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Investigation of Variants In SARS-CoV-2 Infections after Three Doses of COVID-19 Vaccine

Üç Doz COVID-19 Aşı Sonrası Oluşan SARS-CoV-2 Enfeksiyonlarında Varyantların Araştırılması

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Abstract

Aim: Our study focused on retrospectively assessing variant of concern, specified by the World Health Organization (WHO), with one-step reverse transcription and real-time polymerase chain reaction (RT-PCR) test in SARS-CoV-2 positive patients after three doses of attenuated COVID-19 vaccine.

Material and Method: 8.520 samples transported with viral nucleic acid buffer (vNAT) tubes between June 2021 and January 31, 2022, were tested and included in the study. All the patients whose samples were included in our research had 3 doses of CoronaVac (Sinovac Life Science Co, Ltd, Beijing, China). Gender distribution was 4686 (55%) female and 3834 (45%) males. Variant specific genome regions only found in B.1.351, P.1 and B.1.1.7 as well as ORF1ab and N gene regions are investigated by the Bio-Speedy® Emerging Plus kit (Bioeksen AR-GE Technologies, Turkey) used to identify the variants in the study.

Results: All 8.520 samples were SARS-CoV-2 RT-PCR positive. Our study detected alpha and delta variants in 1460 (17.14%) and 3570 (41.9%) patients respectively. 2570 (30.16%) patients did not have any variants according to test results. It was observed that the spread of beta, gamma and other suspicious variants remained at relatively low rates.

Conclusion: The delta variant became dominant from July until to the end of the year. Declining delta variant rates and increasing cases of suspected variants towards the beginning of December 2021 suggest the omicron variant. Therefore, molecular surveillance studies that are planned to take epidemiological data into consideration and to examine the prevalence and gene-based analysis of local and worldwide variants are required.

Keywords: SARS-CoV-2, variants take concern, variant B.1.1.7, variant B.1.351, variant P.1, variant B.1.617.2

Öz

Amaç: Çalışmamızda, üç doz atenüe COVID-19 aşısı sonrası SARS-CoV-2 polimeraz zincir reaksiyonu (PCR) testi pozitif saptanan hastalarda Dünya Sağlık Örgütü (DSÖ) tarafından endişe verici varyantların ("variants of concern – VOCs") dağılımının geriye dönük olarak değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Haziran 2021-31 Ocak 2022 tarihleri arasında laboratuvara rutin çalışma kapsamında viral nükleik asit tamponu (vNAT) tüpü ile gelen ve SARS-CoV-2 RT-PCR testi istenen 8520 örnek çalışmaya dahil edildi. Örneklerin hepsi, 3 doz CoronaVac (Sinovac Life Science Co, Ltd, Beijing, China) aşı geçmişine sahip ve son doz aşıdan en az 28 gün geçtikten sonra alınmış, cinsiyet dağılımı 4686 (%55)'sı kadın 3834 (%45)'ü erkektir. SARS-CoV-2 varyantları; ORF1ab ve N gen bölgelerinin yanı sıra yalnızca B.1.1.7, B.1.351 ve P.1'de bulunan varyant spesifik genom bölgelerini de hedefleyen Bio-Speedy® SARS- CoV-2 Emerging Plus kiti (Bioeksen AR-GE Teknolojileri, Türkiye) ile saptandı.

Bulgular: 8.520 numunenin tamamı SARS-CoV-2 RT-PCR pozitifti. Çalışmamızda sırasıyla 1460 (%17,14) ve 3570 (%41,9) hastada alfa ve delta varyantları saptandı. 2570 (%30,16) hastanın test sonuçlarında göre herhangi bir varyantı yoktu. Beta, gama ve diğer şüpheli varyantların yayılımının nispeten düşük oranlarda kaldığı gözlemlendi.

Sonuç: Çalışmamızda, Temmuz ayından yıl sonuna kadar ise delta varyantının baskın hale geldiği tespit edildi. Bu bağlamda epidemiyolojik veriler ışığında planlanmış, bölgesel ve küresel çapta varyantların sıklığını ve genomik analizlerini irdeleyen moleküler sürveyans çalışmalarının yapılması gerekmektedir.

Anahtar Kelimeler: Sars-CoV-2, SARS-CoV-2, endişe verici varyantlar, varyant B.1.1.7, varyant B.1.351, varyant P.1, varyant B.1.617.2

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INTRODUCTION

The COVID-19 is a significantly infectious disease that initially emerged in China at the very end of 2019. Acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) from the coronavirus family was responsible from the illness. WHO specified the disease as a pandemic on March 11, 2020, after it severely affected almost all countries within weeks.[1] 6.9 million deaths out of approximately 770 million COVID-19 cases have been globally confirmed as of May 2023 according to WHO data.[2] The original strain has mutated since the onset of the pandemic and evolved into several variants. This situation has created threats that may adversely affect the course of the pandemic such as increased risk of transmission, escape from immune response, risk of reinfection, decreased effectiveness of vaccines, and worsening of the clinical picture. WHO primarily categorized mutations as "Variants of Interest (VOIs)", "Variants Under Monitoring (VUMs)" and "Variants of Concern (VOCs)". Alpha (B.1.1.7, UK), Beta (B.1.351, South Africa), Gamma (P.1, Brazil), Delta (B.1.617.2, India), and Omicron (B.1.1.529)[3] are the five concerning variants have emerged so far. Many vaccine studies and subsequent vaccination campaigns against SARS-CoV-2 have started against the pandemic but all the worrying variants have caused a new wave in pandemics resulted in thousands of more deaths worldwide. Therefore, it is critical to identify SARS-CoV-2 variants and follow the mutations as they undergo to eliminate actor of the COVID-19 pandemic.[4] Protection obtained through proper two dose of vaccination gradually declines in severe COVID-19 cases and hospitalization. However, booster vaccination with any of the common mRNA-based vaccines significantly is reported by other research scaling down the reinfection possibility and even if the patient is reinfection and yet the disease can be mildly recovered.[5] Our study aimed to retrospectively examine the distribution of variants in SARS-CoV-2 VOCs positive individuals with one-step reverse transcription and real-time polymerase chain reaction (RT-PCR) after they received 3 doses CoronaVac 600 U/0.5 mL (Sinovac Life Science Co, Ltd, Beijing, China) vaccine and the last dose is at least 28 days before the test.

MATERIAL AND METHOD

The study was carried out with the permission of Yıldırım Beyazit University Yenimahalle Training and Research Hospital Scientific Research Ethics Committee (Date: 11.05.2022, Decision No: 2022-29). All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

The study was carried out retrospectively by covering between June 2021 and January 31, 2022. The study included 8520 samples evaluated as COVID-19 SARS-CoV-2 infection contact follow-up, epidemic management, home patient follow-up and filiation guide as a Scientific Advisory Board study in Ankara Provincial Health Directorate Sample Campus Molecular Diagnosis Laboratory. These samples were transported with a viral nucleic acid buffer (vNAT) tube as routine work by the filiation teams and their test results were positive. All samples obtained from patients who had CoronaVac 600 U/0.5 mL (Sinovac Life Science Co, Ltd, Beijing, China) vaccine (3 doses) and sample collection was at least 28 days after the last dose. Gender distribution was 4686 (55%) females and 3834 (45%) males. Variants was detected with the Bio-Speedy® SARS-CoV-2 Emerging Plus kit (Bioeksen AR-GE Technologies, Turkey). This kit investigates distinct genome regions belonging to the variants with E484K (Gamma and Mu) and L452R (Delta) mutations in the S region and the Nucleocapsid region with D3L (Alfa) mutations in addition to the ORF1ab and N gene regions. Studies were performed on CFX96 DX Real-Time PCR systems (Bio-Rad Laboratories, USA).

Statistical Analysis

Statistical analysis was performed with SPSS 22.0 program (IBM Corp., Armonk, NY, USA). Frequency (n), percentage (%) and mean values were determined in the data analysis.

RESULTS

The average age of 8520 patients was 42.62±14.98 years. 4686 (55%) of them were female and 3834 (45%) were males. 2570 (30.16%) patients did not have any variants while alpha was found in 1460 (17.14%) and delta in 3570 (41.9%). It was observed that the spread of beta, gamma and other suspicious variants remained at relatively low rates (**Table 1**).

Table 1. Distribution of SARS-CoV-2 Variant Types

	SARS-CoV-2 (No variant) n (%)	Alfa Variant n (%)	Delta Variant n (%)	Beta / Gamma Suspicious n (%)	Other Variant Suspicious n (%)
Positive	2570 (30.16%)	1460 (17.14%)	3570 (41.9%)	270 (3.17%)	650 (7.63%)

DISCUSSION

The still ongoing pandemic has started to slow down thanks to the vaccines that emerged because of effective and rapid vaccination studies that were approved for immediate use. It becomes more important whether the vaccines used are effective against variants or not as the strains responsible for overall COVID-19 picture that occurs in vaccinated individuals are frequently variants (VOC)[6] with SARS-CoV-2 variants becoming more common all over the world. Many COVID-19 vaccines have proven to be safe and effective as a booster dose. 7 different vaccines were scrutinized as booster doses in the Cov-Boost trial afer two doses of AstraZeneca or Pfizer vaccines. They are Curevac, AstraZeneca, Moderna, Johnson & Johnson, Novavax, Pfizer and Valneva. The trial showed that all of them enhanced the immunological response.[7]

Vaccination history of positive test subjects (cases) among symptomatic individuals who requested SARS-CoV-2 testing was compared with the vaccination history of negative test

subjects (controls) in a negative case-control study carried out in Alaska. It has been shown that people who have not received the mRNA COVID-19 vaccine reminder dose have an approximately three times higher risk of contracting symptomatic COVID-19 infection compared to people who have received the reminder dose. This analysis also found that reminder dose increased the protection against a COVID-19 reinfection. It has been reported that once-positive patients without a reminder dose have a re-infection risk of 1.6 times compared to those with a reminder dose.[8]

It is known that the immune response of inactivated Sinovac and Sinopharm vaccines is reduced after a certain period like other inactivated vaccines.[9] It seems reasonable to apply a third dose of vaccine to strengthen the immune response for this type vaccines. Moreover, this will support the hypothesis of a third dose vaccine requirement given that it is supported by studies that it is effective against emerging variant viruses.[10] It is thought that China which has received approximately 1.69 billion doses of inactivated vaccines, may change its attitude towards mRNA-based vaccines since she has been keeping a distance from until now due to the low efficacy of these vaccines against new variants (especially the Delta variant) and the increasing number of cases.[11] It is determined that there is a very common SARS-CoV-2 variant positivity in individuals vaccinated with 3 doses of inactivated vaccine in our study similar to this data. Mutations are a natural part of the viruses' life cycle and their adverse effects on the course of epidemics are rare and limited. It is suggested that mutations may help managing existing epidemics and understanding new epidemics.[12] Cellular immune response mediated by T lymphocytes and Natural killer (NK) cells plays a key role in infection control even though spike protein mutations in variant viruses cause evading neutralization.[13] Therefore, vaccines continue to be the greatest arsenal to control variant viruses.

A microneutralization assay was conducted in Israel using Wild-type virus, Beta, Delta and Omicron variant isolates and serum samples from two groups of 20 healthcare professionals. The first group consisted of participants who received two doses of BioNTech vaccine, and the second group consisted of those who received three doses of BioNTech vaccine. Three doses of vaccine resulted in better neutralization of wild-type virus and three variants. Low neutralization efficacy against Wild-type virus and Delta variant was found in an evaluation five months after the second dose. Neutralization against the Omicron variant was four times lower than against the Delta variant even with three vaccine doses. The persistence of the effect of the third vaccine dose against COVID-19 has not yet been determined.[14] We observed that the alpha variant was outweighing in June 2021 while the delta variant became preeminent in July in our study. It has been reported that the infection rate of the delta variant which is defined as one of the VOCs by WHO is approximately twice that of the original virus and that the delta (B.1.617.2) variant is more contagious than previous variants by suppressing globally circulating variants.[15,16] It was observed that the delta variant became dominant in our country as of July 2021 in our study.

Emerging new variants are not only associated with increased contagiousness, distress, and death toll but also, they may deceive diagnostic testing, develop reduced susceptibility to antiviral treatments and have the capacity to cause reinfection in previously vaccinated and surviving individuals. The longer the virus spreads, the higher its probability of mutation increases.[17] Suppression of viral replication through both public health measures along with a fair and widespread vaccine application is critical in reducing the risk of emergence of new variants.[18] In our study, the number of suspected cases of other variants increased in the beginning of December 2021 suggesting the omicron variant which is widely distributed in most countries by the end of the alpha and delta variants' dominance.

CONCLUSION

As far as we know, our study is the first published study on variant prevalence in individuals who are positive after 3 doses of inactivated vaccine. Molecular surveillance studies planned in consideration epidemiological data examining the prevalence and genome-based analysis of local and global variants are required. Pandemic continues due to the fluctuation of incidences with different restrictions applied globally, the risk of reinfection, the fact that the virus is susceptible to new mutations and the acceleration and deceleration in vaccinations. Studies on the spread and genomic analysis of existing variants will allow us to be better prepared for potential outbreaks in addition to the experience we have gained during the COVID-19 pandemic.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Yıldırım Beyazit University Yenimahalle Training and Research Hospital Scientific Research Ethics Committee (Date: 11.05.2022, Decision No: 2022-29).

Informed Consent: All patients signed the free and informed consent form.

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