>

The ability of serum-neutralizing antibodies to attach to the recombinant virus was affected by E484K. Sonia et al discovered that the E484K mutation reduced the

neutralizing activity of human polyclonal sera produced in convalescent (infected with former strains) and vaccinated patients. These mutations are observed in SARS-CoV-2 varieties that belong to the B.1.351 and P.1 lineages, and they proposed that the single E484K mutation in the RBD influences the binding of serum polyclonal neutralizing antibodies from convalescent and vaccinated donors.<sup>22</sup> Furthermore, Collier et al demonstrated that the recent emergence and dissemination of B.1.1.7 viruses carrying the spike E484K mutation causes a considerable loss of neutralization by BNT162b2 mRNA-elicited antibodies, convalescent sera, and mAbs.<sup>23</sup>

The combination of K417N and N501Y completely eliminated this antibody effect. The compensatory mechanism of Lys417Arg appeared to increase the S1 RBD-ACE2 free energy of binding. This may explain the greater spread of the virus in the UK and South Africa.<sup>24</sup>

New salt bridges and hydrogen bonds may occur as a result of mutations in BA.1.1 (K478), BA.2 (R400, R490, and R495), and BA.3 (R397 and H499). Mutations at Receptor-binding Motif (RBM) residues such as Q493R, N501Y, Q498, T478K, and Y505H in Omicron and sub-variants dramatically increased binding affinity with human ACE2. Relative to other mutations, such as K417N, interactions with Omicron variant mutations at residues 493, 496, 498, and 501 appear to restore ACE2 binding efficiency.<sup>25</sup>

Studies have shown that the D614G substitution may result in a higher spread rate in the sub-variant carrying it. Benton et al have shown that Asp614Gly contributes to promoting the opening conformation in the spike of the SARS-CoV-2 in contrast with wild-type, which facilitates the virus binding to ACE2.<sup>26</sup>

D614G did not affect the production, processing, or integration of S proteins into SARS-CoV-2 particles, as demonstrated by the study by Yurkovetskiy et al, although its affinity for ACE2 was lowered as a result of a higher dissociation rate. Cryo-electron microscopy analysis of the S protein trimer revealed that D614G breaks down interprotomer contact and shifts the structure toward an ACE2 binding-competent state, which is hypothesized to be a mechanism for virion membrane fusion with target cells. The neutralizing effectiveness of the antibodies directed against the S protein RBD was unaffected, which is compatible with this more open configuration.<sup>27</sup>

Liu et al reported that P681R mutation facilitates the virus entry to the cell through a cell-surface mediated entry pathway by enhancing cleavage of the whole spike to S1 and S2 parts. According to their findings, the Pro681Arg mutation is the primary mutation responsible for increasing S1/S2 cleavage and enhancing Delta variant replication.<sup>28</sup>

The P.1 lineage also includes a cluster of substitutions near the antigenic areas of the NTD, including L18F, which is known to decrease neutralization by certain antibodies, in addition to changes at positions 417, 484, and 501, which have already been reported.<sup>29</sup> The NTD

supersite also contains or is close to the substitutions T20N and P26S.<sup>30</sup>

Yamamoto et al highly recommended that the H655Y mutation of Omicron S is responsible for the altered usage of the entry pathways by assisting entry through the cathepsin B/L-dependent endosome pathway and impairing entry through metalloproteinase- and TMPRSS2-dependent cell surface pathways without changing the S1/S2 cleavage status.<sup>31</sup>

Each of these mutations can have specific effects, and their accumulation in a strain can directly affect the virulence of the virus. In Table 1, several important mutations can be seen.<sup>13</sup>

### **SARS-CoV-2 strain categorization based on effects of mutations**

The major SARS-CoV-2 strains are divided into two categories by the WHO: strains that are variants of interest (VOI) and those that are variants of concern (VOC). VOC is a variant for which there is evidence of increased transmissibility, more severe illness (such as an increase in hospitalizations or deaths), a notable decrease in neutralization by antibodies produced during prior infection or vaccination, decreased efficacy of treatments or vaccines, or diagnostic detection failures. Variants of VOI strains have genetic markers linked to altered receptor binding, decreased neutralization by neutralizing antibodies, and a markedly elevated risk of transmission (Table 2).

Regarding the effects of vaccines against new SARS-CoV-2 variants, according to published data, the effectiveness of vaccines against mutant strains is thought to be reduced. Because most mutations occur in the S protein of the virus, and because most vaccines induce immune responses against the S protein, these responses have less effect on new mutations. The same is true for antibodies from previous infections.<sup>29,32,33</sup>

However, the extent of this effect varies depending on the vaccine used. Studies have shown that strains with the E484K mutation are more resistant to the effects of vaccine-induced neutralizing antibodies or in the serum of improved patients.<sup>22</sup>

Strain *Alpha* or *B.1.1.7*, was first documented in the United Kingdom by December 2020.<sup>12</sup> According to studies, it is inferred that the Pfizer BioNTech vaccine has shown good performance against this virus<sup>34-36</sup> Also based on the clinical results of the Oxford AstraZeneca vaccine, despite producing much fewer antibodies (nine folds less), the efficacy is 70.4%. This suggests that even with low levels of antibodies, it can provide good immunity against the virus.<sup>37</sup>

A *beta* variant, *B.1.351*, was first reported in South Africa in December 2020<sup>13</sup> and showed significant resistance to antibodies from Pfizer BioNTech and AstraZeneca vaccines. Mutations N501Y, E484K, and K417N/T significantly reduce the strength of antibodies against the virus, even in people who have been fully

**Table 1.** Mutation location and its effects on the virulence of SARS-CoV-2 VOC strains<sup>16</sup>

Variant	Mutation	Location	Function
B.1.1.7 (Alpha)	N501Y	RBD	Increase the RBD affinity for binding to ACE2 receptor. Increase the virus infectivity, transmissibility, and virulence.
	P681H	S1/S2	Facilitate the entry of the virus into the respiratory epithelial cells and increase its transmissibility
	H69-V70del	NTD	Increase the virus infectivity
B.1.351 (Beta)	N501Y	RBD	Increase the virus transmissibility
	K417N, E484K L18F, D80A, D215G, R246I, LAL 242-244 del	RBD NTD	Increase the virus affinity for binding to receptors and escape from the immune system Decrease the effect of neutralizing antibodies
P.1 (B.1.1.28.1) (Gamma)	L18F, K417T, E484K, D614G	S1	Escape from the immune system and reduce immunity due to antibodies. Increase the transmissibility
B.1.617.2 (Delta)	L452R	RBD	Increase the virus transmissibility and the affinity of binding to the ACE2 receptor. Escape from the immune system and reduce immunity due to antibodies.
	P681R	Furin cleavage site	
	D614G	S1	
B.1.1.529 BA.1.1 BA.2 BA.3 (Omicron)	N501Y	RBD	Increase the virus transmissibility Increase the virus infectivity Increase the virus pathogenicity
	N679K, H655Y, P681H	Furin cleavage site	
	R346K	S1	Increase the virus transmissibility Increase the virus infectivity Increase the virus pathogenicity
	T19I, L24del, P25del, P26del, A27S, V213G, T376A, and R408S R216del		

**Table 2.** Potential effects of mutations on the pathogenicity and transmissibility of SARS-CoV-2 variants.<sup>4</sup>

Strains	Characteristics
VOI B.1.525 (Eta) B.1.526 (Lota) B.1.617.1 (Kappa) C.37 (Lambda) B.1.621 (Mu)	Changes in the receptor binding Reduction of the neutralization of antibodies produced by previous covid-19 infection or vaccine injection Reduction of the therapeutics Potential diagnostic effect The anticipable increase in the transmissibility and disease severity Prevalence with a limited spread in the USA or other countries
VOC B.1.1.7 (Alpha) B.1.351 (Beta) P.1 (Gamma) B.1.617.2 (Delta) B.1.1.529 (Omicron)	Increasing transmissibility More severe disease Significant reduction in neutralization by neutralizing antibodies due to vaccine injection or previous infection Reduction in the effectiveness of therapeutics and vaccines Error in diagnosis and vast cross-reaction in targets of diagnostic tests Evidence for reducing vaccines effect in protection against severe disease

vaccinated.<sup>13</sup> The neutralizing effect of antibodies produced by Pfizer BioNTech against this variant was reduced by up to 6.5-folds.<sup>38</sup> The neutralizing effect of the antibodies against 1.351.B decreased by approximately 16-fold in comparison to B.1.1.7.<sup>16</sup>

In the *Gamma* or *P.1* variant, which was reported from Brazil/Japan by Jan-2021 for the first time, due to more mutations in the spike region, it seems to be more resistant to existing vaccines than B.1.351. According to laboratory studies, the performance of these two strains against the Pfizer vaccine is almost the same.<sup>38,39</sup> It can be understood that vaccines do not provide complete protection against these strains, but they are not entirely ineffective.

Regarding the *Delta* variant or *B.1.617.2*, which first emerged in India by Oct-2020, there was a noticeable decrease in the titer of neutralizing antibodies caused by Pfizer vaccine injection in the recipients in comparison with other variants<sup>39</sup>. This reduction is approximately 2.5-fold in people receiving Pfizer and 4.3-fold in those receiving AstraZeneca.<sup>40</sup> In addition, there was a three-fold reduction in the neutralizing antibody titers against B.1.617.2 relative to B.1.1.7.<sup>16</sup>

The situation is slightly different for the *Omicron* variant and *B.1.1.529*. It was first reported in South Africa and soon spread worldwide, causing the return of restrictions on COVID-19 in some countries. Omicron has more mutations than previous variants, leading WHO to place it in the VOC group.<sup>18</sup> Based on clinical observations and its rapid dissemination, Omicron appeared to have a high rate of spread. However, it is not yet clear whether, like Delta, it will lead to an increase in ICU cases.

Sub-lineages of Omicron include BA.1, BA.2, BA.4, and BA.5; the last two, BA.4, and BA.5 become the dominant variants. These two strains have been reported to be more transmissible and resistant to immunity generated by prior variants. The new sub-lineages have more than 30 new mutations that alter the omicron's pathogenicity, infectivity, and transmissibility due to alterations in the omicron spike structure in comparison with the primary strain of Omicron.<sup>25,41</sup>

Studies show that vaccine efficacy decreases 8-127 times due to these mutations as compared to the wild type of SARS-CoV-2, but the administration of booster shots improves this reduction to 12-35 folds. Studies have



revealed that even after three doses of an mRNA vaccine, the efficiency of vaccine-induced immunity against Omicron is only approximately 66.3%, which is less than 85% against the Delta version. Additionally, the more recent subvariants BA.4 and BA.5, significantly evaded neutralizing antibodies produced by both infection and immunization.<sup>41</sup>

Reports from South Africa indicate that the rates of omicron hospitalization are lower than those of delta hospitalization. This risk of hospitalization was approximately 29% lower than that of the other variants. Because of mutations in the S1 region, it seems to be less sensitive to monoclonal neutralizing antibodies in the serum.<sup>42</sup>

An animal study on macaques has shown that injecting booster doses with a specific Omicron Moderna vaccine against the Omicron variant does not provide higher safety and protection than the Moderna vaccine against the primary covid-19 virus.<sup>43</sup>

As reported by the CDC, Omicron sublineages, including three variants BQ.1, BQ.1.1, and XBB.1.5, of which BQ.1 and BQ.1.1, have recently been dominant in the USA. CDC data showed that these variants spread relatively quickly. In addition, it has recently been reported that approximately 49 percent of circulating variants are XBB.1.5.<sup>44</sup>

Despite the variants containing *N679K*, *H655Y*, and *P681H* mutations in the furin cleavage region that appear more infectious,<sup>45</sup> research has shown that these mutations weaken spike protein cleavage and reduce virus entry and proliferation in the alveolar cells of the lung that express *TMPRSS2*. This condition also causes weaker cell-cell connections in lung cells.<sup>46</sup>

The virus uses two pathways to enter the cells: the pathway that requires *TMPRSS2* and *ACE2*, and the pathway that requires only *ACE2*. Therefore, Omicron species use the *ACE2* pathway. *TMPRSS2* is more abundant in the cells of the lower airways, but the Omicron spike is less able to bind to *TMPRSS2* because of the mutations mentioned above, unlike the delta variant.<sup>46,47</sup>

In addition, the entry of Omicron into the cell was not inhibited by drugs targeting *TMPRSS2*. Studies on anti-Omicron antiviral therapies have shown that REGN2 cocktails have virtually no effect against Omicron in vitro, but other studies have shown that molnupiravir and remdesivir retain their effects.<sup>46</sup>

Some studies have reported the formation of new chemical bonds (salt bridges and hydrogen bonds) between RBD and *ACE2* in the Omicron region, and these interactions compensate for the reduced binding affinity of RBD to *ACE2* caused by mutations such as K417N. The omicron genome sequence analysis suggested that Omicron might not be derived from the currently circulating variants of SARS-CoV-2, and it might have a different origin.<sup>18,46</sup>

Studies on the protective efficacy of different vaccines showed that the Sinopharm vaccine demonstrated low

efficacy against Omicron, even after the third shot. In a study, Yu et al reported that virus neutralization by the serum of Sinopharm vaccine recipients after the third dose was effective for the wild-type variant but decreased about 20 times against the Omicron variant.<sup>48</sup>

A large study from the United States found that during the predominance of the Omicron variant, the incidence of hospitalization for covid-19 in non-vaccinated individuals was 23 times higher than that in those fully vaccinated with booster doses. This amount was 5.3 times higher in comparison with vaccinated people without a booster dose. In addition, the incidence of COVID-19 in non-vaccinated individuals was 3.6 and 2 times higher than that in vaccinated individuals with and without the booster, respectively.<sup>49</sup>

Perez-Then E, reported that injection of one dose of Pfizer as a booster to individuals that had received two shots of inactivated vaccine CronaVac leads to increasing neutralizing antibody against Delta and ancestral variant which was similar to the response of individuals who received two shot mRNA vaccine.<sup>50</sup>

However, based on this article's data, the previous booster (2x CronaVac vaccine) was unable to induce sufficient antibodies against Omicron; however, after taking one booster of Pfizer, it reached a detectable level in recipients.<sup>50</sup>

In addition, prior infection in individuals who received 2x CronaVac followed by 1x Pfizer as a booster had no effect on the amount of neutralizing antibodies against Omicron, whereas, in 2x mRNA recipients, prior infection elevated NA significantly.<sup>50</sup>

Ongoing research studies pertaining to COVID-19 and SARS-CoV-2 are currently underway, with the potential to modify our current understanding of the subject matter. As a result, it is important to acknowledge that the information presented in this article is subject to revision as new findings emerge.

## Conclusion

Studies have shown that some mutations play an important role in escaping the virus from the immune system or neutralizing antibodies, more effectively bonding with host cells, or affecting the spreading rate of the virus. The consequences of these changes show themselves in reinfection shortly after individuals are cured, or also in a massive increase in patient numbers.

The results of these studies revealed that the effect of vaccines and neutralizing antibodies in people administered two doses of vaccine is significantly reduced against the Omicron variant, but vaccines are not completely ineffective. According to published reports, a third dose of the vaccine improves the titer of antibodies against this variant. Based on published reports, among the different vaccines evaluated, inactivated vaccines such as CoronaVac Sinopharm showed poorer performance than other vaccines even after a booster dose, whereas mRNA vaccines have shown higher efficiency in different trials.

Also, as a study in China has stated that by prioritizing the administration of booster vaccines to high-risk individuals who have already been vaccinated, not only can their own protection be ensured, but also the potential transmission of the virus to other vulnerable groups can be effectively mitigated.<sup>51</sup>

In general, mutations alter virus behavior, and their accumulation in a variant may lead to different impacts on its transmissibility, virulence, immune escape, and resistance to vaccines, resulting in an increase in the number of patients and the severity of their illness; hence, permanent surveillance of virus genome mutations and new variants is very important in adopting important policies of governments and treatment systems to properly control the virus epidemic and to recognize and predict its possible behavior against vaccines and common treatment methods, as well as to improve these treatments and policies in the future.

#### Acknowledgments

The authors would like to thank the Biotechnology Research Center, Tabriz University of Medical Sciences, for supporting this work (Grant no. 68941).

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#### Competing Interests

The authors declare that they have no conflicts of interest.

#### Consent for Publication

Not applicable.

#### Data Availability Statement

Not applicable.

#### Ethical approval

This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.008).

#### Funding

This work was supported by the Biotechnology Research Center, Tabriz University of Medical Sciences (Grant no. 68941).

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