Correlation Between SARS-CoV-2 Positive Cases Admitted to a Tertiary Care Hospital in Greece and S-gene Mutations

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ABSTRACT

The novel coronavirus SARS-CoV-2 is the cause of the COVID-19 pandemic which rapidly spread worldwide in early 2020. The severity of symptoms of COVID-19 disease, especially among the elderly and high-risk population groups, as well as the complications of the disease, forced the immediate implementation of preventive and protection measures. Moreover, vaccination programs were focused on cooping the virus transmission and spread of the virus in the general population, as well as on the immunization of the community against COVID-19 infection. The circulation of SARS-CoV-2 globally still appears and, as reported to FluNet, was around 10% by the end of February 2024, while the number of new cases and the number of new deaths have decreased. From December 2019, when the first outbreak of the virus was detected in Wuhan, China, until now, SARS-CoV-2 has undergone numerous mutations that have had a major impact on its pathogenesis during the COVID-19 pandemic. Among the SARS-CoV-2 variants and mutations, some are geographically identified, such as D614G, B.1.1.7 in the United Kingdom, B.1.1.28 in Brazil, CAL.20C in Southern California, B.1.351 in South Africa, while B.1.617 and B.1.1.529 have been reported worldwide. The main reason for the identification of SARS-CoV-2 variants and mutations is to investigate any correlation between new mutations and their impact on vaccine efficacy, despite the reported high vaccination rates of the vaccines that were implemented worldwide. The aim of this study is to investigate the correlations between SARS-CoV-2 S-gene target mutations with demographic characteristics, vaccination, hospitalization, and history of previous COVID-19 disease, in patients that were admitted to “Agios Panteleimon” General Hospital of Nikea, Piraeus, Greece, between August 2023 and January 2024.

Keywords: SARS-CoV-2, mutations, strain, variants.

1. INTRODUCTION

COVID-19 due to the SARS-CoV-2 virus has been characterized as the most significant pandemic of the 21st century [1], [2]. It was rapidly transmitted worldwide, affecting the population's health with variable severity of symptoms, mortality, and morbidity, increased need for hospitalization, and is related to lack of the appropriate pharmaceutical substances for disease management and treatment [3], [4].

SARS-CoV-2 is a positive single-stranded RNA virus, that uses this genetic code to reproduce and mutate far more rapidly than a DNA virus [5]. Even minimal changes in the genome sequence of the virus can lead to semantic changes in the target protein structure [6], [7]. The genome of the virus encodes four structural proteins (N, E, M, and S) that give the distinct shape of the virus and are of high importance for viral transmission [7]. Nucleocapsid proteins (N) are responsible for the packaging of the viral RNA. Envelope protein (E) affects viral morphogenesis and promotes the assembly and release of virions. Membrane protein (M) is important for RNA packaging. Spike glycoprotein (S) has a crucial role in the transmission of the virus through its Receptor Binding Domain (RBD).
S proteins consist of S1 and S2 subunits, of which S1 enables the attachment of the virions to the human ACE2 receptor of the host cell membrane.

Except for the structural proteins, the SARS-CoV-2 genome encodes also non-structural proteins such as the Open Reading Frame proteins (ORFs). The most important ORFs are the ORF1a and ORF1b which play a major role in the replication of virions. The RNA-binding (RdRp) domain consists of part of the ORF1ab sequence and has a pivotal role in the transmission of the virus and the process of pathogenesis.

Contrary to N, E, M, and ORF1ab which have a highly conserved sequence among SARS-CoVs, the RBD of Spike protein can have many variations. Until now, more than 50 mutations have been found in the Spike protein, commonly referred to as S-gene dropout. Vaccines and drugs mainly target the four structural proteins mentioned above, as well as the two non-structural proteins ORF1a and ORF1b. Among them, the S gene seems to be more sensitive to mutations resulting in S-gene failure or dropout. Mutations in the S1 subunit seem to affect the interaction with human ACE2 leading to failure of immune response and vaccination effectiveness.

The SARS-CoV-2 Alpha variant is one of the earliest mutated strains from the original SARS-CoV-2 virus. This variant first appeared in September 2020 in the United Kingdom [8]. Alpha variant includes several RBD mutations such as N501Y, and P681H, at positions 69–70 and 144 NTD deletions and several non-spike mutations [9], [10].

The Beta variant was first found in May 2020 in South Africa [11]. Key mutations acquired by the Beta variant include nine mutations on spike protein (N501Y, E484K, and K417N are RBD mutations and NTD deletions at positions 242–244) [10], [11].

The Gamma variant (P.1), which was first found in January 2021 in Brazil, has accumulated more than 22 mutations, with 12 mutations on spike protein. RBD mutations include: L18F, N501Y, E484K and K417T. Also, NTD mutations are found in the Gamma variant. This variant is related to higher rates of hospitalization and increased morbidity, over 3 to 4 times higher in comparison with previously discovered variants [13].

The Delta Variant (B.1.617.2) was first found in India by the end of 2020. Delta variant rapidly became the dominant strain in many countries. WHO announced that the Delta variant is the most transmissible of the variants identified so far [14]. Delta variant has accumulated 23 mutations, with key mutations including Spike protein E484Q and L452R RBD mutation, as well as P681R cleavage site mutation. Several mutations on ORF3 and ORF7 have been reported by conducted studies [15]. This variant shows higher transmission and infectivity levels, compared to other variants, which is associated with the prevalence of the Delta variant among young ages according to Scottish reports. Infection with the Delta Variant is also associated with higher infectious viral loads compared to the Alpha variant, in both vaccinated and unvaccinated individuals [16].

The Omicron variant (B.1.1.529), first found in November 2021, has more than 50 mutations, and 30 genetic changes in spike protein, with most of the mutations involved in immune escape or having higher transmissibility [17]–[19].

The so far mentioned mutations of SARS-CoV-2 refer to D614G point mutation within the spike genome of the virus that had a rapid spread through the population, since late February 2020 [20]–[23]. The N501Y mutation enhances ACE2 proximity and replication of the virus and has been detected in the United Kingdom (SARS-CoV-2 variant, B.1.1.7/VUI-202,012/01) and started by early October 2020 in South Africa (SARS-CoV-2 variant, the B.1.351/501Y.V2) [24]–[26]. The N501Y virus variant spread from Africa to Scotland, France, Sweden, Switzerland, and South Korea in December 2020, and Australia in January 2021. Other mutations in S protein such as K417N, and E484K, may impact the effectiveness of COVID-19 vaccines [27]. Moreover, L452R, B.1.617.1, B.1.427/9, K417N/T, N439K, Y453F, S477N, T478K, F490S, S494P are all related to RBD mutations and enhancing RBD binding to the ACE2 receptor [9]. E484K mutation in spike protein concerns the initiation of the viral entry process since it occurs in critical sites of the receptor binding pattern of the RBD. This mutation was first discovered in the South African variant and the Brazilian variant later [28]–[30].

Vaccines and drugs mainly target the four structural proteins mentioned above, as well as the two non-structural proteins ORF1a and ORF1b. Among them, the S gene seems to be more sensitive to mutations resulting in S-gene failure or dropout.

The aim of this study is to investigate the correlations between SARS-CoV-2 S-gene target mutations with demographic characteristics, vaccination, hospitalization, and history of previous COVID-19 disease, in patients that were admitted to “Agios Panteleimon” General Hospital of Nikaia, Piraeus, Greece, between August 2023 and January 2024.

2. Materials and Methods

The study included 110 patients admitted to the emergency department of General Hospital “Agios Panteleimon” Nikaia Piraeus, with symptoms of COVID-19 disease and were found positive for SARS-CoV-2. Participants were thoroughly informed regarding the objectives and procedures of the study, following which they provided their formal consent. After the implementation of RT-PCR in nasopharyngeal specimens, the positive samples were further analyzed for mutations. The study is in conformance with the Helsinki Declaration requirements and GDPR requirements. Data pertaining to the participants were systematically collected at the point of admission, utilizing referral notes alongside the Hospital Information System. This process of data accumulation extended from August 2023 to January 2024. The methodology for SARS-CoV-2 RNA extraction and detection involved the use of the Xiamen Zeesan Sanity 2.0 System, an integrative, automated platform designated for molecular diagnostics. Nasopharyngeal specimens in 3 ml virus transport media Citoswab by Citotest Labware Manufacturing were collected for this purpose. Handling of the specimens was
performed according to the manufacturer’s instructions. For the extraction and purification of viral RNA, the integrated system uses the magnetic particle methodology. Next, a multiplex TaqMan probe-based one-step real-time RT-PCR completes the targeting of the conserved region of ORF1ab and N genes of SARS-CoV-2. RNase P served as an internal control in all specimens to identify potential false negatives, with the assay demonstrating a sensitivity of 200 copies/ml. Next, all 110 positive samples went through S gene dropout detection using the real-time RT-PCR method to investigate possible mutations. The study was executed within the hospital’s molecular laboratory, which operates under the auspices of Schem EQA (Lab Scala) quality control standards.

3. RESULTS

The study included 110 patients; 47 (42.7%) were male and 63 (57.3%) were female. Patient ages ranged from 14 to 99 years. The age of male patients ranged from 10 to 95 years (mean value 66.72 years), while the age of female patients ranged from 17 to 96 years (mean value 67.10 years). The median values were equal to 67.00 years and 71.00 respectively. There is no significant difference in the mean age value between the two groups; p-value = 0.921; 95% CI for difference: (61.13; 72.32). Age distribution in the study sample is presented in Fig. 1.

Out of the patients included in the study, 83 (73.6%) were vaccinated against COVID-19 and 29 (26.4%) were not vaccinated. Among vaccinated patients 36 were male and 45 were female. Out of the patients under study 63 (56.4%) patients, 24 male and 39 female, were hospitalized. Moreover, 64 patients (58.2%), 31 male and 33 female, had a history of previous disease.

The mutations detected in the patients under study were distributed according to the Table I.

The most prevalent was JN.1 detected in 22 cases, followed by JN.1.1 detected in 15 cases, JN.1.4 detected in 10 cases, and KC.1 detected in 8 cases. 8 samples need retesting due to poor sample quality. The mutations detected in the study are presented in Fig. 2.

We performed a chi-square test of independence to observe all the possible associations between the following parameters: gender, age, hospitalization, mutation, previous disease, vaccination, and month of the infection. The results and the respective p-values are presented in the following Table II.

We observed significant associations between hospitalization with previous disease (p-value = 0.018) and between mutation with the month of infection (p-value = 0.000).

4. DISCUSSION

It is evidenced that SARS-CoV-2 has undergone several mutations which is often a phenomenon among viruses (cov-lineages.org). Viruses change through continual mutations, and often these mutations may result in a new variant of the viruses, that enhances virus spread, transmission, virulence, and infectivity [31], [32]. Co-existence of other viruses’ circulation such as RSV,
Influenza A, and B as well as high transmission of SARS-CoV-2 accelerate mutations of its genome [33].

The mutations that have undergone the SARS-CoV-2 virus, may play an important role in the virulence, the manifestation of severe symptoms of the disease, the reduction of the efficacy of the vaccine, and the ability of viral transmission [34], [35].

The results of the present study show that women (mean age 67.10) were mostly affected (57.3%) during the period of time under study. Most of the cases (73.6%) were vaccinated, and female individuals had the higher vaccination rate. 56.4% of patients were hospitalized, while women were mostly hospitalized, 58.2% of cases mentioned that had a history of previous COVID-19 disease [36]–[38].

The appearance of omicron strain mutations in our study shows that between August 2023 and January 2024 JN.1 (20%), JN1.1 (13.64%), and JN 1.4 (9.09%) were the dominant variants, and our results are relevant to https://www.covid19genomics.dk/statistics; CDC, 2024 and in accordance to cov-lineages.org. The latest epidemiological data from the CDC reported that COVID-19 activity during the above-mentioned period of time increased as the prevalence of the JN.1 Variant Continues to Rise [39]. Our results show that JN.1, JN1.1 JN 1.4 cause more severe disease, since the need for hospitalization of patients was high (56.4%) and that JN.1 was the most widely circulating variant of SARS-CoV-2 in Greece. CDC recognizes that JN.1 is strongly distinct and causes a wave of new infections, so this fact raises the probability that WHO will declare JN.1 as the next variant of concern leading to a new phase of the pandemic [40]. Even though JN.1 displaces other circulating strains, the prevalence of KC.1 (7.27%), XBB.1.16.11 (4.55%), and XBB.1.5.104 (4.55%) were also recognized in our study. This evidence also demonstrates the transmissibility, immunity, and infection severity of the omicron strain in our cases [41]–[47].

Despite the high vaccination rate (73.6%) of patients, the percentage of hospitalizations from COVID-19 was concerning (56.4%). That leads to serious thoughts about the severity of symptoms, as well as the efficacy of vaccines, since the implemented vaccination program in Greece includes 4 doses [17], [37], [41], [48], [49].

5. Conclusion

The JN.1 variant of SARS-CoV-2 was detected in August 2023, and since then has spread rapidly worldwide, becoming the dominant lineage in EE countries and leading to a new big wave of COVID-19 up to February 2024. WHO in January 2023 strongly stated that “COVID-19 was a continuing global health threat, causing far too much preventable disease with worrying potential for long-term health consequences”.

The JN.1 (or BA.2.86.1.1) comes from lineage BA.2 and first appeared in the middle of 2023, originating from omicron sub-variant BA.2 in 2022.

The previously reported infection with COVID-19 in patients may interfere with step-change variants of SARS-CoV-2, thus the virus can avoid immunity.

The undertaken mutations in SARS-CoV-2 variants and the numerous detected lineages demonstrate different transmissibility, symptoms, illness severity, and response to implemented immunization correlated to demographic characteristics of the patients, in different countries globally.

Vaccines and drugs mainly target the four structural proteins mentioned above, as well as the two non-structural proteins.
proteins ORF1a and ORF1b. Among them, S gene seems to be more sensitive to mutations resulting in S-gene failure or dropout [50]. This indicates that the S gene is more likely to give false negative results when used as the only target for RT-PCR testing compared to other viral proteins [51]. Mutations in the S1 subunit seem to affect the interaction with human ACE2 leading to failure of immune response and vaccination effectiveness [49].

**AUTHOR CONTRIBUTIONS**
Antonia Mourtzikou conceived and designed the study wrote the initial and final drafts of the article, made the final editing, and supervised the study. Marilena Stamouli statistically analyzed and interpreted the extracted data. Elpida Toka wrote the instructions for the assay as well as the methodology and provided scientific and logistic support. Georgia Kalliora provided research scope and literature references. Antonia Mourtzikou, Christina Setiopopulo, and Haritini Gotsi carried out the molecular tests. Ioanna Petraki contributed to data collection details. Maria Kimouli carried out the project administration.

**CONFLICT OF INTEREST**
All authors declare no conflict of interest.

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