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CLINICAL MICROBIOLOGY - RESEARCH PAPER

Impact of SARS-CoV-2 Delta and Omicron variants on viral burden and cycle threshold in BNT162b2-vaccinated 12–18 years group

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Abstract

The SARS-CoV-2 pandemic continues to impact the medical, economic, social, and political areas worldwide. Although it has been claimed that children are the most responsible for the outbreaks as of September 2021, the statistics showed controversary. Although it showed no difference in viral load and Ct values between symptomatic children and symptomatic adults, or between asymptomatic children and asymptomatic adults, the molecular mechanism remains unclear. Here, we aimed to investigate the effects of different strains on infection by comparing viral load levels in pediatric patients aged 12–18 years, infected with different variants of SARS-CoV-2, and vaccinated with full-dose BNT162b2. In this retrospective study, a total of 200 patients aged 12–18 years, who were diagnosed with COVID-19 in our hospital, and vaccinated with full-dose BNT162b2, were analyzed according to their gender, age, viral load, and cycle threshold values. Viral RNA levels were evaluated using Ct values, a semi-quantitative proxy of viral load. While the findings did not show a significant difference between gender and age ($P=0.886$ and $P=0.897$, respectively), a significant difference was found between the Ct and viral load ($P<0.0001$). In conclusion, SARS-CoV-2 viral load was higher in cases infected with SARS-CoV-2 Delta variant than SARS-CoV-2 Omicron variant (mean Ct = 23.05 ± 4.06, viral load = 7.8 × 105 copies/ml and mean Ct = 28.04 ± 3.02, viral load = 7.8 × 103 copies/ml, respectively). These findings indicated that the Delta variant had high viral load and our result could be one of the causes the Delta variant was more effective in the pandemic severity than the other variants in the October–December periods when the Delta variant was dominant in Northern Cyprus. During the same period, the severity of the disease was higher, with higher hospitalization and death rates.

Keywords SARS-CoV-2 · Delta · Omicron · BNT162b2 · Pediatrics · COVID-19

Introduction

The SARS-CoV-2 virus was first reported by China in late 2019 [1]. As it continues to spread in full swing, it was declared a "pandemic" by the World Health Organization on March 11, 2020, and its effects are still ongoing. While it has caused crises in the health, social, and economic fields worldwide, it has also caused mobility in the field of science due to the necessity of vaccine studies [2]. Although measures such as masks, hand hygiene, and social distancing have been taken, no definite and clear solution has been found in terms of public health. According to current data, it still maintains its dangerous picture with the number of cases exceeding 500 million and the number of deaths exceeding 6 million [3].

The coronavirus family is large, with positive single-stranded RNA viruses. It has been reported by many articles that the 4 coronaviruses, called NL63, 229E, HK1, and OC43, cause colds in humans [4]. All these 4 coronaviruses are considered to be of zoonotic origin and it is suggested that OC43, which is from the beta-coronavirus family, is the main responsible for the "Russian flu" pandemic that occurred between 1889 and 1990 [5]. In addition to SARS and MERS, coronavirus has not been a priority target of vaccine studies because it causes mild

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symptomatic diseases in humans. Also, the need that will be developed for the common cold to cover and be specific to 4 different viruses has made it difficult to study the vaccine [6]. The genome of the SARS-CoV-2 virus has 30,000 bases. There are 11 known protein-coding genes and 12 proteins expressed from these genes. Nucleocapsid (N), Envelope (E), Membrane (M), and Spike (S) are some examples of structural proteins encoded in the viral genome. It has been reported that any mutations that occur in genes associated with these proteins or that will change the structure and/or function of these proteins affect the ability of viral entry, fusion, and virus survival [7]. There are 12 functional ORF regions of the virus. After the SARS-CoV epidemic in 2002–2004, vaccine studies were developed preclinically and subjected to a phase 1 trial [8]. However, due to the major mutations occurring in the ORF region of the virus, the vaccine studies have become stationary again with the disappearance of the virus. In April 2022, with the increase in numbers of cases and deaths, rigorous scientific work has led to the innovation of various types of quick and efficient vaccines for SARS-CoV-2, which were catalyzed by the dedication of healthcare workers and scientists on a global-scale infectious. Most vaccines are subject to phase trials and Pfizer/BioNTech and Moderna (BNT162b2 and mRNA-1273) by mRNA-based vaccines in phase III studies with a high percentage (95%) have led to the efficiency. Janssen (AstraZeneca) and AZD1222 vaccine (AZD1222) were the first to identify adenovirus vector–based vaccines from the University of Oxford/AstraZeneca revealed high levels of protection of 70.4% and 66.9% relatively [9, 10]. Inactive vaccines such as CoronaVac and Sinovac, which were one of the first vaccines to be administered, have been reported to have a different percentage of effectiveness by different countries. Turkey reported efficiency of 83.5%, and Chile reported efficiency of 65.9% whereas WHO issued effectiveness for CoronVac remained at 51%. The variants of this condition and the conflicting percentages of effectiveness that SARS-CoV-2 has due to the mutations it has undergone are held responsible [11, 12]. Therefore, countries are urged by the world health organization to carry out their independent studies to further evaluate this vaccine. Accompanied by this information, it seems that the interaction of the variant with the vaccine should still be investigated.

North Cyprus experienced its first COVID-19 case and lockdown in March 2020 [13]. Vaccine rollout in North Cyprus started with the inactivated virus vaccine CoronaVac. Priority was given to healthcare workers and those above the age of 65 years. CoronaVac implementation was followed with BNT162b2 vaccine for individuals 65 years old and AstraZeneca/Oxford for the 55–65 year group. As of September 2021, children/pediatric groups have been suggested as the biggest responsible for the epidemic. However, according to studies conducted, children are responsible for less than 20% of SARS-CoV-2 cases. Again, according to studies, the number of children between the ages of 12–18 who were hospitalized due to SARS-CoV-2 does not exceed the number of children hospitalized because of the seasonal flu. Accordingly, it was reported that there were no statistically significant results when the values of the viral load/cycle threshold of asymptomatic children and asymptomatic adults or symptomatic children and symptomatic adults were compared. Therefore, in our study, we aimed to investigate the effects of different strains on infection by comparing the levels of viral load in pediatric patients aged 12–18 years with full-dose BNT162B2 vaccine infected with different variants of SARS-CoV-2 in pediatric patients aged 12–18 years.

Materials and methods

Totally, two-hundred teenage patients, who had been fully vaccinated with the BNT162b2 vaccine, SARS-CoV-2-positive, which had been infected with the Delta or Omicron (BA.1) VOCs, were included in the study. The COVID-19 patients were diagnosed with SARS-CoV-2 between November 2021 and March 2022, and they had symptomatic and asymptomatic infections. The COVID-19 patients > 18 aged were excluded from the study.

Detection of VOCs was done by using to Multiplex SARS-CoV-2 VOC RT-qPCR detection kit (Nicosia, Northern Cyprus). Following the viral RNA isolation, samples of all individuals were screened for Spike (S) gene mutations including H69-70 deletion, N501Y, K417N, T478K, Y144del, and P681R mutations to differentiate the VOCs of SARS-CoV-2 between Delta (B.1.617.2) and Omicron (BA.1). We considered the VOCs as follows;

- Delta, if the specimens are positive for T478K and P681R mutations and negative for the mutation H69-70 deletion, N501Y, K417N, and Y144del mutations.
- Omicron, if the specimens are positive for the H69-70 deletion, N501Y, T478K, K417N, and Y144del mutations and negative for the P681R mutation.

Whole-genome sequencing has been performed on a few of the specimens to confirm the VOCs which were detected by next-generation sequencing technique (GISAID reference numbers EPI_ISL_12574367, EPI_ISL_12574374, EPI_ISL_12574370, EPI_ISL_12574375, EPI_ISL_12574368, EPI_ISL_12574373, EPI_ISL_12574369, EPI_ISL_12574371, EPI_ISL_12574372, EPI_ISL_12574000).

The prediction of the viral load was defined by using cycler thresholds (Ct) that is the value of the first PCR cycle the viral RNA amplification is detected. The viral load level was inversely correlated with Ct value which is a low viral load characterized by a high Ct and a high viral load characterized by low Ct values.

Table 1 The effect of SARS-CoV-2 variants on viral load and cycle threshold in pediatric patients aged 12–18 years with full-dose vaccination

SARS-CoV-2 variants/ parameter	Sex					Age		Ct value	
	N = 100		*P* value	OR	95% CI	Mean	*P* value	Mean	*P* value
Delta	M	44	0.886	1.010	0.414–2.460	14.6 ± 2.11	0.897	23.9 ± 2.6	***< 0.001****
	F	56				15.6 ± 2.02		22.2 ± 4.0	
Omicron BA.1	M	45				14.5 ± 1.8		28.3 ± 3.2	
	F	55				15.2 ± 1.9		28.4 ± 3.1	

N, number; *M*, male; *F*, female; *OR*, odds ratio; *CI*, confidence interval; *Ct*, cycle threshold. *$p < 0.05$ considered as significant.

Statistical analyses

Differences in categorical variables were examined by chi-square (χ^2) tests. The quantitative variables were examined using Student's-*t* test or Mann–Whitney *U* test according to normal or abnormal distributions. One-way ANOVA test followed by Bonferroni's test the post hoc test for multiple comparisons was used. The results were expressed as the mean ± standard deviation, or percentage, wherever appropriate. The odds ratios (ORs) and 95% confidence intervals (CIs) by the use of binary logistic regression. Statistical significance was considered when $p < 0.05$.

Results

One hundred SARS-CoV-2 teenage patients who were infected by Delta VOC and 100 SARS-CoV-2 teenage patients who were infected by Omicron (BA.1) VOC have been included in the study. The Ct value from RT-qPCR which was used for SARS-CoV-2 detection was recorded for the estimated viral load of all of the patients.

Vaccination was completed between the 1st of September and the 1st of October 2021 for the cohort between 0 and 12 ages. The adult group was selected among people who were vaccinated between the 1st of November 2021 and the 31st of January 2022.

The mean age of the patients was similar in both groups (Delta patients 14.80 ± 1.78, Omicron (BA.1) patients 15.09 ± 2.12) ($p > 0.05$). Also, the distributions of gender were similar between groups; 44 (44%) male, 56 (56%) female for Delta, 45 (45%) male, 55 (55%) female for Omicron (BA.1) patients ($p > 0.05$).

The analyses of the viral load between the two groups for viral load showed that patients of Delta VOC (Ct = 23.05 ± 4.06) had higher viral load and low Ct values compared to patients of Omicron (BA.1) VOC (Ct = 28.04 ± 3.02), and the differences in viral load were significant ($p = 0.001$). When we analyzed viral load according to gender, we observed no significance between female and male Omicron (BA.1) patients and also between female and male Delta patients. But the comparison of the viral load between two VOCs according to gender remained significant (Table 1).

Discussion

SARS-CoV-2 Omicron and Delta strains had been shown to have dominance during October–December 2021 period in Cyprus. The preliminary data and clinical outcomes indicate that the Omicron variant has milder symptoms and a lower rate of hospitalization than the Delta variant [14]. BNT162b2 vaccination was known for its enormous neutralizing antibody capacity against SARS-CoV-2 variants [15]. It is not yet known whether these two different variants have an effect on viral load levels in patients aged 12–18 years and vaccinated with a full dose of BNT162b2. For this reason, 100 children with Delta VOC, and 100 children with Omicron VOC, were included in the study with evenly distributed genders to investigate the effect of BNT162b2 vaccination on viral load between these two VOCs.

The data revealed that there is no difference in the viral load between different genders in BNT162b2-vaccinated children, correlating with the study of Marks et al. [16] that was participated among vaccinated adults.

Viral load is an important parameter influencing the infectiousness of viruses along with their immune evasion capabilities and binding affinities to the responsible receptors [17]. The present study revealed that the viral load of the vaccinated children infected with the Delta variant is higher than the children infected with the Omicron variant correlating with the study of Puhach et al. [18] that was participated among adult patients. BNT162b2 vaccination is well known for its high effectiveness against the Delta variant–infected children; however, it has a moderate effect on the Omicron variant [19, 20]. The lower vaccine efficacy was also demonstrated by a pre-print study participated in adolescents [21].

The lower level of viral load with milder symptoms and hospitalization in the Omicron variant could be attributed to high viral circulation that can be characterized by a higher probability of asymptomatic infection in children. This might contribute to a larger portion of hidden infections, with a subsequent increase in the rate of a protective immune response [20]. The transmission rate of the SARS-CoV-2 Omicron variant is

known to be higher. This could suggest that other mechanisms than increased infectious viral load contribute to the high infectiousness of the SARS-CoV-2 Omicron variant [18].

The cycle threshold (Ct) comparison data revealed that the SARS-CoV-2 Delta variant has a significantly lower value when compared to the Omicron variant in children. This indicates that the Delta variant progresses with a higher viral load than the Omicron variant. This could be due to the fact that the viral load of the Omicron variant is known to be lower than the Delta variant in vaccinated individuals, a similar trend with adults [18]. In another way, this might also be attributed to the hidden infections between children that might provide a higher immunity against the particular variant [20].

In conclusion, this study provides strong evidence of higher infectiousness of the SARS-CoV-2 Omicron variant in comparison to the Delta variant in fully BNT162b2-vaccinated children between 12 and 18 ages. Even though the viral load of the SARS-CoV-2 Delta variant samples of fully BNT162b2-vaccinated children is higher than the SARS-CoV-2 Delta variant, the rate of transmission is known to be greater in the Omicron variant. These data altogether emphasized that children between 12 and 18 ages who are vaccinated with BNT162b2 could be infected with SARS-CoV-2 but would have milder symptomatic diseases.

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