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The evaluation of SARS-CoV-2 mutations at the early stage of the pandemic in Istanbul population

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Abstract

Background Determination of SARS-CoV-2 variant is significant to prevent the spreads of COVID-19 disease.

Methods We aimed to evaluate the variants of SARS-CoV-2 rate in positive patients in Kanuni Sultan Suleyman Training and Research Hospital (KSS-TRH), Istanbul, Türkiye between 1st January and 30th November 2021 by using RT-PCR method.

Results Herein, 825,169 patients were evaluated (male:58.53% and female:41.47%) whether COVID-19 positive or not [(+):21.3% and (−):78.7%] and 175,367 patient was described as positive (53.2%-female and 46.8%-male) by RT-PCR. COVID-19 positive rate is observed highest in the 6–15- and 66–75-year age range. The frequencies were obtained as SARS-CoV-2 positive (without mutation of B.1.1.7 [B.1.1.7 (U.K), E484K, L452R, B.1.351 (S. Africa/Brazil) spike mutations] as 66.1% (n: 115,899), B.1.1.7 Variant as 23.2% (n:40,686), Delta mutation (L452R) variant as 9.8% (n:17,182), B.1.351 variant as 0.8% (n:1370) and E484K as 0.1% (n: 230). In April 2021, general SARS-CoV-2 and B.1.1.7 variant were dominantly observed. Up to July 2021, B.1.617.2 (Delta variant/ Indian variant) and E484K has been not observed. B.1.351 variant of SARS-CoV-2 has been started in February 2021 at the rarest ratio and March 2021 is the top point. September 2021 is the pick point of E484K. African/Brazil variant of SARS-CoV-2 has been started in February 2021 at the rarest ratio and March 2021 is the top point. September 2021 is the pick point of E484K. When the gender type is compared within the variants, women were found to be more prevalent in all varieties.

Conclusions The meaning of these mutations is very important to understand the transmission capacity of the COVID-19 disease, pandemic episode, and diagnosis of the virus with mutation types. Understanding the variant type is important for monitoring herd immunity and the spread of the disease.

Keywords SARS-CoV-2, COVID-19, Delta, Epsilon, Kappa, Mutation, RT-PCR

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Introduction

The novel Coronavirus (CoV) outbreak was firstly reported in Wuhan city of China, in December 2019 and then spread globally severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible RNA virus that causes "coronavirus disease 2019" (COVID-19) [1–3]. COVID-19 pandemic has been announced by World Health Organization (WHO) in February 2020 [4] and the International Committee on Taxonomy of Viruses (ICTV) named the virus as SARS-CoV-2 [5]. This emerging disease is transmitted person-to-person with droplets, contaminated objects and direct or indirect contacts with mucous membranes in eyes or noses. Disease has an incubation period in which 6.4 days reported [6, 7].

In Türkiye, the first case of COVID-19 was reported on March 11, 2020 [8]. The SARS-CoV-2 pandemic has significantly affected the populace in Turkey, a rustic appearing as a bridge among Asia and Europe continents [9]. In June 2021, the capacity to laboratory diagnose of SARS-CoV-2 infection by real-time reverse transcription–polymerase chain reaction (RT-PCR) become multiplied to all eighty-one provinces of Türkiye, growing the range of laboratories capable of carry out molecular analysis to 482 [10, 11].

Up to now (20.03.2024), 17,004,677 q-RT- PCR positive cases have been reported in Türkiye [8]. According to Turkish Ministry of Health data, between January 1, 2021, and December 1, 2021, a total of 83,507,861 cases were examined and 6,586,936 samples were found positive by q-RT- PCR in Türkiye [12]. This our study covers the period between January 1 and November 30, 2021. In that time 825,169 cases were examined 175,357 patients were found SARS CoV-2 RT-PCR RNA positive in Kanuni Sultan Suleyman—Training and Research Hospital (KSS-TRH), Istanbul, Türkiye.

Phylogenetic evaluation of newly generated sequences mixed with epidemiological and scientific statistics changed into a key tool to recognize the transmissibility pathway of this pandemic virus and to manipulate public fitness measures towards COVID-19 disease [13]. The excessive charge of viral replication is related to the emergence of recent viral variants that generally include mutations of their spike protein [14, 15]. Viral variants growth the performance of viral transmission, cellular tropism, and pathogenicity, and break out immune recognition [16]. The ample and rapidly availability of viral genomic information has had a profound impact at the reaction to the COVID-19 pandemic, from monitoring and tracing of transmission [17, 18], to vaccine and drug development [19]. The WHO proposes a viral class via using the Greek alphabet [20]. During the pandemic waves, numerous SARS-CoV-2 variants had been diagnosed and ultimately categorized as variants of concern (VOC), variants of interest (VOI), and variants under monitoring (VUM) through the WHO [21]. To pick out variants that present a higher health risk, the WHO describe two most important varieties of viral variants: VOC and VOI. Thus, Alpha, Beta, Gamma, Delta, and Omicron are defined as VOCs, in contrast, the variants Lambda and Mu are classified as VOIs [22].

In March 2020, the Global Initiative on Sharing All Influenza Data (GISAID) database commenced to proportion SARS-CoV-2 genomes from Turkey, contributing to the overall performance of molecular characterization and to assess the superiority and diffusion of variants on this unique intercontinental geographic area [23]. Understanding the variant type of SARS-CoV-2 is crucial for tracking herd immunity and the unfold of the disease.

The United States (US) Centers for Disease Control and Prevention (CDC) outline VOCs as people with elevated transmissibility, virulence, severity with inside the signs of disease, reduced performance of antibodies (neutralization and diagnosis), and decreased effectiveness of remedies and protection induced by available vaccines [24].

Delta variant is highly transmissible and also responsible for more severe COVID-19 disease. Additionally, this VOC multiplied the hospitalizations and infected subjects, especially with inside the older age group, irrespective of vaccination status [25, 26]. Delta dominated the COVID-19 epidemic worldwide until Omicron variant emerged in November 2021 [27]. In contrast, viral variants with mutations that alternate the receptor binding affinity—increase the transmissibility (high community transmission) and the severity of the illness produced, affecting the affinity of the antibodies via way of means of the spike protein, and favoring the immune escape—and the effectiveness of the diagnosis are the ones taken into consideration as VOIs [24].

Studies have demonstrated the negative impact of these variants on transmission, vaccines, and therapeutics [28, 29]. As these variants have also spread globally, they have generated significant public health worldwide [30].

Istanbul is a province of Turkey located between Asia and Europe with a population of approximately 16 million. It is also an important transfer center in international air transportation. It is a touristic destination with intense human movements. KSS-TRH is one of the largest hospitals in Istanbul. During the first period of the epidemic, it was appointed as a COVID 19 laboratory diagnosis center by the Turkish Ministry of Health. During the COVID 19 epidemic, it was very important to monitor the mutations of the SARS COV2 virus with laboratory diagnosis and follow the situations of the epidemic.

In this study, SARS COV-2 virus variants were investigated by PCR in the nasopharyngeal swab samples of patients who applied to KSS-TRH hospital with suspicion of COVID 19 between January 1, 2021 and November 30, 2021, and possible mutations were tried to be detected. A laboratory surveillance study was conducted by KSS—TRH, COVID 19 Laboratory Diagnosis Center researchers in 18–28 January 2021, during the first period of the epidemic by Kömürcü et al. According to results, in January, all variants are described as B.1.1.7 type [31].

The study aimed to investigate the status of mutations of SARS-CoV-2 observed after N501Y. Herein, we try to evaluate Delta, Epsilon and Kappa variants in SARS-CoV-2 positive patients to contribute to the literature in the global pandemic that affects Istanbul population in a retrospective approach with the comparison of age, gender, sequence analysis, and seasonal effect on mutation. The data presented in this study will make a great contribution to the COVID-19 literature in terms of epidemic control and management.

Material and method

a) *Subject*: Nasopharyngeal swabs samples of SARS-CoV-2 patients transferred to KSS-TRH, Istanbul, Türkiye, were utilized and collected by trained personnel between 1st January 2021 and 30 November 2021. The swabs were placed in viral transport medium (VTM) solution tube upon collection. Molecular tests were run on the same day. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki and study protocol was reviewed and approved by Ethics Committee of KSS-TRH, Istanbul, Türkiye.

b) *Real Time PCR Tests*: Patients' sample were tested by using two different kits as Bio-Speedy SARS-CoV-2 Emerging Plus (Bioeksen, Türkiye) and Diagnovital Diagno5plex Ns SARS-COV-2 Real-Time PCR Kit (RTA Laboratories, Türkiye). The kit protocols did not require any extra RNA extraction step due to Viral Transport Medium (VTM) solution with nucleic acid extraction property. During the experiment, only swab samples in VTM solution was enough with vigorous vortexing for RNA extraction. Samples obtained with the swab was putted in the VTM. Since RNA molecules are very susceptible to nuclease digestion that may be coming from degrading cells, it was transported the samples in an icebox and isolate nucleic acids within 4 h.

c) *Bio-Speedy SARS-CoV-2 Emerging Plus Kit*: Bio-Speedy SARS-CoV-2 Emerging Plus kit (Bioeksen, Türkiye) has five channels. FAM, HEX, ROX, Cy5 and Cy5.5 channels are utilized to investigate the SARS-CoV-2 variants. Channels refer to ORF1ab + N, RNaseP mRNA (Internal Control), Spike (S) E484K mutation, Nucleocapsid (N) D3L mutation, Spike (S) L452R mutation, respectively. The threshold set was arranged as 200 according to the kit protocol for the Biorad CFX96 (Bio-Rad Laboratories, USA) platform. HEX channel is utilized as internal control. If the FAM, ROX, Cy5 Cq values are smaller or equal 33 (≤ 33), result mean that positive, otherwise the result is considered negative**.**

d) *Diagnovital Diagno5plex NS SARS-COV-2 Real-Time PCR Kit*: Diagnovital Diagno5plex Ns SARS-COV-2 Real-Time PCR Kit (RTA Laboratories, Türkiye). FAM, HEX, ROX, Cy5 and Cy5.5 channels was used for the detection of variants of SARS-CoV-2. The threshold set was arranged as 200 according to the kit protocol for the Biorad CFX96 (Bio-Rad Laboratories, USA) platform. Internal control is read in HEX channel as RNAseP gene and should be HEX ≤ 36. On the other hand, positive control should be amplified in all channels as FAM, HEX, ROX, Cy5 and Cy5.5 ≤ 38. Positive curves should be sigmoid curve and under and equal 38 Cq.

e) *Next Generation Sequencing Analysis*: The Next Generation Sequencing (NGS) study was carried out at the Ankara General Directorate of Public Health National Virology Reference Laboratory. For this purpose, SARS-CoV-2 samples that were detected as strongly positive in KSS-TRH were selected and sent to reference laboratory. The variants of SARS-CoV-2 with mutations, genomes are analysed in next generation sequencing (NGS) and the bioinformatic analysis was utilized in National Center for Biotechnology Information (NCBI) and Global Initiative on Sharing Avian Influenza Data (GISAID). The main application scenario for CoVsurver is to highlight phenotypically or epidemiologically interesting candidate amino acid (aa) changes for further research and should ideally be combined with experimental testing and verification of any predicted phenotypes. Result for comparison with reference selection is hCoV-19/Wuhan/WIV04/2019 [28].

f) *Statistical Analysis*: Statistical package program (SPSS 25.0 version) was used in the analysis of the data obtained because of the research. While analysing the obtained data, descriptive statistical methods (frequency, percentage and mean) were used and they were turned into tables and graphics. Moreover, ratio charts were scaled to 1000.

Results

Data was collected because of the applications of patients who applied to KSS-TRH COVID-19 diagnostic laboratory between 1st January 2021 and 31st November 2021. In addition, mutations on the data were examined. A total of 825,169 patient samples were included in the study. The distribution of 825,169 samples, which were PCR analysed in the laboratory according to gender if was determined that 58,53% so 482,964 were male (400,919 of 61.7% are COVID-19 negative and 82,045 of 46,8% are COVID-19 positive), and 41,47%, 342,205 were female (248,883 of 38.3% are COVID-19 negative and 93,322 of 53.2% are COVID-19 positive). In total it was determined that 175,367 of 21,3% of the participants were COVID-19(+), and 649,802 of 78,7% were COVID-19(−).

In this study, we evaluate the SARS-CoV-2 variants based on both RT-PCR and NGS technology results. 825,169 patients are evaluated (male ratio as 58.53% and female ratio as 41.7%) whether or not COVID-19 positive or not [(+) results as 21.3% and (−) results as 78.7% of the all patients] and 175,367 patients were described as COVID-19(+). In this context, according to the test results, it was determined that 53.2% of the male participants were female and 46.8% of the male participants were COVID-19(+). All patients are compared according to the age and age of between 6 and 55 is applied our laboratory with a COVID-19 suspect. Within this distribution, it was discovered that the 26–35 age group, or the young population, had the highest test number rate and intensity of COVID-19(+) rate. At the same time the intensity of the COVID-19(+) rate is observed highest in the 6–15 age range, followed by the 66–75 age range. The most test capacity is performed in January and the highest COVID-19(+) rate is obtained in April and May 2021. It happens with emerging variant suspect of COVID-19.

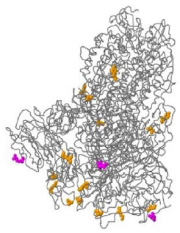
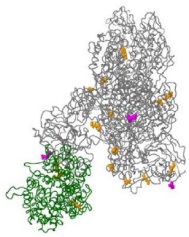
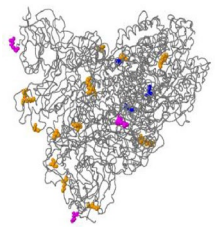
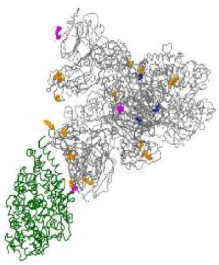
The protein structure of the having mutation patients swab samples are shown in Fig. 1A. The left column refers to the mutation in Spike glycoprotein (PDB: 6acc, EM 3.6 Angstrom) with RBD in down conformation. % aa identity: 99.529%/# aa changes: 6 while right column demonstrates mutations in Spike glycoprotein (PDB: 6acj, EM 4.2 Angstrom) in complex with host cell receptor ACE2 (green ribbon). % aa identity: 99.450%/# aa changes: 7.

Moreover, the list of mutation is displayed in loop/termini region nearest residue as Table 1 and Table 2. Herein, NGS technology was preferred to verify with RT-PCR gold standard technique for the identification of the Mutation.

The NGS was completed in the Turkish Ministry of Health, National Virology Reference Laboratory of the Public Health Directorate, Ankara. Sequence alignments are performed. The comparison between general SARS-CoV-2 and mutations are demonstrated in Fig. 1. FASTA format is created by using NCBI program and then all sequences are aligned to create mutation changes in GISIAD program. The splitting RNA within multiple segments, adapters, sequence libraries, and recombining is part of the NGS process to obtain a genomic sequence as similar with capillary electrophoresis. The NGS is a speed process and has accuracy while the cost of sequencing is declined. Millions of fragments in massively parallel are aligned in NGS processes.

The age distributions of 825,169 participants who underwent PCR analysis in the laboratory are shown in the Fig. 2A. Based on the annual statistics, it was discovered that the patients who applied for the test were mostly between the ages of 6 and 55. Within this distribution, it was discovered that the 26–35 age group, or the young population, had the highest test rate and concentration. When each age is compared based on the frequency, age of under 5 as 1.0%, between 6 and 15 as 5.5%, between 16 and 25 as 23.8%, between 26 and 35 as 25.6%, between 36 and 45 as 19.8%, between 46 and 55 as 13.1%, between 56 and 65 as 6.8%, between 66 and 75 as 2.8%, and higher than 76 as 1.6%. In Fig. 2B, the status of being COVID-19 (+) according to the age distribution of the subjects who underwent PCR analysis in the laboratory are given. In this context, the intensity of the COVID-19 (+) rate is highest in the 6–15 age range, followed by the 66–75 age range. The monthly average age of the participants who underwent PCR analysis is shown in the Fig. 2C. The average age in January-2021 was determined to be 37.37, the highest average age in a year. In the months that followed, it was discovered that the average age decreased over time, with the lowest average age occurring in October 2021. During the year, the average age of the subjects who had the 2021 COVID PCR analysis was 35.72. The Fig. 2D shows the monthly average age of the participants who underwent PCR analysis in the laboratory and were found to be COVID-19 (+). The annual average age of COVID-19 (+) is 36.59. It was determined that the average age was 39.74 in January-2021. It is the highest average age on average in 2021. In the following months, it was determined that the average age decreased as time progressed, and it was determined that the lowest average age in October-2021 was 32.59.

The seasonal distribution of 825,169 participants who underwent PCR analysis in the laboratory is shown in Fig. 3A. According to these data, Winter season (January–February-2021) as 79,583 number (9.6% frequency), Spring season (March–April–May-2021) as 300,713 number (34.4% frequency), Summer season (June–July–August-2021) as 170,029 number (20.6% frequency), Autumn season (September–October–November-2021) as 274,844 number (33.3% frequency). In this context, the maximum number of applications are noticed in the

A	Spike glycoprotein (PDB: 6acc, EM 3.6 Angstrom) with RBD in down conformation. % AA identity: 99.529% / # aa changes: 6	Spike glycoprotein (PDB: 6acj, EM 4.2 Angstrom) in complex with host cell receptor ACE2 (green ribbon). % AA identity: 99.450% / # aa changes: 7
List of variations displayed in structure (nearest residue if in loop/termini region) T19R(20) L452R T478K N501Y D614G P681R(674)		
List of variations displayed in structure (nearest residue if in loop/termini region) T19R(20) L452R T478K N501Y D614G P681R(674) D950N		

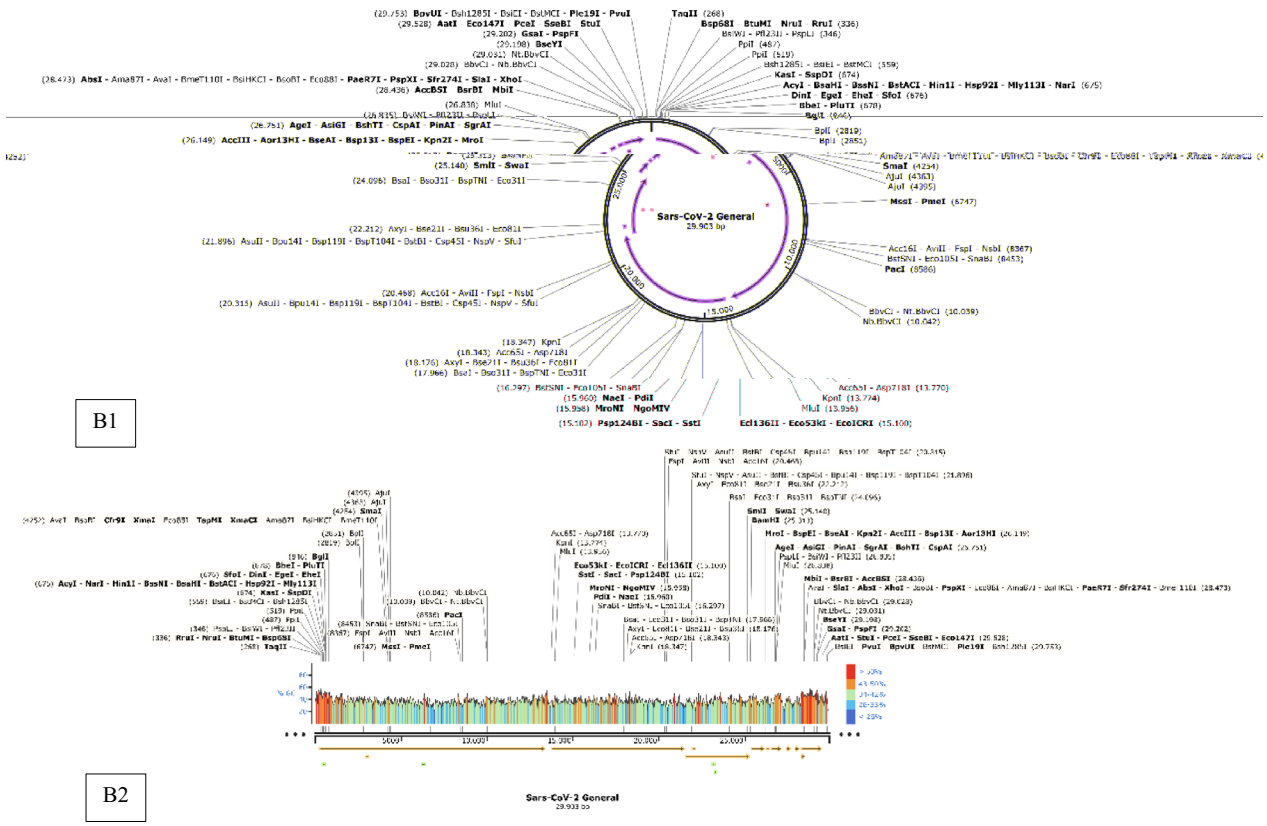


Fig. 1 **A** 3D structural visualization of the spike glycoprotein with aa changes identified in the query sequences shown as colored balls, **B1**. and **B2** the comparison of general SARS-CoV-2 and mutation sequence: SARS-CoV-2 General Map Spiral Image and Line Image, **C1** and **C2** the comparison of general SARS-CoV-2 and mutation sequence: List of variations displayed in structure (nearest residue if in loop/termini region) T19R (20) L452R T478K N501Y D614G P681R (674) Spiral Image and Line Image, **D1** and **D2** the comparison of general SARS-CoV-2 and mutation sequence: List of variations displayed in structure (nearest residue if in loop/termini region) T19R (20) L452R T478K N501Y D614G P681R (674) D950N Spiral Image and Line Image (designed by Y.Artik)

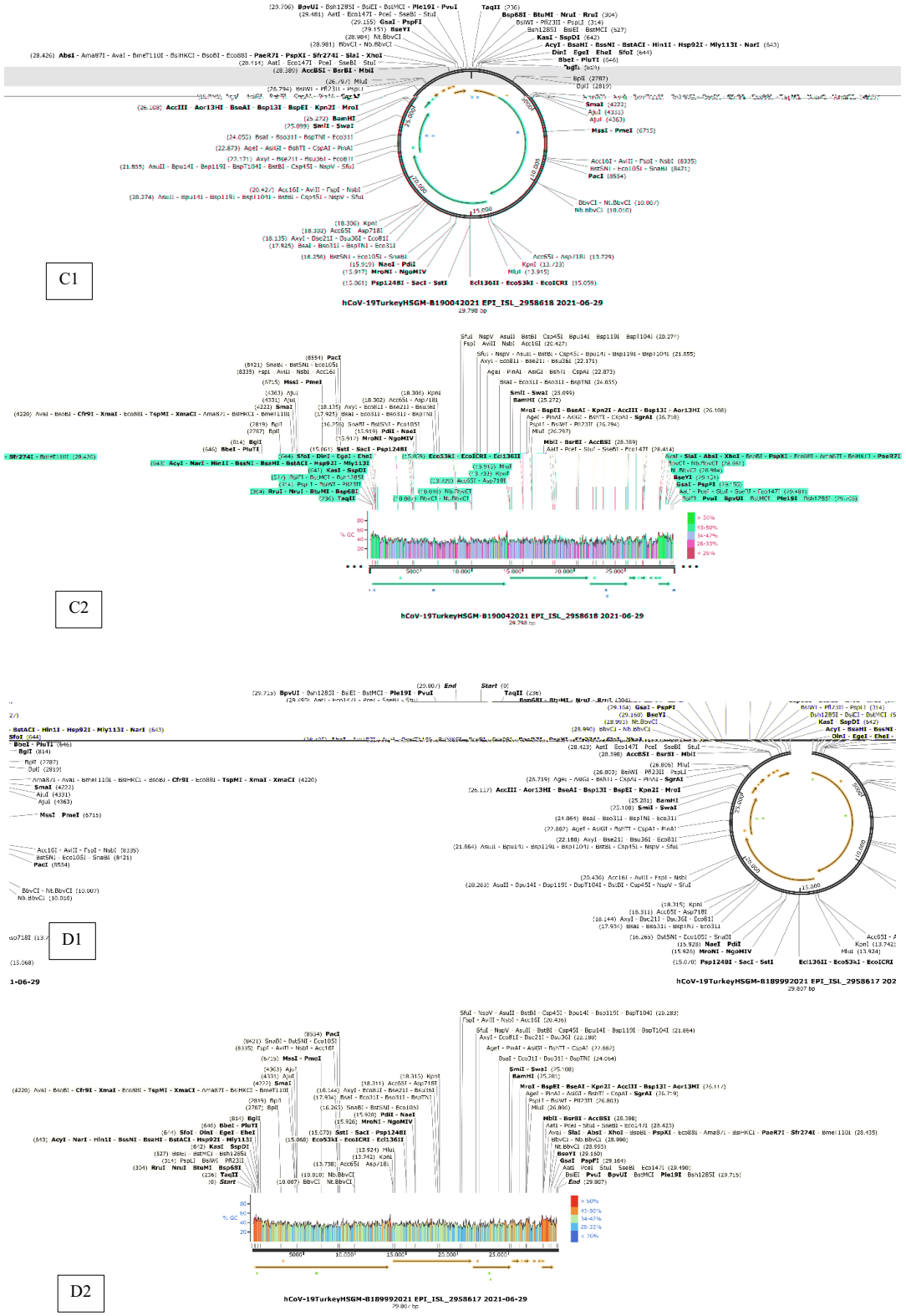


Fig. 1 continued

Table 1 The mutation test changes: List of variations displayed in structure (nearest residue if in loop/termini region) T19R (20) L452R T478K N501Y D614G P681R (674)

Query	Clade	Best reference hit	% Id	% Coverage	#Δs	List of aa changes
hCoV-19/Türkiye/HSGM-B19004/2021 EPI_ISL_2958618|2021-06-29	GK	NSP1 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP2 hCoV-19/Wuhan/WIV04/2019	99.7	100	2	R27C, P129L
		NSP3 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP4 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP5 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP6 hCoV-19/Wuhan/WIV04/2019	99.7	99.0	4	T77A, S106del, G107del, F108del
		NSP7 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP8 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP9 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP10 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP11 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP12 hCoV-19/Wuhan/WIV04/2019	99.9	99.0	1	P323L$^{\#o}$
		NSP13 hCoV-19/Wuhan/WIV04/2019	99.8	100	1	P77L
		NSP14 hCoV-19/Wuhan/WIV04/2019	99.8	100	1	A394V
		NSP15 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP16 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		Spike hCoV-19/Wuhan/WIV04/2019	99.5	100	6	T19R, L452R$^{\$\#a}$, T478K$^{\$\#rao}$, N501Y$^{\$\#rao}$, D614G$^{\$\#lo}$, P681R$^{\$}$
		NS3 hCoV-19/Wuhan/WIV04/2019	99.6	100	1	S26L
		E hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		M hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NS6 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NS7a hCoV-19/Wuhan/WIV04/2019	98.3	100	2	V82A$^{\$}$, T120I
		NS7b hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NS8 hCoV-19/Wuhan/WIV04/2019	100	97.5	0	No aa changes
		N hCoV-19/Wuhan/WIV04/2019	99.0	100	4	D63G$^{\$\#o}$, R203M, G215C, D377Y

Spring-2021 season, and subsequently there are fluctuations (winter-2021. The monthly distribution of 825,169 subjects who underwent PCR analysis in the laboratory are given in Fig. 3B. In this context, the number of people applying for the test is increasing, the highest number of applications is seen in April-2021, and then it is seen that it increases in October. The frequencies are measured as January-2021 as 5.1% (41,909), February-2021 as 4.6% (37,674), March-2021 as 11.3% (93,167), April-2021 as 17.4% (143,284), May-2021 as 7.8% (64,262), June-2021 as 5.4% (44,606), July-2021 as 5.9% (48,301), August-2021 as 9.3% (77,122), September-2021 as 10.6% (87,541), October-2021 as 12.2% (100,336), November-2021 as 10.5% (86,967). In Fig. 3C, the status of being COVID-19 (+) according to the months of the subjects who underwent qualitative PCR analysis in the laboratory between January 1st, 2021—November 30th, 2021, are given. In this context, the months with the highest COVID-19 (+) rate are April and May, respectively. In Fig. 3D, the COVID-19 (+) density of the subjects who underwent qualitative PCR analysis in the laboratory was measured according to the seasons. In this context, it was determined that the season with the highest COVID-19 (+) rate was spring.

The mutation/variant distributions of 175,367 patients whose qualitative analysis results were COVID-19(+) out of 825,169 subjects who underwent PCR analysis in the laboratory between January 1st, 2021 and November 30th, 2021 are shown in Fig. 4A. According to this distribution, the British variant is encountered first, followed by the Delta, B.1.351 variant. The "E484K Variant" is considered a rare variant/mutation. The frequencies are obtained as SARS-CoV-2 positive as 66.1% (11,589), B.1.1.7 (U.K.) Variant as 23.2% (40,686), Delta Mutation Variant (L452R) as 9.8% (17,182), B.1.351 (South Africa/Brazil) Variant as 0.8% (1370) and E484K as 0.1% (230). In Fig. 4B, the distribution of 115,899 patients without mutations out of 175,367 patients whose qualitative analysis results were found to be COVID-19(+) among 825,169 subjects are given. According to this distribution, it has been increasing since the first month of the year, the most positive was observed in April-2021 and then

Table 2 The mutation test changes: list of variations displayed in structure (nearest residue if in loop/termini region) T19R (20) L452R T478K N501Y D614G P681R (674) D950N

Query	Clade	Best reference hit	% Id	% Coverage	#Δs	List of aa changes
hCoV-19/Türkiye/HSGM-B18999/2021 EPI_ISL_2958617|2021-06-29	GK	NSP1 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP2 hCoV-19/Wuhan/WIV04/2019	99.7	100	2	R27C, K81N
		NSP3 hCoV-19/Wuhan/WIV04/2019	99.9	100	1	A488S$^{\#o}$
		NSP4 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP5 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP6 hCoV-19/Wuhan/WIV04/2019	99.7	100	1	T77A
		NSP7 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP8 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP9 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP10 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP11 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP12 hCoV-19/Wuhan/WIV04/2019	99.9	99.0	1	P323L$^{\#o}$
		NSP13 hCoV-19/Wuhan/WIV04/2019	99.8	100	1	P77L
		NSP14 hCoV-19/Wuhan/WIV04/2019	99.8	100	1	A394V
		NSP15 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NSP16 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		Spike hCoV-19/Wuhan/WIV04/2019	99.5	100	7	T19R, L452R$^{\$\#a}$, T478K$^{\$\#rao}$, N501Y$^{\$\#rao}$, D614G$^{\$\#lo}$, P681R$^{\$}$, D950N$^{\#o}$
		NS3 hCoV-19/Wuhan/WIV04/2019	99.6	100	1	S26L
		E hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		M hCoV-19/Wuhan/WIV04/2019	99.5	100	1	I82T
		NS6 hCoV-19/Wuhan/WIV04/2019	100	100	0	No aa changes
		NS7a hCoV-19/Wuhan/WIV04/2019	98.3	100	2	V82A$^{\$}$, T120I
		NS7b hCoV-19/Wuhan/WIV04/2019	97.7	100	1	T40I
		NS8 hCoV-19/Wuhan/WIV04/2019	100	97.5	0	No aa changes
		N hCoV-19/Wuhan/WIV04/2019	99.0	100	4	D63G$^{\$\#o}$, R203M, G215C, D377Y

it decreased. Then it rises again in October-2021. In this direction, it is seen that the month with the lowest mutation without mutation is in June-2021. On the other side, in the Fig. 4C, the monthly distribution of 40,686 British Variants out of 175,367 patients are given. According to this distribution, it is seen that it has emerged from February-2021 of the year. It has been increasing since February-2021, and the highest number of UK variants were found in April-2021 and then it decreased. Towards the end of the year, the UK variant appears to have dropped. In Fig. 4D, the monthly distribution of 17,182 Delta Mutation Variant (L452R) out of 175,367 patients whose qualitative analysis results were found to be COVID-19(+) among 825,169 subjects are given. According to this distribution, it is seen that L452R Variant has been encountered since July-2021 of the year. It has been increasing since August-2021 and it is seen that it reaches the highest point in October-2021. In Fig. 4E, the distribution of 1370 B.1.351 variant out of 175,367 patients are given. According to this distribution, it is seen that it has emerged from February-2021 of the year. It has been increasing since February-2021 and the most B.1.351 variant was found in March-2021 and then it decreases. It is seen that the UK variant has dropped towards the end of the year. The distribution of 230 E484K Variants among 825,169 participants who underwent PCR analysis in the laboratory is given in Fig. 4F, according to months, out of 175,367 patients whose qualitative analysis data were found to be COVID-19(+). L452R Variant has been encountered since July-2021 of the year, according to this distribution. It has been increasing since September-2021 and it is seen that it reaches the highest point in October-2021. The qualitative analysis results of the gender of 825,169 participants who underwent PCR analysis between January 1, 2021 and November 30, 2021 were evaluated in the Fig. 4G, as well as the association between gender and COVID-19(+) variations. As a result, women were found to be more prevalent in all varieties.

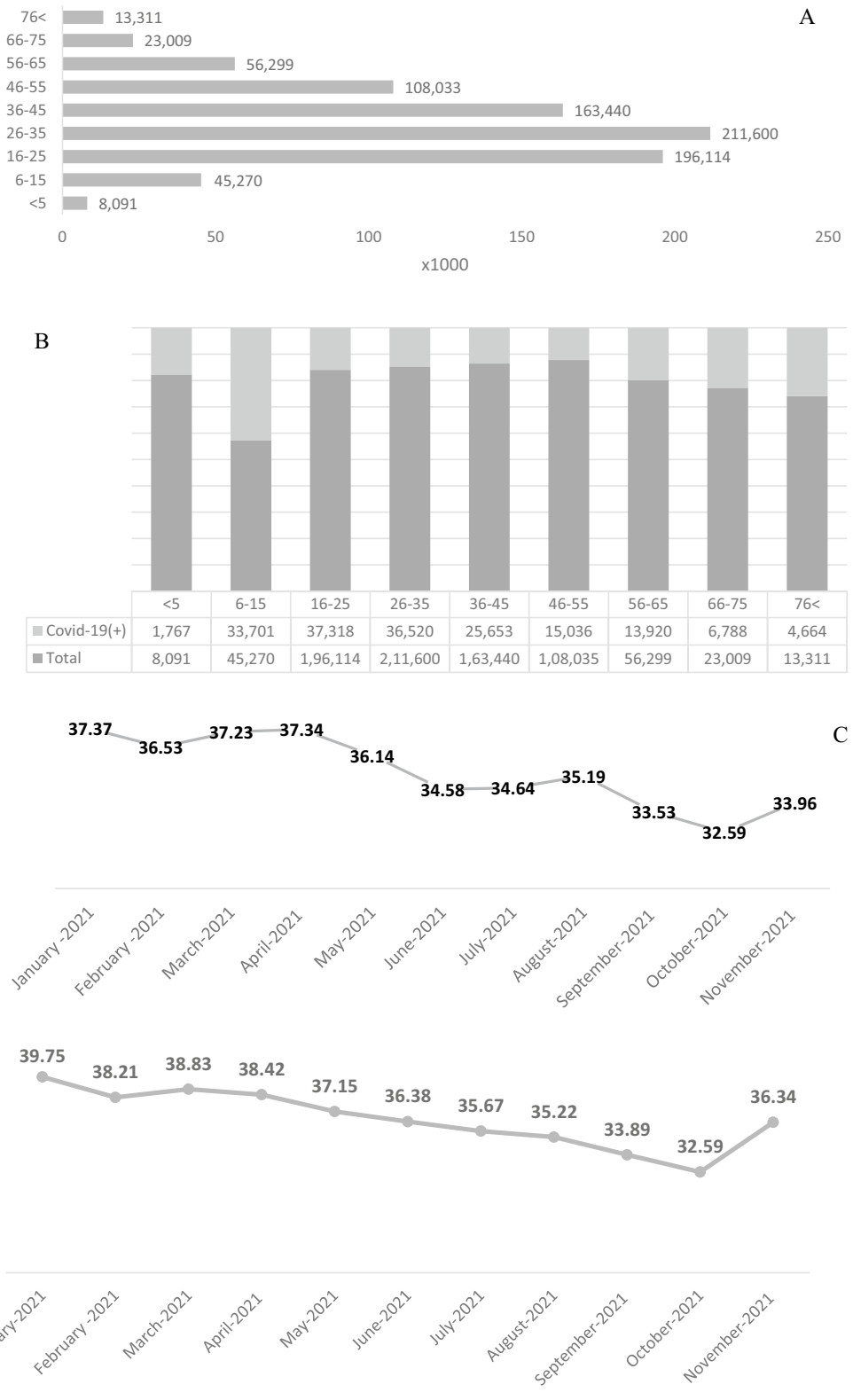


Fig. 2 **A** Frequency Percent of Number of Tests (x axis show ages and y axis show number of PCR tests). **B** Frequency Percent of Number of PCR by Age/COVID-19 PCR (+). **C** Monthly Average Age Charts of Monthly Test Applicants (x axis shows mean of ages). **D** Monthly Test COVID-19(+) Monthly Average Age Charts (x axis shows mean of ages)

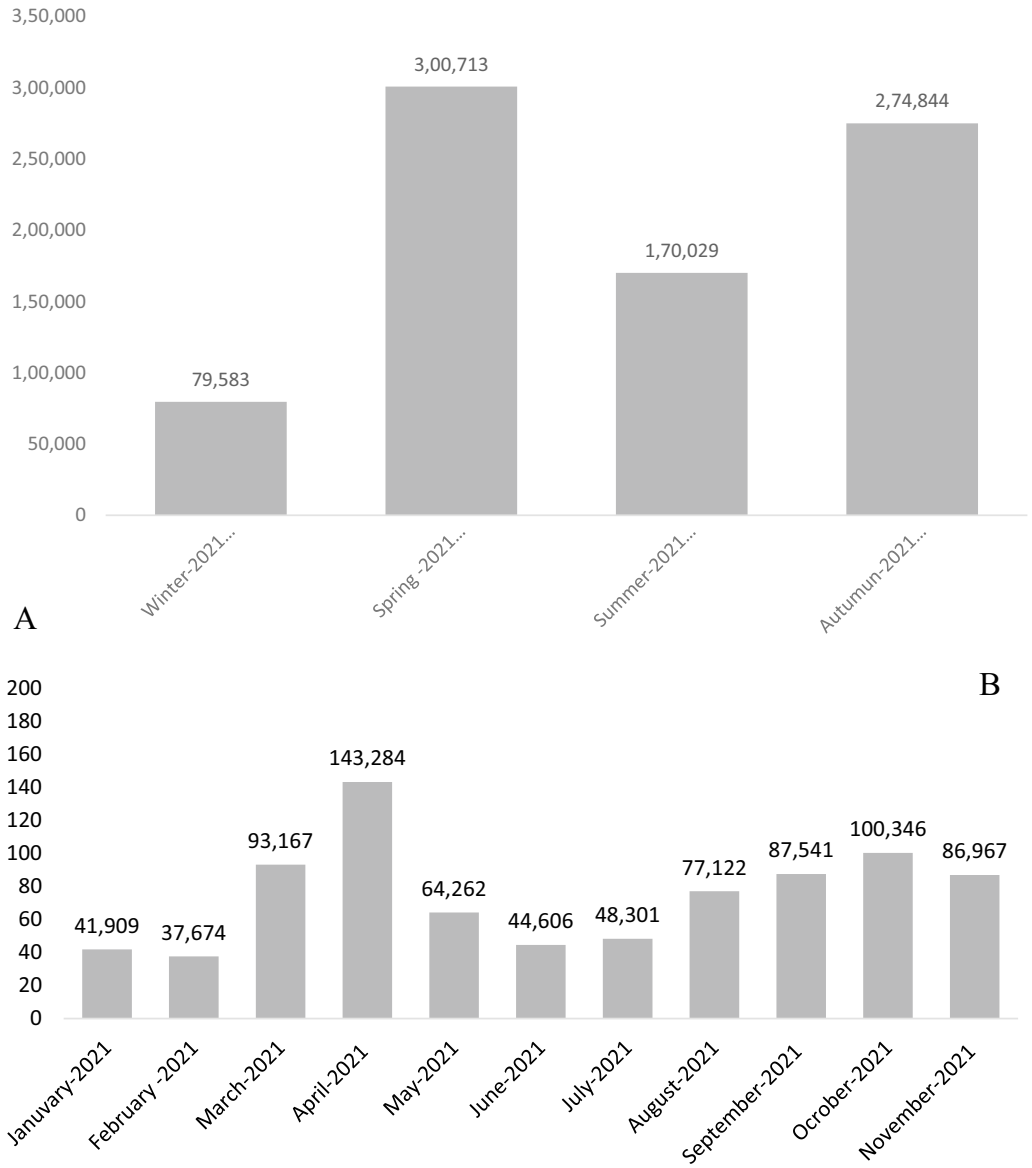
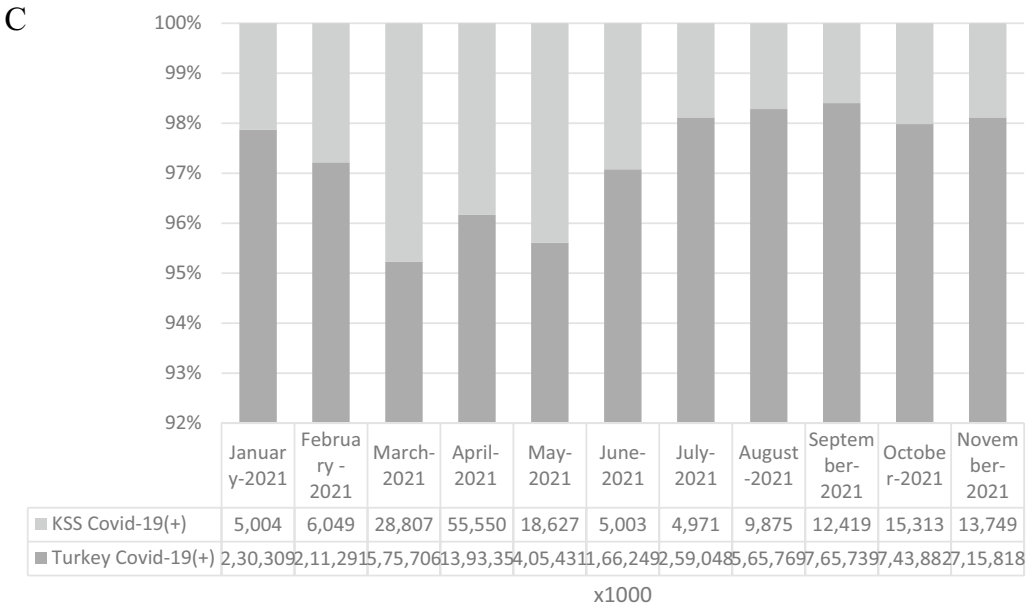


Fig. 3 **A** Frequency Percent Graphs of Number of Tests by Monthly Number of Tests (x axis shows number of PCR tests). **B** Number of Tests (x axis shows number of PCR tests, × 1000). **C** Total Test numbers by Months/COVID-19 PCR (+) in KSS- TRH and Türkiye. **D** Seasonal COVID-19 PCR positive case numbers and positivity rate (%) in KSS- TRH

Discussion

Recently world fights with the newly emerged coronavirus family member, SARS-CoV-2 and disease are COVID-19 [1, 2, 32]. In recent studies shown that actual therapy against the disease is limited. Antivirals, such as remdesivir and nirmatrelvir-ritonavir, have proved to be most useful earlier in illness and for less severe disease. Immunomodulatory therapies, such as dexamethasone and interleukin-6 or Janus kinase inhibitors, are most useful in severe disease or critical illness [33]. Thus, diagnosis of the disease was the main starting point. Up to now, sequence analysis has been utilized however which are time-consuming and require special instruments and specialized experts. Therefore, RT-PCR is preferred as a gold standard method to detect the diagnosis and follow-up of the COVID-19 disease [34].

Many different types of SARS-CoV-2 variants are described such as Alpha, Epsilon, Gamma, B.1.1.7 (U.K.), Brazil, Delta or even Kappa variants [35]. The genetic variability of SARS-CoV-2 determined the accumulation of mutations over time, influencing transmission, severity of disease, and vaccination efficacy



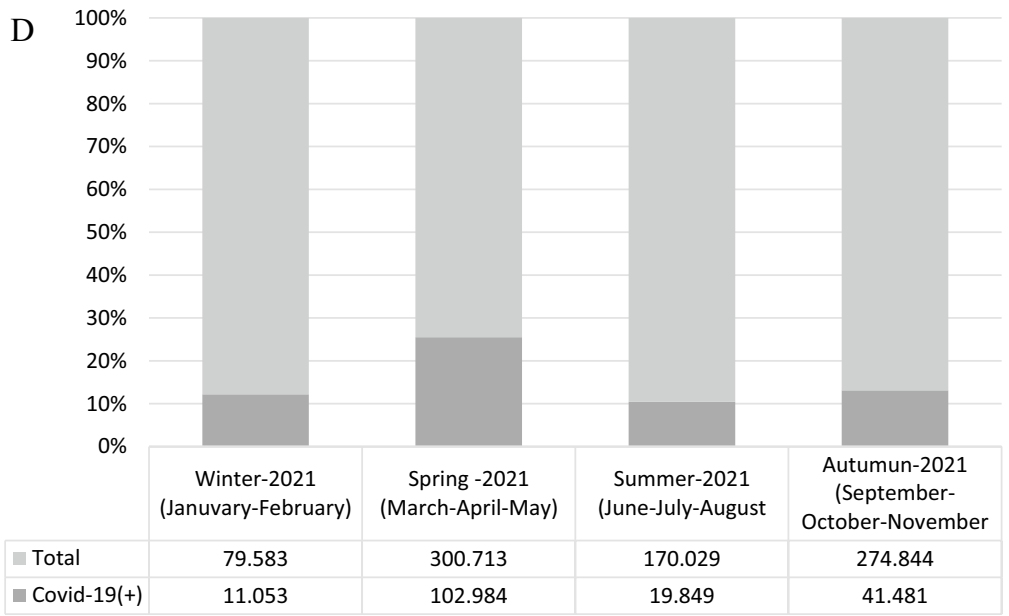


Fig. 3 continued

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Fig. 4 **A** Frequency-Percent Table and Graph of COVID-19 PCR (+)'s by Variants (x axis shows number of variants). **B** Frequency-Percent of COVID-19(+) PCR's by SARS-CoV-2-Positive (x axis shows number of positivity). **C** Frequency-Percentage of COVID-19(+)'s by UK Variant (x axis shows number of UK variant). **D** Percentage of COVID-19 PCR (+)'s by Delta Variant (L452R) (x axis shows number of L452R variant). **E** Frequency-Percent of COVID-19 PCR (+)'s by South Africa/Brazil Variant (x axis shows number of South Africa/Brazil variant). **F** Frequency-Percentage of COVID-19 PCR (+)'s by E484K Variant (x axis shows number of E484K variant). **G** Association between gender and COVID-19 PCR (+) variations (x axis shows number of variants)

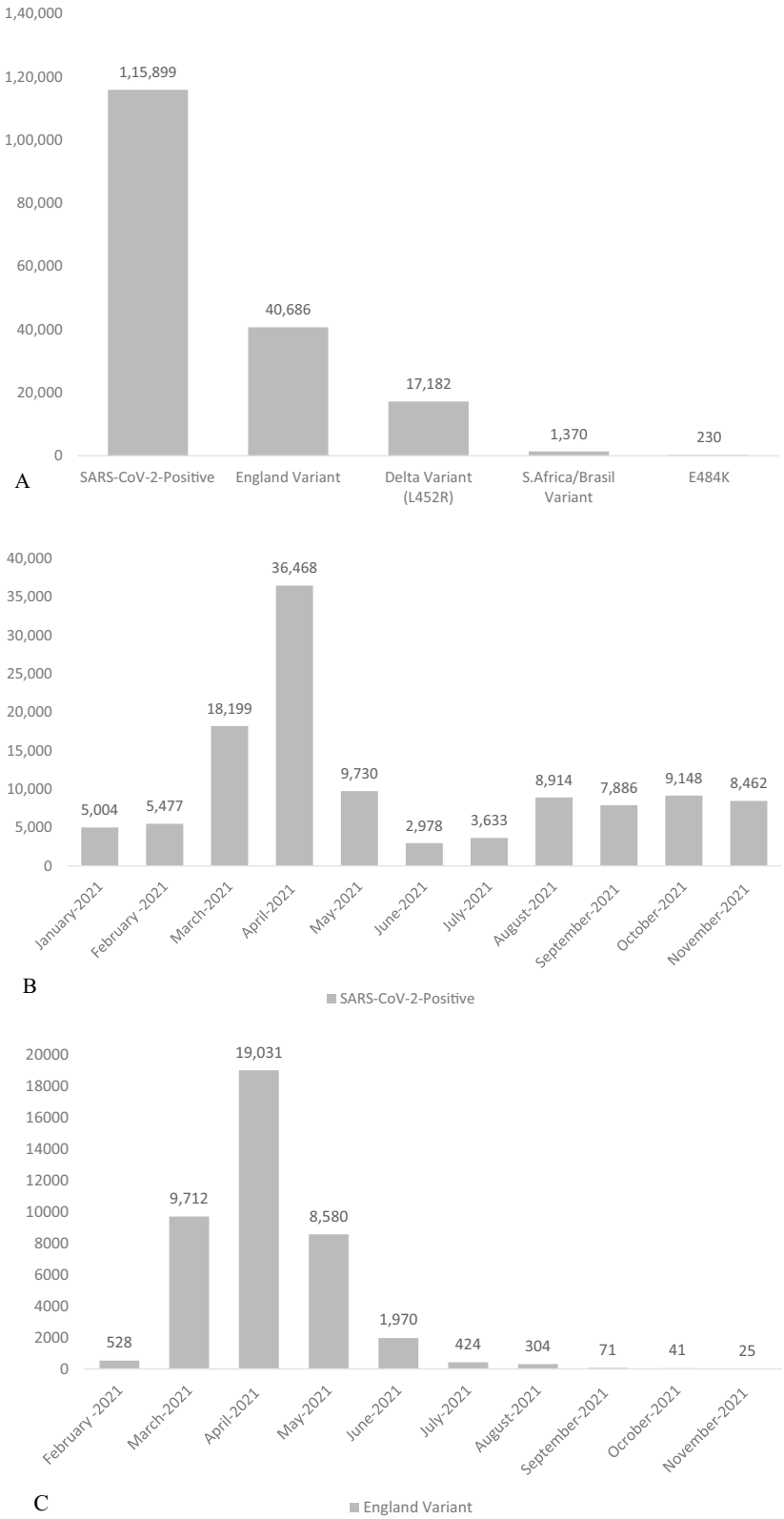
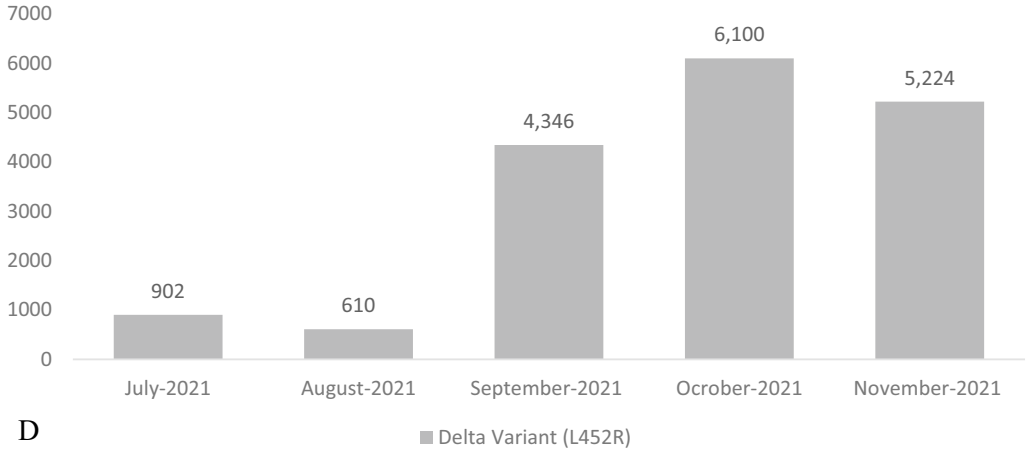


Fig. 4 (See legend on previous page.)



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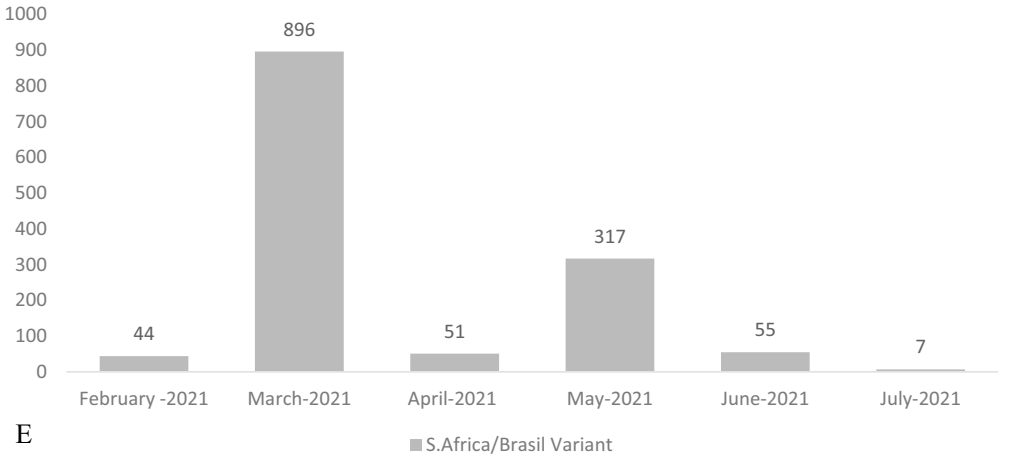

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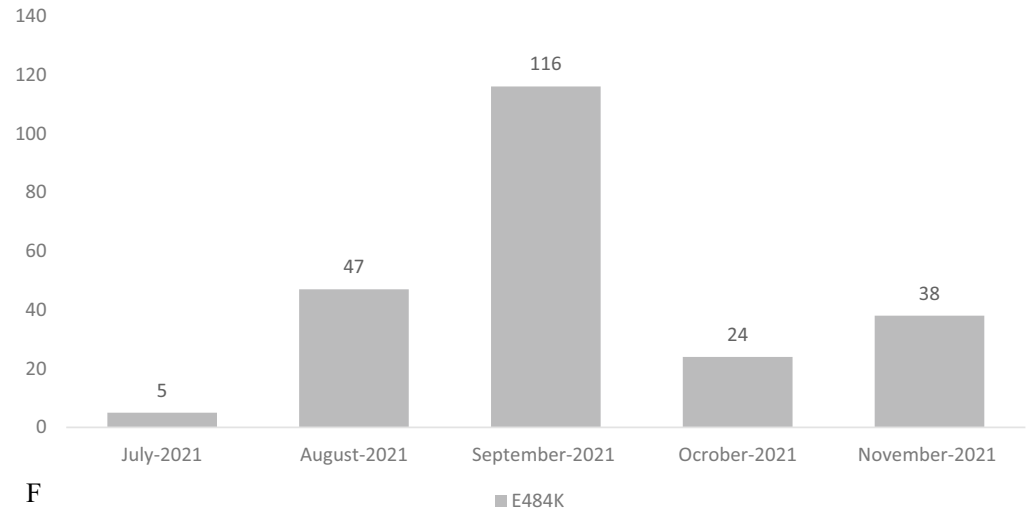
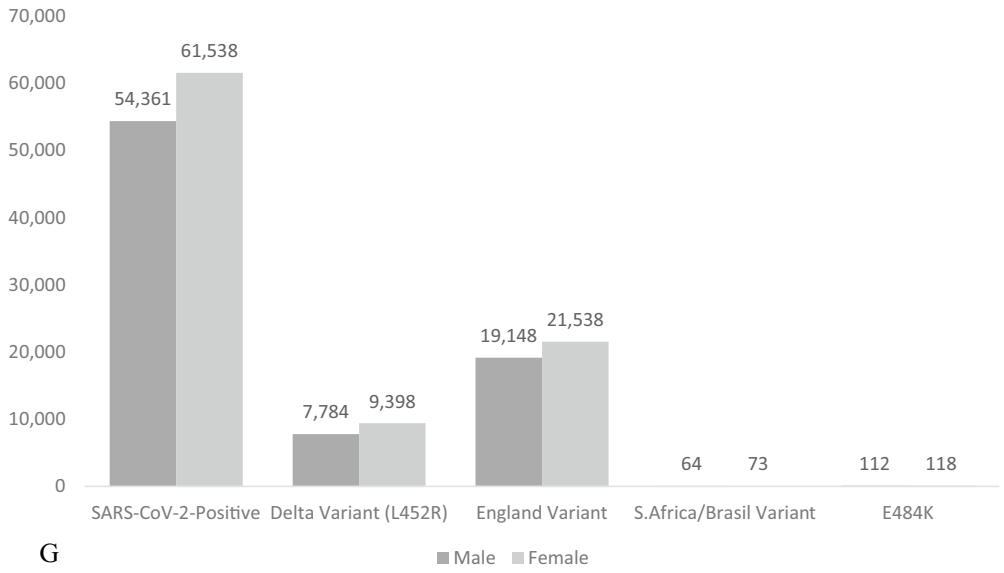


F

Fig. 4 continued



G

Fig. 4 continued

[36, 37]. Since the beginning of the pandemic, clinical and demographic characteristics linked to sequence information were noted to better understand outbreak episodes and local dynamic evolution [38, 39]. The US CDC has been categorized variants of concerns as a variant with one or more mutations which is important to understand how the virus infects people easily and spreads from person to person. If the SARS-CoV-2 variant has a clear association with unusual events, it is called a VOC, and if the variant is still being investigated for its association with unusual events, it is called a VOI. B.1.1.7, also known as the British variant, contains an unusually high mutation rate. This feature provides this variant with high contagiousness [28, 29, 37]. It has been reported that this variant has little evasion effect from post-vaccine neutralizing antibodies for all vaccines [40].

The unaware of their COVID-19 status are actual problem for the virus transmission which is highly related with the mutations and variants of the SARS-CoV-2. SARS-CoV-2 has showed increased mutations rates encourage its spread that provide it to spread in the face of rising population immunity while maintaining their replication fitness. Sequence analysis reported most of the mutation is found in the spike protein of the virus [41].

Additionally, D614G; the RBD mutation N501Y; the RBD mutation E484K; other RBD mutations; NTD mutations; mutations proximal to the S1/S2 furin cleavage site; and non- spike mutations are demonstrated with several [35]. Thus, SARS-CoV-2 variants are classified according to their lineage and component mutations [1, 17, 42].

The variants of SARS-CoV-2 is classified by transmissibility, disease severity the response to vaccination by produced immunoglobulin IgG antibody, reduced effectiveness of treatments, or diagnostic detection failures, and termed as VOC or VOI [43–45].

In the early 2020, India, two actual variant was emerged sharing with common ancestor as Delta (B.1.617.2) and Kappa (B.1.617.1) [44]. In the United States, the Delta variant was first identified in March 2021 and after a while Delta plus is described [45]. These variants show mutations as L452R in RBD region, P681R in roximal furin cleavage site, and other various mutations within orf3, orf7a, and the nucleocapsid gene [46]. While B.1.617.1 included the RBD mutation (E484Q), the B.1.617.2 showed the RBD mutation (T478K) [35].

According to the US CDC data, signature Spike mutations in the aggregated Delta and Delta Plus variant include T19R, (V70F*), T95I, G142D, E156-, F157-, R158G, (A222V*), (W258L*), (K417N*), L452R, T478K, D614G, P681R, and D950N [47]. Especially, B.1.617.2 of SARS-CoV-2 shows the high transmission ability, and low vaccine coverage in which 54 countries are affected rapidly [48].

Recent studies reported that both vaccinated and unvaccinated infected people demonstrate similar Delta viral loads. Alpha, Beta, Gamma, Delta, Delta Plus, Epsilon, Eta, Theta, Iota, Kappa, and Lambda are listed in these variant class [49]. These questions recalled whether vaccination controls Delta spread as effectively.

By late November of 2020, the Epsilon (B.1.429/B.1.427) lineage raised in the US state of California. The sequence analyses are reported that the

epsilon variant is defined with 4 additional amino acid mutations, including two spike protein mutations, S13I and W152C, located in the signal peptide and N-terminal domain, respectively [50]. Additionally, L452R and S13I/W152C mutations were found Epsilon variant [51]. On the other side, in 2021 March, new variant is described termed with lineage B.1.617.1 and then the Kappa variant of interest by the WHO. Generally, E484Q, L452R and P681R co-mutation in the Spike glycoprotein (S protein) is observed with this variant. Additionally, it is termed as co-mutation of L452R and P681R [52].

Kappa variant emerged ~ 35% of all sequenced cases in India. Although the multiple mutations are found in S protein of the virus to increase the viral fitness, variant has not dominantly spread globally. Kappa and Delta variants share the identical substitutional mutation (L452R) [53]. The mutations were observed at similar positions (484) in Kappa, Beta and Gamma, with differing amino acid changes [54]. Thus, the meaning of these mutations is very important to understand the transmission capacity of the COVID-19 disease, pandemic episode, and diagnosis of the virus with mutation types.

The Nucleocapsid (N) D3L mutation of B.1.1.7 is lineage specific and is used for detection of this variant. The E484K mutation significantly reduces antibody neutralization. This mutation significantly increased the transmission rate of SARS-CoV-2 in America and India. It has also been reported that this mutation has the effect of avoiding neutralizing antibodies formed after BNT162b2 vaccination. The basic reproduction number (R0), is intended to be an indicator of the contagiousness or transmissibility of infectious and parasitic agents. R0 number of B.1.617.2, which is the average number of people who will contract a contagious disease from one person with that disease, is between 5 and 8. These means that if $R0 > 1$, the epidemic will grow, and if $R0 < 1$, the epidemic will reverse [55]. Variants containing the E484K mutation (such as the Beta and Gamma variants), reduced neutralization of post-vaccine sera, whereas there was a minimal effect on the Alpha variant. Garcia-Beltran et al. utilized a lentiviral vector-based SARS-CoV-2 pseudovirus neutralization assay and compared the neutralizing capacity of BNT162b2 vaccine and mRNA-1273 vaccine post-vaccination sera against SARS-CoV-2 variants of interest or concern [56]. In conclusion, studies on post-vaccine sera showed that E484K alone, or combined with other mutations or variants containing E484K, reduced the neutralization titer, regardless of the vaccine platform used in different studies [57–60]. Due to the availability of L452R and E484Q mutations, the B.1.617.1 has showed high capability to evade humoral immunity than B.1.617.2 [59].

Additionally, it exhibits reduced susceptibility to "Casirivimab" and to "Bamlanivimab". B.1.617.2 were lower in ChAdOx1 vaccines than in BNT162b2 vaccine while B.1.617.2 had higher replication and spike-mediated entry than B.1.617.1 [60]. The UK has been exhibited a high vaccination ratio. However, although there was high vaccination coverage, the B.1.617.2 variant spread dominantly and rapidly. Spread ability of Delta variant has been higher than Alpha and Epsilon variant as 90%, globally [57]. Some medical experts supposed that the actual problem of high transmission ability of B.1.617.2 variant could be related to the country's decision to delay second doses of vaccine.

Lopez Bernal et al. were explained that one dose of either the Pfizer-BioNTech BNT162b2 vaccine or the AstraZeneca-Oxford ChAdOx1 nCoV-19 vaccine is insufficient to conserve against symptomatic infection with the B.1.617.2 variant. On the other hand, two doses showed the effectivity to 88% and 67%, respectively with these two vaccines which are still lower protection against than Alpha variant offered by both vaccines [61].

The present version PCR assays, regularly desired with inside the initial detection of SARS-CoV-2 mutations, permit the prediction of lines that want to be showed via way of means of NGS [62]. Alpha (September 2020), Beta (May 2020), Gamma (November 2020), and Delta (October 2020) were previously circulating VOCs, while Omicron (November 2021) is the only VOCs in circulation on the World [30].

In Türkiye, the most common lineages were found as B.1.1.7, B.1.617.2, B.1.1.529, and B.1.1.529.1 by Sayan et al. [62] from April 2021 to February 2022. According to Sayan's Study, In September 2021, B.1.617.2 lineage (n = 28, 100%) were the dominant in Turkiye. Other lineages were not detected among the sequenced strains. In Turkiye, Delta was circulating in December 2021 with a higher rate (n = 27, 84%) while a new strain named Omicron (n = 5, 16%) was reported for the first time in the same month [62].

In May 2021, the prevalent variant circulating in Turkiye was Alpha (B.1.1.7), followed by the Beta (B.1.351) and Gamma (P.1) VOCs and, by a low percentage, of the Epsilon (B.1.427 and B.1.429) VOI [24]. In June 2021, COVID-19 cases increased rapidly (around four million subjects infected) and over 40,000 deaths were observed [23]. The hallmark of the fourth wave was the appearance of the Delta (B.1.617.2) lineages [63]. In August 2021, more than 20,000 people were infected per day in Turkiye [26]. Delta variant dominated the epidemic worldwide until Omicron emerged in November 2021 [64].

In this study, during the investigation of variant types of SARS-CoV-2, the British variant is raised first, followed by the Delta, B.1.351 (South Afica/Brasil) variant

between January 1 and November 30 2021 in Istanbul population.

In this study, The "E484K Variant" is considered a rare variant/mutation in the early period of the pandemic. The E484K mutation is no longer a rare and has been detected in several lineages and VOCs in the later period of pandemic. The frequencies are obtained as SARS-CoV-2 positive as 66.1% (115.899) and B.1.1.7 Variant as 23.2% (40.686), Delta Variant (L452R) as 9.8% (17.182), B.1.351 variant as 0.8% (1.370) and E484K as 0.1% (230) in our study.

To understand the transmission ability of the COVID-19 disease, variants are important starting point. Herein, we evaluate the four main type of the variant varieties. In April 2021, SARS-CoV-2 positive patients are dominantly observed and same time B.1.1.7 variant of the SARS-CoV-2. However, after the June 2021, B.1.1.7 variant has declined, and November 2021 ratios has been zero. Up to July 2021, Delta variant (L452R) has been not observed. It has been increasing since September-2021 and October 2021 is the higher point of the transmission of Delta Mutation Variant (L452R). B.1.351 variant of SARS-CoV-2 has been started in February 2021 at the rarest ratio and March 2021 is the top point. After the July 2021, the variant has been observed with a decline. On the other hand, E484K has not been observed up to July 2021 as L452R variant and September 2021 is the pick point. Up to end of the October 2021, there was a fluctuation for this variant type. When the gender type is compared within the variants, women were found to be more prevalent in all varieties. As of June 2021, detection of variants is insufficient.

Conclusion

Following the global impact of the variants, SARS-CoV-2 variants (delta, epsilon, kappa, etc.) played an important role in the transmission of COVID-19 disease. It has been reported that the delta variant is the most common and affects the severity of the disease the most. During the COVID-19 pandemic period, most delta variant positive patients experienced severe disease. In fact, even though patients were vaccinated (one or more times), the delta variant appeared to affect the population.

Therefore, it was interpreted that these variants may increase transmission rates of the virus and/or increase the risk of re-infection. Understanding the variant type of SARS-CoV-2 is important for monitoring herd immunity and the spread of the infection. For this reason, management, and control of the epidemic risk during the pandemic period in Istanbul, which connects the European and Asian continents and is an international transfer location, was very important in terms of minimizing the global risk. In conclusion, this study provides additional information and data into SARS-CoV-2 epidemiology and surveillance in Türkiye.

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Author contributions

YU analysed the data, wrote the article, and vouched for it. SZMK provided support on academic consultancy and administrative process management throughout the entire research process and in the analysis of PCR results. YA developed the protocol, summarized, and analysed the data, wrote the article, and vouched for it. NPC analysed the data, wrote the article, and vouched for it. AT collected the data. KŞ provided support on academic consultancy the research process.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study protocol was reviewed and approved by Ethics Committee of KSS-TRH, Istanbul, Türkiye No: 2021.11.305, Subject No: KAEK/2021.11.305 Date: 26.11.2021-09:45-E-80929729-000-20628 and Republic of Türkiye, Ministry of Health, COVID-19 Scientific Research Studies Approval No: YakupArtik-2021-11-03T01_31_31. Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare no competing interests.

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