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Whole Genome Sequence Analysis of Six SARS-CoV-2 Positive Patients Followed in a Tertiary University Hospital

Üçüncü Basamak Üniversite Hastanesinde Takip Edilen Altı SARS-CoV-2 Pozitif Hastanın Tüm Genom Dizi Analizi

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Abstract

Aim: In this study, we aimed to determine mutations in the gene sequence of this virus, by performing whole genome sequence analysis from patient samples found positive by actual RT-PCR for SARS-CoV-2.

Material and Method: The study included six adult patient samples with different clinical manifestations with positive PCR tests for SARS-CoV-2, between June 01, 2020, and March 12, 2021. Sequence knowledge of all samples/testers has been loaded into the GISEAD (Global Initiative on Sharing All Influenza Data) data system. Clade Analysis, Genome Analysis, Variant Analysis, and Phylogenetic Tree Analysis were conducted.

Results: 3 of the patients were women (female), and three were men (male), with the mean age of 42.5 years old (between 20-61). Totally 71 mutations were specified in 6 adult patients. By the Pangolin lineage, three of the patients were B.1.177, two were B.1, one was of B1.36 lineage. By the Pango lineage, two of the patients were B.1.609, one was B.177, one was B.1.36. By the Nexstrain Clade, four of the patients were 20A and two were of 19A lineage. No D614G mutation was detected in any of the patients. While five patients recovered, one patient with metastatic lung adenocarcinoma died.

Conclusion; The patients were detected in the commonly found 'Non-VOC' group. Therefore, variants could not be associated with the clinical status and prognosis of the patients. However, it is thought that the data obtained contribute to both global and national SARS-CoV-2 data.

Keywords: Genome sequence analysis, SARS-CoV-2, Konya, mutation

Öz

Amaç: Bu çalışmada Gerçek RT-PCR ile SARS-CoV-2 için pozitif bulunan hasta örneklerinden tam genom dizi analizi yaparak bu virüsün gen dizisindeki mutasyonları belirlemeyi amaçladık.

Gereç ve Yöntem: Çalışma, 01 Haziran 2020 - 12 Mart 2021 tarihleri arasında SARS-CoV-2 için pozitif PCR testleri olan farklı klinik belirtilere sahip altı yetişkin hasta örneğini içermektedir. Tüm numunelerin/test edicilerin sekans bilgisi, GISEAD (Tüm İnfluenza Verilerini Paylaşma Küresel Girişimi) veri sistemine yüklenmiştir. Clade Analizi, Genom Analizi, Varyant Analizi ve Filogenetik Ağaç Analizi yapılmıştır.

Bulgular: Hastaların 3'ü kadın (kadın), 3'ü erkek (erkek) olup, yaş ortalaması 42,5 (20 - 61 arası) idi. 6 yetişkin hastada toplam 71 mutasyon belirlendi. Pangolin soyuna göre, hastaların üçü B.1.177, ikisi B.1, biri B1.36 soyundandı. Pango soyuna göre hastaların ikisi B.1.609, biri B.177, biri B.1.36 idi. Nexstrain Clade'e göre hastaların dördü 20A ve ikisi 19A soyundandı. Hiçbir hastada D614G mutasyonu saptanmadı. Beş hasta iyileşirken, metastatik akciğer adenokarsinomu olan bir hasta hayatını kaybetti.

Sonuç; Hastalar yaygın olarak bulunan 'VOC olmayan' grupta tespit edildi. Bu nedenle varyantlar, hastaların klinik durumu ve prognozu ile ilişkilendirilememiştir. Ancak elde edilen verilerin hem küresel hem de ulusal SARS-CoV-2 verilerine katkı sağladığı düşünülüyor.

Anahtar Kelimeler: Genom dizi analizi, SARS-CoV-2, Konya, mutasyon

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INTRODUCTION

In the year December 2019, incidents of pneumonia of obscure origination were determined in the province of Wuhan of Hubei city of Peoples Republics of China, and almost identical incidents outspread to other cities of China and later to all world, causing the pandemic. The causative agent of this outbreak was called SARS-CoV-2 because of its resemblance to the Severe Acute Respiratory Syndrome Agent-Coronavirus (SARS-CoV). The World Health Organization (WHO) named as Coronavirus disease-2019 (COVID-19). On the date January 30, 2020, WHO proclaimed the epidemic as a worldwide emergency situation and a pandemic on 11 March 2020. And the first incident in Turkey was reported on 11 March 2020. Incidents have correspondingly been seen in province Konya and also they are still being seen. SARS-CoV-2 infection characteristically starts with fever, persistent dry cough, fatigue, and progresses to shortness of breath.[1]

Generally the most valid method for the virological diagnosis of active infection is present reverse transcriptase polymerase chain reaction (rRT-PCR) for identifying viral RNA.[2] Genomic sequence analysis can be used to quickly and accurately identify the transmission routes of the pathogen.[3] The first genome analysis on COVID-19 was published on January 10, 2020, by the researchers team directed by Yong-Zhen Zhang.[4] The achievement of COVID-19 diagnostic test kits, antibody tests, and protein-targeted medications likely depends on genomic variants. And for antibody tests, the sensitivity of the test can be greatly reduced if a mutation affects protein recognition. Consequently, mutation profiles of isolates circulating in abundance in the region should be considered in order to adapt these tests.[3]

The phylogenetic characterization of this virus is crucial to contribute to the knowledge of viral variation to identify the most suitable regions to be used as vaccine targets or antivirals. Studies on this subject in our region are limited. Therefore, in this study, by performing gene sequence analysis from patient samples found positive for SARS-CoV-2 PCR (RT-PCR), it was aimed to contribute to the epidemiological data of our country, to compare the studied strains with other strains registered in the gene bank, to add new sequence information to the gene bank, and to provide information about the clonal relationship and origin of the mutations in the gene sequence of this virus, which spread from Wuhan to Konya.

MATERIAL AND METHOD

This academic paper was permitted by the Turkey Health Ministry (Dated 17.06.2020 and numbered 2020-06-12T13_47_55) and the University Ethics Committee (decision no. 2020/2694). Characteristics of the patients, such as age, gender, hematological and biochemical factors, were recorded.

In our hospital's Microbiology laboratory, nasopharyngeal swabs from patients, which is a standard protocol for routine diagnosis, were taken into a viral transport medium, and the existence of SARS-CoV-2 was inspected. Viral RNA extraction from these testers was completed with a nucleic acid isolation equipment (RINATM, Bio Eksen, Turkey) in accordance with the producer's directives. The RT-PCR response was conducted with the commercial equipment (BioSpeedy SARS-CoV-2 dual gene, Bio Eksen, Turkey) using the Rotorgene Q (Qiagen, Germany) heat cycler. Diagnosis with the equipment was made by RT-PCR directing the SARS-CoV-2 particular N and orf1ab gene area. After a test run was completed, the response curves were understood according to the procedures of the equipment procedure process. Values less than the cycle threshold (Ct) 38 ($Ct < 38$) were considered positive.

For further analysis, 6 SARS-CoV-2 positive samples with high virus-related load (cycle threshold < 20) were randomly chosen. RNA samples were saved in a freezing compartment at -80°C till additional processing. At the following phase, sequence study of the whole genome of the virus was executed with next-gen sequencing apparatuses; followed by variant analysis, genome analysis, bioinformatics analysis, clade analysis, and phylogenetic tree analysis.

Sequence Analysis

SARS-CoV-2 virus whole genome sequencing was conducted on Oxford Nanopore Technologies, Cat. #MIN-101B (MinION TM) instrument using Oxford Nanopore Technology. RNA samples with full nucleic acid isolation were transformed to cDNA by contrary transcription. Subsequently the contrary transcription phase, with 2 diverse primer pools in the ARTIC nCoV-2019 V3 panel, ½ minute of first denaturation at 98°C, 15 seconds of denaturation at 98°C with 35 cycles, and 5 minutes of attachment and elongation at 65°C PCR step was performed. Afterwards PCR, the same samples worked with 2 diverse primer pools were gathered in one tube. Agencourt AMPure XP beads equipment (Beckman Coulter, Kat. #A6388) was used for refinement. Concentration measurement was performed with Qubit™ four Fluorometer (Thermo Sciences, Cat. # Q33238) before the tip preparation phase. The NEBNext End Repair/dA-tailing Module (New England Bio-labs, Cat. #E7546) set was used for tip reparation and dA tail generation. After NEBNext Ultra II End Prep enzyme mixture was added, the samples were keept warm (incubation), for 5 min at 20°C and 5 min at 65°C. For barcoding, NEB Blunt/TA Ligase Master (New-England Bio-labs, #M0367) was mixed with nuclease-free water, tipped DNA and native barcodes. The mixed substance was kept warm at 20°C for 20 minutes and at 65°C for 10 minutes. After keeping warm (incubation), all barcoded samples were gathered in a tube. The Agencourt AMPure XP beads equipment (Cat. # A63880, Beckman Coulter) was used for refinement, and the concentration was measured with a Qubit TM-4 Fluorometer (Thermo Science, Cat. #Q33238) before the adapter ligation phase. For the adapter ligation step, NEBNext Quick T4 DNA Ligase (New-England Bio-labs,

Cat. # M0202), AMII (Adapter Mix II), and NEBNext Quick Ligation Reaction Buffer (5X) were added to the pool sample tube. The combination was then kept (incubated) at room heat for 1/3 hour, and purification was completed using Agencourt AMPure XP beads. Finally, the concentration of the DNA library to be loaded was measured with the Qubit TM 4 Fluorometer (Thermo Science, Cat. #Q33238). Afterwards the SpotON Flow Cell (Oxford Nanopore Technologies; Cat. # FLO-MIN106D) was made ready for loading with the mixture of Flush Tether (FLT) and Flush Buffer (FB) in the Flow Cell Priming Equipment (Oxford Nanopore Technologies; Cat. # EXP-FLP002), the arranged DNA archive was loaded into the flow cell, and sequence analysis was started using the MinKNOW program.

Bioinformatics Analysis

This analysis comprises of 2 parts. In the first part, the genome of the virus was removed from the generated FASTQ information. At this stage, mutations in the virus were also identified. At this stage, the FASTQ information were charted to the reference genome with Minimap 2 and the BAM file was produced. The BAM file, after needed quality control and filtering with Medaka, Samtools and Longshot tools, mutation discovery was made and the file containing the mutations in VCF format was produced. Then, the genome of the virus was produced with the BAM and VCF file produced with the help of Bcftools. In the second stage of the analysis, clade analysis was performed for each sample. In these clade analyzes, Nextstrain clade analysis was performed and then Pangolin linage was added. Clade analysis is actually a sequence-based classification.

RESULTS

Three of the patients were women (female) and 3 were men (male), with the mean age of 42.5 years-old (between 20-61). Laboratory values of the patients at the time of admittance are presented in **Table 1**, and genome sequence analysis results are presented in **Table 2**. The numerical values of the laboratory results of the patients at the time of application.

In this study, a total of 71 mutations were detected in six adult patients reported from the Konya region. By the Pangolin lineage, three patients were B.1.177, two were B.1, and one was of B1.36 lineage. By the Pango lineage, two patients were B.1.609, one was B.177, and one was B.1.36. By the Nexstrain Clade, four patients were 20A, and two were of 19A lineage. Different mutations were detected in the N regions of the first, second and third patients. The most common mutation detected in the 1st, 2nd, 3rd, and 6th patients was NSP12 (P323L). NSP12 (M601I) mutation was found in the fourth patient, NSP12 (T739I) mutation was found in the sixth patient, and N501Y mutation was found in the Spike protein in the fifth patient. The first, second, third and fifth patients had mutations in the ORF1a regions; Mutations in ORF1b regions were detected in patients 1, 2, 3, 4 and 6. mutations were detected in the ORF3a regions of the second and fourth patients. Mutations were detected in the ORF8 region of the 1st and 5th patients. No D614G mutation was detected in the patients. While five patients recovered completely, by the pangolin lineage, the fourth patient with metastatic lung adenocarcinoma, descendant of B.1.177, died.

Displaying patient genomes on the global phylogenetic tree is shown in **Figure 1**.

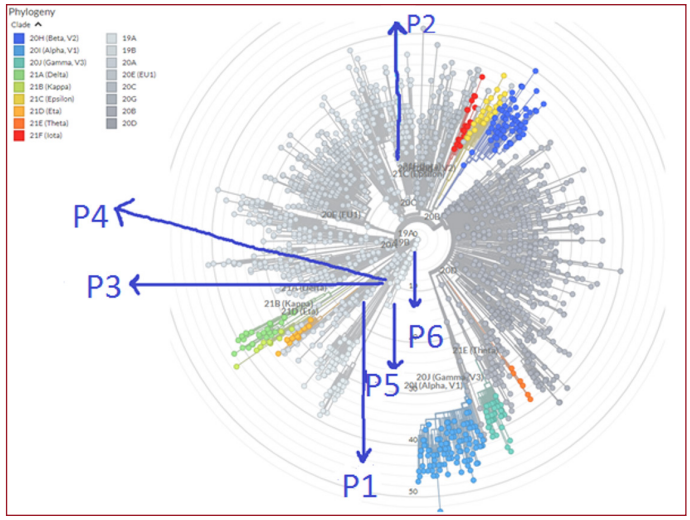

Figure 1. Imaging of the genomes in this study on the global phylogenetic tree (P:Patient). The image was created using the auspice.us website and sample files with json extension.

Table 1. Laboratory Results of the Patients at the Time of Application

Patient number	1	2	3	4	5	6
Hemoglobin (gr/dl)	12.6	13.3	16	10.4	12	16
Leukocytes(/µl)	7040	5950	6170	8980	3030	5920
Neutrophil (/µl)	4680	5230	4530	7220	2210	3030
Lymphocyte(/µl)	1260	590	790	480	180	2360
Platelets (/µl)	320000	233000	172000	156000	148000	147000
C Reactive Protein (mg/L)	8.5	9.2	3.6	170.9	20.9	2.3
Sedimentation (mg/h)		17		56	25	
Procalcitonin (µg /L)		0.032		1.26	0.062	
Fibrinogen (mg/dl)	356	345	175	403	244	309
D Dimer (ng /ml)	86	178	54	2103	695	186
Ferritin (µg /L)	66.5	85.6	86.8	4718	437.6	242

Leukocytes;4000-10000 /µl Lymphocyte; 800-5500 /µl C -reactive protein (CRP); 0-0.5 mg/L Sedimentation;0-20 mg/h D -dimer;0-243ng/mL Ferritin; 13-150 ug /L

Table 2. Genome sequence analysis of the patients

	Patient	2. Patient	3. Patient	4. Patient	5. Patient	6. Patient
Age/Gender	30/F	58/F	20/M	61/M	26/F	60/M
CT Value	12.8	12.98	14.66	14.30	11.95	15.75
Number of mutations	18	12	11th	10	17	3
GISEAD access number	EPI_ISL_5403171	EPI_ISL_5403187	EPI_ISL_5403086	EPI_ISL_5403204	EPI_ISL_5403201	EPI_ISL_5403199
Sampling time	07.12.2020	12.09.2020	27.11.2020	12.03.2021	09.12.2020	12.07.2020
Pangolin lineage	B.1.177	B.1.36	B.1.177	B.1.177	B.1	B.1
Pango lineage	B.1 77	B.1.36	B.1.609	B	B	B.1.609
Amino Acid Changes (GISEAD)	Spike Q14H, NR203K, NS8 G8stop, NSP1 S34T, NSP3 S1286I, NSP12 P323L	N S194L, NS3 S177I, NSP4 F390L, NSP12 P323L	Spike D1084Y, N R203K, NSP3 V1795F, NSP12 P323L, NSP15 V66L	N N196T, NSP12 M601I	Spike A570D, Spike D1118H, Spike N501Y, Spike P681H, N D3N, N M1V, NS8 Q27stop, NS8 R52I, NSP2 L550F, NSP3 I1412T	NN196T, NR203, NS3 T151I, NSP12 P323L, NSP12 T739I
Nextrain clade	20A	20A	20A	20A	19A	19A
Changes	C241T, C313T, T365A, C466T, C3037T, G6576T, C6941T, G10870C, C14408T, G20373T, C20451T, G21604T, G27915T, G28881A	C241T, C3037T, C9724A, C14408T, C18877T, C22444T, C25276A, G25922T, C26735T, C27059T, C28854T, G29777T	C241T, C3037T, G8102T, C14408T, G19816T, T19839C, G24812T, G28881A, T29464C	C241T, C3037T, C14408T, C15656T, C25844T, A28860C, G28881A	C2453T, C3037T, C5986T, T6954C, T16548A, C20148T, A23063T, C23271A, C23604A, G24914C, C27972T, G28048T, G28280C	C3037T, G15243T, A28860C
Deletion	-	-	-	-	-	-
Insertion	27864:T, 28881:AC	-	28881:AC	28881:AC	28280:TA	-
pcrPrimerChanges	ChinaCDC_N_F: G28881A	-	ChinaCDC_N_F: G28881A	ChinaCDC_N_F: G28881A		
AA Changes	N:R203K, ORF1a:S34T, ORF1a:S2104I, ORF1b:P314L, ORF8:G8, Q:Q14H	N:S194L, ORF1a:F315L ORF1b:P314, ORF3a:S177I	N:R203K, ORF1a:V2613, ORF1b:P314, ORF1b:V211L, Q:D1084Y	N:N196T, N:R203K, ORF1b:P31, ORF1b:T73I, ORF3a:T15I	N:D3H, ORF1a:L730F, ORF1a:I2230T, ORF8:Q27, ORF8:R52I, Q:N501Y, Q:A570D, Q:P681H, Q:D1118H	N:N196T, ORF1b:M592I
AA deletions	-	-	-	-	-	-
Average Depth (Coverage) Amount	1,331X	1,661X	1,088X	1,494X	12,992X	8,796X
Reference Genome Coverage Ratio (%)	99.86	99.78	99.77	99.78	99.03	99.84

F: Female M: Male NSP: Nonstructural protein AA: amino acid

DISCUSSION

As of 9 September 2023, 770,437,327 confirmed incidents and 6,956,900 deaths have been informed worldwide by WHO.[5] As of March, 2023, there were 17,232,066 incidents and 102,174 deaths announced by the Turkish Ministry of Health.[1] There are full genome sequence analyses of 15,968,508 SARS-CoV-2 viruses in the GISAID's database on Distribution Entire Influenza Information (Global Initiative), 101,592 of which are from Turkey. 30 viruses, including six viruses we uploaded, are from Konya.[6]

Coronavirus disease can be seen at any age,[7] and the mean age of the adult patients included in our paper was 42.5 years (20-61 years).

Clinical findings in coronavirus infection be able to range from asymptomatic to acute respiratory distress syndrome and multi-organ dysfunction.[8] Four of six patients were outpatients with home isolation, and the fifth patient was followed up in the hospital and discharged with recovery. The fourth patient with metastatic lung adenocarcinoma, whose general condition deteriorated while being followed in the ward and died in the intensive care unit, was of B.1.177 lineage by the Pangolin lineage, and mutations were detected in the N, ORF1b and ORF3a regions. The cause of death was associated with metastatic lung cancer, since the SARS-CoV-2 agent of this deceased patient did not have variants of concern ("Variant of Concern";VOC). VOCs are variants with

increased infectiousness of SARS-CoV-2 or that may adversely affect the epidemiology of COVID-19, variants with increased disease properties or a change in the clinical picture, or variants that reduce the effectiveness of communal health measures or raise concerns by reducing the effectiveness of existing diagnostic tests, vaccines or drugs. VOC (WHO, 22.06.2021) variant ALFA (B.1.1.7) was earliest noticed in England, BETA (B.1.351) earliest in the South Africa, GAMMA (P.1) earliest in the Brazil, DELTA (B. 1.617.2) was first noticed in India.[9] None of the variations obtained in our study were found to be VOCs, so no relationship could be established between the variant and clinical findings.

Laboratory tests other than RT-PCR are non-particular in coronavirus infection. The white blood cell calculation is standard or low. There may be lymphopenia and mild thrombocytopenia. C-reactive protein (CRP) and sedimentation (SED) are generally high. But procalcitonin (PCT) levels are generally standard level. A high PCT level may show a bacterial coinfection. Alanine aminotransferase, Aspartate aminotransferase, D-dimer may be high. Ferritin is an acute period reactant, and high serum ferritin level has been associated with organ harm to a larger extent in severe COVID-19 patients.[10,11] In our study, the leukocyte, neutrophil and thrombocyte values of the patients were normal. The CRP, SED, D-Dimer, and ferritin levels of the fourth and fifth patients were high, and the laboratory parameters of the patients were found at different values.

Mutations develop in SARS-CoV-2, although less frequently compared to other RNA viruses.[12] In a paper, 549 and 53 distinctive variations were noticed from 47 SARS-CoV-2 isolates.[13] In the paper prepared by Khailany et al., 156 total and 116 distinctive variations were found in 95 SARS-CoV-2 isolates.[14] In our paper, 71 mutations were detected in six adult patients.

Looking at the SARS-CoV-2 genome, the C>T nucleotide transformation is the most common nucleotide change in the genome.[15] In the study of Karamese et al., it was reported that C>T base change was the most common.[13] In our study, the most common base change was found as C>T. There were 7 C>T base changes in the initial patient, 8 in the second, 3 in the 3rd, 5 in the 4th, 5 in the 5th, and 1 in the 6th patient.

The source of B.1.177 Lineage is the United Kingdom 64%, Spain 10%, Germany 5%, Switzerland 4%, and Italy 4%.[16] Patients 1, 3, and 4 are from lineage B.1.177. This lineage is predominantly European and is thought to have spread as a consequence of the opening of the boundaries in the summertime of 2020. It is most common in the United Kingdom 62%, Spain 12%, Germany 4%, Switzerland 4% and Italy 4%. The earliest was found on 02.02.2020.[17]

B.1.36 Lineage originates from India 29%, Canada 14%, UK 13%, Denmark 8%, Hong Kong 5%.[16] The genome of the second patient is of the B.1.36 lineage, the earliest detected in the world in February 2020 and is most common in India 33%, Canada 31%, UK 8%, Denmark 4%, Hong Kong 3%.[17]

B.1 Lineage appeared in Europe at the beginning of 2020. The B.1 lineage is the recognized dominant worldwide lineage and has been sectioned into more than 70 sublineages. All variants in the B.1 clade share a specific mutation called D614G. This mutation is one of the first to be detected in the US in the early stages of the pandemic, after initially circulating in Europe. There is evidence that variants with the D614G mutation spread faster than viruses without this mutation.[16] The D614G mutation is assumed to be dominant for the reason that it provides more uniform spread of the virus.[18] D614G has been shown to be the most common mutation in the spike glycoprotein.[13] It is thought that this mutation may affect the effectiveness of the vaccine.[19] The presence of mutations in the protein in all strains with the D614G mutation is almost also responsible for the response in replication (ORF1ab P4715L; RdRp P323L), which may affect the virus's rate of replication. This protein is the target of antiviral drugs such as remdesivir and favipiravir, and because it is susceptible to mutations, treatment-resistant strains can emerge rapidly. These variants, which are formed by mutations in the receptor binding region of the spike protein, also facilitate the binding of ACE2 to the receptor on the host cell surface.[20] In the study of Adebali et al., 23 out of 30 genomes have the D614G mutation. The D614G mutation appears to be mutated in 2 synonyms in ORF1ab.[3] In a study we conducted in pediatric patients in Konya, D614G mutation was detected.[21] However, D614G mutation was not detected in this study. The 5th and 6th patients are of the B.1 lineage. The B.1 lineage is a large EU (European) lineage, the starting point of which coarsely corresponds to the outbreak of Northern Italy in the first three months of 2020. It is most common in the US 46%, the UK 9%, Turkey 8%, France 4% and Canada 3%. The earliest was found on 01.01.2020.[17]

In a paper comparing mutation profiles by illness rigorousness, D614G and P323L mutations in SARS-CoV-2 were found to be associated with severe COVID-19 incidents.[22] P323L mutation was detected in NSP12 in patients one, two, three, and six. The disease progressed mildly in these patients.

The most ample amino acid substitutions, P314L (23/30) (ORF1b) and D614G (Spike), were distributed worldwide and were not particularly enriched in Turkey. ORF1Av378I and ORF9S194L were found in two of 30 isolates and demonstrate a high frequency (15 times overall) in Turkey.[3] In the fifth patient, it was observed that proline was converted to histidine at amino acid 681 (P681H) in spike protein. This mutation has been reported to have unique and emergent features with an important exponential increase in global rate of recurrence. The P681H mutation is typical of new-type SARS-CoV-2 variations from the UK and Nigeria.[23] In another study we conducted in pediatric patients, P681H mutation was found in four of six patients.[21]. In our paper, P681H mutation was detected in the fifth patient.

241>C-T, one of the 5'-UTR mutations of the most common SARS-CoV-2 genome worldwide, is also present in all sequences in the study of Sahin et al.[24] In our paper, C241T mutation was noticed in the first, second, third and fourth patients.

According to the available literature, ORF genes have a very important role for the duration of COVID-19.[25] In the paper of Adebali et al., P314L mutation was found in the ORF1b gene area in 23 of 30 samples.[3] In our study, P314L mutation was detected in the ORF1b gene area without D614G mutation in the first and second patients. In the thesis study of Soyak at 21 isolates from our country, Omicron BA.2 subvariants were detected in 12 patients, Omicron BA.1 subvariants in four patients, Omicron BA.5 subvariants in four patients, and Delta variant in one patient. The mutations c.1841A>G (D614G), c.425G>A (G142D), c.9764C>T, NSP4 (T492I), and c.14144C>T (NSP12 P323L) were detected in all isolates analyzed.[26] In our study, mutations were detected in the ORF1a:S34T, ORF1a:S2104I, ORF1b:P314L, ORF8:G8 regions in the first patient, in the ORF1a:F3153L, ORF1b:P314L, ORF3a:S177I regions in the second patient, in the ORF1a:V2613, ORF1b:P314, ORF1b:V211L regions in the third patient, in the ORF1b:P31, ORF1b:T73I, ORF3a:T15I regions in the fourth patient, in the ORF1a:L730F, ORF1a:I2230T, ORF8:Q27, ORF8:R52I regions in the fifth patient, and in the ORF1b:M592I region in the sixth patient.

CONCLUSIONS

In this study, 71 mutations were detected in six adult patients reported from the Konya region. By the Pangolin lineage, three of the patients were B.1.177, two were B.1, one was of B1.36 lineage. By the Pango lineage, two of the patients were B.1.609, one was B.177, one was B.1.36. By the Nexstrain Clade, four of the patients were 20A and two were of 19A lineage. Different mutations were detected in the N regions of the first, second and third patients. In order to investigate the phylogenetic features of SARS-CoV-2 infections in detail, studies should be conducted with more samples from different regions of Turkey. Regular gene sequencing during the pandemic will be beneficial as it will reveal new variants along with known variants. At the same time, we think that genetic sequence analyzes, including our research, can help to understand the dynamics of the virus and to develop vaccines.

ETHICAL DECLARATIONS

Ethics Committee Approval: This academic paper was permitted by the Turkey Health Ministry (Dated 17.06.2020 and numbered 2020-06-12T13_47_55) and the University Ethics Committee (decision no. 2020/2694).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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