Impact of B.1.617 and RBD SARS-CoV-2 Variants on Vaccine Efficacy: An In-silico Approach

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Abstract

The existing panels of COVID-19 vaccines are based on the spike protein of earlier SARS-CoV-2 strain that emerged in Wuhan, China. However, the evolving nature of SARS-CoV-2 has resulted in emergence of new variants, thereby, posing a greater challenge in the management of the disease. India faced a deadlier second wave of infections very recently and genomic surveillance revealed that B.1.617 variant and its sub lineages are responsible for majority of the cases. These are highly infectious and possibly more lethal and therefore labelled as variants of concern by WHO. Hence, it's crucial to determine if the current vaccines available can be effective against these variants. To address this, we performed molecular dynamics (MD) simulation on B.1.617 along with K417G variants and other RBD variants. We studied structural alteration of the spike protein and factors affecting antibody neutralization and immune escape. We found in seven of the 12 variants studied, there was a structural alteration in RBD region further affecting its stability and function. Docking analysis of RBD variants and wild type strain demonstrated increase in binding affinity with ACE2 (angiotensin 2 altered enzymes) receptor in these variants. Molecular interaction with CR3022 antibody revealed that binding affinity was less in comparison to wild type, with B.1.617 showing the least binding affinity. These findings from the extensive simulations provides novel mechanistic insights on the conformational dynamics and improves our understanding of the enhanced properties of these variants in terms of infectivity, transmissibility, neutralization potential, virulence and host-viral replication fitness.

1. Introduction

COVID-19, a serious and continuously spreading pandemic affecting the world, creates severe ailments and apparently everlasting health problems. While Monoclonal antibody (mAb) therapy has recently gained approval for emergency use; a few vaccines have exhibited potential & defensive effects upon COVID-19, mostly targeting the trimeric spike glycoprotein, which is involved in host cell interaction and gives passage to cell entry as well as the essential target for neutralizing antibodies. Essentially those were aimed against the earlier SARS-CoV-2 strain that emerged in 2019 in Wuhan China^{1,2}. Due to the perceived ease of transmission and expansive mutations in spike proteins, the speedy evolution of new variants of SARS-CoV-2 is of high concern. The data of daily new cases of Covid-19 suggests that the virus is spreading more quickly during the second wave in India. According to recent reports by the BBC, Hindustan Times, and Irish Times, several reports of feasible re-infection have been depicted in recent months in 2021. Globally, deaths due to Covid-19 are rising again, on average around 12,000 per day, and new cases are also increasing, excelling at 700,000 per day. In Brazil, where there are about 3,000 deaths per day, or in recent weeks, a quarter of the world's lives were lost. The situation is equally grim in India, where cases are again taking a quick surge after the weeks of February. The Asian nation has disclosed > 14 million cases of Covid-19 and over 174,300 deaths so far. A new variant with two changes - E484Q and L452R, (B.1.617) has been reported in India. India is returning to a deadlier new wave of infections after reporting a B.1.617, making it the second most affected country in the world, surpassing Brazil. Among the sub-lineages of B.1.617.2 (*akadelta*) variant, 50% more transmissible than B1.117 has shown

to drive majority of the cases in the country and also likely shows immune-evasion properties. According to Times of India, The New Indian Express and The Wire news reports several cases of post-vaccination re-infection have also been observed in some parts of the country. This brings us to the question, is there any chance that the re-infection is caused by the new variant? The answer is yet to be explored. This new variant was found in > 200 samples from Maharashtra only, which became the first hot spot region during the second national wave that started in the last few months. By 7 April, Maharashtra alone accounted for more than half of new cases in the country, convincing experts to doubt that the variant might be blamed, however more sequencing data from across the country is required for the validation.

According to the Irish Times, in India, over 180,000 new infections occurred in a 24-hour period in the previous week, taking the total number of cases to over 13.9 million. There is still a great need to explore the functional aspects or potential efficacy of currently available vaccine therapy against SARS-CoV-2 variants. For better management of this second wave, we need to re-evaluate the threshold efficacy of the vaccine explore the functional aspects or potential efficacy of vaccine therapy currently available, on the B.1.617, and thereby look for strategic changes for the better efficacious vaccine in the future. It was noted that several mutations of the receptor-binding domain (RBD), are essential for the interaction of Human angiotensin 2 altered enzymes (ACE2)³ and antibodies, as well as region that neutralizes antibodies. It is therefore imperative to understand up to what extent the recently emerged SARS-CoV-2 variants affect the current vaccination program. The RBD can exist in two conformities, alluded to as "up" and "down" i.e. receptor accessible and receptor in-accessible⁴. The *in-silico* investigation revealed that ACE2 and potential antibodies bind in a similar area on the spike protein^{5,6}. An antibody becomes very effective when forestalling viral spread by impeding the ACE2 binding site in the RBD. CR3022 antibody showed the most elevated binding affinity with SARS-CoV-2 protein RBD^{7,8}.

Here, in this study, we retrieved 28 different spike protein variants, and out of these 28 variants, 12 variants belong to the RBD region only. Here, we focused on RBD variants that affect the ability of CR3022 and ACE2 to bind with the SARS-CoV-2 RBD and used molecular dynamics (MD) simulations to understand the conformational dynamics.

2. Material And Methods

2.1 Retrieval of crystal structures

Crystal structures of spike protein (PDBID-7AD1), ACE2 (PDBID-6ACG) and antibody CR3022 (PDBID 6YLA) were retrieved from PDB RCSB (<https://www.rcsb.org/>). All water molecules and hetero-atoms were removed by using Discovery studio visualization software (BIOVIA 2020).

(<http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php>).

2.2 Homology modeling and Energy minimization

Based on high similarity, 7AD1 (crystal structure of SARS-CoV-2) was selected as template for homology modeling of RBD mutant variants using the SWISS-MODEL⁹. Energy minimization and structural analysis of RBD mutant variants were done with UCSF Chimera¹⁰. Evaluation of the modeled structure was done by PDB-Sum (<http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html>).

2.3 Docking analysis

Docking of RBD mutant variants with selected targets (ACE2 receptor and antibody structure CR3022) was carried out by PatchDock server¹¹ by choosing parameter RMSD esteem 4.0 and complex type as default. Docking investigation was based on geometric shape complementarities score. Higher score indicates higher binding affinity. Outcome of the results is based on the docking scores and interaction at the RBD regions. Protein-protein and antibody-protein interactions were visualized by LigPlot plus v2.2¹².

Molecular interactions of antibody CR3022 and ACE2 receptor with RBD variants were performed by antibody script under antibody loop numbering scheme i.e. KABAT Scheme and DIMPLOT script algorithm package built into LigPlot plus v2.2 respectively.

2.4 Molecular Dynamics Simulation

The equilibrium and the dynamic behavior of wild and mutant variants of RBD Spike protein was studied by using GROMACS¹³.

MD simulation brings about time-dependent conformational changes and adjustment of protein, which opens to the alteration in unique nature after establishment of mutation in protein. We used GROMOS96 54a7 force field¹⁴ for MD simulation study. We added solvent water around protein to facilitate from spc216.gro as a non-exclusive equilibrated 3-point dissolvable water model in a dodecahedron. Here, we kept the protein in the centre at least 1.0 nm from the case edges.

Further, the steepest descent algorithm was utilized for energy minimization, to remove the steric conflicts and unstable conformations. Further we equilibrate the system via NVT ensemble (constant Number of particles, Volume and Temperature) and NPTensemble (constant Number of particles, Pressure and Temperature). After achieving equilibrium process, we moved for MD run to 10ns. Data analysis was done by Gromacs tools i.e. gmx rms for RMSD (Root Mean Square Deviation), gmxrmsf for RMSF (Root Mean Square Fluctuation), gmx gyrate for radius of gyration (Rg), gmxhbond for H-bond (for intra-protein H-bonds and for H-bonds between protein and water), and gmxsasa for SASA (solvent accessible surface). We further used GRACE software for data visualization.

3. Results

3.1 Docking analysis

We retrieved 28 variant mutants (**Fig. 1**) in spike protein identified to date. We found 12 variants/mutants in the RBD region. The RBD region is important for ACE2 and Antibody interactions. A few RBD variants have already shown to affect the vaccine efficacy as documented earlier by wet lab and dry lab results (Table 1), however, the vaccine efficacy against the B.1.617 and K417G variants is yet to be elucidated. We have done structural analysis of all 12 RBD mutant variants and compared them with wild type. We found that seven mutant variants (F486L, Q493N, B.1.617 (L452R & E484Q), R408I, L455Y, K417G and E484K) have structural changes in RBD region (**Fig. 2**). We analyzed interactions between RBD variants and ACE2 receptor. Moreover, we also checked the interactions between antibody and RBD variants. We found that seven structurally changed variants (F486L, Q493N, B.1.617, R408I, L455Y, K417G and E484K) have high docking score against ACE2 receptor compared with wild type and less docking score against antibody (CR3022) unlike wild type (Table 2). Out of seven variants, B.1.617 (B.1.617 < Q493N < E484K < K486L < L455Y < R408I < K417G) demonstrates lowest binding energy against antibody. Molecular interactions of antibody and ACE2 receptor with RBD variants are depicted in **Supplementary File1**. Our *in-silico* study suggests that the B.1.617 and K417G variants may affect vaccine efficacy.

Table 1

Table showing studies related to vaccine efficacy hampered by RBD based variants detected by different methodology.

Mutant Strain	Method of assessment	Finding	Reference
N501Y, A570D, N439K	Phenotype accessing by convalescent sera, mAB, serum sample from phase-I trial of mRNA-1273 vaccine (Moderna) & NVX-CoV2373 (Novavax)	Remains sensitive to neutralization, unaffected to current vaccine	[15]
E484K	By convulsive plasma and vaccinated sera	Reduced neutralization, hampers vaccine efficacy	[17]
Q498Y, Q493N, L455Y, F486L, N501T	Computation Algorithms & MD Simulation	Destabilized RBD & HR1 domain of Spike, affecting receptor binding site	[23]
R408I (B.1.1.7)	<i>In silico</i> approach by Informational Spectrum Method	RBD binding with ACE2 got affected, resistance to vaccine based on wild type SARS-CoV-2	[24]
G614, 501Y.V1, 501Y.V2	Neutralization accessing for DNA vaccine INO-4800 using sera from ferrets	Comparable Neutralization titres against G614 & 501Y.V1 while 4 fold lower against 501Y.V2	[25]
K417G	NA (Not Available)	NA	NA
B.1.617 (L452R & E484Q)	NA	NA	NA

Table 2
Prediction of RBD based variants interaction with antibody and ACE2 receptor.

Interaction between Ab (CR3022_6YLA)-RBD_variants	Docking score	Interaction between ACE2-RBD_variants	Docking score	Structure hampered in the region of RBD
F486L	18538	F486L	19150	YES
B.1.617 strain (L452R &E484Q)	17370	B.1.617 (L452R &E484Q)	18434	YES
Q493N	17722	Q493N	17814	YES
R408I	18984	R408I	17656	YES
L455Y	18758	L455Y	19032	YES
K417G	19428	K417G	18734	YES
E484K	17848	E484K	18014	YES
A570D	20342	A570D	17856	NO
N501T	20286	N501T	17602	NO
N501Y	21498	N501Y	17600	NO
Q498Y	22218	Q498Y	17102	NO
N439K	20556	N439K	17174	NO
Spike_Wild	21050	Spike_Wild	17910	NO

3.2 Molecular Dynamics (MD) Simulations

To examine the dynamic behavior, MD simulation runs for 10 ns to contemplate the structural stability of RBD mutant variants (F486L, Q493N, B.1.617, R408I, L455Y, K417G and E484K) in comparison to wild type. Various parameters studied all through the simulation trajectory, such as RMSD, Rg, RMSF, SASA, total number of intra-molecular hydrogen bonds of protein and H-bond between protein and water with the time dependent function of MD to examine the functional and structural impact of a mutant on wild protein.

The RMSD and RMSF (**Fig. 3 and Fig. 4**) of C-alpha chain atoms of all RBD mutant variants showed significant fluctuations in stability as well as in flexibility in comparison to Wild one. Rg and SASA analysis (**Fig. 5 and Fig. 6**) depicted much fluctuation between mutant and wild type. The fluctuations of hydrogen bonds are easily shown in all RBD mutant variants as compared to wild type (total intra-molecular H-bond and H-bond between protein and water) (**Fig. 7 and Fig. 8**) (Table 3). These molecular simulation data showed hampered structural stability and complexity of all seven RBD mutant variants as compared to wild type.

Table 3

Comparative studies of total intra-molecular H-bond and H-bond between protein and water of all RBD mutant variants along with wild type. *HD: Hydrogen Donor HA: Hydrogen Acceptor.

Variants	Total-Intramolecular hydrogen bonding		H-bond between protein and water	
	HD*	HA*	HD*	HA*
Wild Type	397	767	20501	20871
B.1.617 strain	401	770	23601	23970
E484K	398	766	26606	26974
F486L	397	767	23200	23570
K417G	396	766	28484	28854
L455Y	398	768	25553	25923
Q493N	397	767	25576	25946
R408I	394	764	25532	25902

4. Discussion

The newly emerged variants make our fight against global pandemic tougher as these variants might provide an evolutionary advantage, are more transmissible and harder for immune systems to detect. The 20I / 501Y.V1 variant of the lineage B.1.1.7, first discovered in the UK, has eight major mutations in the spike genes that may affect vaccine efficiency, antibody therapy, and pose a threat of re-infection. In addition to remaining susceptible to antibody neutralization, the B.1.1.7 (alpha) variant does not seem to be a major burden for available vaccines^{15,16}.

B.1.351 (Beta variant), a variant first encountered in South Africa, is of greater concern as this variant is incompliant to NTD mAbs neutralization, mainly due to E484K mutations. In addition, we have also evidence that B.1.351 was more opposing to neutralization by convulsive plasma (9.4-fold) and vaccinated sera (10.3–12.4-fold)¹⁷. The SARS-CoV-2 P.1, the Brazilian variant of B.1.1.28 lineage, has 10 mutations in spike gene viz. D614G, T20N, D138Y, L18F, R190S, and P26S in the NTD and K417T, E484K and N501Y in the RBD region and H655Y within the furin cleavage site. It shares mutations similar to B.1.35. P.1 on the same 3 RBD residues which are resistant to neutralization by the RBD targeted mAbs. Shared E484K mutation is the main culprit, which emerged in more than 50 lines independently along with B.1.526, recently identified in New York. A significant loss of neutralizing activity has been shown by vaccinated serum and convalescent plasma towards P.1, but the decrease is not as good as compared to what was found against B.1.351. Accordingly, the risk of re-infection by P.1 or dropped efficacy of vaccine protection may not be severe like B.1.351¹⁸.

The mRNA-1273 vaccine's neutralizing activity towards number of variants like B.1.351, B.1.1.7 + E484K, B.1.1.7, P.1, B.1.427 / B.1.429, D614G, 20A.EU2, 20E [EU1], N439K-D614G, and previously identified mutant in Denmark mink cluster 5 were identified and found to have the same neutrality level as Wuhan-Hu-1 (D1414)¹⁹. Limited loss in antibody neutralizing activity against B.1.1.7 while significant loss against B.1.35 was shown by the AstraZeneca ChAdOx1 vaccine, thus maintaining its efficacy towards B.1.1.7 and demonstrating a major loss of efficacy against the benign version of B.1.151. Although the efficacy against B.1.1.7 was found to have retained by the BNT162b2 Pfizer / BioNTech COVID-19 vaccine. The Novavax vaccine (NVX-CoV2373) reported differential protective immunity in the clinical trials i.e. 96%, 60%, and 86% against parental strain, B.1.351 and B.1.1.7, respectively²⁰. Many SARS-CoV-2 variants have been detected in the last few weeks of March in India. India is experiencing a sharp rise in coronavirus infections from between March and April 2021. Based on world meter statistics data <https://www.worldometers.info/>, we observed sudden enhancement of total, active, daily new cases, and death rate in the months of March and April indicates the beginning of second wave in India (**Fig. 9**). We also observed a sudden rise in the confirmed and active cases in all State/Union territory (UT) of India with sudden rise from March to April 2021 according to covid-19 India tracker (<http://www.covid19india.org>) (**Fig. 10 and Fig. 11**). Based on these studies, it can be hypothesized that the B.1.617 may also one of the causes of the sudden increment of cases in all state and UT of India. It might be possible that B.1.617 plays a major role in speedy spreading of infections in India.

The consequences of the current examination propose that the new B.1.617 (along with 6 others RBD mutant strain) inside the receptor-restricting site could lessen the immunization adequacy and higher the probability of reinfections by influencing the SARS-CoV-2 connection with the CR3022 antibody and ACE2 receptor. Our docking analysis observed that the binding affinity of mutant strain with ACE2 receptor is increased and is low with antibody compared to other variants.

The RMSD, RMSF, Rg, SASA, Intramolecular H-bond and H-bond between protein and water molecules were plotted to analyze the stability as well as flexibility of structurally hampered mutant RBD variants. Comparison of wild with mutant RBD protein, significant RMSD fluctuations were observed in all variants (B.1.617 0.25–0.5 nm, E484K nm 0.25–0.4 nm, F486L 0.2–0.3 nm, K417G 0.2–0.4 nm, L455Y 0.2–0.4 nm, L455Y 0.2–0.4 nm, Q493N 0.2–0.4 nm and R408I 0.25–0.45 nm). However more fluctuation was observed in B.1.617 as compared to others. The RMSD output showed that the protein stability could be influenced. We observed lower RMSF values of variant in comparisons to wild that confirms the compressed behavior of mutant trajectory. Higher value of Rg was noticed in all mutants' cases which indicate the possibility of lower compactness of protein. High fluctuations of SASA revealed that the protein structure and consequently protein function might be hampered. Fluctuations of total intramolecular H-bond and H-bond between protein and water have been found in all structurally hampered RBD mutant variants which signify the rigidity of protein might be influenced²¹.

Previous study has disclosed that the residues F486, L455, Q493, and N501 in the RBD spike protein form a major binding domain for the human ACE2 receptor²². A few mutants' viz. L455Y, Q493N, R408I, Q498Y, F486L, N501T within the RBD region (319–591), and D936Y& A930V within HR1 site (912–984) have

also been studied by *in silico* analysis to investigate the basic structure of spike glycoprotein. After comparing MD simulations in mutants and WT, a significant destabilizing outcome of mutations on the HR1 and RBD domains was revealed. Researchers revealed compromised stability of the overall spike protein structures by investigating the effect of framed mutations, before binding to the receptor²³.

5. Conclusion

In this severe COVID-19 pandemic, *in silico* studies can be used to stimulate the proceeding of development of therapeutic agents for the treatment of the disease. In this study, we performed molecular docking and simulation-based screening of recently emerged B.1.617 and previously reported RBD variants of COVID-19 to compare the binding and functional stability of spike proteins. Results of the present study suggest that the B.1.617 strain and K417G within the receptor-binding site could reduce the vaccine efficacy and increase the chances of reinfection by affecting the SARS-CoV-2 interaction with the CR3022 antibody and ACE2 receptor. We have examined the impact of B.1.617 and earlier reported RBD variants on the spike glycoprotein's structural stability by *in silico* analysis along with molecular simulation data and found structural alteration in the RBD domain in seven mutant variants. Further molecular interaction study of CR3022 antibody and ACE2 receptor with the RBD variants and comparison with wild type strain revealed the reduced binding affinity of all seven mutant versions with antibody, besides B.1.617 found to have the lowest affinity among all the RBD variants. These findings infer the possibilities of antigenic drift, ensuing incompatibility of current vaccine for B.1.617 strain. This B.1.617 strain seems to be a major burden for the available vaccine that could reduce the vaccine efficacy drastically and so may increase the chances of re-infection. These findings from extensive MD simulations provide novel mechanistic insights about the conformational plasticity and is crucial in understanding the interaction between the emerging variants and CR3022 antibody and ACE2 receptor. This information can be further harnessed for improvement of the available vaccines and aid in vaccine development. However, the results must be taken with caution as more research is still needed to explicate the exact consequences of the B.1.617 strain of SARS-CoV-2.

Declarations

Conflict of Interest

No Conflicts of interest to declare

Author Contributions

PR: Conceptualize and performed the experiments, data analysis, and writing-original draft. N: writing-original draft, and data analysis. CD: Data analysis and reviewing-original draft. GJ: reviewing-original draft and data analysis. CBM: reviewing-original draft and data analysis. PD: conceptualization, supervision, and reviewing-original draft. All authors contributed to the article and approved the submitted version.

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Figures

NTD: N-terminal domain, RBD: receptor-binding domain, RBM: receptor-binding motif, SD1 and SD2: Subdomain1 and 2, S1 and S2: Protease Cleavage Site 1 and 2, FP: fusion peptide, HR1 and HR2 heptad-repeat regions 1 and 2, CH: Central Helix, CD: Connector Domain, TM: transmembrane region, CT: cytoplasmic tail, HM: Homotrimeric assembly, 6HB: Six Helix bundle

Figure 1

We retrieved 28 variant mutants (Figure1) in spike protein identified to date. We found 12 variants/mutants in the RBD region. The RBD region is important for ACE2 and Antibody interactions. A few RBD variants have already shown to affect the vaccine efficacy as documented earlier by wet lab and dry lab results (Table 1), however, the vaccine efficacy against the B.1.617 and K417G variants is yet to be elucidated.

Figure 2

We have done structural analysis of all 12 RBD mutant variants and compared them with wild type. We found that seven mutant variants (F486L, Q493N, B.1.617 (L452R & E484Q), R408I, L455Y, K417G and E484K) have structural changes in RBD region (Figure2).

Figure 3

The RMSD and RMSF (Figure3 and Figure 4) of C-alpha chain atoms of all RBD mutant variants showed significant fluctuations in stability as well as in flexibility in comparison to Wild one.

Figure 4

The RMSD and RMSF (Figure3 and Figure 4) of C-alpha chain atoms of all RBD mutant variants showed significant fluctuations in stability as well as in flexibility in comparison to Wild one.

Figure 5

R_g and SASA analysis (Figure 5 and Figure 6) depicted much fluctuation between mutant and wild type.

Figure 6

Rg and SASA analysis (Figure 5 and Figure 6) depicted much fluctuation between mutant and wild type.

Figure 7

The fluctuations of hydrogen bonds are easily shown in all RBD mutant variants as compared to wild type (total intra-molecular H-bond and H-bond between protein and water) (Figure 7 and Figure 8) (Table3).

Figure 8

The fluctuations of hydrogen bonds are easily shown in all RBD mutant variants as compared to wild type (total intra-molecular H-bond and H-bond between protein and water) (Figure 7 and Figure 8) (Table3).

Figure 9

India is experiencing a sharp rise in coronavirus infections from between March and April 2021. Based on world meter statistics data <https://www.worldometers.info/>, we observed sudden enhancement of total, active, daily new cases, and death rate in the months of March and April indicates the beginning of second wave in India (Figure9).

Figure 10

We also observed a sudden rise in the confirmed and active cases in all State/Union territory (UT) of India with sudden rise from March to April 2021 according to covid-19 India tracker (<http://www.covid19india.org>) (Figure10 and Figure 11).

Figure 11

We also observed a sudden rise in the confirmed and active cases in all State/Union territory (UT) of India with sudden rise from March to April 2021 according to covid-19 India tracker (<http://www.covid19india.org>) (Figure10 and Figure 11).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFileDoubleMutant.docx](#)