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Coronavirus Pandemic

Whole genome sequencing and analysis of the clinical implications of SARS-CoV-2 strains

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Abstract

Introduction: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants that emerged shortly after the coronavirus disease 2019 (COVID-19) pandemic began have altered epidemiological and clinical findings; and these variants changed the course of this health crisis.

Methodology: Whole-genome sequencing was performed on SARS-CoV-2 strains isolated from 21 patients with COVID-19. The frequency of structural changes in the virus and their effects on clinical findings of the disease were analyzed.

Results: The spike Q493R mutation was detected more frequently in patients who had received four or more doses of a COVID-19 vaccine ($p = 0.043$). The clinical effect of the spike R346K and A263T mutations (reported in Türkiye for the first time) detected in a patient who had received four doses of the vaccine in the 3 months prior to being infected with COVID-19 could be related to escape from the antibody response. The spike R21T mutation may increase the virus's entry into intestinal cells; and, as a result it may be responsible for severe clinical course and gastrointestinal symptoms. The patient infected with the Omicron BA.2 subvariant with the spike L452M mutation exhibited a significant increase in inflammatory parameters; suggesting that this mutation may trigger an excessive immune response and hyperinflammation.

Conclusions: This is the first study based in Türkiye that evaluated the clinical impact of variations in the sequences of SARS-CoV-2 variants. There is a need for further investigation into the clinical impact of these results in a larger population spread over more centers, and more sequencing studies.

Key words: COVID-19; SARS-CoV-2; whole genome sequencing.

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Introduction

Since its initial detection in Wuhan, China, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has undergone several genetic modifications that resulted in new variants over time. The rise of these new variants has altered epidemiological and clinical findings, thus changing the course of the coronavirus disease 2019 (COVID-19) pandemic. The emergence of these variants has also impacted COVID-19 vaccination and treatment strategies [1]. Five lineages of SARS-CoV-2 have been considered variants of concern (VOC) by the World Health Organization (WHO): Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529). Omicron is currently the only circulating VOC. More recently, Omicron subvariants and sublineages—BQ.1, BQ.1.1, BA.4.6, BF.7, BA.2.75.2, XBB.1, and BF.7—have been identified and have attracted global attention [2]. According to the statistics released by the European Centre for Disease Prevention and Control, the estimated distribution of the VOCs from 25 September to 8 October 2023 were: 66% (59%–72%) for XBB.1.5 + F456L, 28% (20%–34%) for XBB.1.5, and 2% (1%–3%) for BA.2.75.

Analysis of the whole-genome sequences of SARS-CoV-2 isolates allows scientists to identify new variants and mutations [3]. According to the WHO 2021 report, determining changes in the RNA sequence contributes to understanding factors such as the evolution, spread, and infectivity of SARS-CoV-2; clinical findings related to COVID-19; and the effectiveness of vaccines. This information is highly relevant to control the COVID-19 pandemic. The spread of the SARS-CoV-2 variants can be monitored by comparing viral genomes and constructing molecular phylogenetic trees at the national and global levels [4]. This study analyzed the whole-genome sequences of SARS-CoV-2 samples collected at various time points to investigate how changes in variants and mutations alter clinical findings.

Methodology

General data

This prospective clinical study was performed between January and November 2022. A total of 21 patients who applied to the Pamukkale University Department of Infectious Diseases and Clinical Microbiology Clinic were included. The inclusion criteria were: (1) SARS-CoV-2 nucleic acid was detected by real-time fluorescent reverse-transcription polymerase chain reaction (RT-PCR, Rotor Gene Q, Qiagen, Germantown, USA); (2) age ≥ 18 years; (3) provided informed consent to participate in the study; and (4) the disease manifestation met the diagnostic criteria of the 'Diagnosis and Treatment Protocol for Coronavirus Disease' [5] and the WHO criteria [6] .

Individuals who were prioritized for the sequencing of SARS-CoV-2, included those who had received the COVID-19 vaccine within the last 3 months but were infected by SARS-CoV-2, had prolonged RT-PCR positivity (> 4 weeks), a history of recurrent COVID-19, and immunocompromised individuals.

The exclusion criteria were: (1) age < 18 years; (2) pregnant women; and (3) patients with unknown COVID-19 vaccination status.

The general clinical data and test results of laboratory indexes of the patients were collected.

Laboratory tests

Throat or nose swab samples were collected for RT-PCR (Rotor Gene Q, Qiagen, Germantown, USA). The threshold cycle (Ct) of the *ORF* and *N* genes of SARS-CoV-2 were detected according to the manufacturer's instructions. Serum samples were collected from patients who had received ≥ 4 doses of vaccine to determine the spike IgG class antibody titers during COVID-19 infection; a value ≥ 50 AU/mL was interpreted as positive for the anti-SARS-CoV-2 IgG antibody.

The SARS-CoV-2 sequences were analyzed based on the Global Initiative on Sharing All Influenza Data (GISAID) [7] to determine the molecular relationships and evolution of the whole-genome sequences of the 21 SARS-CoV-2 isolates. The phylogenetic tree, mutation frequency graph, and three-dimensional (3D) views of the spike protein for the whole-genome sequences were created by using the GISAID website. The frequencies of the identified mutations, both globally and in Türkiye, were obtained from the GISAID data [7].

Statistical analysis

The data were analyzed using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as the mean ± standard deviation, and categorical variables were presented as a number and percentage. In the case of continuous variables, the data were assessed to determine whether it met the assumptions for parametric testing; when it did not, the Mann–Whitney U test was employed. Correlations were assessed using Spearman or Pearson correlation analyses for parametric and non-parametric data, respectively. The categorical variables were assessed using the Chi-squared test. A p value below 0.05 was deemed statistically significant

Ethical considerations

This study was conducted in compliance with the Declaration of Helsinki and was approved by the Pamukkale University Ethics Committee (Ethics No. 60116787-020/56297).

Table 1. Demographic characteristics of the patients.

Demographic data	(n, %)
Gender (male/female)	13/8 (61.9/38.1)
Age (mean, min–max) years	65.3 ± 20.7 (26–97)
18–70 years old	8 (38.1)
≥ 70 years old	13 (61.9)
Inpatients	18 (85.7)
Outpatients	3 (14.3)

Results

A total of 21 patients who were diagnosed with COVID-19 between January and November 2022 were included in the study. The whole-genome sequences of their SARS-CoV-2 isolates were obtained. The samples were collected at the following times: 7 (33.3%) in February–March, 8 (38%) in April–May, 4 (19%) in

Table 2. Coronavirus disease 2019 (COVID-19) vaccination status of the patients.

Case no.	Vaccination status
1	2 Sinovac-CoronaVac
2	2 Sinovac-CoronaVac
3	2 Sinovac-CoronaVac 2 Pfizer-BioNTech BNT162b2
4	2 Sinovac-CoronaVac 2 Pfizer-BioNTech BNT162b2
5	3 Sinovac-CoronaVac 1 Pfizer-BioNTech BNT162b2
6	2 Sinovac-CoronaVac 2 Pfizer-BioNTech BNT162b2
7	2 Sinovac-CoronaVac 3 Pfizer-BioNTech BNT162b2
8	2 Sinovac-CoronaVac 1 Pfizer-BioNTech BNT162b2
9	4 Sinovac-CoronaVac
10	4 Sinovac-CoronaVac
11	4 Sinovac-CoronaVac
12	2 Sinovac-CoronaVac 2 Pfizer-BioNTech BNT162b2
13	Unvaccinated
14	2 Sinovac-CoronaVac 2 Pfizer-BioNTech BNT162b2
15	3 Pfizer-BioNTech BNT162b2
16	4 Sinovac-CoronaVac
17	2 Sinovac-CoronaVac 2 Pfizer-BioNTech BNT162b2
18	3 Sinovac-CoronaVac
19	4 Sinovac-CoronaVac
20	4 Sinovac-CoronaVac 1 Pfizer-BioNTech BNT162b2
21	Unvaccinated

Table 3. Clinical progression of fully vaccinated and other patients.

Vaccination with ≥ 4 doses	Mild cases (n, %)	Severe and moderate Cases (n, %)	*p* value
Yes	4 (66.7%)	2 (13.3%)	$p = 0.031$
No	2 (33.3%)	13 (86.7%)	

June–July, and 2 (9.5%) in October. Of the 21 patients included in the study, 13 (61.9%) were men. The mean age was 65.3 ± 20.7 (range 26–97) years. Eight (38%) patients were 18–70 years old, while 13 (62%) patients were ≥ 70 years old. In total, 18 (85.7%) patients were followed and treated in hospital wards or intensive care units, while 3 (14.3%) patients were followed as outpatients. Table 1 presents the demographic data of the patients, and Table 2 summarizes the vaccination histories.

Patients were considered to be fully vaccinated if they had received at least 2 doses of Sinovac-CoronaVac® and at least 2 doses of Pfizer-BioNTech BNT162b2®, for a minimum of 4 total doses. As shown in Table 3, 6 (28.5%) patients who had developed an antibody response were compared with other patients in terms of the clinical condition. The patients who had received at least 4 doses of the vaccine and had developed an antibody response, exhibited a milder clinical course compared with those who had not developed an antibody response in terms of development of severe to moderate illness ($p = 0.031$). Furthermore, the duration of PCR positivity was shorter (median = 6 and 26.9 days, in patients with ≥ 4 vaccine doses who developed antibodies, and in those without antibody response, respectively, $p = 0.04$) in this patient group. There were no differences in terms of the presenting symptoms, demographic data, mortality, mutation, and variant analysis.

Six patients (28.6%) exhibited mild clinical symptoms, 7 (33.3%) had moderate clinical symptoms, and 8 (38.1%) presented with severe symptoms (Table 4). Two patients had previously been infected with COVID-19 once. Six (28.5%) patients died due to COVID-19.

The patients with prolonged RT-PCR positivity (> 4 weeks) had a higher incidence of malignancy ($p = 0.016$) and a history of lymphoma ($p = 0.006$), compared to other patients. The patients with prolonged PCR positivity were more likely to be in the moderate to severe disease group. There was no significant difference in the frequency of mutations between patients with severe or mild clinical courses. Eight patients within the study cohort had received ≥ 4 vaccine doses and were subsequently diagnosed with COVID-19, 7 patients required intensive care monitoring despite receiving ≥ 2 vaccine doses, 6 patients had COVID-19 RT-PCR positivity persisting for > 4 weeks, and 9 patients had additional comorbidities that could lead to immunosuppression (7 patients had malignancy, 1 patient had human immunodeficiency virus [HIV], 1 patient was an organ transplant recipient, and 1 patient had systemic lupus erythematosus [SLE]).

Sequencing of the 21 samples yielded a total of 20,570,027 read pairs to generate the SARS-CoV-2

Table 4. Demographic and clinical data of the patients with rare mutations.

Case no.	Gender	Age (years)	Comorbidities	Clinic	Novel mutations	Survival status
1	Male	73	Lymphoma, HIV	Moderate	–	Deceased
2	Male	54	Lymphoma	Severe	–	Deceased
3	Female	79	CAD	Mild	–	Survivor
4	Male	73	Bladder cancer, DM, CKD, obesity	Severe	–	Survivor
5	Male	75	DM	Moderate	–	Survivor
6	Male	78	DM, CAD, CKD	Mild	–	Survivor
7	Female	30	–	Mild	Spike A263T	Survivor
8	Male	76	Lymphoma	Severe	–	Deceased
9	Male	75	DM, CKD, lymphoma	Moderate	–	Survivor
10	Male	89	HT	Moderate	NSP2 L18I	Survivor
11	Female	66	HT, CAD, COPD, CKD, obesity	Severe	–	Deceased
12	Male	85	DM, HT, prostate cancer, CKD	Moderate	NSP2 V599I	Survivor
13	Female	97	HT	Moderate	NSP14 V40I	Survivor
14	Male	32	–	Mild	–	Survivor
15	Male	50	HT	Mild	–	Survivor
16	Male	70	DM, CAD	Severe	–	Deceased
17	Female	26	–	Mild	–	Survivor
18	Male	70	CAD, lymphoma	Moderate	NSP2 F156L	Survivor
19	Female	80	DM, HT	Severe	–	Survivor
20	Female	67	HT, CAD, obesity	Severe	–	Deceased
21	Female	28	SLE	Severe	–	Survivor

CAD: coronary artery disease; CKD: chronic kidney disease; COPD; chronic obstructive pulmonary disease; DM: diabetes mellitus; HIV: human immunodeficiency virus; HT: hypertension; SLE: systemic lupus erythematosus.

Table 5. Analysis of demographic characteristics of the patients and the variants of the infecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Case no.	Gender	Age (years)	Variant	Subvariant	Duration of PCR Positivity (days)	Detection period
1	Male	73	Omicron (BA.1-like)	BA.1.14	33	February 2022
2	Male	54	Delta (B.1.617.2-like)	AY.121	64	February 2022
3	Female	79	Omicron (BA.2-like)	BA.2.12	–	February 2022
4	Male	73	Omicron (BA.2-like)	BA.2	15	March 2022
5	Male	75	Omicron (BA.2-like)	BA.2	–	March 2022
6	Male	78	Omicron (BA.1-like)	BA.1.18	–	February 2022
7	Female	30	Omicron (BA.1-like)	BA.1.1	–	March 2022
8	Male	76	Omicron (BA.1-like)	BA.1.1	45	April 2022
9	Male	75	Omicron (BA.2-like)	BA.2	134	April 2022
10	Male	89	Omicron (BA.2-like)	BA.2	–	April 2022
11	Female	66	Omicron (BA.2-like)	BA.2.9	–	April 2022
12	Male	85	Omicron (BA.2-like)	BA.2	11	April 2022
13	Female	97	Omicron (BA.2-like)	BA.2	29	April 2022
14	Male	32	Omicron (BA.5-like)	BE.1.1	–	July 2022
15	Male	50	Omicron (BA.5-like)	BA.5.2	–	June 2022
16	Male	70	Omicron (BA.2-like)	BA.2	–	June 2022
17	Female	26	Omicron (BA.2-like)	BA.2	–	May2022
18	Male	70	Omicron (BA.2-like)	BA.2.1	93	June 2022
19	Female	80	Omicron (BA.2-like)	BA.2	–	May 2022
20	Female	67	Omicron (BA.5-like)	BA.5.6	14	October 2022
21	Female	28	Omicron (BA.5-like)	BA.5.6	15	October 2022

genomes (with > 96% coverage). The Omicron BA.2 subvariant was detected in 12 patients, the Omicron BA.1 subvariant in 4 patients, the Omicron BA.5 subvariant in 4 patients, and the Delta variant in 1 patient.

Among the 12 patients with the BA.2 subvariant, 9 had BA.2.0, 1 had BA.2.1, 1 had BA.2.12, and 1 had BA.2.19. Among the 4 patients with the BA.1 subvariant, 2 had BA.1.1, 1 had BA.1.14, and 1 had BA.1.18. The 4 patients with the BA.5 subvariant included 2 with BA.5.6, 1 with BA.5.2, and 1 with BE.1.1. The patient with the Delta variant had the AY.121 genome sequence (Table 5).

The mutations that were detected in all the isolates included: c.1841A>G (D614G), c.425G>A (G142D), c.9764C>T NSP4 (T492I), and c.14144C>T (NSP12 P323L). Additionally, there were a total of 13 spike mutations, 1 M mutation, and 66 N mutations; each observed only once. The majority of these variants were in the surface glycoprotein and ORF1ab regions. Correlation analysis between the detected mutations and the frequencies of these mutations in sequences from throughout the world revealed a strong positive correlation ($r(163) = 0.81$, $p < 0.001$). Cases with the BA.1, BA.2, Delta, and BA.5 subvariants were analyzed for mutations that were specific to that variant or mutations that had been rarely found in that variant (Table 4).

The genomes of the Omicron BA.1 subvariant isolated from the 4 patients were identical, aside from the spike A263T mutation detected in 1 patient and the spike R346K mutation detected in 2 other patients. The spike A67V, del211, and ins214EPE mutations were found in these Omicron BA.1 isolates; and were not present in the other isolates. Two of these patients experienced a severe clinical course, with PCR positivity reported for > 4 weeks (33 and 45 days); unfortunately, both the patients died. These patients were 73 and 76 years old, and both had a history of lymphoma. One of the patients was also being monitored for acquired immune deficiency syndrome (AIDS) and had a history of receiving 2 doses of Sinovac-CoronaVac®. The other patient had received 3 doses of Sinovac-CoronaVac®. Distinctively, the SARS-CoV-2 isolates from these two patients carried the spike R346K mutation.

The other 2 patients with the Omicron BA.1 subvariant—aged 30 and 78 years—had a mild clinical course. The younger patient had no known underlying chronic conditions and was managed on an outpatient basis. This individual had received 2 doses of the Sinovac-CoronaVac® and 3 doses of the Pfizer-BioNTech BNT162b2®, and had a high antibody titer (30,024 AU/mL). Notably, the isolate from this patient carried a rare (present in only 0.01% of all samples with spike sequences) spike A263T mutation, which had not been previously reported in Türkiye.

As mentioned earlier, 12 patients were infected by the Omicron BA.2 subvariant. Among these patients, 2 had a mild clinical course, 6 had a moderate clinical course, and 4 had a severe clinical course. Two of the patients with severe clinical course died. One of these cases had a rare spike R21T mutation (present in only 0.07% of all samples with spike sequences), which had

previously been reported only once in Türkiye. In addition, the patient who died had another rare mutation, spike L452M, which had been reported only twice in Türkiye [7]. This mutation was present in only 0.21% of all samples with spike sequences. The patient had received 3 doses of Sinovac-CoronaVac® and 1 dose of Pfizer-BioNTech BNT162b2®.

Two out of the 4 patients infected by the Omicron BA.5 subvariant exhibited a mild clinical course, while the other 2 experienced a severe clinical presentation. Both patients with severe clinical symptoms were undergoing immunosuppressive treatment due to rheumatic diseases. These patients were infected by Omicron BA.5.6. The first patient was a 28-year-old individual with chronic renal failure due to SLE, requiring hemodialysis. This patient had no history of vaccination against COVID-19 and exhibited extensive lung involvement. The second patient was a 67-year-old woman who had received 3 doses of Sinovac-CoronaVac® and 2 doses of Pfizer-BioNTech BNT162b2®. Despite vaccination, she experienced a severe clinical course that ultimately led to her death. Both the patients were infected by Omicron BA.5.6 with the same rare mutations: NSP13 A389V (0.33% of all samples with the NSP13 sequence), NSP14 M315I (0.13% of all samples with the NSP14 sequence), and NSP16 T140I (0.50% of all samples with the NSP16 sequence).

Discussion

In this study, the patients were infected by the predominant SARS-CoV-2 variants at the time when the samples were collected. The dominant Delta variant was replaced by the Omicron BA.1 and BA.2 subvariants in February–March 2022. The samples taken at this time yielded the Delta variant from 1 patient, the BA.1 subvariant from 3 patients, and the BA.2 subvariant from 3 patients. The Omicron BA.2 variant dominated globally between April and June 2022, while the Omicron BA.5 subvariant was observed in June and July 2022. As a result, 9 of the 10 samples collected from April to June 2022 were the BA.2 subvariant (and the other sample was the BA.1 subvariant), while the BA.5 subvariant was detected in the 2 samples taken at the end of June and the beginning of July. The Omicron BA.5 and its subvariants dominated throughout the world in October 2022 with a rate of 77.1% [8]; and the Omicron BA.5 subvariant was detected in both samples collected at this time.

Individuals who were fully vaccinated (i.e., they had received at least 4 doses of vaccine) and developed an antibody response after vaccination tended to have a milder clinical course of the disease and a shorter duration of RT-PCR positivity. Similarly, in a study in the United Kingdom that involved 1,610 healthcare workers who received the Pfizer-BioNTech BNT162b2® vaccine, the duration of PCR positivity was shorter in vaccinated individuals compared with the control group. Vaccinated individuals also experienced milder symptoms and had a lower hospitalization rate [9]. Another study reported that vaccination reduced asymptomatic infections, decreased transmissibility, and led to a shorter duration of nucleic acid amplification test (NAAT) positivity [10]. Significant progress has been made in the fight against COVID-19 through vaccines and antibody therapies. However, in order to monitor the ability of the variants to escape vaccine-induced immunity, it is necessary to track neutralizing antibody titers in patients with asymptomatic and symptomatic infections after vaccination, and to perform viral RNA sequencing studies [11].

Patients with prolonged PCR positivity (> 4 weeks) were compared with other patients. This group had a higher incidence of malignancies and a history of lymphoma. Patients with a longer duration of PCR positivity were more likely to be in the moderate to severe illness group. Chemotherapeutic agents used to treat malignancies, corticosteroids, and the immune dysregulation caused by the disease itself can disrupt the immune response against COVID-19, leading to prolonged viral shedding [12,13]. It is important to emphasize compliance with infection control measures and isolation periods for these patients due to the risk of prolonged infectivity and the emergence of strains capable of evading immune responses. Similarly, some authors suggest that prolonged shedding may be an indicator of severe illness and could impact prognosis [14].

It is critical to understand the role of immunosuppression in the mutation of SARS-CoV-2 to gain insights into how individuals with suppressed immune systems handle the infection. Many studies have suggested that multi-mutational SARS-CoV-2 variants can emerge from persistent COVID-19 cases and that multi-step evolutionary leaps can occur in the context of partial immune control [15–17]. In this study, priority was given to include immunosuppressed patients because these patients could serve as a reservoir for genetic changes.

The most common mutations in the patient cohort were spike D614G, spike G142D, NSP4 T492I, and NSP12 P323L; which were present in all patients. According to the GISAID 2023 [7] data, the spike

D614G and NSP12 P232L mutations are the most prevalent mutations throughout the world, with percentages of 99.2% and 97.2%, respectively, consistent with the results of the present study. A 2022 global study on the frequency of mutations reported that the most detected spike mutation in Asia, Africa, the Americas, and overall, was D614G [18].

Among the patients infected by the Omicron BA.1 subvariant, 2 died, and the other 2 cases exhibited changes in their clinical presentation even though they had received 4 doses of vaccine within the last 3 months. These BA.1 isolates specifically carried the spike A67V, del211, and ins214EPE mutations, which cause structural changes in the spike protein [19]. The spike A67V mutation works in conjunction with the H69-V70 deletion, altering the antigenic region and affecting viral infectivity [20,21]. Additionally, ins214EPE, in combination with Y145D, is associated with a 7-fold increase in resistance to antibodies. The A67V mutation has also been linked to a decrease in serum neutralization following recovery and vaccination [22]. In this study, these three mutations were associated with increased viral infectivity in patients and resistance to the immune responses.

The Omicron BA.1 subvariant was isolated in a patient with a mild clinical course. This isolate also carried another rare mutation, namely spike A263T. This rare mutation has only been reported 1,163 times throughout the world, and accounts for 0.01% of all samples with the spike sequence. According to COVID-19 data from the GISAID [7], there have been no previous reports of this mutation from Türkiye or neighboring countries. There is no available literature regarding the clinical effects of this mutation. The single patient infected by Omicron BA.1 carrying this mutation was a 30-year-old woman, with no comorbidities, who experienced a mild clinical course, and was managed on an outpatient basis. The patient had received 2 doses of Sinovac-CoronaVac® and 3 doses of Pfizer-BioNTech BNT162b2®, one month prior to testing and had a high antibody level (30,024 AU/mL). The unique features of this case—the patient was young and had no comorbidities, and the spike A263T mutation is rare—raises the possibility that its clinical impact may be more related to antibody escape rather than infectivity and transmissibility.

The rare spike R21T mutation was identified in a patient infected by the Omicron BA.2 subvariant who experienced a severe clinical course with widespread lung involvement and gastrointestinal symptoms following COVID-19 infection and 4 doses of Sinovac-CoronaVac®. This mutation was associated with a fatal outcome. According to the WHO May 2021 COVID-19 Weekly Epidemiological Update [23], the spike R21T mutation, which accompanied the B.1.617.1 (Kappa variant), increases the virus's ability to enter human and intestinal cells, and it shows resistance to the monoclonal antibody Bamlanivimab. These findings are consistent with the present study.

There was a distinct spike L452M mutation in another patient infected by the Omicron BA.2 subvariant. This patient had diabetes mellitus and coronary artery disease. The patient died following a hyperinflammatory clinical course (with an IL-6 level of 1355 AU/mL) and unresponsiveness to an IL-1 receptor antagonist (Anakinra). Studies have indicated that the spike L452M mutation enhances the binding affinity between angiotensin-converting enzyme 2 (ACE2) and the receptor binding domain (RBD), and can adversely affect the efficacy of spike glycoprotein–based vaccines against SARS-CoV-2 [24]. The L452M mutation can potentially lead to immune evasion and enhanced virus–cell binding, contributing to increased infectivity and/or pathogenicity of SARS-CoV-2 and hyperinflammation.

A 28-year-old woman with a severe clinical course and acute respiratory distress syndrome (ARDS)-like lung involvement was infected by the Omicron BA.5 subvariant. She presented a hyperinflammatory profile (with an IL-6 level of 125 AU/mL). She had not been vaccinated and was receiving hemodialysis support due to chronic renal failure associated with SLE. The isolate carried the NSP13 A389V, NSP14 M315I, and NSP16 T140I mutations, which were not observed in the other patients. There are no clinical data available regarding these mutations. However, studies suggest that the NSP13, NSP14, and NSP15 proteins are among the most potent viral interferon antagonists during the early infection period of SARS-CoV-2 [25]. These mutations effectively suppress early interferon production and subsequently contribute to exaggerated inflammatory responses and severe lung immunopathology due to delayed interferon signaling [26]. The patient's comorbidities—chronic kidney disease and SLE—and unvaccinated status likely led to hyperinflammation and ARDS-like lung involvement due to this subvariant.

Conclusions

The COVID-19 pandemic continues to impact the global population, and the emergence of new viral variants remains inevitable. Recently, EG.5 (Eris) has become the dominant SARS-CoV-2 variant throughout the world. EG.5 has an additional spike F456L mutation compared to the other subvariants. Preliminary

evidence suggests that this might help EG.5 evade neutralization by antibodies in body fluids.

Whole-genome sequencing plays a crucial role. It helps to determine the virus's infectivity, disease-related symptoms, and the effectiveness of COVID-19 vaccines; and to identify regional epidemiological variations and sources. Considering the protein alterations identified in this study and their potential implications for immune evasion and effects on host messenger RNA (mRNA), the development of vaccines and drugs targeting these proteins could hold promise for the control of COVID-19.

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Authors contributions

Conceptualization, FS and TS; methodology, FS, TS and OT; software, FS, AC and OT; validation, AC and OT; formal analysis, FS, TS and HT; investigation, TS and FS; resources, FS and HT; data curation, FS and TS; writing—original draft preparation, FS and TS; writing—review and editing, FS and TS; project administration, FS and TS; funding acquisition, FS and TS. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

No conflict of interest is declared.

References

1. Angius F, Puxeddu S, Zaimi S, Canton S, Nematollahzadeh S, Pibiri A, Delogu I, Alvisi G, Moi ML, Manzin A (2023) SARS-CoV-2 evolution: implications for diagnosis, treatment, vaccine effectiveness and development. Vaccines 11: 1235. doi: 10.3390/vaccines11071235.
2. Sah R, Rais MA, Mohanty A, Chopra H, Chandran D, Bin Emran T, Dhama K (2023) Omicron (B.1.1.529) variant and its subvariants and lineages may lead to another COVID-19 wave in the world? An overview of current evidence and counteracting strategies. Int J Surg Open 55: 100625. doi: 10.1016/j.ijso.2023.100625.
3. Bray N, Sopwith W, Edmunds M, Vansteenhouse H, Feenstra JDM, Jacobs P, Rajput K, O'Connell AM, Smith ML, Blomquist P, Hatziioanou D, Elson R, Vivancos R, Gallagher E, Wigglesworth MJ, Dominiczak A, Hopkins S, Lake IR (2024) RT-PCR genotyping assays to identify SARS-CoV-2 variants in England in 2021: a design and retrospective evaluation study. Lancet Microbe 5: e173–e180. doi: 10.1016/S2666-5247(23)00320-8.
4. Centers for Disease Control and Prevention (2022) Science brief: emerging SARS-CoV-2 variants. May 30, 2022. Available: https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/scientific-brief-emerging-variants.html?CDC_AA_refVal=https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html. Accessed: 15 March 2023.
5. National Health Commission of the People's Republic of China (2020) Diagnosis and treatment protocol for COVID-19 patients (tentative 8th edition). Available: https://en.nhc.gov.cn/2020-09/07/c_81565.htm. Accessed: 15 March 2023.
6. World Health Organization (2020) Therapeutics and COVID-19: living guideline (WHO/2019-nCoV/therapeutics/2020.5). World Health Organization. Available: https://iris.who.int/bitstream/handle/10665/338480/9789240018440-eng.pdf. Accessed: 15 March 2023.
7. Global Initiative on Sharing All Influenza Data (GISAID) (2023) GISAID database. Available: https://gisaid.org/. Accessed: 15 March 2023.
8. Xiong W, Peng L, Tsang TK, Cowling BJ (2023) Epidemiology of SARS-CoV-2 Omicron BA.5 infections, Macau, June-July 2022. Emerg Infect Dis 29: 453–456. doi: 10.3201/eid2902.221243.
9. Hall VJ, Foulkes S, Saei A, Andrews N, Oguti B, Charlett A, Wellington E., Stowe J, Gillson N, Atti A, Islam J, Karagiannis I, Munro K, Khawam J, Chand M, Brown C, Ramsay M, Lopez-Bernal J, Hopkins S (2021) Effectiveness of BNT162b2 mRNA vaccine against infection and COVID-19 vaccine coverage in healthcare workers in England, multicentre prospective cohort study (the SIREN Study). Lancet 397: 1725–1735. doi: 10.1016/S0140-6736(21)00790-X.
10. Emary KRW, Golubchik T, Aley PK, Ariani CV, Angus B, Bibi S, Blane B, Bonsall D, Cicconi P, Charlton S, Clutterbuck EA, Collins AM, Cox T, Darton TC, Dold C, Douglas AD, Duncan CJA, Ewer KJ, Flaxman AL, Faust SN, Ferreira DM, Feng S, Finn A, Folegatti PM, Fuskova M, Galiza E, Goodman AL, Green CM, Green CA, Greenland M, Hallis B, Heath PT, Hay J, Hill HC, Jenkin D, Kerridge S, Lazarus R, Libri V, Lillie PJ, Ludden C, Marchevsky NG, Minassian AM, McGregor AC, Mujadidi YF, Phillips DJ, Plested E, Pollock KM, Robinson H, Smith A, Song R, Snape MD, Sutherland RK, Thomson EC, Toshner M, Turner DPJ, Vekemans J, Villafana

TL, Williams CJ, Hill AVS, Lambe T, Gilbert SC, Voysey M, Ramasamy MN, Pollard AJ (2021) Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. Lancet 397: 1351-1362. doi: 10.1016/S0140-6736(21)00628-0.

11. Hacisuleyman E, Hale C, Saito Y, Blachere NE, Bergh M, Conlon EG, Schaefer-Babajew D, DaSilva J, Muecksch F, Gaebler C, Lifton R, Nussenzweig MC, Hatziioannou T, Bieniasz PD, Darnel RB (2021) Vaccine breakthrough infections with SARS-CoV-2 variants. New Engl J Med 384: 2212–2218. doi: 10.1056/NEJMoa2105000.
12. Lee CY, Shah MK, Hoyos D, Solovyov A, Douglas M, Taur Y, Maslak P, Babady NE, Greenbaum B, Kamboj M, Vardhana SA (2022) Prolonged SARS-CoV-2 infection in patients with lymphoid malignancies. Cancer Discov 12: 62–73. doi: 10.1158/2159-8290.CD-21-1033.
13. Baang JH, Smith C, Mirabelli C, Valesano AL, Manthei DM, Bachman MA, Wobus CE, Adams M, Washer L, Martin ET, Lauring AS (2021) Prolonged severe acute respiratory syndrome coronavirus 2 replication in an immunocompromised patient. J Infect Dis 223: 23–27. doi: 10.1093/infdis/jiaa666.
14. Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, Peiris M, Poon LLM, Zhang W (2020) Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis 20: 656–657. doi: 10.1016/S1473-3099(20)30232-2.
15. Corey L, Beyrer C, Cohen MS, Michael NL, Bedford T, Rolland M (2021) SARS-CoV-2 variants in patients with immunosuppression. New Engl J Med 385: 562–566. doi: 10.1056/NEJMsb2104756.
16. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, Solomon IH, Kuo HH, Boucau J, Bowman K, Adhikari UD, Winkler ML, Mueller AA, Hsu TY, Desjardins M, Baden LR, Chan BT, Walker BD, Lichterfeld M, Brigl M, Kwon DS, Kanjilal S, Richardson ET, Jonsson AH, Alter G, Barczak AK, Hanage WP, Yu XG, Gaiha GD, Seaman MS, Cernadas M, Li JZ (2020) Persistence and evolution of SARS-CoV-2 in an immunocompromised host. New Engl J Med 383: 2291–2293. doi: 10.1056/NEJMc2031364.
17. Truong TT, Ryutov A, Pandey U, Yee R, Goldberg L, Bhojwani D, Aguayo-Hiraldo P, Pinsky BA, Pekosz A, Shen L, Boyd SD, Wirz OF, Röltgen K, Bootwalla M, Maglinte DT, Ostrow D, Ruble D, Han JH, Biegel JA, Li M, Huang C, Sahoo MK, Pannaraj PS, O'Gorman M, Judkins AR, Gai X, Dien Bard J (2021) Increased viral variants in children and young adults with impaired humoral immunity and persistent SARS-CoV-2 infection: a consecutive case series. EBioMedicine, 67: 103355. doi: 10.1101/2021.02.27.21252099.
18. Abavisani M, Rahimian K, Mahdavi B, Tokhanbigli S, Mollapour Siasakht M, Farhadi A,Kodori M, Mahmanzar M, Meshkat Z (2022) Mutations in SARS-CoV-2 structural proteins: a global analysis. Virol J 19: 220. doi: 10.1186/s12985-022-01951-7.
19. Asif A, Ali M, Khan MA, Akram W, Majeed F, Ali A, Kamran M (2022) The comparison of mutational progression in SARS-CoV-2: a short updated overview. J Mol Pathol 3: 201–218. doi: 10.3390/jmp3040018.
20. Ozer EA, Afolaranmi OA, Ayodele OA, Ogunkunle TO, Fagbami AH, Oyewole IO (2021) Coincident rapid expansion of two SARS-CoV-2 lineages with enhanced infectivity in Nigeria. medRxiv. doi: 10.1101/2021.04.09.21255206.
21. Cui Z, Liu P, Wang N, Wang L, Fan K, Zhu Q, Wang K, Chen R, Feng R, Jia Z, Yang M, Xu G, Zhu B, Fu W, Chu T, Feng L, Wang Y, Pei X, Yang P, Xie XS, Cao L, Cao Y, Wang X (2022) Structural and functional characterizations of infectivity and immune evasion of SARS-CoV-2 Omicron. Cell 185: 860–871.e13. doi: 10.1016/j.cell.2022.01.019.
22. Shahnshah MFH, Alswat KA, Al-Shehri MA, Alkharsah KR (2022) Comparative analysis of B.1.617.2 (Delta) variant of SARS-CoV-2. Journal of Microbiology and Infectious Diseases 12: 20–26. doi: 10.5799/jmid.1086226.
23. Khairnar K, Rahangdale S, Tomar SS, Tamboli E, Ravikumar S, Salsekar L (2023) L452M in spike glycoprotein indicative of zoonotic links of SARS-CoV-2 XBC.1. Authorea Preprints. doi: 10.22541/au.167357764.45753521/v1.
24. World Health Organization (2021) COVID-19 weekly epidemiological update — 25 May 2021. Available: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---25-may-2021. Accessed: 15 March 2023.
25. Yuen CK, Lam JY, Wong WM, Mak LF, Wang X, Chu H, Cai JP, Jin DY, To KK, Chan JF, Yuen KY, Kok KH (2020) SARS-CoV-2 nsp13, nsp14, nsp15 and orf6 function as potent interferon antagonists. Emerg Microbes Infect 9: 1418–1428. doi: 10.1080/22221751.2020.1780953.
26. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, Perlman S (2016) Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe 19: 181–193. doi: 10.1016/j.chom.2016.01.007.