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Vaccine-elicited immune pressure and SARS-CoV-2 mutational dynamics in breakthrough infections

Sarwareddy Kartik Kumar [a], Srinivas Sathrasala [b], Jandhyala Sai Krishna [c], Patnam Sreekanth [a], Anula Divyash Singh [a], M.S. Ratnamani [d], Iravathy Goud Kalal [e], Karthik Bharadwaj Tallapaka [c], Gajjela Praveen Kumar [f], Manda Venkata Sasidhar [a,g,*,1], Swarna Deepak Kuragayala [h,1,*]

[a] Apollo Hospitals Educational and Research Foundation (AHERF), Apollo Hospitals, Jubilee Hills, Hyderabad 500033, India
[b] Department of Critical Care Medicine, Apollo Hospitals, Apollo Health City, 500033, India.
[c] CSIR - Centre for Cellular and Molecular Biology, Uppal Road, Habsiguda, Hyderabad 500007, India
[d] Department of Microbiology, Apollo Hospitals Hyderabad, India
[e] Department of Cytogenetics & Molecular Biology, Apollo Health City, Jubilee Hills, Hyderabad, Andhra Pradesh 500033, India
[f] Apollo Research & Innovations (ARI), Apollo Hospitals Enterprise Limited, Hyderabad, India
[g] Urvogelbio Private Limited, AHERF, Jubilee Hills, Hyderabad 500033, India
[h] Department of General Medicine, Apollo Institute of Medical Sciences and Research, Hyderabad, Telangana, India

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ABSTRACT

Breakthrough infections post-vaccination raise concerns regarding vaccine efficacy and viral adaptability. This study examines the relationship between viral mutations, vaccine efficacy, and breakthrough infections in a cohort of 42 individuals positive for SARS-CoV-2 (Delta variant) from March to July 2022. The cohort included vaccinated (Covishield™ $n = 18$, Covaxin™ $n = 16$) and unvaccinated individuals ($n = 8$). Sequencing and mutation analysis revealed higher mutation rates in vaccinated individuals. Immune escape mutations (T19R, L452R, T478K, D614G, P681R, and D950N) were prevalent among vaccinated individuals. The receptor binding domain (RBD) exhibited amino acid substitutions enhancing spike-ACE2 binding. Serum Spike-IgG was present in all patients, indicating acquired immunity. Consequently, enhanced immunological barriers in vaccinated individuals led to an elevated mutation rate as SARS-CoV-2 sought to evade immunity and augment spike protein-ACE2 receptor affinity. Identified mutations showed a negative correlation with kidney health, evidenced by higher serum creatinine levels ($P = 0.0043$). These findings underscore the potential implications for managing and responding to future outbreaks, highlighting the necessity for ongoing viral mutation surveillance and analysis.

1. Introduction

The COVID-19 pandemic, triggered by the SARS-CoV-2 virus, has left an indelible mark on global health, economies, and routine life. The virus's rapid global propagation since its emergence in late 2019 has resulted in many infections and fatalities (Hiscott et al., 2020; Mofijur et al., 2021). In response to this crisis, the scientific community, in an unprecedented effort, developed and deployed a range of vaccines to reduce disease severity, hospitalisations, and deaths (Strain et al., 2022). However, while these vaccines have been instrumental in controlling the virus spread, they are not entirely foolproof. Cases of COVID-19 in fully vaccinated individuals referred to as breakthrough infections, have been reported (Tan et al., 2023b). While less severe and less frequent than infections in unvaccinated individuals, these instances are critical in understanding the enduring efficacy of vaccines (Lipsitch et al., 2022).

A significant factor in the fight against COVID-19 has been the emergence of SARS-CoV-2 variants, each originating and causing infection surges in different countries before spreading (Keni et al.,

Abbriviations: RBD, Receptor-binding domain; ACE2, Angiotensin-converting enzyme 2; CVD, Cardio vascular disease; CKD, Chronic Kidney Disease; AKI, Acute Kidney Injury.

* Corresponding authors at: Apollo Hospitals Educational and Research Foundation (AHERF), Apollo Hospitals, Jubilee Hills, Hyderabad 500033, India.
E-mail addresses: sasidhar@aherf.net (M.V. Sasidhar), drksdeepak@gmail.com (S.D. Kuragayala).

[1] Contributed equally as corresponding authors.

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2020) globally. These variants carry unique mutations, some of which can alter the virus characteristics, including its transmissibility and virulence. In the specific context of India, hybrid immunity, a combination of vaccine-induced immunity and immunity from previous infection during the Delta variant second wave, has potentially resulted in less severe Omicron variant infections (Acuña-Castillo et al., 2022; Karyakarte et al., 2022; Petrone et al., 2023). However, the occurrence of breakthrough infections, though a small percentage, raises pertinent questions.

Vaccines such as Covishield™ and Covaxin™, which are widely administered in India, induce an immune response through different mechanisms: Covishield™ uses a chimpanzee adenoviral vector to deliver the spike protein genetic code, and Covaxin™ uses an inactivated virus (Chavda et al., 2022; Das et al., 2022). These vaccines stimulate B and T cell responses and produce antibodies against the SARS-CoV-2 virus. Despite their proven efficacy against various SARS-CoV-2 strains, the appearance of new virus mutations might affect their effectiveness, leading to breakthrough infections in approximately 10 % of vaccinated individuals (Lee et al., 2022). The spike protein is the main target of developed COVID-19 vaccines, as it facilitates virus entry into human cells (Das et al., 2022). Spike protein mutations, especially in the receptor-binding domain (RBD), can alter its affinity for the ACE2 receptor, potentially increasing infectivity. Other amino acid mutations in the spike protein can cause changes in its physicochemical properties. It can reduce antibodies Field's neutralizationability (Ozono et al., 2021; da Costa et al., 2022; Liu et al., 2022; Pondé, 2022). Consequently, it is plausible that breakthrough infections in vaccinated individuals could involve viruses with adaptive spike protein mutations, enabling them to exploit viral entry mechanisms more efficiently. However, a notable gap persists in the scientific understanding of the intricate relationships between mutation frequency and breakthrough infections.

This study investigates the frequency of mutations, particularly in the spike protein, in Covishield™ and Covaxin™-vaccinated individuals who experienced breakthrough infections in Hyderabad, India. Understanding the nature and impact of these mutations is crucial for assessing their influence on vaccine efficacy and informing public health strategies. This knowledge will aid in improving the quality of life and effectively preventing future infection waves.

2. Material and methods

2.1. Study design

This study involved a cohort of 50 individuals who tested positive for SARS-CoV-2 via RT-PCR for a second time between March and July 2022. The nasal swab samples were collected at Apollo Hospitals, Hyderabad, India. Patient selection was based on clearly defined inclusion criteria, including testing positive for SARS-CoV-2 a second time, absence of other acute infectious diseases, and informed consent to participate in the study. The cohort was categorized into two groups: vaccinated ($n = 34$) and unvaccinated ($n = 8$). The vaccinated group was further subdivided based on the vaccine administered: Covaxin™ ($n = 16$) and Covishield™ ($n = 18$). In the exclusion criteria, additional samples from Comirnaty (n = 1) and Spikevax (n = 1 sample) were excluded due to their limited representation in the dataset. Unfortunately, six samples were also excluded due to insufficient vaccination details. Consequently, although the initial cohort comprised 50 patients, the subsequent correlation analysis involved data from 42 patients. The severity profile of the final cohort, comprising 42 individuals, revealed a distribution of 25 mild cases, 13 moderate cases, and 4 severe cases.

Demographic data, including gender, age, and disease severity, were analyzed from electronic medical records. Disease severity was assessed based on defined clinical parameters, including symptoms, hospitalization requirements, and need for oxygen support or intensive care. Samples were labeled with anonymous codes and sent to CSIR-CCMB for whole-genome sequencing using Illumina NovaSeq 6000 sequencing system (San Diego, CA, USA). Mutation analysis was performed using the ViralVar tool (Version 1.0, Chicago, USA), with sequencing data formatted into the required input format. Further, differences in serum IgG levels in vaccinated and unvaccinated individuals were estimated to assess the COVID-specific immunity and kidney function markers were analyzed as COVID is well known to affect kidney health. The study protocol was reviewed and approved by the Institutional Ethics Committee at Apollo Hospitals (IEC no.AHJ-ACD-078/09–21). All individuals included in this study provided informed consent.

2.2. Sequencing and library preparation

The viral RNA was meticulously extracted from nasopharyngeal swabs following the manufacturer's protocols, and these RNA templates were utilized for sequencing full-length SARS-CoV-2 genomes using the Illumina COVIDSeq method (RUO Version, San Diego, CA, USA) following manufacturer guidelines. The process involved synthesizing and amplifying first-strand cDNA using two primer pools, followed by an amplification step to add a 10-base index and sequencing adapter. Purification using paramagnetic beads ensured the selection of appropriately sized fragments for sequencing, quantification, normalization, and sequencing on a NovaSeq 6000 (Illumina) produced 100-bp paired-end reads. Base calling, quality assessment, read trimming, mapping to the reference genome (NC_045512.2), consensus sequence generation, coverage calculation, and mutation identification were performed using the specified software tools and parameters, including bcl2fastq (v2.20.0.422), FastQC (v0.11.9), Trimmomatic (v0.32), HISAT2 (v2.1.0), bcftools (v1.11), SAMtools (v1.11), and Nextclade (v1.1.0) (Munigela et al., 2022).

2.3. Mutation analysis

Mutation analysis was performed using the ViralVar tool, with sequencing data formatted into the required input format. The study identified distinct mutation events at each genomic position or protein residue relative to the reference sequence (NCBI: NC_045512.2). Frequencies of insertions, deletions, and substitutions were calculated. The most common mutations, referred to as top-line mutations, were ranked, and their percentages were normalized based on the number of patients. To visualize the accumulation of mutations in the spike RBD region, the distribution of mutations was mapped onto the protein's 3D structure (Alisoltani et al., 2022).

2.4. Clinical data collection

Laboratory data containing various parameters, including biochemistry, hematology, microbiology, pathology, etc., were collected from an electronic medical record system. The patients' blood samples were collected on the first day of their hospitalization for comprehensive analysis. The correlation analysis was performed using statistical methods, including one-way Analysis of Variance (ANOVA), student's *t*-test, and Kruskal-Wallis test. This data has been entered and managed by trained physicians and technicians (Koyyada et al., 2022).

2.5. Statistical analysis

Patient data were processed using statistical analysis softwares, including SPSS, version 24 (Chicago, USA) and GraphPad PRISM, version 8 (*Boston, MA, USA*). Significance levels were established at $p < 0.05$ through one-way ANOVA, Chi-square test, student's t-test, and Kruskal-Wallis test. The ViralVar tool employs the binomial test to pinpoint specific proteins in the uploaded dataset with noteworthy differences in mutation frequencies.

3. Results

3.1. Patient demographics

This study encompassed a cohort of 42 individuals re-infected with the SARS-CoV-2 Delta variant, split into unvaccinated ($n = 8$, average age 49.12 years), Covishield™ vaccinated ($n = 18$, average age 56.2 years), and Covaxin™ vaccinated groups ($n = 16$, average age 57.31 years). Gender distribution consisted of 27 males and 15 females. The unvaccinated group had equal numbers of both genders, while the Covishield™ and Covaxin™ vaccinated groups comprised 13 and 10 males and 5 and 6 females, respectively. Considering the severity of the infection, 25 individuals experienced mild symptoms, 13 moderate, and four severe. The unvaccinated group had 4 mild cases, 3 moderate cases, and 1 severe case. For the Covishield™ vaccinated group, the distribution was 12 mild, 4 moderate, and 2 severe, while the Covaxin™ vaccinated group reported 9 mild, 6 moderate, and 1 severe cases. Furthermore, within the cohort, diabetes (31.8 %) and hypertension (43 %) stood out as the predominant comorbidities. On the other hand, instances of CVD, CKD, and obesity were comparatively lower. Interestingly, no cases of asthma were identified among the patients in this study (Table 1).

3.2. Correlation between vaccination status and mutation rates

Our analysis has demonstrated a significant correlation between vaccination status and mutation rates in SARS-CoV-2. Samples from individuals vaccinated with Covaxin™ and Covishield™ displayed higher mutation rates than those from unvaccinated individuals. This trend was observed in the prevalence of the top ten mutations, particularly within the spike and N proteins, which were significantly more common in both vaccinated groups (Fig. 1). A deeper dive into the mutation profile revealed specific nucleotide substitutions and amino acid changes. The most frequently observed nucleotide substitutions were found at positions A23403G, C14408T, and C241T (Supplementary Fig. 1A). The most common amino acid substitutions included D614G, P314L, 5UTR241, and F106F (Supplementary Fig. 1C). Regarding the types of nucleotide changes, C-T substitutions were the most prevalent across all samples, followed by G-T, A-G, and T-C substitutions (Supplementary Fig. 1B). These findings suggest an intriguing link between vaccination and increased mutation rates within the studied population.

3.3. Identification of notable amino acid substitutions through ViralVar analysis

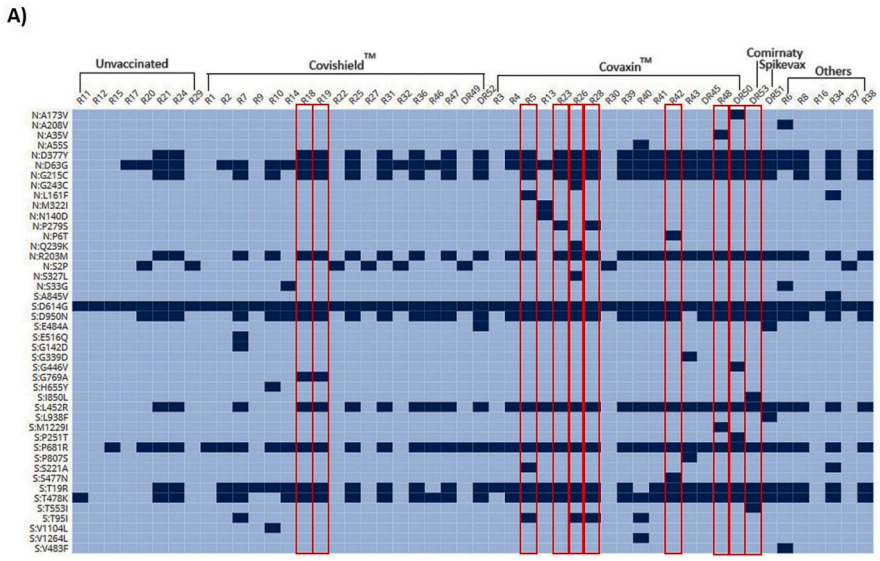
To investigate the frequency of spike protein mutations associated with elevated mutation rates in Covishield™ and Covaxin™ samples in comparison to the reference sequence of SARS-CoV-2 isolate Wuhan-Hu-1 (NC_045512.2), we conducted a comprehensive ViralVar analysis. This analysis identified several prevalent mutations (T19R, L452R, T478K, D614G, P681R, and D950N) across all samples (Fig. 2A). The frequency of these mutations was substantially higher in the Covaxin™ group, followed by the Covishield™ group. The overall mutation rates were 73 % for Covaxin™, 51 % for Covishield™, and 44 % for unvaccinated samples (Fig. 2B). These findings suggest a potential association between vaccination status and an increased prevalence of spike protein mutations in breakthrough infections (Cherian et al., 2021; Koyyada et al., 2022). In both vaccinated groups, we observed several unique mutations (Fig. 2C). The Covishield™ group included T95I, G124D, E494, E516Q, H655Y, and V1104L. The Covaxin™ group exhibited T95I, S221A, G339D, S477N, P807S, and V1264L. These unique mutations may suggest selection pressure and potential viral adaptation to enhance viral entry into vaccinated hosts (López-Cortés et al., 2022; Mahmood et al., 2022). However, the functional impact of these unique mutations on viral entry and other aspects of the viral lifecycle remain to be determined.

3.4. Mapping of amino acid variants in modelled spike protein

To understand the potential functional implications of the observed mutations, we utilized the 3D structure analysis feature in the ViralVar tool to map these mutations onto the spike protein 3D structure. Our dataset consistently observed two mutations, L452R and T478K, across all samples (Fig. 3). These mutations have been previously associated with enhanced infectivity and increased virus pathogenesis (Cherian et al., 2021; Shah and Woo, 2021). In samples related to the Covishield™ vaccine, we identified significant neutralizing resistance-causing mutations, namely E516Q and E484A (Fig. 3B). Conversely, in the Covaxin™-related samples, we found distinctive mutations, S477N and G339D (Fig. 3C), also known to possess neutralizing activity (Liu et al., 2021; Ghosh et al., 2022). These findings hint at the potential for these mutations to modulate the neutralizing response against COVID-19 in vaccinated individuals (Harvey et al., 2021; Kumar et al., 2022). This observation suggests that these mutations may present challenges to the efficacy of the current vaccines.

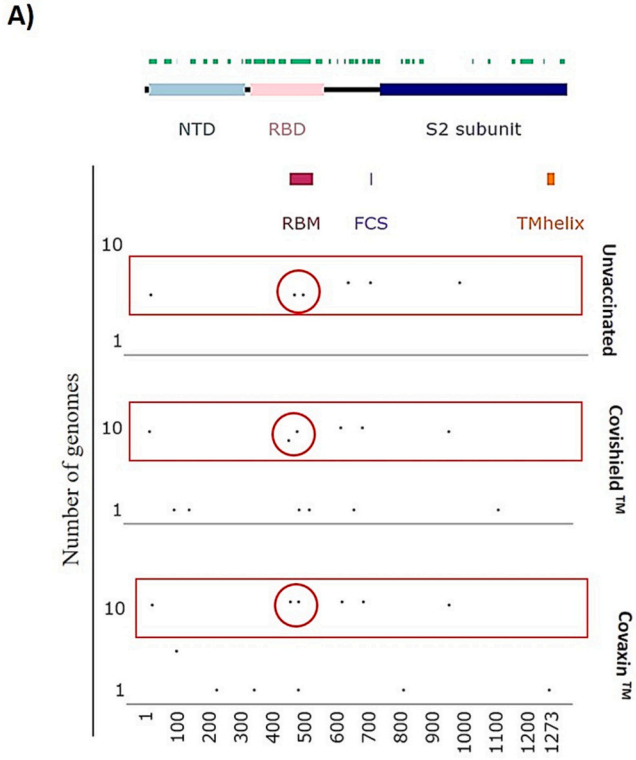
Table 1
Demographic profile of COVID-19 patients included in the study.

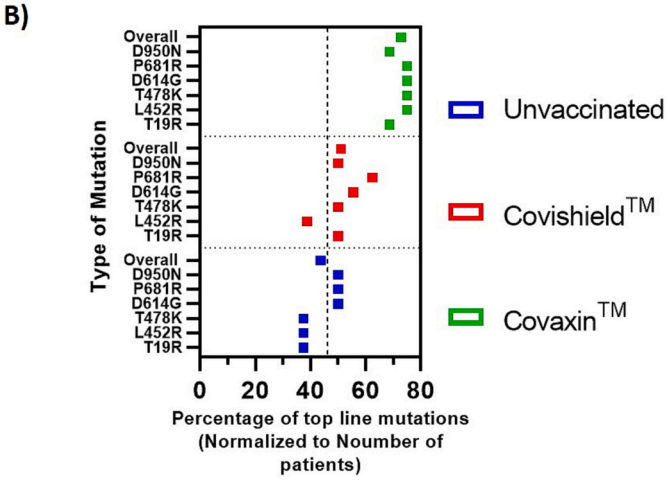
	Total	Unvaccinated	Vaccinated (Covishield™)	Vaccinated (Covaxin™)	*P*-Value (Using one-way ANOVA and Chi-square test)
Number of patients	42	8	18	16	–
Patient Age in years (Mean ± SD)	55.68 ± 13.74	49.12 ± 20.26	56.2 ± 8.05	57.31 ± 15.39	0.3761
Gender					0.5414
Male	27	4	13	10	–
Female	15	4	5	6	–
Severity					0.8472
Mild	25	4	12	9	–
Moderate	13	3	4	6	–
Severe	4	1	2	1	–
Comorbidities (Percentages)					
Diabetes	14 (31.8 %)	4 (50 %)	4 (22 %)	6 (37.5 %)	0.7128
Hypertension	19 (43 %)	3 (19.5 %)	10 (55.5 %)	6 (37.5 %)	0.8522
CVD	4 (9 %)	1 (12.5 %)	0	3 (18.75 %)	0.5489
CKD	3 (6.8 %)	1 (12.5 %)	2 (11 %)	0	0.7350
Obesity	3 (6.8 %)	1 (12.5 %)	2 (11.1 %)	0	0.5085
Asthma	0	0	0	0	–



S No	Sample ID	Vaccine name
1	R5	Covaxin ™
2	R28	Covaxin ™
3	R42	Covaxin ™
4	R26	Covaxin ™
5	DR50	Covaxin ™
6	R48	Covaxin ™
7	DR53	Comirnaty
8	R18	Covishield ™
9	R19	Covishield ™
10	R23	Covaxin™

Fig. 1. Correlation of SARS-CoV-2 mutations and vaccination status in breakthrough infections. (A) This panel comprehensively depicts the mutational landscape in the Spike and N proteins among all collected samples. The focus is on samples with the highest mutational burden, delineating the prevalence and distribution of mutations. (B) This panel correlates the vaccination status of individuals (Covaxin™, Covishield™, or unvaccinated) with the mutation rates, specifically highlighting samples with the highest mutation frequencies. The figure underscores the interplay between vaccination and mutation rates in breakthrough infections.





C)

S. No	Covishield ™	Covaxin ™
1	T95I	T95I
3	G142D	S221A
4	E484A	G339D
5	E516Q	S477N
6	H655Y	P807S
7	V1104L	V1264L

Fig. 2. Comparative analysis of SARS-CoV-2 mutations in unvaccinated, Covishield™, and Covaxin™ groups. (A) This panel displays ViralVar-generated plots, providing a detailed overview of mutation frequencies at each amino acid position for unvaccinated, Covishield™, and Covaxin™ samples. (B) This panel illustrates the distribution of top-line mutations and the corresponding overall mutation rates across all models, highlighting the disparities between the vaccinated (Covishield™ and Covaxin™) and unvaccinated cohorts. (C) This panel identifies amino acid residues uniquely mutated in vaccinated samples that are not typically observed in the unvaccinated group, indicating potential distinct mutation trends in individuals' post-vaccination.

3.5. Serum IgG levels in vaccinated and unvaccinated patients

In our analysis of enzyme-linked fluorescence assay (ELFA) data, we found that both vaccinated and unvaccinated individuals with breakthrough infections displayed detectable levels of spike-IgG (Fig. 4), indicating the presence of COVID-19-specific immunity (Alharbi et al., 2022; Ebrahim et al., 2022). Despite vaccination, the virus exhibits an increased mutation frequency, suggesting a potential counteraction

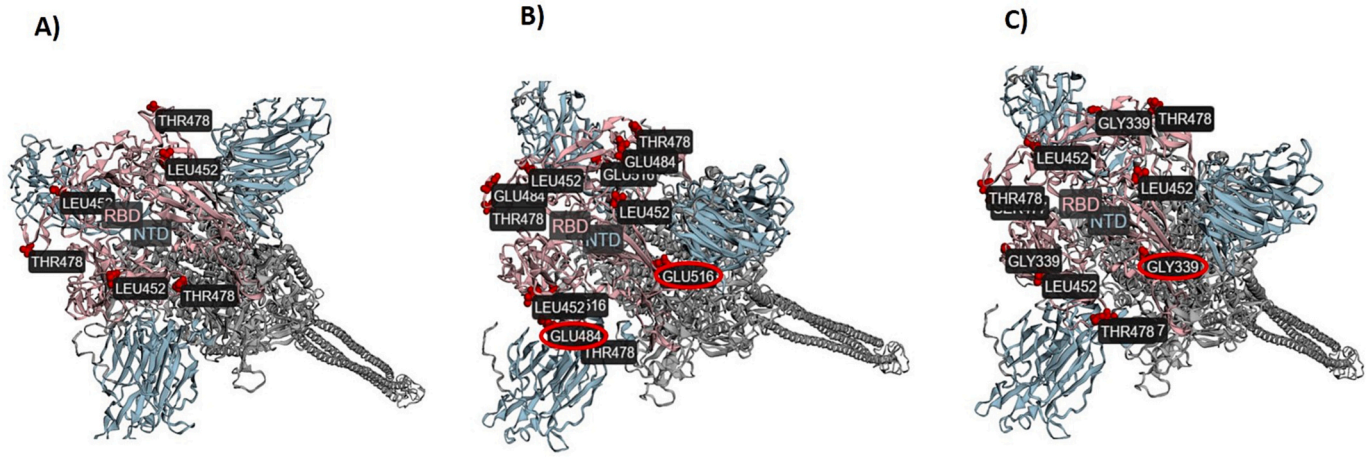


Fig. 3. 3D models of spike protein highlighting RBD mutations across unvaccinated, Covishield™, and Covaxin™ groups. (A-Unvaccinated, B-Covishield™, C-Covaxin™). These panels showcase 3D representations of the spike protein, focusing on the RBD mutations detected in Covishield™ and Covaxin™vaccinated samples. Models were constructed utilising the iTeaser feature in ViralVar. Modifications are exclusive to Covishield™, and Covaxin™ samples are accentuated in red, while the RBD domain within the protein structure is delineated in magenta. It's worth noting that the S477N mutation, although identified in the Covaxin™sample, needs to be visually represented in the 3D model due to its positioning in the protein internal trimeric region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

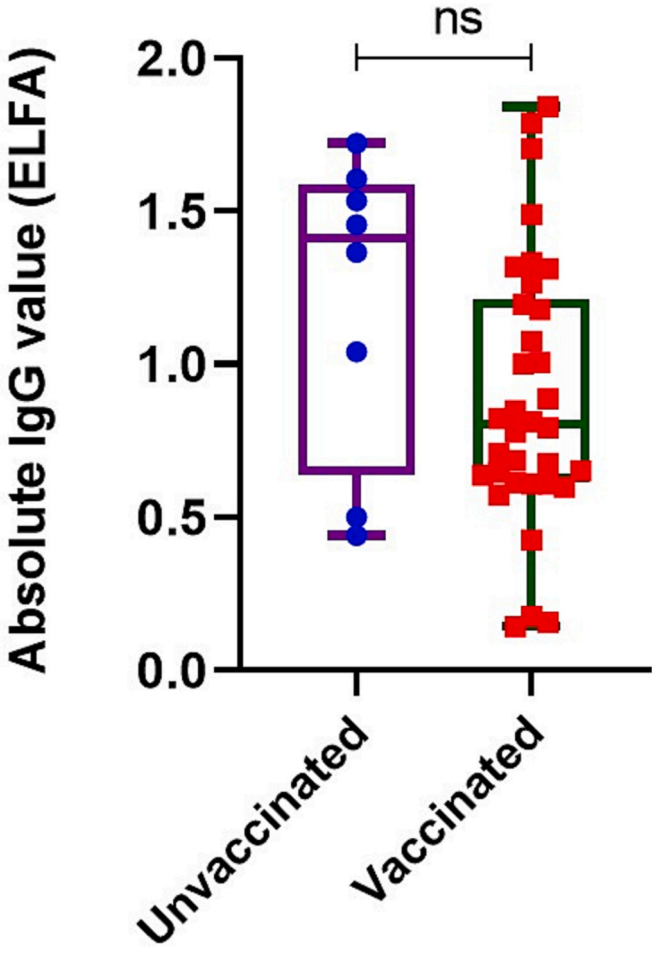


Fig. 4. Comparison of serum IgG levels in vaccinated and vaccinated groups. This figure showcases a comparative analysis of serum IgG levels between unvaccinated and vaccinated (Covishield™and Covaxin™) groups (*P*-value: 0.0795), as quantified by ELFA. The statistical analysis was conducted using the student's *t*-test. The visual representation of the data offers valuable insights into the humoral immune response following SARS-CoV-2 infections in the context of vaccination status.

mechanism to the protective effect of neutralizing antibodies. In the context of vaccination, these mutations may enhance the virus affinity for the human ACE2 receptor (Han et al., 2022). Notably, in the samples associated with the Covishield™ vaccine, we identified significant neutralizing resistance-causing mutations, namely E516Q and E484A. In contrast, characteristic mutations, S477N and G339D, which also possess neutralizing activity, were found in the samples associated with the Covaxin™ vaccine.

3.6. Elevated mutation rates appear to correlate with adverse kidney function markers

Our investigation explored the relationship between mutations and widely used indicators of renal function, such as creatinine, urea, and urea nitrogen, given COVID-19 established propensity to affect kidney health (Sabaghian et al., 2022; Tan et al., 2023a). Interestingly, serum creatinine levels were significantly elevated among vaccinated individuals (*P*-value 0.0043, Unvaccinated vs. vaccinated). Notably, this pattern was more pronounced within subgroups with higher and lower mutation rates (*P*-value 0.0060), implying a credible link between these mutations and an elevated susceptibility to kidney impairment (Fig. 5). Conversely, other parameters (Supplementary Fig. 2), including urea and urea nitrogen, did not demonstrate significant distinctions between unvaccinated and vaccinated groups (*P*- values: 0.94 and 0.75) or low and high mutation rate groups (*P*-values: 0.97 and 0.56). Thus, creatinine can be one of the importantmarkers for understanding the health status of patients with mutations. This underscores the intricate interplay between mutation rates, kidney function markers, and COVID-19 infection, necessitating further exploration for thorough validation and nuanced understanding.

4. Discussion

The evolving nature of SARS-CoV-2 has resulted in many variants, each with its unique mutational profile. Specific variants, characterised by extensive mutations in the spike protein, have raised global concern due to their potential impact on vaccine efficacy and the likelihood of breakthrough infections (Das et al., 2022; Han and Ye, 2022; He et al., 2023). In light of this, we undertook an in-depth analysis of the mutational landscape in vaccinated individuals, specifically those vaccinated with Covishield™ and Covaxin™ who had breakthrough infections.

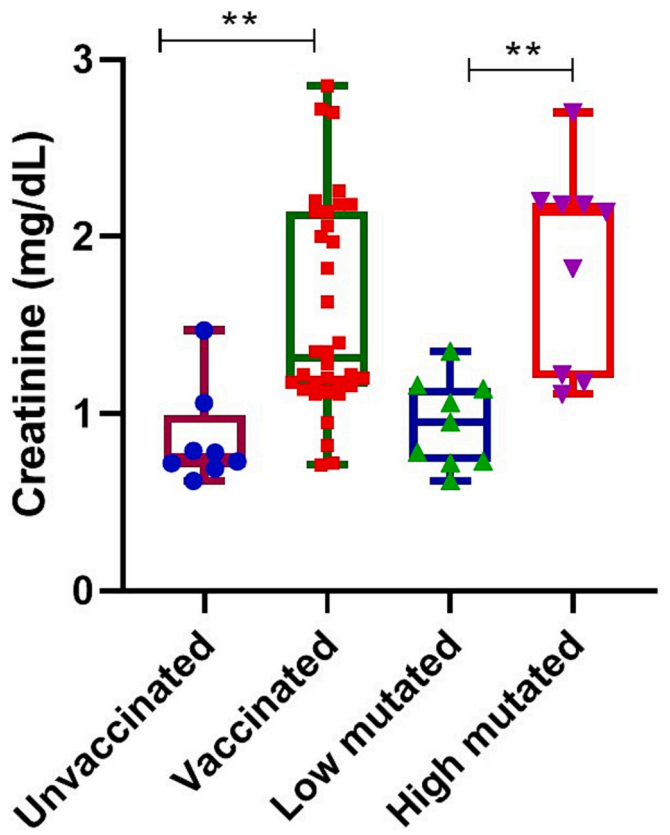


Fig. 5. Serum creatinine levels in patient groups. The figure demonstrates significant differences in serum creatinine levels between unvaccinated and vaccinated groups ($P = 0.0043$) and lower versus higher mutation subgroups ($P = 0.0060$). The statistical analysis was performed employing the Kruskal-Wallis test. Notably, significant differences are observed in both contexts, highlighting the potential influence of vaccination and mutation rates on creatinine levels.

Our mutation data revealed that the Delta variant had undergone more mutations in vaccinated individuals than in unvaccinated individuals. However, it is crucial to note that the virulence of the virus was not higher in either group. This phenomenon could arise from hybrid immunity acting as a safeguard against virus (Altarawneh et al., 2023; Grant et al., 2023). Among the mutations identified, D614G and P681R/H were significantly more prevalent in vaccinated individuals. These mutations, linked to enhanced viral entry and fusion, could alter the viral phenotype, increasing its virulence. These findings align with prior studies, including that by Khatri et al. (2023), emphasising the importance of these mutations in the viral pathogenicity (Khatri et al., 2023). Furthermore, we identified mutations (T19R, L452R, T478K, and D950N) that could impact spike protein dynamics and potentially reduce the effectiveness of antibody neutralisation (Planas et al., 2021; Mahmood et al., 2022; Furusawa et al., 2023). These mutations were more prevalent in vaccinated individuals, suggesting a potential vaccine-induced selective pressure. Interestingly, these findings align with previous reviews on the challenges of upcoming vaccinations in India, highlighting the impact of spike protein changes associated with mutations (Altarawneh et al., 2023). Additionally, L452R, T478K, and S477N mutations have been reported to strengthen the spike-ACE2 binding. To this end, the S477N mutation was exclusively present in Covaxin™ samples at the RBD region, while the L452R and T478K mutations were common binding site mutations across all samples (Cherian et al., 2021; Wang et al., 2021).

Furthermore, our analysis identified other significantly important mutations in vaccinated samples, such as G339D and E484A at the RBD region of the spike protein. These less conserved mutations were predicted to interact with neutralizing antibodies, potentially contributing to immune escape. Another novel mutation, E516Q, was observed in Covishield™ vaccinated samples, showing potential implications in facilitating binding with ACE2 based on similar observations from the South Africa beta variant study (Tegally et al., 2021; Chakraborty et al., 2022; Ghosh et al., 2022). However, more data on this mutation, specifically in the Delta variant, is needed. Overall, the spike protein of the Delta variant appears to bind effectively to the receptor through L452R and T478K mutations. However, in vaccinated individuals, the virus undergoes further modifications at the binding site, such as E516Q, E484A, S477N, and G339D, to strengthen the RBD's interaction with ACE2. The higher selection pressure in vaccinated individuals might drive these additional mutations to help the virus escape from neutralizing antibodies and cause resistance (Chakraborty et al., 2022; Mahmood et al., 2022).

Our ELFA data analysis revealed the presence of spike-IgG antibodies in both vaccinated and unvaccinated individuals, indicating a robust immune response to COVID-19 (Tretyn et al., 2021; Jahan et al., 2022). Despite this immune response, the virus appears to counteract this defence mechanism by enhancing the frequency of mutations, potentially increasing the virus's affinity for the human ACE2 receptor and evading the neutralizing effect of the antibodies. This suggests a complex interplay between the virus and host immunity, especially in the context of vaccination (Jahan et al., 2022). Furthermore, our analysis of commonly associated kidney function markers has revealed elevated creatinine levels in both the vaccinated group and the subgroup with many mutations. This suggests that monitoring creatinine levels in COVID-19 patients during the early stages of hospitalization could serve as a valuable ongoing assessment (Uribarri et al., 2020). This observation also implies that the identified mutations might contribute to an increased risk of kidney injury, as the prevalence of AKI in COVID patients has been reflected in previous studies (Temiz et al., 2022; Tummala et al., 2022).

Our study provides compelling evidence supporting the hypothesis that vaccines may exert selective pressure on viruses. Thereby, SARS-CoV-2 undergoes significant mutations to enhance the spike protein binding ability to ACE2. This adaptation enables the virus to evade neutralizing antibodies despite their substantial presence, leading to breakthrough infections. These findings underscore the critical importance of continuous surveillance and further research to optimize vaccine strategies and effectively combat emerging variants.

However, our study has limitations. The limited sample size and reliance on a single isolated hospital, although suitable for drawing preliminary conclusions, may constrain the broader generalizability of our findings. Furthermore, the cross-sectional study design partially restricts our ability to infer changes over time. Therefore, more extensive longitudinal studies are needed to validate our findings and provide a more comprehensive understanding of the interplay between vaccination and viral mutation. Furthermore, future research should delve deeper into the biological mechanisms underpinning the observed differences in biomarker levels and how these correlate with specific viral mutations and patient health outcomes. Despite the observed increased mutation rates, vaccination remains a critical tool in the fight against COVID-19 due to its demonstrated effectiveness in preventing severe disease, hospitalization, and death.

5. Conclusions

Our study provides a comprehensive analysis of the mutational landscape of SARS-CoV-2 in the context of vaccination and presents novel insights into the potential impact of these mutations on clinical biomarkers. We observed higher mutation rates in vaccinated individuals and identified specific mutations that were more prevalent in causing immune escape and strengthening ACE2 binding affinity. These adaptive mutations may facilitate viral entry and infection despite the substantial presence of COVID-specific (IgG) antibodies. Additionally,

we found a positive correlation between these mutations and traditional kidney function marker creatinine, raising concerns about their implications for kidney health. Therefore, continuous viral surveillance and adaptability in vaccine strategies are needed to manage these evolving mutations.

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CRediT authorship contribution statement

Sarwareddy Kartik Kumar: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Srinivas Sathrasala:** Formal analysis, Data curation. **Jandhyala Sai Krishna:** Visualization, Software. **Patnam Sreekanth:** Methodology, Data curation. **Anula Divyash Singh:** Methodology, Data curation. **M.S. Ratnamani:** Formal analysis, Data curation. **Iravathy Goud Kalal:** Data curation. **Karthik Bharadwaj Tallapaka:** Visualization, Software. **Gajjela Praveen Kumar:** Data curation. **Manda Venkata Sasidhar:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Conceptualization. **Swarna Deepak Kuragayala:** Writing – review & editing, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare there is no conflict of interests.

Data availability

Data will be made available on request.

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