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RESEARCH ARTICLE

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The effectiveness of inactivated SARS-CoV-2 vaccine (CoronaVac) on antibody response in participants aged 65 years and older

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Abstract

In this study, it was aimed to determine the antibody responses after the two doses of inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccinations in people who were above 65 years old and to evaluate the factors affecting this response. A total of 235 participants aged 65 years and older were included. Blood samples were taken and data about age, gender, comorbid diseases, and presence of side effects after vaccination were noted. Anti-SARS-CoV-2 QuantiVac ELISA (IgG) test kit (catalogue number: EI-2606-9601-10-G, Euroimmun) was used. The mean age was 70.38 ± 4.76. Approximately 120 of 235 participants had at least one comorbid disease. The mean levels of anti-SARS-CoV-2 IgG antibody after 4 weeks from the first and second doses of vaccine were 37.70 ± 57.08 IU/ml, and 194.61 ± 174.88 IU/ml, respectively. Additionally, 134 of 235 participants (57.02%) had under 25.6 IU/ml antibody level (negative) after 4 weeks from the first vaccine dose while this rate was 11.48% (n = 27) after 4 weeks from the second vaccine dose. The 19 (70.4%) participants who had under had 25.6 IU/ml antibody level after 4 weeks from the first dose of vaccine had at least one comorbid disease including diabetes mellitus, and 8 (29.6%) participants had no comorbid disease (F = 2.352, p = 0.006). Lower rates of antibody response were detected in participants aged 65 years and older and those with comorbidities both in our study and similar studies in the current literature. Further studies should evaluate whether the low antibody titers are really associated with age and comorbidities or not. Finally, prospective studies are needed to determine how long the immunity provided by SARS-CoV-2 vaccines will continue.

KEYWORDS
65 years, antibody response, CoronaVac, SARS-CoV-2, vaccine

1 | INTRODUCTION

A novel virus-associated disease named coronavirus disease-2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was firstly declared in December 2019 in Wuhan, China.[1] Since December 2019, there have been more than 180 million confirmed infections and over 3.9 million deaths reported worldwide.[2] Although clinical signs and symptoms of patients infected with SARS-CoV-2 are heterogeneous, cough, fever, and dyspnea are the most common symptoms. The clinical diagnosis of COVID-19 is mainly based on epidemiological history, clinical symptoms, computed tomography (CT) scan, and nucleic

acid detection by a real-time polymerase chain reaction technique.[3,4]

SARS-CoV-2 is an enveloped, single-stranded RNA beta coronavirus that belongs to the family Coronaviridae.[5] It has four major structural proteins; envelope (E), membrane (M), nucleocapsid (N), and spike (S) protein. The S and N proteins are the principal immunogens used for the detection of anti-SARS-CoV-2 specific antibodies.[6,7] The S protein consists of two subunits. The first subunit (S1) mediates the attachment of the virus to human cells via its receptor-binding domain (RBD), and the second one (S2) mediates membrane fusion for viral entry. Antibodies that bind to the S protein can neutralize coronaviruses.[6]

Sinovac-CoronaVac, developed by Sinovac/China National Pharmaceutical Group in Beijing/China, is an inactivated COVID-19 vaccine. The vaccine was highly immunogenic in healthy adults aged 60 years and above in Phase I/II clinical trials.[8] According to the results, the vaccine showed good safety and immunogenicity, comparable with that observed in healthy adults aged 18–59 years in the prior studies.[9,10] Following the positive data obtained from Phase III studies, the inactivated SARS-CoV-2 vaccine has been started to be applied in our country since January 15, 2021. Until now, approximately 28 million people have been vaccinated with two doses of COVID-19 vaccine including Sinovac and BioNTech vaccines.[11]

Currently, our understanding of antibody responses in people who is above 65 years old following COVID-19 vaccination is limited. In this study, it was aimed to determine the antibody responses after the two doses of inactivated COVID 19 vaccinations in people who are above 65 years old and to evaluate the factors affecting this response.

2 | MATERIALS AND METHODS

2.1 | Ethical statement and permissions

This study was approved by both the Republic of Turkey Ministry of Health COVID-19 Scientific Research Evaluation Commission (approval date: 12.02.2021; number: 2021-02-10516-23-51) and the Local Ethics Committee of Kafkas University Faculty of Medicine (approval date: 11.03.2021; number: 2021/29).

The authors assert that all procedures contributing to this study comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. A written informed consent form was signed by each participant.

2.2 | Sample and data collection

A total of 422 participants were included in this study. The inclusion criteria were being above 65 years old and being vaccinated with two doses of the SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Sciences Co. Ltd.). The blood samples were collected after 4 weeks from the first dose and 4 weeks from the second dose. Approximately 187 patients whose blood samples could not be obtained 4 weeks after the second dose of vaccination were excluded from the study. Finally, the study was completed with 235 participants.

Blood samples (approximately 6–7 ml) from the participants were taken into blood tubes with EDTA and centrifuged at 3500 rpm for 10 min. The serum samples were separated and stored at −80°C till the study day. The data about age, gender, comorbid diseases, and the presence of side effects after vaccination were obtained from the patients.

2.3 | ELISA for antibodies against SARS-CoV-2

An Anti-SARS-CoV-2 QuantiVac enzyme-linked immunosorbent assay (ELISA) (IgG) test kit (catalogue number: EI-2606-9601-10-G, Euroimmun) which applies a recombinant S1 subunit of the SARS-CoV-2 spike protein, enabling detection of IgG antibodies was used. Each kit contains a 96-well microplate with six break-off reagent wells coated with recombinant structural protein of SARS-CoV-2. All kit contents and 96-well ELISA microplates were brought to room temperature before the study. The first well was negative control, the second well was a positive control, and 3–8 wells were calibrators (1, 10, 20, 40, 80, and 120 RU/ml, respectively). 100 µl diluted samples (1:101) were added to each sample wells. After incubation (at 37°C, for 60 min) and washing with phosphate-buffered saline, a 100 µl enzyme conjugate (peroxidase-labeled anti-human IgG) was added to each well. After further washing protocol, 100 µl of chromogen/substrate solution was added and incubated at 37°C for 30 min under darkened conditions. Finally, a 100 µl stop solution was added to each well and the plate was read at a wavelength of 450 nm by a Multiskan™ GO UV/Vis microplate spectrophotometer (Thermo Fisher Scientific).

Results were evaluated by calculating a ratio of the OD of the samples over the OD of the calibrators ranging from 1 to 120 RU/ml. Quantitative results obtained in RU/ml were converted to International Units (IU/ml) by multiplying 3.2 in accordance with WHO specifications. If the ratio was under 25.6 IU/ml, it was considered as negative; if it was between 25.6 and 35.2 IU/ml, it was considered as borderline positive; and if it was above 35.2 IU/ml, it was considered as positive.

2.4 | Statistical analysis

The data were analyzed using the IBM SPSS version 21.0 statistical software (IBM). The "number (n)," "percentage (%)," "mean," "standard deviation (*SD*)," and minimum and maximum values were given for descriptive statistics. The independent samples *t*-test or Mann–Whitney U test was used to compare numerical variables. The continuous independent variables with parametric distributions were analyzed using the one-way analysis of variance or Kruskal–Wallis tests. All the p values were based on a two-sided test of statistical significance and significance was accepted at the level of $p < 0.05$.

TABLE 1 The descriptive statistics of all participants

	Number (*n*)	Percentage (%)
Gender		
Male	124	52.8
Female	111	47.2
Comorbid diseases		
At least one	120	51.1
No	115	48.9
Comorbid diseases		
DM	49	20.9
HT	58	24.7
Asthma	7	3.0
COPD	6	2.5
Side effects of vaccination		
At least one	42	17.9
No	193	82.1
Side effects of vaccination		
Headache	19	8.1
Pain at the injection site	8	3.4
Joint pain	2	0.9
Fatigue	2	0.9
Headache + joint pain	3	1.3
Headache + pain at the injection site	7	3.0
Fever	1	0.4

Abbreviations: Cardiac Dis., cardiac disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HT, hypertension.

3 | RESULTS

Out of the 235 participants, 124 (52.8%) were male and 111 (47.2%) were female. The average age of participants was 70.38 ± 4.76 (min: 65, max:85). 120 of 235 (51.1%) participants had at least one comorbid disease and 42 of 235 (17.9%) of those declared at least one side effect after vaccination (Table 1). The mean levels of anti-SARS-CoV-2 IgG antibody after 4 weeks from the first and second doses of vaccine were 37.70 ± 57.08 IU/ml (min: 0, max: 317.25), and 194.61 ± 174.88 IU/ml (min: 0, max: 677.82), respectively. There was no statistically significant difference between gender and mean antibody level ($Z = -0.993$, $p = 0.321$).

On the other hand, 134 of 235 participants (57.02%) had under 25.6 IU/ml antibody level and were evaluated as negative after the 4 weeks from the first vaccine dose while this rate was 11.48% ($n = 27$) after 4 weeks from the second vaccine dose.

TABLE 2 The statistical analyses of variables concerning mean antibody level

Variables			*F*/*Z*	*p*
Gender			−0.0993	0.321
At least one comorbid disease	X	No comorbid disease	2.352	**0.006**
Participants with DM	X	Participants without DM	−4.524	**0.000**
Participants with HT	X	Participants without HT	−0.042	0.067
Participants with asthma	X	Participants without asthma	−0.412	0.680
Participants with COPD	X	Participants without COPD	−0.257	0.797

Abbreviations: COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HT, hypertension.

Nineteen (70.4%) participants who had under had 25.6 IU/ml antibody level after 4 weeks from the first dose of vaccine had at least one comorbid disease including diabetes mellitus (DM), and 8 (29.6%) participants had no comorbid disease.

There was a significant difference between the mean antibody level of participants who had at least one comorbid disease and those who had not ($F = 2.352$, $p = 0.006$). The participants who had DM had lower antibody levels, and a significant difference was detected between the participants who had DM and who had not ($Z = -4.524$, $p = 0.000$) concerning mean antibody levels (Table 2).

4 | DISCUSSION

Quantitative determination of anti-SARS-CoV-2 antibodies is crucial for the estimation of the humoral response and may help the monitoring of antibody response in vaccinated individuals.[12] In this study, we aimed to detect anti-SARS-CoV-2 IgG antibodies in individuals who were above 65 years old. The mean age of participants was 70.38 ± 4.76. The mean values of anti-SARS-CoV-2 IgG antibody after 4 weeks from the first and second doses of vaccine were 37.70 ± 57.08 IU/ml (min: 0, max: 317.25), and 194.61 ± 174.88 IU/ml (min: 0, max: 677.82), respectively. Additionally, the rates of participants whose antibody levels were detected after 4 weeks from the first and second doses of vaccine were 42.98% and 88.52%, respectively.

Most vaccine studies are based on a strategy of inducing neutralizing antibodies that bind specifically to the spike protein of SARS-CoV-2 and prevent the virus from interacting with its target cell. Plaque reduction neutralization tests (PRNT) are the gold standard virological reference method for the detection of neutralizing antibodies specific to SARS-CoV-2.[13] Additionally, PRNT is also recommended by the World Health Organization (WHO) during the SARS-CoV-2 outbreak as an accepted confirmatory test for detecting

humoral response.[13,14] However, the incubation time of 5–7 days and the need for a biosafety level 3 (BSL3) laboratory are the major disadvantages of PRNT and make it difficult for routine testing.[15] At that point, it is necessary to find a fast, simple, and high accuracy test as an alternative to PRNT for detecting humoral response to SARS-CoV-2 antibody. For this study, we used an anti-SARS-CoV-2 QuantiVac ELISA (IgG) test kit (Euroimmun) (EI IgG ELISA) which uses a recombinant protein of the S1 domain as a target.

There are different kinds of supplementary serological tests that used different antigenic targets such as nucleocapsid (N) protein, S protein, S1 subunit of S protein, and RBD.[16] Those tests had high sensitivity rates (between 88.1% and 98.8%) when compared with PRNT. However, it has been reported by Patel et al.[17] that antibodies against N antigen do not have a neutralizing effect on SARS-CoV-2, as the N protein is in the envelope structure. Therefore, it is recommended that serological tests targeting surface structures such as S1 antigen and RBD should be preferred in cases where neutralization tests cannot be performed. In this study, we used the Euroimmun anti-SARS-CoV-2 ELISA test kit which targeting S1 surface protein. It was shown that this test kit had high sensitivity for detecting anti-SARS-CoV-2 antibodies with a rate of 97.8%.[17,18]

There are limited studies in the current literature about the antibody response rate in elderly people.[9,19–21] A study reported that the CoronaVac vaccine is immunogenic in adults aged above 60 years. The results showed that the neutralizing antibodies were low before the second vaccination; however, the seroconversion rates reached over 95% after the two-dose vaccination.[9] Additionally, Bayram et al.[12] reported that after the first dose, anti-spike antibodies were detected in 9 of 24 (37.5%), and after the second dose they were detected in 22 of 23 participants (95.7%). Another study reported a significant decline in vaccine effectiveness among individuals 70–74, 75–79, and more than or equal to 80 years of age.[20] These findings were similar to other studies performed in other countries such as Denmark, the USA, and Israel.[22–24] Data from 34 participants with an average age of 78.8 showed that 61.8% and 85.3% efficiency of SARS-CoV-2 vaccine with first and second doses, respectively.[21] Our study showed similarity in terms of SARS-CoV-2 vaccine efficiency. The rates of participants whose antibody levels were detected after 4 weeks from the first and second doses of vaccine were 42.98% and 88.52%, respectively.

People older than 60 years have an increased risk of severe illness and death from COVID-19, especially those with comorbidities. The immune response to infection or vaccination is usually reduced in older adults due to immune senescence.[25] Grupper et al.[25] claimed that age was an important factor in the humoral response induced after vaccination regardless of chronic medical conditions. Geisen et al.[26] also reported that significantly lower levels of anti-SARS-CoV-2 antibody were detected in patients with chronic inflammatory diseases even after two-dose vaccination.

In our study, there was a significant difference between the mean antibody level of participants who had at least one comorbid disease and those who did not ($p = 0.006$). Additionally, participants with DM had lower antibody response and a significant difference was detected between the participants who had DM and those who did not ($p = 0.000$). Ranzani et al.[20] reported that comorbidities could lead to lower antibody response ($p = 0.001$). They were also reported lower antibody responses in patients with DM, but not significantly different from those without DM. Another study pointed out that patients who had immune-related diseases including DM had significantly lower levels of antibody titers.[27] On the other hand, two studies from Italy revealed that the presence of DM did not affect the antibody response against the SARS-CoV-2 spike protein.[28,29]

5 | CONCLUSION

Although the use of serological tests instead of PRNT for confirmatory and diagnostic purposes has not been recommended yet, they can be used to determine the quantity of antibodies, especially in screening to understand the epidemiology of COVID-19. For this reason, it is very important to determine the performance of serological test kits that detect antibodies with different principles and put them into use for screening and diagnostic purposes for public health. Further studies for better understanding the efficiency of serological test kits should be performed.

On the other hand, lower rates of antibody response were detected in participants aged more than 65 years and those with comorbidities both in our study and similar studies in the current literature. Further studies should evaluate whether the low antibody titers are really associated with age and comorbidities or not. Finally, prospective studies are needed to determine how long the immunity provided by SARS-CoV-2 vaccines will continue.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included in the article.

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