ARAŞTIRMA MAKALESİ/ORIGINAL RESEARCH

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Comparison of Prognosis Between SARS-CoV-2 Wild and Variant Lineages in Kocaeli Province, Turkey

Kocaeli İli, Türkiye'deki SARS-CoV-2 Yabani ve Varyant Soyları Arasındaki Prognoz Karşılaştırması

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ÖZ

GİRİŞ ve AMAÇ: Son zamanlarda, SARS-CoV-2'nin genetik varyantları dünya çapında giderek daha yaygın hale geliyor. RNA virüsleri, insanlar arasında yayılırken sürekli olarak genomik mutasyonlar biriktirir. Bu çalışmada, Kocaeli, Türkiye'deki COVID-19 hastaları arasında SARS-CoV-2 varyantlarının ve vahşi tiplerin farklı sonuçlarla ilişkili olup olmadığını karşılaştırmayı amaçladık.

YÖNTEM ve GEREÇLER: Kesin COVID-19 teşhisi konan 14 yaşından büyük hastalar dahil edildi. Nazofaringeal örnekler B.1.1.7, B.1.351 ve P.1 suşlarını ayırt etmek için tasarlanmış Bio-Speedy® SARS-CoV-2 Variant Plus kiti (Bioeksen Inc., İstanbul, Türkiye) ile tarandı. Pozitif tespit edilen örnekler, yeni nesil dizileme ile doğrulandı.

BULGULAR: Çalışmamızda 53 varyant ve 33 vahşi tip soy enfekte COVID-19 hastası değerlendirildi. 59 hastadan alınan SARS-CoV-2 pozitif numunelere, B.1.1.7 soyuna ait 52, Güney Afrika B.1.351 soyuna ve 6'sı vahşi soylara ait sonraki genom dizilimi sonrasında bir soy atanmıştır. Varyant grubunda 46 (%86,8) hastada hafif hastalık mevcuttu.

TARTIŞMA ve SONUÇ: Varyant soya sahip hastaların hafif hastalığı olduğunu bulduk. Bu grupta yatış ve yoğun bakım gereksinimleri daha azdı. Virüsün epidemiyolojisi için altın standart olan dizileme yöntemi ile SARS-CoV-2'nin yeni genetik varyantlarının saptanması önemlidir.

Anahtar Kelimeler: SARS-CoV-2, COVID-19, prognoz, SARS-CoV-2 varyantları

ABSTRACT

INTRODUCTION: Recently, genetic variants of SARS-CoV-2 are becoming increasingly common around the world. RNA viruses constantly accumulate genomic mutations as they spread among humans. In this study, we aimed to compare whether SARS-CoV-2 variants and wild types are associated with different outcomes among COVID-19 patients in Kocaeli, Turkey.

METHODS: Patients aged >14 years with a definitive COVID-19 diagnosis were included. The nasopharyngeal samples were scanned with the Bio-Speedy® SARS-CoV-2 Variant Plus kit (Bioeksen Inc., Istanbul, Turkey), designed to distinguish B.1.1.7, B.1.351, and P.1 strains. Positive detected samples were confirmed by next-generation sequencing.

RESULTS: 53 variants and 33 wild-type lineages infected COVID-19 patients were evaluated in our study. SARS-CoV-2 positive samples from 59 patients were assigned a lineage following next-genome sequencing 52 belonging to the B.1.1.7 lineage, 1 to the South African B.1.351lineage, and 6 to the wild lineage. In the variant group, 46 (86.8%) patients had mild disease.

DISCUSSION AND CONCLUSION: We found that patients with variant lineage had mild disease. Hospitalization and intensive care requirements were less in this group. It is important to detect new genetic variants of SARS-CoV-2 with the sequencing method which is the gold standard for the epidemiology of the virus.

Keywords: SARS-CoV-2, COVID-19, prognosis, SARS-CoV-2 variants

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INTRODUCTION

In late December 2019, COVID-19 disease, which was defined in a group of patients with respiratory symptoms, spread out quickly to the world. It has become a global health problem and on March 11, 2020, the World Health Organization (WHO) declared this outbreak a pandemic. (1). As of May 13, 2021, there were 159,949,065 confirmed cases of COVID-19 including 3,322,439 deaths. (2). According to the Turkish Ministry of Health data, in Turkey 5,072,462 cases were reported and 43,821 people have died by 13 May 2021 (3).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an enveloped RNA virus from the Betacoronaviridae family that causes Covid-19 disease. Recently, genetic variants of SARS-CoV-2 have been identified and variants pose the biggest challenge ahead of ending the pandemic. RNA viruses constantly accumulate genomic mutations as they spread among humans due to a lack of proofreading mechanisms (4). SARS-CoV-2, like other RNA viruses, is prone to develop mutations over time as it spreads between hosts (5). Fortunately, no antigenic drift has been detected so far for SARS-CoV-2. However, mutations identified in the spike protein of the virus raise concerns about the rate of transmission and immunological resistance (6).

The Centers for Disease Control and Prevention (CDC) closely monitors variants that emerge during the pandemic to improve immunity and disease prognosis. The CDC classified the variants into 3 groups: Variant of Interest (VOI), Variant of Concern (VOC), and Variant of High Consequence (VOHC) (7). Among them, only VOC lineages are considered important because of their impact on public health. First, in December 2020, the B.1.1.7 variant was defined after the increase in cases in Kent, England. It soon became the dominant genome globally and is now the most common lineage seen in the United States (66%) (8). Subsequently, B.1.351 lineage was seen in South Africa. Then, in early January, P.1 strain was first identified in passengers arriving from Brazil. B.1.427 and B.1.429 lineages were first identified in California in February 2021. These variants share one specific mutation called D614G. There is evidence that variants with this mutation spread more quickly than viruses without this mutation (9).

In this study, we aimed to determine whether SARS-CoV-2 variants and wild types are associated with different outcomes among COVID-19 patients in Kocaeli, Turkey.

MATERIALS AND METHODS

Subjects, samples, and sequencing

The nasopharyngeal samples collected at Kocaeli State Hospital Laboratory tested positive for SARS-CoV-2 by quantitative PCR with cycle threshold (Ct) below 32 were subjected to this study. Viral RNA was extracted by a full-automatic rotary nucleic acid magnetic particle extraction system The Auto Extractor GeneRotex96 (Tianlong Science and Technology Co. Xi'an City, China) has been used for SARS-CoV-2 RNA isolation from the nasal/oropharyngeal swab samples.

Subsequently, the collected samples were scanned with the Bio-Speedy® SARS-CoV-2 Variant Plus kit (Bioeksen Inc., Istanbul, Turkey), a one-step reverse transcription, and real-time PCR (RT-qPCR) test designed to distinguish B.1.1.7, B.1.351 and P.1 strains. Positive detected variant samples were confirmed by next-generation sequencing (NGS).
SARS-CoV-2 spike gene, glycoprotein receptor binding domain has been sequenced. The sequence primer pairs: R: 5'- acacctgtgcctgttaaacca - 3' and F: 5'gacaaagttttcagatcctcagttttaca - 3'(~1535 bp). NGS sequencing was carried out on the Miseq (Illumina Inc, Ca, USA) platform. Spike NGS PCR amplification protocol was in the following conditions: at 45C for 10 min, at 95C for 2 min, then for 40 cycles; 95C for 10s, 57C for the 30s, and 72C for 30 s.
Alignment of the resulting sequences was performed with Miseq Reporter based on BWA software (http://bio-bwa.sourceforge.net/).

Patients aged >14 years with a definitive COVID-19 diagnosis were included. Demographic information and prognosis of the patients were collected using the hospital data system. The need for hospitalization was evaluated as moderate prognosis, application of anti-cytokines or pulse steroid therapy, non-invasive mechanical ventilation support, and treatment in the intensive care unit as severe prognosis.

Ethical approval

The study protocol was approved by the Ethics Committee of Near East University with the number YDU/2021/93-1383.

Statistical analysis

IBM SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA) program was used for analysis. The median (min-max), mean (standard deviation) and number (percentage) of the data were indicated. Comparisons were made with the Pearson chi-square test.

RESULTS

Eighty-six patients were included in our study. SARS-CoV-2 PCR was positive in the nasopharyngeal swab taken from all patients. Of these patients, 59 patients were found to be suspected of mutation with the test Biospeedy SARS-CoV-2 Variant Plus (Bioeksen Inc., Istanbul, Turkey). However, when the verification method, Next-Generation Sequencing (NGS) was studied, Six samples were found to have a wild lineage. Sequence analysis of 53 patients revealed variant lineages. 27 patients were included in the study as patients who were scanned with Biospeedy SARS-CoV-2 Variant Plus and had no mutations. NGS could not be performed on these patients due to financial reasons. As a result, 53 variants and 33 wild - type lineage - infected COVID-19 patients were evaluated in our study.

When the SARS-CoV-2 positive samples taken from 59 patients were examined; B.1.1.7 lineage was found in 52 patients, B.1.351 lineage in 1 patient, and wild lineage in 6 patients.

The mean age of our patients was 40 and the male gender was predominant (46,53%). Ct values of all patients were below 32. Thoracic tomography was performed in 15 patients. Of these, 12 were interpreted as category 2 (compatible with COVID) and 3 as normal. The characteristics of the patient group are shown in Table 1. Four of the patients had asymptomatic disease. The comparison of accompanying symptoms in wild and variant types is shown in Figure 1. One patient had a single dose of inactive coronavirus vaccine after that she had COVID-19 disease with variant lineage and, one person had wild type COVID-19 disease after two doses of inactive coronavirus vaccine. Antibody tests were not performed in any of the patients. When evaluated according to the prognosis classification, a total of 75 patients had overcome the disease with mild prognosis. On the other hand, 46 (86.8%) of those who were in the variant group had mild disease. Two people who died were also infected with wild lineage. The treatment and prognosis differences of the variant and wild groups are shown in Table 2.

Table 1. Demographic and Medical Findings of the Patients

Characteristic	**Patient**
Age, mean ± SD	40 ±15
Gender, M/F, n(%)	46 (53,5)/40 (46,5)
SARS-CoV-2 PCR Ct, mean ±SD	24,7± 3,2
Symptomatic patient, n (%)	80 (93)
Wild/variant lineage, n (%)	33 (38)/53 (61)
Hospitalized patient, n (%)	11 (12,8)
Comorbidity, n (%)	21
	4
Asthma	1
Arrhythmia	2
DM	5
HT	3
Hypothyroidism	1
Glaucoma	1
CAD	1
Malignancy	1
Migraine	1
CVS	1
Ulcerative colitis	

Abbreviations: F/M; female/ male, Ct: cycle threshold, DM: diabetes mellitus, HT: hypertension, CAD: coronary artery disease, CVS: cerebrovascular stroke

Table 2. Cohort Characteristics by SARS-CoV-2 Lineage

Characteristic	Wild lineage	Variant lineage	p-value
Gender, M/F, n (%)	16 (48,5) / 17 (51,5)	30 (56,6) / 23 (43,4)	0.609
Age, mean±SD	40±18	40±14	0.803
BMI±SD	27,2±5,8	25,8±4,8	0.262
SARS-CoV-2 PCR Ct (25^{th}-75^{th} per)	25,41 (23-28)	24 (22,5-26)	0.130
Hospitalized patient, n	4	7	1.000
Treatment experience, n(%)			
Favipiravir	29	42	0.979
Acetyl salicylic acid	5	10	0.953
Enoxaparin	5	6	0.429
Prednisolone 40 mg	2	7	-
Prednisolone 250 mg	2	1	-
Tocilizumab	1	0	-
.Prognosis, n (%)			
Mild	29 (87,9)	46 (86,8)	0.585
Moderate	2 (6,1)	6 (11,3)	
Severe	2 (6,1)	1 (1,9)	

Abbreviations: M/F; male/female, Ct: cycle threshold, BMI: Body Mass Index, per percentile

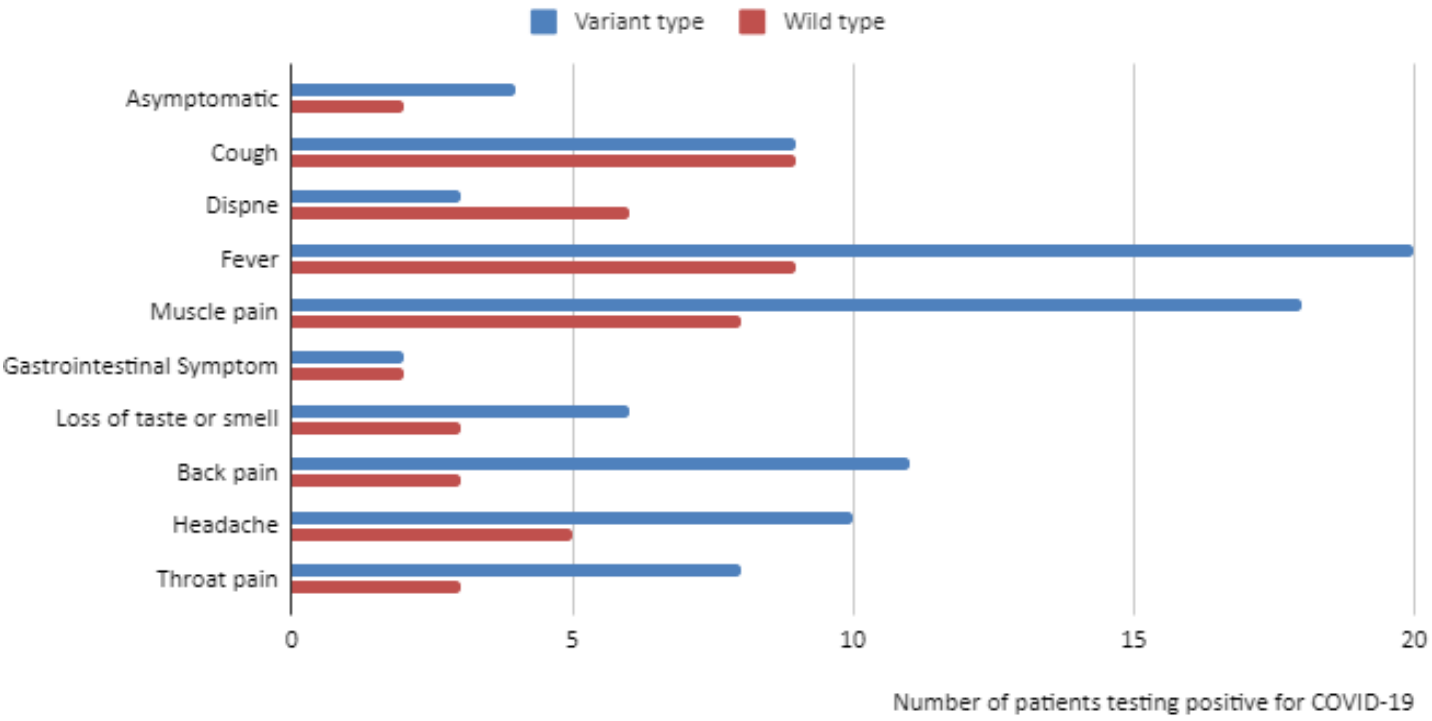

Figure 1. The Comparison of Accompanying Symptoms in Wild and Variant Type Infected COVID-19 Patients

DISCUSSION

We aimed to compare the prognosis between wild lineage and variant of concerns. Although we could not compare variants among themselves due to the small sample size, we found that patients with variant lineage had mild disease. Hospitalization and intensive care requirements were less in this group. In addition, the two people who died had wild lineage. When variants were first detected, it was stated in the literature that viruses with D614G mutation may be more virulent and increase the severity of the disease (10). As the number of cases increased, it was understood that until now, there was no evidence that this new B.1.1.7 showed better clinical outcomes (11).

Previous studies in the UK show increased contagiousness of up to 71% compared to wild lineage (12). The relatively higher incidence of cough symptoms in patients infected with B.1.1.7 may explain the increased tranfsmission observed in this variant (11). However, in our study, no significant difference was found between the groups in terms of cough symptoms. Fever, sore throat, and muscle pain symptoms are prominent in the variant group. This confirms the literature information that variant infected COVID-19 patients mostly present with common cold-like disease (13).

In our study, patients with wild lineage and variant lineage hospitalization ratios were 12% and 13% respectively. Likewise, in a case-control study, B.1.1.7 and other variants were compared in terms of hospitalization risk and no statistically significant difference was found (14). Similarly, Korber et al. showed no significant increase when comparing D614G status and hospitalization rate (15).

The limitations of our study are the inability to sequence all samples due to financial reasons and insufficient blood and imaging results since the patient group was selected from the outpatient clinic.

In conclusion, it is important to detect new genetic variants of SARS-CoV-2 in a global pandemic setting. Because the sequencing method is the gold standard method, there is a need for widespread use of this method and further studies are needed on the epidemiology of the virus.

Ethics Committe Approval: The study protocol was approved by the Ethics Committee of Near East University with the number YDU/2021/93-1383.

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Informed Consent: The study was designed as retrospective.

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