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RESEARCH ARTICLE

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The evaluation of potential global impact of the N501Y mutation in SARS-COV-2 positive patients

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Abstract

Rapid and reliable detection of severe acute respiratory syndrome coronavirus 2 mutations are significant to control the contagion and spread rate of the virus. We aimed to evaluate the N501Y mutation rate in randomly chosen positive patients with the polymerase chain reaction (PCR). The evaluation and analysis of the data with a retrospective approach in cases with mutations, in terms of public health, will contribute to the literature on the global pandemic that affects our society. Public health authorities will take the necessary precautions and evaluate the current situation. The N501Y mutation was detected in patients with positive Covid-19 PCR test results. The positive samples were examined based on the 6-carboxy-fluorescein (FAM) channel in reverse transcription PCR (RT-PCR) quantitation cycle (Cq) values as low Cq (<25), medium Cq (25–32), and high Cq (32–38) groups. In the study, 2757 (19.7%) of 13 972 cases were detected as mutation suspects and 159 (5.8%) of them were found to have mutations. The ages of the cases with mutations ranged from 1 to 88 years (mean age of 40.99 ± 17.55). 49.7% ($n = 79$) of the cases with mutations were male, and 50.3% ($n = 80$) were female. When the RT-PCR-Cq results were examined, it was seen that it varied between 11.3 and 35.03, with an average of 20.75 ± 3.32.

KEYWORDS

coronavirus, N501Y mutation, polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), SARS-CoV-2

1 | INTRODUCTION

Three types of coronavirus have plagued humans since the early 21st century; severe acute respiratory syndrome coronavirus (SARS-CoV), the Middle East respiratory syndrome coronavirus (MERS-CoV), and finally severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] SARS-CoV-2 first reported in the Wuhan city of China in December 2019, has caused a pandemic all over the world, which infected more than 146 billion and killed more than 3 billion people.[2] The disease of the SARS-CoV-2 is called COVID-19. Globally, there have been 192 284 207 confirmed cases of COVID-19 including 4 136 518 deaths reported by the World Health Organization (WHO). As of July 19, 2021, a total of 3 568 861 733 vaccine doses have been administered.[3] The SARS-CoV-2 transmits from person to person through droplets, contaminated objects, and also direct contact. The symptoms of COVID-19 are described as fever, throat, tiredness, cough, and sore.[4] The average incubation time was detected as 5.2 days and this information was updated as 6.4 days with new studies.[5] The coronavirus family are positively stranded enveloped RNA viruses. They can be mainly classified into four genera such as beta, alpha, gamma, and delta-coronavirus.[6] SARS-CoV-1, SARS-CoV-2, and MERS-CoV are the type beta of coronavirus and only affected on

mammalian. The major structural form of the SARS-CoV-2 is the 30 kb genome with 14 open reading frames encoded to the spike protein (S), nucleocapsid protein (N), a small membrane protein (SM), and membrane glycoprotein (M) with an additional membrane glycoprotein (HE).[7] The spike protein provides the coronavirus entry into host cells by first binding to a specific host receptor and then fusing viral and host membranes. When the spike protein interacts with a host receptor part, the spike protein is divided into two subunits; first an amino-terminal subunit (S1) and a carboxyl-terminal subunit (S2) by host furin-like proteases.[8] The C-terminal of the S1 subunit (S1 CTD) consists of the receptor-binding domain (RBD) which takes the critical role in recognizing and binding the host receptor. Furthermore, it is responsible for the zoonotic transmission of coronaviruses and determining cell tropism. On the other hand, the S2 subunit involves a hydrophobic fusion loop and two heptad repeat regions (HR1 and HR2) which are significant for membrane charge fusion as seen in Figure 1.[9] The CTD1 (N-terminal domain in CTD) of the spike protein is a receptor binding domain. The conformational changes emerge in this domain with the changes from the "down" conformation to the "up" conformation. These conformational changes cause the conversion of the inactivated state to the activated state to allow for receptor binding. The first step of viral entry within the host cell is the interaction of spike RBD of SARS-CoV-2 and ACE2 receptor of the host.[10] Furthermore, most of the mutation is observed in these regions.

The major challenges against the control of pandemic all over the world are the mutations in SARS-CoV-2 genome. Nowadays, various types of variants of SARS-CoV-2 have been reported. In September 2020, the first variant was described in the United Kingdom (UK) (B.1.1.7 lineage) and another variant (B.1.351 lineage) in October 2020 in the Republic of South Africa (RSA). Afterwhile, it spread to various places globally such as South Africa,[11] Brazil,[12] and the United States.[13] For both variants, the mutation in the RBD region of SARS-CoV-2 is described by the N501Y mutation. On the other hand, in B.1.351 lineage, the K417N, and E484K mutations are found in the RBD region.[14]

Three mutations in the S protein of the novel variant (N501Y, HV69-70del, and P681H) have potential biological implications. P681H is adjacent to the furin cleavage site, a location known to be of biological importance. HV69-70del located in the N-terminal domain (NTD) has been identified in variants associated with immune escape in immunocompromised patients.[15] The N501Y is located in the receptor binding motif (RBM) of the C-terminal domain (CTD) and has been found to increase its binding affinity to human ACE2 receptor as shown in Figure 1. The SARS-CoV-2 Variant of Concern 202012/01 (VOC-202012/01), also known as strain B.1.1.7 and N501Y, associated with a significant increase in the rate of COVID-19 infection in the United Kingdom. VOC-202012/01 is defined by 23 mutations: 13 nonsynonymous mutations (which change proteins), 4 deletions (which change proteins), and 6 synonyms (which do not change proteins). This is an unusually large number of mutations in a single cluster, particularly the S protein, and is predicted to increase the rate of spread of SARS-CoV-2 by 70% ratio.[16] Thus, with this study, we aim to evaluate the N501Y mutation rate in randomly chosen 13 972 positive COVID-19 PCR test result patients to contribute to the literature in the global pandemic that affects our society with a retrospective approach with the comparison of age, gender, and Cq scales of patients.

2 | MATERIALS AND METHODS

2.1 | Sample collection, transportation, and storage

Nasopharyngeal swabs of SARS-CoV-2 patients were collected by trained personnel and transferred to Kanuni Sultan Suleyman Training and Research Hospital in a VTM solution tube. A total of 13 992 randomly selected patients' samples which were clinically showed positive symptoms were tested with Bio-Speedy (SARS CoV-2 Double Gene RT-qPCR kit [Version 1]). After the test of a gold standard method of RT-qPCR, positive patients were examined with the mutation kit of a Bio-Speedy SARS CoV-2 N501Y

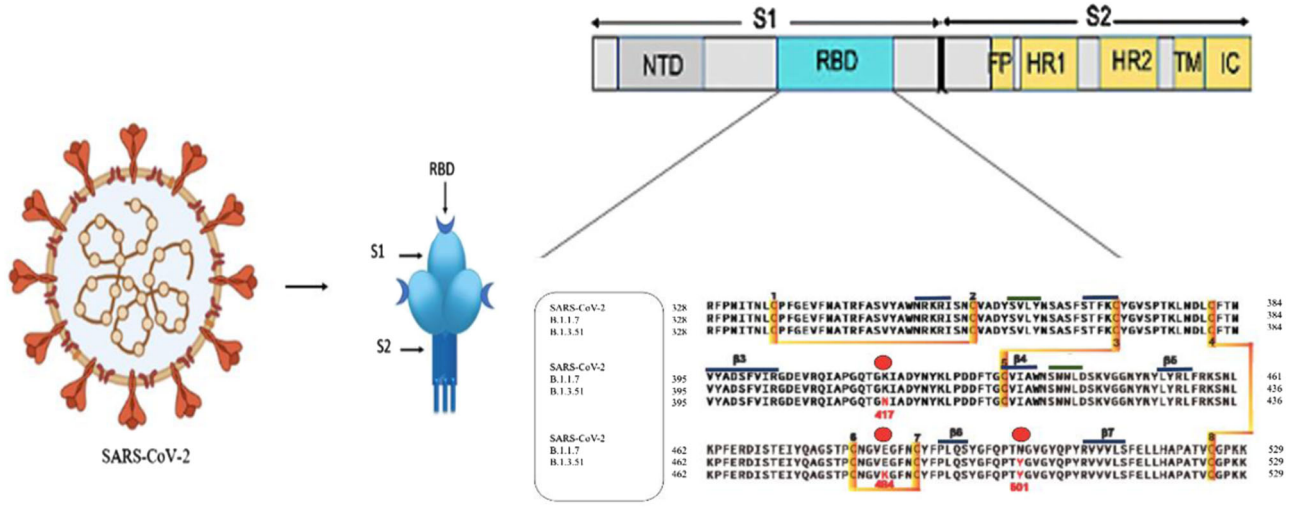

FIGURE 1 Structure and mutation region of SARS-CoV-2. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Mutation Kit. The Cq values were evaluated based on kit protocol.

2.2 | RT-PCR tests

The utilized kit protocol did not require any extra RNA extraction step because of the use of VTM solution with nucleic acid extraction property. During the experiment, only swap samples with VTM solution was enough vigorous vortexing for RNA extraction. For the first detection within 13 972 patients, Bio-Speedy (SARS CoV-2 Double Gene RT-qPCR Kit [Version 1]) is used. The primers of the kit were designed based on the conserved regions of ORF1ab and RNaseP genes of SARS-CoV-2. Fam and phosphoramidite (Hex) channels were used for ORFlab and RNaseP gene, respectively. During the experiment, Biorad CFX96 platforms were utilized. According to the kit protocol, 5 µl patient samples with VTM were added to a 15 µl ready kit mixture to achieve 20 µl PCR mixture in total. Thermal cycle parameters of RT-PCR amplification were as follows: 52°C for 5 min for reverse transcription, 95°C for 10 s for holding, then 40 cycles of 95°C for 1 s and 55°C for 30 s for denaturation, annealing, and extension, respectively.

2.3 | N501Y mutation RT-PCR tests

For the detection of N501Y mutation within 2757 (19.7%) of 13,972 patients' samples, a Bio-Speedy (SARS CoV-2 N501Y Mutation Kit) was used. Variants arising from B.1.1.7 (UK), B.1.351 (South Africa), and P.1 (Brazil) strains drew attention to their features. The N501Y mutation in the SARS-CoV-2 Spike (S) protein is present in all three emerging lineages. It is also important because it is characterized by increased binding affinity to human ACE2. FAM, Rox, Cy5 Red channels were used. The conserved region (6-carboxy-fluorescein [FAM]) of the Orf1ab gene is targeted for the detection of all SARS-CoV-2 strains. Human RNase-P messenger RNA (mRNA) (HEX) was targeted as an internal control. The N501Y mutation (6-carbocyl-X-Rhoddamine [ROX]) has been targeted to detect N501Y containing variants. The mutation-specific B.1.1.7 strain (carboxylic acid [Cy5]) was targeted for differentiation of VOC-202012/01 from other strains (B.1.351, P.1) containing the N501Y mutation. Similarly, Biorad CFX96 platforms were utilized. The threshold level for calculating the number of Cq is 200 RFU for Biorad CFX96. According to the kit protocol, 2.5 µl patient samples with VTM were added to a 7.5 µl ready kit mixture to achieve a 10 µl PCR mixture in totally. Thermal cycle parameters of RT-PCR amplification were as follows: 52°C for 5 min for reverse transcription, 95°C for 10 s for holding, then 40 cycles of 95°C for 1 s and 60°C for 1 s for denaturation, annealing, and extension, respectively. Moreover, the test interpretation is given in Table 1.

2.4 | Test interpretation

The threshold set was arranged as 200 according to the kit protocol for the Biorad CFX96 platform. The positive results of SARS-CoV-2 made sense as sigmoids with Cq values below 32 for FAM channel irrespective of Hex values. Nonsigmoidal signals and sigmoidal signals with Cq values above 32 in the FAM channel and sigmoidal signals with Cq values below 32 in the Hex channel were interpreted as negative based on the kit protocol. Nonsigmoidal signals and sigmoids below 32 Cq on both Fam and Hex channels were interpreted as an invalid result. The test also targets a conserved region of SARS-CoV-2 Orf1ab as an internal control. The samples containing the N501Y mutation are concluded as VOC-202012/01 positive, according to the kit protocol. The limit of detection of the kit for Orf1ab gene target is 500 copies/ml, while that of the N501, Y501 targets are 5000 copies/ml. Additionally, for the N501Y mutation test interpretation, various situations were obtained as summarized in Table 2.

2.5 | Statistical analyzes

Number Cruncher Statistical System Statistical Software program was used for statistical analysis. When evaluating the study data,

TABLE 1 RT-qPCR interpretation of the N501Y mutation

Situation	FAM	ROX	CY5	Result
1	–	–	–	(1) SARS-CoV-2 negative
2	+	–	–	(1) SARS-CoV-2 positive; (2) B.1.1.7 and all N501Y containing variants are negative
3	+	+	+	(1) SARS-CoV-2 positive, (2) VOC-202012/01 positive, (3) all N501Y containing strains except B.1.1.7 negative
4	+	+	-	(1) SARS-CoV-2 positive, (2) B.1.1.7 negative, (3) If the difference between Cq-FAM and Cq-ROX is <8, strains containing N501Y mutations other than B.1.1.7 (B.1.135 and P.1) is concluded as positive. (4) If the difference between Cq-FAM and Cq-ROX is ≥8, it is concluded as negative for all variants containing N501Y.
5	+	–	+	(1) SARS-CoV-2 positive, (2) All variants containing N501Y negative (3) If the difference between Cq-FAM and Cq-Cy5 is <8, it results in B.1.1.7 positive but VOC-202012/01 negative. (Rare but possible case) (4) If the difference between Cq-FAM and Cq-Cy5 is ≥8, B.1.1.7 is concluded as negative

Abbreviations: FAM, 6-carboxy-fluorescein; RT-qPCR, quantitative reverse transcription PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 2 The N501Y mutation test interpretation

	Samples		Negative control		Test interpretation
	N501	Y501	N501	Y501	
Situation 1	Cq>Cq		Neg	Neg	Y501 positive = N501Y mutation positive
Situation 2	Cq<Cq		Neg	Neg	N501 positive = N501Y mutation negative
Situation 3	Neg Cq		Neg	Neg	Y501 positive = N501Y mutation positive
Situation 4	Cq Neg		Neg	Neg	N501 positive = N501Y mutation negative
Situation 5	Cq=Cq		Neg	Neg	Y501 negative = N501Y mutation negative
Situation 6	Cq/Neg	Cq/Neg	Cq	Neg	It may be contamination
Situation 7	Cq/Neg	Cq/Neg	Neg	Cq	
Situation 8	Cq/Neg	Cq/Neg	Cq	Cq	
Situation 9	Neg	Neg	Neg	Neg	Problem in kit

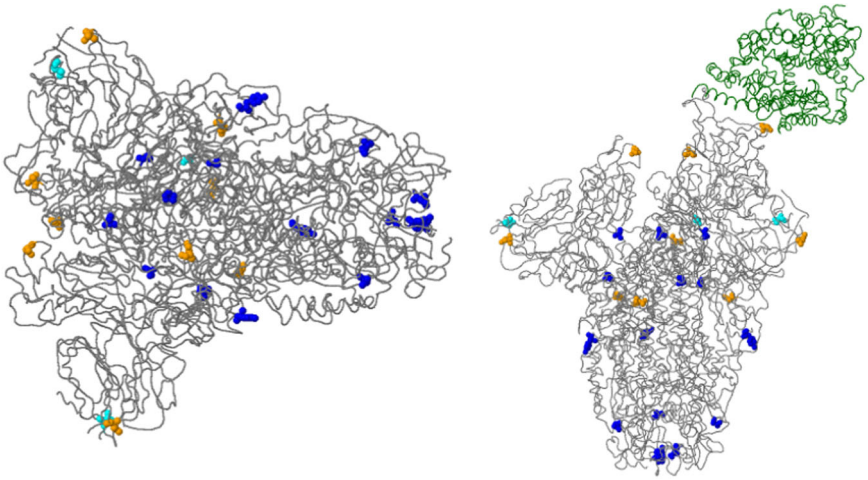

FIGURE 2 3D structural visualization of the spike glycoprotein with aa changes identified in the query sequences shown as colored balls

descriptive statistical methods (mean, *SD*, median, frequency, ratio), as well as Shapiro–Wilk test and box plot graphics, were used for the compliance of the variables with the normal distribution. Mann–Whitney *U* test was used for the comparisons between groups of nonnormally distributed parameters. Spearman's correlation analysis was used to evaluate the relationships between age and test results. Significance was evaluated at the $p < 0.05$ level.

3 | RESULTS

Domain architecture of the SARS-CoV-2 spike monomer is showed in Figure 1 as RBD region, NTD, N-terminal domain; SD1, subdomain 1; SD2, subdomain 2; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane region; CT, C-terminal. The spike protein of coronavirus is a class I fusion protein, which is synthesized as a single polypeptide chain precursor involving three segments: a large ectodomain, a single-pass transmembrane anchor, and a short intracellular tail as shown in Figure 1. Sequence alignment of RBD from SARS-CoV-2, B.1.1.7, and B.1.351 variants spike proteins are (the N501Y, K417N, and E484K mutations) shown in red with circle. The interface of ACE2 (cyan) in complex with Spike RBD from SARS-CoV-2 green stick and B.1.1.7 lineage orange lines are described in Figure 1. The 501, 417, and 484 residues in RBD and RBD mutants were indicated in ball and sticks.

Moreover, the N501Y mutation patient's genome are analyzed in next generation sequencing (NGS) and the bioinformatic analysis was utilized in NCBI and GISAID. The protein structure of the having mutation patients swab samples are shown in Figure 2.

Photo on the Left: Spike glycoprotein (PDB: 6acc, EM 3.6 Angstrom) with RBD in down conformation. % AA identity: 99.214% Photo on the Right: Spike glycoprotein (PDB: 6acj, EM 4.2 Angstrom) in complex with host cell receptor ACE2 (green ribbon). # aa changes: 10. List of variations displayed in structure (nearest residue if in loop/termini region): H69del V70del(69) Y144del(143) N501Y A570D D614G P681H(674) T716I S982A D1118H as seen in Table 3.

TABLE 3 The N501Y mutation test changes

Query	Clade	Best reference hit	%id	%coverage	#Δs	List of aa changes
hCoV-19/Turkey/HSGM-2469/2021 EPI_ISL_1073743|2021-01-28	GRY	NSP1 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
		NSP2 hCoV-19/Wuhan/WIV04/2019	99.8%	100%	1	**R27C**
		NSP3 hCoV-19/Wuhan/WIV04/2019	99.8%	100%	3	**T183I**, **A890D**, **I1412T**
		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	100%	99.0%	3	**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	100%	100%	0	no aa changes
		NSP14 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
		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	99.4%	99.8%	10	**H69del**$^{\$}$, **V70del**$^{\$}$, **Y144del**$^{\$\#a}$, **N501Y**$^{\$\#rao}$, **A570D**$^{\#o}$, **D614G**$^{\$\#lo}$, **P681H**$^{\$}$, **T716I**, **S982A**$^{\#o}$, **D1118H**$^{\#o}$
		NS3 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
		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	100%	100%	0	no aa changes
		NS7b hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
		NS8 hCoV-19/Wuhan/WIV04/2019	98.3%	99.2%	3	**Q27stop**$^{\#o}$, **R52I**$^{\#o}$, **Y73C**
		N hCoV-19/Wuhan/WIV04/2019	99.0%	100%	4	**D3L**, **R203K**, **G204R**, **S235F**

In the study, NGS technology was preferred in addition to RT-PCR studies for the identification of the N501Y mutation. The NGS procedure was performed by the Republic of Turkey Ministry National Virology Reference Laboratory of the Public Health Directorate. Sequences were first converted to fasta format. Later, the Gisiad program was used to examine the mutation changes. The NGS process includes splitting RNA into multiple segments, adding adapters, sequencing libraries, and recombining them to create a genomic sequence. In principle, it is similar to capillary electrophoresis. The key difference is that NGS sequences millions of fragments in massively parallel, increasing speed and accuracy while reducing the cost of sequencing. Thus, 159 mutation sample of the 13 972 samples are analyzed with NGS and results are compared with the normal sequence of SARS-CoV-2 as shown in Figure 3.

Between 18 and 28 January 2757 (19.7%) of 13 972 cases were detected as mutation suspects and 159 (5.8%) of them were

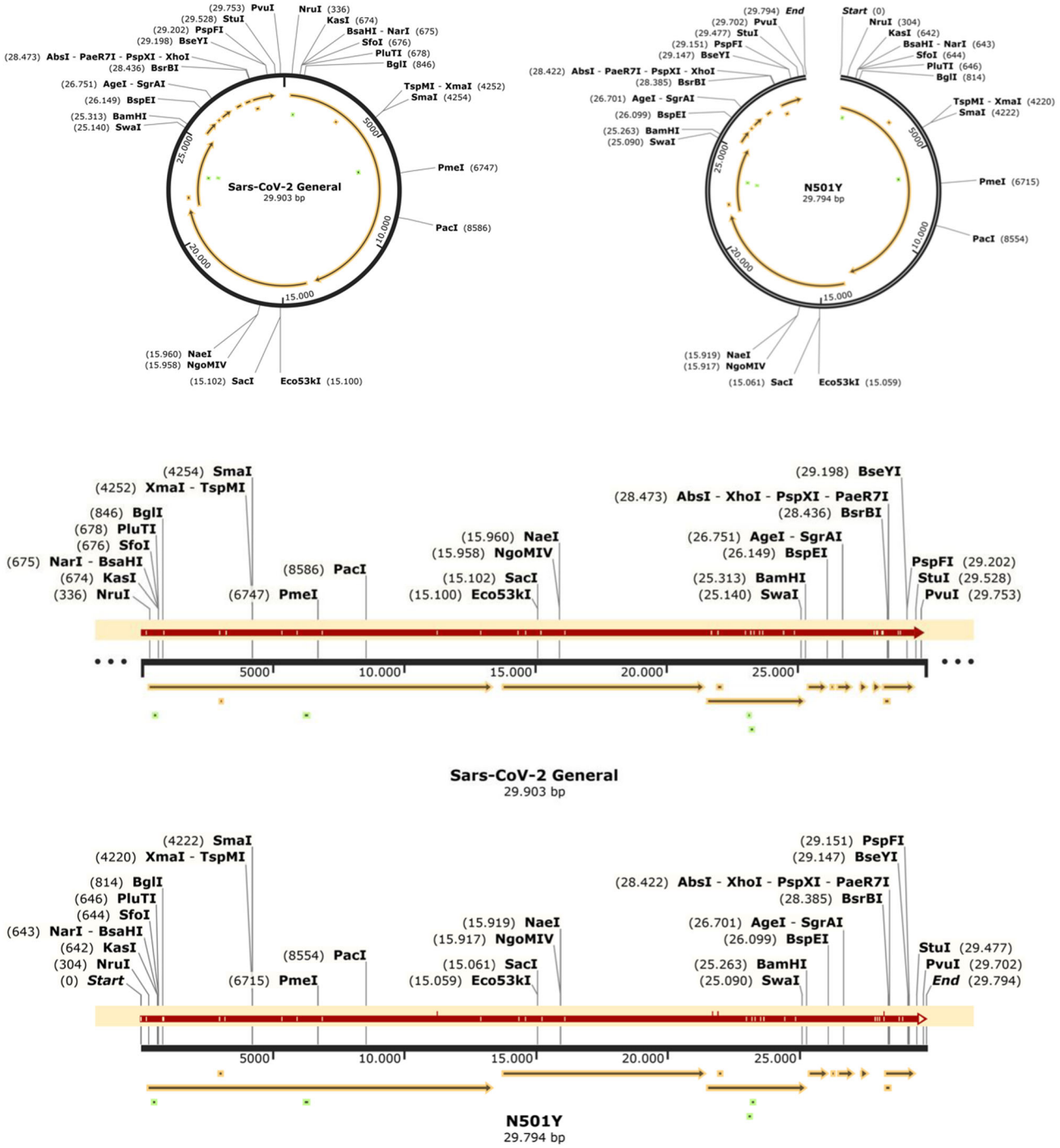

FIGURE 3 The comparison of general SARS-CoV-2 and N501Y mutation sequence. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

found to have mutations. The ages of 13 972 cases tested ranged from 0 to 100, with an average of 35.21 ± 15.73 years. Of the 13 972 cases tested, 70.5% (n = 9849) were male and 29.5% (n = 4123) were female and 88.9% (n = 12426) had negative results and 10.8% (n = 1515) had positive results. The test of 0.003% (n = 5) of the cases has still not been concluded. The sample given by 0.007% (n = 1) of the cases was found inappropriate. Finally, a new sample was requested from 0.1% of the cases (n = 20) as summarized in Table 4.

The mean age of the cases whose test results were negative was 34.52 ± 15.13 and ranged from 0 to 100, and 73.4% (n = 9121) were male and 26.6% (n = 305) were female. The mean age of the cases with positive test results was 40.72 ± 19.06 years and ranged from 0 to 96. 46.9% (n = 711) were male and 53.1% (n = 804) were female according to the RT-qPCR results of patients as summarized in Table 5.

A total of 2757 of 13 972 cases were found to be suspicious for mutation and were tested with the Bio-Speedy SARS CoV-2 N501Y Mutation Kit while a mutation was detected in 159 cases. The ages of the cases with mutations ranged from 1 to 88 years, with a mean age of 40.99 ± 17.55. 49.7% (n = 79) of the cases with mutation were male and 50.3% (n = 80) were female. When the PCR-Cq results are examined, it is seen that it varies between 11.3 and 35.03, and its average is 20.75 ± 3.32 as summarized in Table 6. The variant of N501Y mutation (N501 and Y501) was also determined with the kit protocol. While the average result of N501 is 30.97 ± 4.70, Y501 is calculated as 22.70 ± 3.27.

Moreover, when the N501 variant type Cq results are examined in N501Y mutation results, it is seen that it varies between 0 and 39.86 average is 30.97 ± 4.71. When the Y501 variant type Cq results are examined, it is seen that it varies between 13.6 and 37.27, and its average is 22.70 ± 3.27 as summarized in Figure 2. Although the positivity Cq value hovers around the 20 s, this value is approximately 30–35 for the N501 variant and between 20 and 25 for the Y501 variant. The Cq values of female and male patients have been also calculated. Dispersion is shown with a plot graph. The circular sign refers to females, while the triangle sign shows to the male population. According to the graph, the age of Cq female variants is lower than the Cq male variants.

The correlation coefficient between mutation relationship age and PCR-Cq, N501 and Y501 variant type results was calculated by using the "r" equation (Spearman's Correlation Coefficient). The low correlation was calculated as 0.139 (the PCR-Cq value decreases with increasing age) between the ages of the subjects participating in the study and PCR-Cq measurements which means that there was no statistical significance ($p > 0.05$). No statistically significant correlation also was calculated between the ages of the subjects participating in the study and N501 variant type measurements ($p > 0.05$). Moreover, no statistically significant correlation was defined between the ages of the subjects participating in the study and Y501 variant type measurements ($p > 0.05$) as summarized in Table 7. A statistically significant difference was not found between the PCR-Cq, N501 and Y501 mutation values of the cases according to the genders ($p > 0.05$) by using a Mann–Whitney U test.

According to the results, while the mean age of positive female patients was 20.87 ± 3.23, the mean age of the female with N501Y mutation was calculated as 30.92 ± 5.61. In addition, the mean age of the female with Y501 mutation is 22.76 ± 3.29. Considering the total positive rate, the mean age of positive male patients is 20.64 ± 3.44. Although the mean age of men with N501 variant was 31.03 ± 3.6, the mean age of men with Y501 variant was calculated as

TABLE 4 Demographic information of the cases

	Total patient (n = 13 972)	
Age		
Average ± Ss	35.21 ± 15.73	
Median (Min-Max)	30 (0–100)	
	n	**%**
Gender		
Male	9849	70.5
Female	4123	29.5
Results		
Negative	12 426	88.9
Positive	1515	10.8
Awaiting results	5	0.003
Inappropriate sample	1	0.007
New sample	20	0.1

TABLE 5 Comparison of test results according to demographic information

***N* = 13972**	**Negative**	**Positive**	**Awaiting results**	**Inappropriate sample**	**New sample**
Age					
Average ± Ss	34.52 ± 15.13	40.72 ± 19.06	34.4 ± 14.29	49 ± 0	39.5 ± 17.59
Median (Min-Max)	30 (0–100)	39 (0–96)	25 (22–50)	49 (49–49)	31.5 (14–79)
Gender					
Male	9121 (73.4)	711 (46.9)	3 (60)	1 (100)	11 (55)
Female	3305 (26.6)	804 (53.1)	2 (40)	0 (0)	9 (45)

TABLE 6 Demographic information of cases with mutation detected

	Mutation detected samples (n = 159)	
Age		
Average ± Ss	40.99 ± 17.75	
Median (Min-Max)	41 (1–88)	
	n	**%**
Gender		
Male	79	49.7
Female	80	50.3
PCR-Cq		
Averaget ± Ss	20.75 ± 3.39	
Median (Min-Max)	20.7 (11.3–35.0)	
N501		
Average ± Ss	30.97 ± 4.70	
Median (Min-Max)	31.3 (0–39.86)	
Y501		
Average ± Ss	22.70 ± 3.27	
Median (Min-Max)	22.4 (13.6–37.3)	

TABLE 7 In cases with mutation; relationship between age and PCR-Cq, N501, and Y501 results

	Age
PCR-Cq	
r	−0.139
p	0.080
N501	
r	−0.050
p	0.529
Y501	
r	−0.118
p	0.137

Note: *r* = Spearman's correlation coefficient.

22.66 ± 3.28 years as summarized in Table 8. The results have been calculated with a Mann–Whitney *U* test.

TABLE 8 Evaluation of PCR-Cq, N501, and Y501 results of cases with mutations according to gender

	MALE (n = 79)	FEMALE (n = 80)	p
PCR-Cq			
Avarage ± Ss	20.64 ± 3.44	20.87 ± 3.23	0.700[a]
Median (Min-Max)	21 (13–35)	20.5 (11.3–30.3)	
N501			
Avarage ± Ss	31.03 ± 3.6	30.92 ± 5.61	0.705[a]
Median (Min-Max)	30.9 (19.2–39.9)	31.8 (0–39.8)	
Y501			
Avarage ± Ss	22.66 ± 3.28	22.76 ± 3.29	0.736[a]
Median (Min-Max)	22.1 (16.2–37.3)	22.7 (13.6–32.3)	

[a]Mann–Whitney *U* test.

4 | DISCUSSION

Over one and half decades, people have faced five different pandemics caused by viral infections that are SARS, Swine flu, Ebola, MERS, and ultimately COVID-19. To curb these pandemic episodes, early diagnosis is the first step to initiate effective therapy and also to manage the spreading of infections. Surveillance is a significant approach for pandemic response. Although new SARS-CoV-2 variants can be most accurately identified by genomic sequencing, this approach is time-consuming and expensive. A simple and more rapid screen for the key SARS-CoV-2 mutations is ensured by RT-qPCR. Thus, the RT-qPCR is the gold standard method to detect infections.[17]

The emergence of SARS-CoV-2 variants concerning phenotypic mutations is the public health interest. Nowadays, many various variant types of SARS-CoV-2 have been described. In particular, the N501Y mutation has exhibited potential risk firstly in the United Kingdom and after a while all over the world. Moreover, the variant type of N501Y mutations was described as N501 and Y501, respectively. N501Y mutation is one of the important mutation types among other types of mutation of SARS-CoV-2 and is also termed with VOC-202012/01.

Three mutations in the S protein of the novel variant (N501Y, HV69-70del, and P681H) have potential biological implications.[15] The N501Y is located in the RBM of the CTD and has been found to increase its binding affinity to human ACE2; as mutations in the CTD of the S protein (aa 333-527) are most likely to alter the receptor recognition properties of SARS-CoV-2. As shown in Figure 2, 10 exact amino acid changes were described for N501Y mutation. Additionally, the difference between general SARS-CoV-2 and N501Y mutation sequence is determined by sequence alignment with NGS technology as shown in Figure 3. The possibility of other mutations increase the transmissibility of the virus in combination with N501Y.[18] 85% of the sequenced SARS-CoV-2 genomes containing N501Y are VOC-202012/01. All genomes containing N501Y sequenced in the UK since September 2020, belong to VOC-202012/01. Therefore, only N501Y screening will be decisive in areas with a low prevalence of VOCs outside of South Africa. Detection of more than one mutation is necessary for the detection of VOC in South Africa, as 11% of total sequences in South Africa contain N501Y and none of them belong to VOC-202012/01. The new variant containing N501Y detected in South Africa is designated 501Y.V2.[19] Analyses showed that 501Y.V2 spreads faster than variants without

N501Y. In other words, all SARS-CoV-2 variants containing N501Y are important in terms of epidemiology and their prevalence should be monitored.[20] Due to significantly raised concerns about the higher transmission rate and the escape from neutralizing antibodies of mutation N501Y, this study focuses on describing of N501Y mutation type in a wide range of nasal swab samples of patients. The defined mutations were also classified with the variant types of N501Y mutation as N501 and Y501, respectively.

The mutation in SARS-CoV-2 not only affected just one form. The variants of the SARS-CoV-2 with an asparagine-to-tyrosine substitution at position 501 (N501Y) in the RBD show enhanced infectivity compared to wild-type. Moreover, SARS-Cov-2 variants include the two Alpha variants (B.1.1.7, United Kingdom and B.1.1.7 with the additional E484K mutation), the Beta variant (B.1.351, South Africa), and the Gamma variant (P.1, Brazil). In this study, we evaluated between 18 and 28 January 2757 13 972 cases. According to our results, in January, all variants are described as B.1.1.7 type. However, in our laboratory, during the 9 months, all variants were examined gradually. Although during the 5 months, B.1.1.7 variant types are dominantly observed, in the last 3 months these variant types showed a significant decline. On the other hand, B.1.351 variant type is almost hardly seen. However, in last 3 months, E484 mutation type showed itself dominantly as shown in Figure 4.

When the Cq results were examined in N501Y mutation results, it was seen that they varied between 0 and 39.86. the average was 30.97 ± 4.71, of which the Y501 variant type varied between 13.6 and 37.27, and its average was 22.70 ± 3.27. The Cq values of each variant type of N501Y mutation are also the information about much more positivity. According to the results (Figure 5), Y501 showed a lower Cq value than N501. In the literature Leung et. al.[14] studied N501Y transmissibility and based on the results showed that 501Y is estimated to present an R0 1.75 times higher than 501 N, meaning it is 75% more transmissible compared with the 501 N strain.[14] In the female population, positivity is higher than males throughout all ages. Moreover, the mutation rate in the female population was higher in

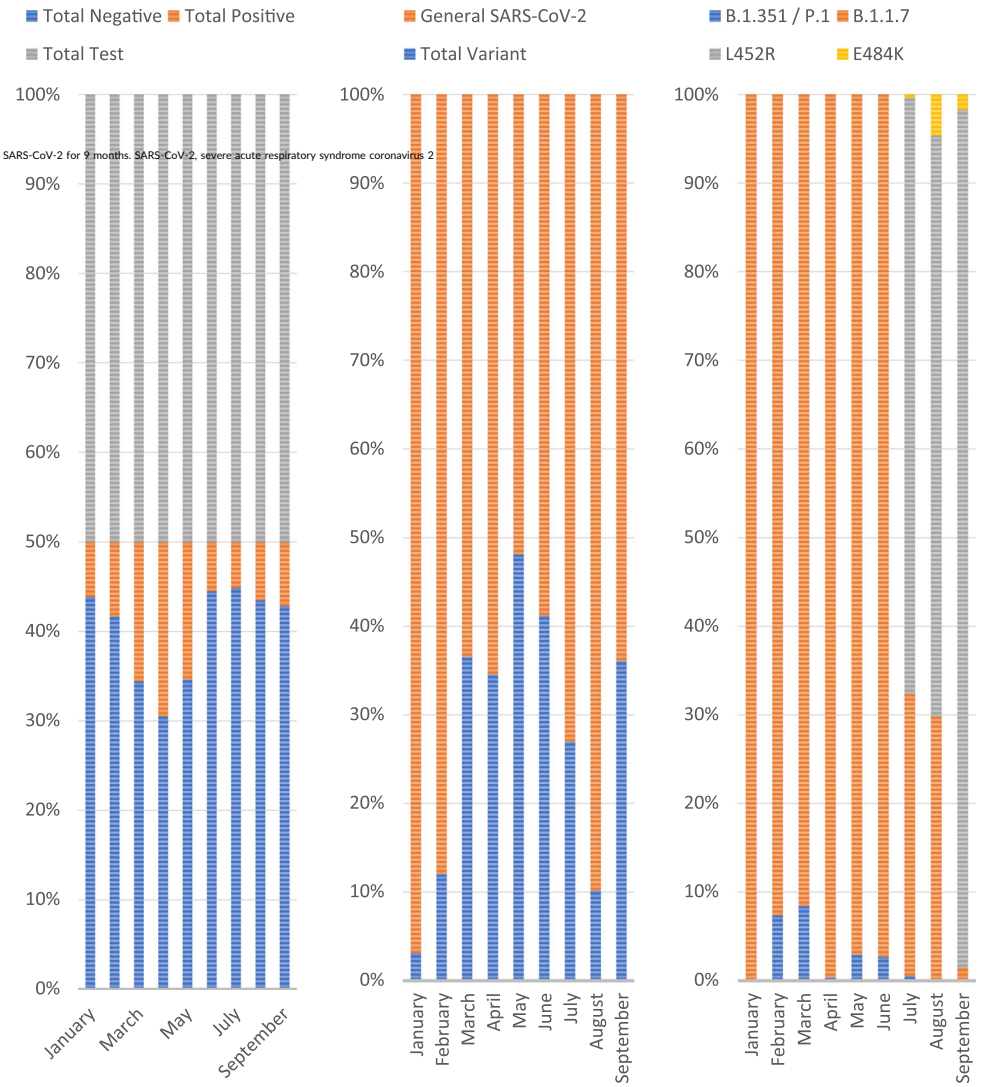

FIGURE 4 Distribution of variant types of SARS-CoV-2 for 9 months. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

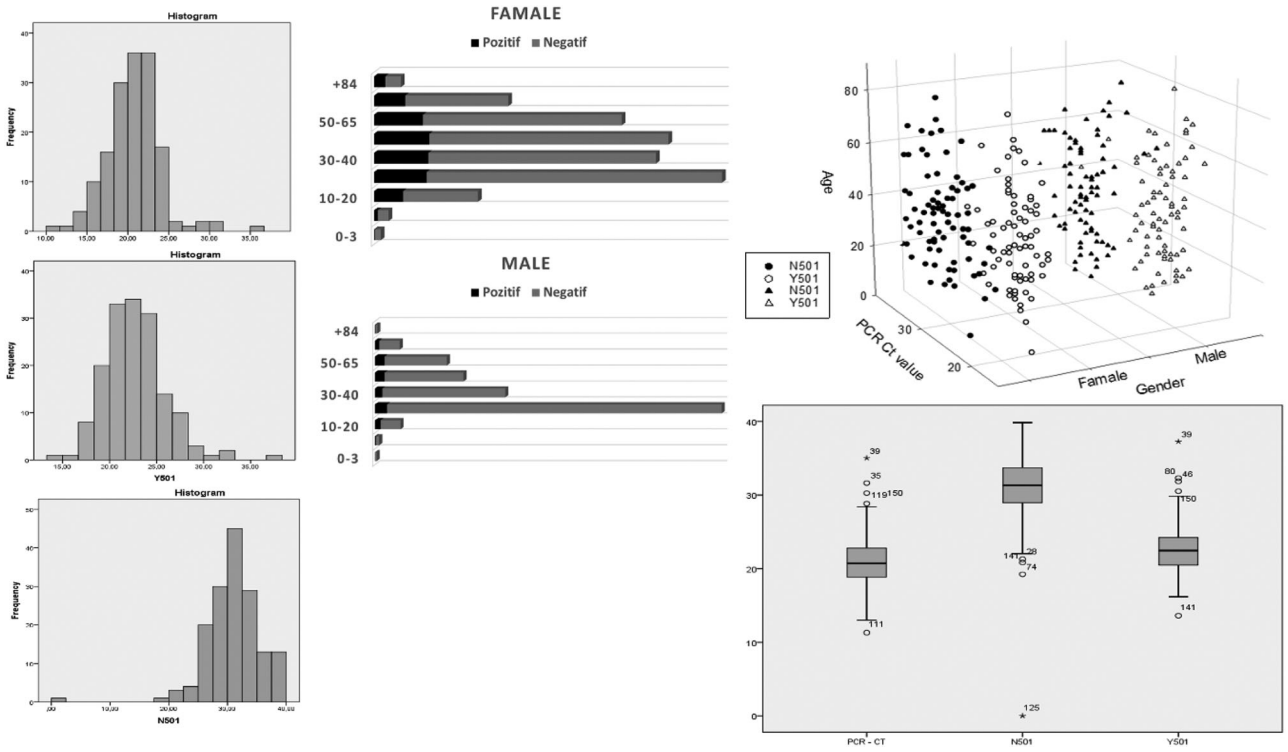

FIGURE 5 Y501 distribution of cases with mutations, PCR-Cq, N501, and Y501 distribution of cases with mutations, female/male age and % positivity distribution of total tests

the age range. For the male population, +84 and 0–3 ages of males have not shown significant values. Additionally, Cq value rates of the N501 variant have dispersed at a higher rate than Y501 according to the histograms (Figure 2). The Cq values of the variant type of N501Y mutation showed differences among females and males. The age of the female variants are lower than of the males. According to the results, also the comparison between age scales and genders was evaluated retrospectively. Thus, the N501Y mutation type of SARS-CoV-2 retrospective approach will be created by way of evaluation and analysis of the data in terms of public health. Moreover, recent studies showed that the FDA-authorized mRNA vaccines continued to induce a high level of neutralization against B.1.1.7 variant, but a lower level against B.1.351 variants.[21] Therefore, we aimed that these mutations are within the RBD region, understanding the new variants' binding mechanism to ACE2 receptor is of great value. With this study, the data of mutation type of SARS-CoV-2 and statistically informative preliminary study, and risk management studies can be carried out for another variant in the light of these data.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Selen Zeliha Mart Kömürcü: she provided support on academic consultancy and administrative process management throughout the entire research process and in the analysis of PCR results. **Yakup Artik**: developed the protocol, summarized and analyzed the data, wrote the article and vouched for it. **Nevra Pelin Cesur**: development of protocol, methodology, review and regulation. **Arzu Tanriverdi**: data collection of PCR results, collection of experimental materials and laboratory application. **Derya Çakir Erdoğan**: in the analysis of the PCR results. **Şule Çelİk**: in the analysis of the PCR results. **Elif Yilmaz Güleç**: in the analysis of the PCR results, contributions have been made.

ETHICS STATEMENT

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 Kanuni Sultan Suleyman Training and Research Hospital No: 2021.04.127, Subject No: KAEK/2021.04.127 Date: 08.04.2021—10:08—E-80929729-000-6924 and Republic of Turkey, Ministry of Health, Covid-19 Scientific Research Studies Approval No: YakupArtik-2021-03-13T12_59_15.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article [and/or] its Supporting Informations Material Files. Further enquiries can be directed to the corresponding author.

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