SCIENCE

IgG-RBD Response Due to Inactivated SARS-CoV-2 Vaccine: Alteration in D-Dimer and Fibrinogen Concentrations, Association with Comorbidities and Adverse Effects

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Abbreviations: IgG-RBD, immunoglobulin G-receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization; COVID-19, coronavirus disease 2019; IHME, Institute for Health Metrics and Evaluation; S, spike; ACE2, angiotensin-converting enzyme 2; RBD, receptor-binding domain; RT-PCR, reverse transcription-polymerase chain reaction; COPD, chronic obstructive pulmonary disease; AU/mL, arbitrary units per milliliter; CLSI, Clinical and Laboratory Standards Institute.

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ABSTRACT

Objective: To examine the immunoglobulin G-receptor-binding domain (IgG-RBD) response and changes in fibrinogen and D-dimer concentrations in individuals with a past coronavirus infection and followed by CoronaVac.

Methods: The study consisted of a total of 116 participants. Blood samples were drawn from subjects 21–25 days after they received first and second doses of CoronaVac as well as from individuals with a past infection. Fibrinogen, D-dimer, and IgG-RBD concentrations were measured.

Results: The IgG concentrations of the vaccinated subjects were significantly higher ($P < .001$), fibrinogen levels were lower ($P < .001$), and D-dimer levels increased following the second vaccination compared with the first vaccination ($P = .083$). No difference was obtained in IgG-RBD between vaccinated and previously infected individuals ($P = .063$). The differences in fibrinogen and D-dimer were statistically nonsignificant between both groups.

Conclusion: The CoronaVac vaccine appears to be safe and effective. It is essential for individuals to take personal protective measures, such as using masks and distancing.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was accepted as a pandemic by the World Health Organization (WHO) on March 11, 2020, and has spread globally, presenting high morbidity and mortality. The clinical findings and severity of coronavirus disease 2019 (COVID-19) show variations from asymptomatic cases to mild-to-moderate and severe cases, and studies have demonstrated the relationship of disease severity with advanced age and underlying comorbidities.[1] However, severe infections may not be limited to risk groups: severe cases have also been seen in young people.[2]

According to the reports of the Institute for Health Metrics and Evaluation (IHME), the cumulative total COVID-19 death rate was reported as approximately 91.7 per 100,000 by May 2021, and an increased disparity has been reported in terms of mortality and morbidity rates among countries, and even subcommunities within countries, depending on the testing strategies, capacities, and healthcare policies of the countries.[3]

To date, no specific therapeutic approaches have emerged for COVID-19 infections beyond the preventive strategies, including widespread testing, distancing, and isolation; and thus, SARS-CoV-2 vaccination became the priority of global health services. With preventive strategies and vaccination of a sufficient number of people, individual protection is ensured, and consequently, herd immunity prevents the global spread of the virus and reduces morbidity and mortality.[4] Most vaccine studies were developed primarily against the SARS-CoV-2 spike (S) protein because of the increased T cell responses targeting the SARS-CoV-2 S protein on the viral membrane in patients with past SARS-CoV-2 infection.[5] The S protein consists of S1 and S2 subunits. The S1 region binds to angiotensin-converting enzyme 2 (ACE2) receptors on the host membrane by its receptor-binding domain (RBD); and the S2 region is responsible for virus-membrane fusion and facilitates viral entry.[6,7] The RBD of the S protein has been shown to be a primary target of neutralizing antibodies. In an experimental study, it was shown that the

RBD has a role in stimulating the neutralizing antibody response and protecting against SARS-CoV-2.[8]

Vaccines against SARS-CoV-2 are classified according to the different technologies in which they are developed: live attenuated vaccines, inactivated vaccines, soluble protein vaccines, viral vectors, nanoparticles, and DNA or RNA vaccines.[9,10] CoronaVac (Sinovac Life Sciences), is an inactivated vaccine that has been used in China, Brazil, and Turkey against SARS-CoV-2 during the pandemic. It is produced in African green monkey kidney cells (Vero E6), then chemically inactivated using β-propiolactone and formulated with a specific adjuvant, CpG oligonucleotide, and aluminum hydroxide.

mRNA vaccines BNT162b2 and mRNA-1273—Pfizer, BioNTech, and Moderna—were the first approved vaccines in the world that were developed by modifying RNA to code the SARS-CoV-2 S protein. Inversely, DNA vaccines were produced by cloning the S protein gene into bacterial plasmids.[11] Live-attenuated vaccines (SARS-CoV-2-VAC) (trial numbers NCT04619628 and MV-014–212) are developed by either using an avirulent strain of the virus or by creating a genetically weakened form of the virus and stimulating mucosal and cellular immunity without adjuvants.[12] However, different antibody kinetics have been demonstrated due to the variability in assay type—qualitative or quantitative manner—or target antigen.[13] In all these vaccines, neutralizing antibodies and Th1-driven CD4+T cell responses are essential for protective immunity against SARS-CoV-2.[14] However, an immunologic cutoff for the vaccines for protection against SARS-CoV-2 infection has not yet been clarified.[15]

SARS-CoV-2 infection activates the coagulation system together with immune responses associated with the severity of the disease.[16,17] Several studies have revealed similar or altered results for coagulation parameters during SARS-CoV-2 infection.[18] Therefore, we aimed to examine the IgG-RBD response and changes in fibrinogen and D-dimer concentrations in individuals who were previously infected with coronavirus or vaccinated following the first and second doses of inactivated CoronaVac vaccine. We also aimed to evaluate the relationship of the IgG response in terms of age, sex, comorbidities, and local or systemic adverse effects.

Materials and Methods

Subjects

This cross-sectional study was carried out between December 2020 and April 2021 by medical biochemists, laboratory technicians, phlebotomists, and administrative staff working in the central laboratory of Istanbul Medical Faculty who were vaccinated with CoronaVac vaccine in 2 doses, 5 μL per injection, 28 days apart, intramuscularly in the deltoid muscle. Blood samples were drawn from the subjects on days 21–25 after the first and second doses of vaccination (n = 91) and from individuals who had a previous coronavirus infection 14–21 days after the beginning of the symptoms, as a comparison group (n = 25). The diagnosis of COVID-19 infection was made using real-time reverse transcription polymerase chain reaction (RT-PCR) with nasopharyngeal and throat swabs using a SARS-CoV-2 Double Gene RT-qPCR kit (Bio-Speedy R&D Technologies).

For the evaluation of age and comorbidities related to the antibody response, all groups were classified according to their age groups: group 1, 20–40 years; group 2, 40–60 years; and group 3, ≥60 years. The comorbidities of the subjects were evaluated in 5 groups: hypertension and cardiovascular disease, diabetes mellitus, asthma/chronic obstructive pulmonary disease (COPD), hypo/hyperthyroidism, and other autoimmune diseases (eg, rheumatoid arthritis, Sjögren's disease). While immunocompetent individuals of both sexes were included in the study, the exclusion criteria included pregnancy, organ failures such as liver or kidney failure, and immune system deficiency. Written informed consent was obtained from each participant, and the clinical trial protocol was approved by the Ethical Committee of Istanbul University (#2021-26).

Methods

Blood samples were drawn into 3.2% sodium citrate tubes for fibrinogen, D-dimer, and serum separator tubes (BD Vacutainer) for IgG-RBD measurements. Tubes were centrifuged at 2000*g* for 15 minutes. Supernatant serum samples were aliquoted and stored frozen at −80°C until measuring of IgG-RBD concentrations. But, fibrinogen and D-dimer were measured in the separated plasma samples on days 21–25, after first and second dose of vaccination, or 14–21 days after a past infection within 2 hours on the same day.

IgG antibodies against the RBD of the S protein (SARS-CoV-2 RBD IgG) were quantitatively assessed by chemiluminescence immunoassay using Maglumi SARS-CoV-2-S-RBD-IgG kit (LOT#:270210111) on a Maglumi 2000 analyzer (SNIBE, Shenzhen New Industries Biomedical Engineering). In this assay, IgG test results of ≥1.10 arbitrary units per milliliter (AU/mL) are considered reactive. The reproducibility was between 5.5% and 6.2%.

Fibrinogen and D-dimer measurements were performed in accordance with the manufacturer's recommendations using a Sysmex CN 6000 coagulation analyzer with original reagents from Siemens (Sysmex). D-dimer measurements were made using the immunoturbidimetric method with the INNOVANCE D-dimer assay (LOT#:561598), and fibrinogen was measured using the Clauss clotting method with the Dade Thrombin reagent (LOT#:565102). Precision results were between 2.3% and 5.2% for fibrinogen and 2.5% and 6% for D-dimer. All precision studies for IgG-RBD, fibrinogen, and D-dimer were performed in our laboratory according to Clinical and Laboratory Standards Institute (CLSI) document EP5-A2.[19] Within-run precision was performed by repeatedly (n = 20) analyzing the manufacturer's 2-level controls, while the between-day precision was analyzed using the 2-level controls on 20 consecutive days.

The participants were asked about any adverse events during their visit for blood sampling, and they also filled out a questionnaire 7 days after vaccination, which questioned whether they experienced adverse effects, including both systemic and local effects, along with all relevant factors related to the participants. Systemic adverse effects were headache, fatigue, fever, diarrhea, arthralgia, myalgia, and nausea; and local adverse effects were local pain, swelling, tenderness, redness, warmth, and swollen lymph glands on the same side.

Statistical Evaluation

The data were analyzed using the SPSS 21 software package (SPSS). The results are expressed as median (Q1–Q3). The normality of the data distribution was evaluated using the Kolmogorov–Smirnov test. A χ^2 or Fisher's exact test were used to assess the differences in the categorical variables between the groups. Kruskal–Wallis test and Mann–Whitney *U* test were performed to compare the unpaired samples. Correlation

analyses were performed using Spearman's test. Statistical significance was defined as $P < .05$.

Results

The characteristics of the study population are presented in **TABLE 1**. The study consisted of a total of 116 participants, 63.8% female and 36.2% male. The average age was 44 (range, 21–83) years for women and 44 (range, 24–70) years for men. Twenty-five of 116 subjects had a previous infection (21.6%). The percentage of age groups was presented in **TABLE 2**. In terms of age, 39.7% of the participants were aged 20–40 years, 47.4% were aged 40–60 years, and 12.9% were aged ≥60 years.

The antibody concentrations of the vaccinated subjects were significantly higher after the second vaccination compared with that of the first vaccination (0.42 [0.18–3.11] vs 29.99 [9.43–95.50] AU/mL, [$P < .001$]). The fibrinogen concentrations were significantly lower (307 [264.3–356] vs 334 [279.6–375.4] mg/dL, [$P < .001$]); however, D-dimer levels were increased following the second vaccination compared with the first vaccination (250 [190–240] and 310 [200–430] µg/L, [$P = .083$]), respectively (**TABLE 1**).

Of the participants, 21.6% had a previous coronavirus infection. When the IgG concentrations of vaccinated individuals were compared with those of previously infected individuals, no statistically significant difference was obtained ($P = .063$). Also, the differences in fibrinogen and D-dimer concentrations were statistically nonsignificant between both groups.

When we evaluated IgG-RBD concentrations across the age groups (**TABLE 2**), the first and second IgG concentrations were the highest in the 20–40 year age group and were the lowest in the ≥60 year age group. The second IgG concentrations were higher in the 20–40 year age group compared with those of 40–60 year and ≥60 year age groups. The second IgG concentration of the 40–60 year age group was also higher than that of the ≥60 year age group, but the differences were statistically not significant. Within the age groups, antibody concentrations on the 25th day following the second dose were also higher compared with the first antibody concentration ($P = .001$ for the 20–40 year age group; $P < .001$ for 40–60 year age group; and $P = .028$ for the ≥60 year age group). Across the age groups, the fibrinogen levels also showed statistically significant alterations for all age groups (40–60 years $P = .009$ and $P = .011$ for ≥60 years).

The local and general adverse effects are presented in **FIGURE 1**. Fifteen percent of the subjects had systemic adverse effects, and 15% had local adverse effects after the second dose. After the first vaccination, no statistically significant differences were obtained in the IgG, D-dimer, and fibrinogen concentrations. However, after the second vaccination, statistically significant changes were obtained only in D-dimer levels in subjects with local and general adverse effects compared with the subjects with no adverse effects ($P = .017$, for both), but the IgG and fibrinogen concentrations were higher in subjects with no adverse effects.

We also evaluated the relationship of comorbidities in individuals with IgG-RBD levels. Of the entire group, 12.1% had hypertension/cardiovascular disease, 4.4% had diabetes mellitus, 5.5% had hypo/hyperthyroidism, and 3.3% had asthma/COPD. Only D-dimer concentrations were significantly higher in the subjects with any of the chronic diseases, (290 µg/L vs 375 µg/L; $P = .020$) compared with the healthy subjects. Also, in the subjects with hypertension/cardiovascular diseases, the median IgG concentrations were lower than those of healthy subjects

TABLE 1. Anti S-RBD IgG Antibody, D-Dimer, and Fibrinogen Concentrations at 21–25 Days Following First and Second Dose of SARS-CoV-2 Vaccination (CoronaVac)

Status	Age, y (Median [Q1-Q3])	Anti S-IgG-RBD (AU/mL)			Fibrinogen (mg/dL)			D-Dimer (µg/L)		
		After First Dose of Vaccination	After Second Dose of Vaccination	*P*	After First Dose of Vaccination	After Second Dose of Vaccination	*P*	After First Dose of Vaccination	After Second Dose of Vaccination	*P*
Vaccinated (n = 91)	44 (34–56)	0.42 (0.18–3.11)	29.99 (9.43–95.50)	<.001[a]	334.4 (279.6–375.4)	307 (264.3–356)	<.001[a]	250 (190–420)	310 (200–430)	.083
COVID-19 inf (n = 25)	40 (36–46)	-	13.26 (6.857–50.38)		-	260 (238.9–278)		-	190 (190–270)	
All groups (n = 116)	44 (35–52)	1.02 (0.24–7.67)	31.46 (10.2–99.63)		327.9 (279.6–375.4)	302.8 (264–353.3)		245 (190–405)	310 (200–430)	
Women (n = 61)	44 (34–51)	0.54 (0.21–4.75)	37.35 (10.28–111.3)	<.001[a]	335.1 (298.6–390)	312.4 (279–364.6)	.003[a] .029[b]	270 (190–440)	330 (250–440)	.169 .016[b]
Men (n = 30)	44 (36–55)	0.39 (0.17–1.01)	21.8 (8.66–58.83)	<.001[a]	312 (253.7–370.5)	279.5 (249.1–334.2)	.015[a]	195 (190–325)	220 (190–355)	.408

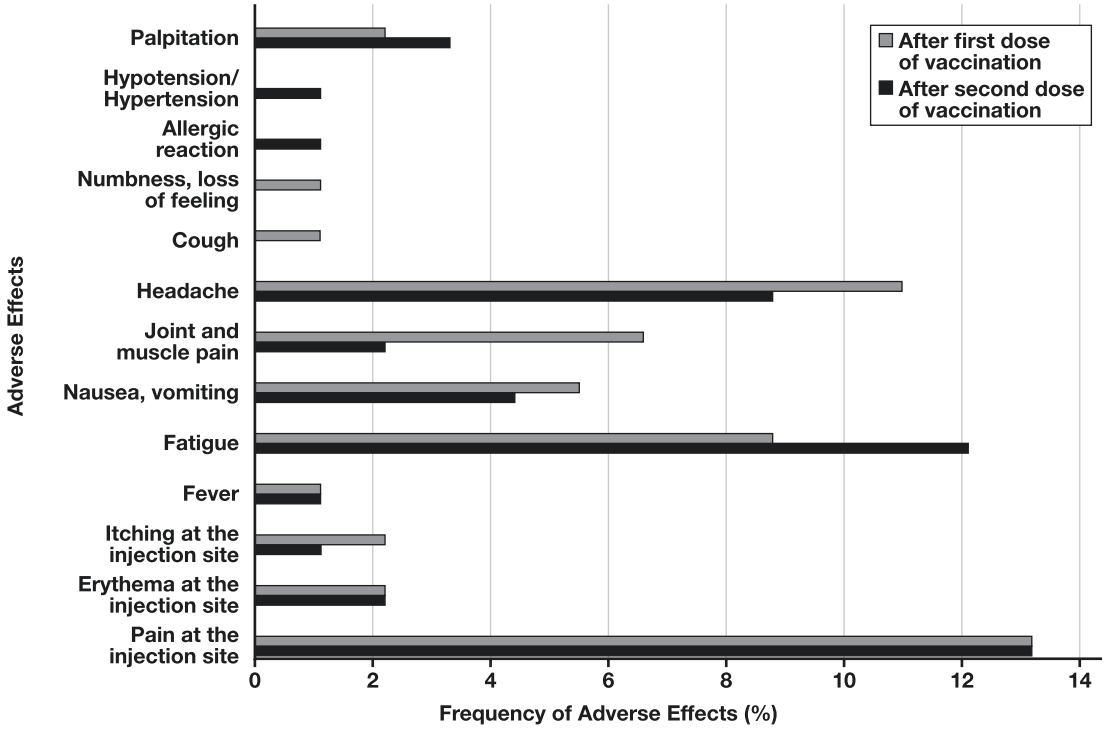
[a]*In comparison with vaccination doses;* P < *.05.*
[b]*In comparison with men after second dose of vaccination;* P < *.05.*

TABLE 2. Anti S-RBD IgG Antibody, Fibrinogen, and D-Dimer Levels Across the Age Groups and Following SARS-CoV-2 First and Second Vaccinations

Age Groups (y)	Anti S-RBD IgG (AU/mL)			Fibrinogen (mg/dL)			D-Dimer (μg/L)		
	After First Dose of Vaccination	After Second Dose of Vaccination	*P*	After First Dose of Vaccination	After Second Dose of Vaccination	*P*	After First Dose of Vaccination	After Second Dose of Vaccination	*P*
20–40 (n = 33)	0.39 (0.17–4.46)	46.11 (16.05–186)	<.001[a]	290.5 (260.0–381.2)	302.8 (264.0–372.0)	.109	195 (190–340)	280 (190–360)	.570
40–60 (n = 43)	0.53 (0.20–5.16)	32.02 (10.45–92.24)	<.001[a]	335.2 (310.0–375.4)	309.9 (278–353.3)	.011[a]	275 (190–430)	310 (200–400)	.290
>60 (n = 15)	0.33 (0.11–0.66)	13.15 (7.21–25.85)	.005[a]	364.7 (290.4–394.1)	293.3 (251.8–353.3)	.022[a]	310 (200–440)	435 (270–690)	.046[a]

[a]*In comparison with vaccination doses;* P < *.05.*

FIGURE 1. The percentages of local and systemic adverse effects following the first and second administration of CoronaVac vaccine in 91 subjects.



following the second dose of vaccine (34.9 AU/mL vs 12.1 AU/mL, *P* = .006); and a 58.1% increase was detected in D-dimer concentrations after the second dose. The percentage of participants with IgG concentrations higher than 1 AU/mL and 5 AU/mL after the first vaccination was almost the same as the normotensive patients. After the second vaccination, these percentages were significantly different compared with those with no hypertension (63.6% vs 90.4%; *P* = .034 and *P* = .031, respectively). In patients with diabetes mellitus, hypo/hyperthyroidism, or asthma, no statistically significant changes were obtained in IgG, fibrinogen, and D-dimer concentrations compared with healthy subjects.

Also, weak association was found between age and IgG (*P* = .021, *r* = −0.252), with D-dimer (*P* = .020, *r* = 0.255) following the second vaccination using Spearman's test.

Discussion

In this study, we evaluated the IgG antibody concentrations against the RBD of SARS-CoV-2 S protein using chemiluminescence assays following the first and second dose of CoronaVac. IgG-RBD concentrations on the 25th day of the second vaccination were significantly higher than the first vaccination. IgG responses against SARS-CoV-2 after vaccination may vary significantly depending on age, sex, previous COVID-19 infection, and the health conditions of the individual.[20,21] The IgG concentrations of vaccinated individuals did not differ significantly when compared with previously infected individuals. However, there are studies revealing stronger T cell responses and higher antibody concentrations in subjects with known past SARS-CoV-2 infections compared with vaccinated subjects using SARS-CoV-2 IgG and IgG Quant II, contrary to our findings.[22] While IgG-RBD concentrations did not increase in 13% of the subjects in our study group after 2 doses of CoronaVac vaccine, this rate was 5% in the study of Muena et al.[23] In another randomized, placebo-controlled, phase 3 clinical trial study conducted in Turkey, it was reported that the percentage of seronegativity increased from 5% to 14% over the age of 50, and seropositive cases decreased with age.[24]

We also found the highest IgG-RBD concentrations in the 20–40 year age group and the lowest in the ≥60 year age group, in accordance with

other studies.[20,23,24] The IgG levels of the younger group were significantly higher compared with the 40–60 and ≥60 year age groups. Despite the pathogenesis, immunosenescence is multifactorial; the decreased ability to respond to antigens due to reduced plasmablasts and lower memory T cell function are the main reasons for the low antibody levels, decreased response to vaccines, and increased susceptibility to infectious diseases.[25] In another study performed with 2 doses of CoronaVac vaccine, the association between neutralizing antibody levels and antireceptor binding of IgG has been shown in subjects aged 18–59 years; however, there is insufficient evidence for immunosenescence in subjects aged ≥60 years.[26]

Several studies have demonstrated the role of T cells in the stimulation of the immune system and the association of CD4+CD25+T cells with the IgG concentration against SARS-CoV-2.[27,28] Supporting these results, Goel et al[29] showed stimulation of preexisting memory cells specific to the SARS-CoV-2 antigen after the first dose of vaccine, in subjects who recovered from the coronavirus infection, and an increase in antibody and memory cells in vaccinated subjects after 2 doses of vaccine. They also showed diminished memory cell responses with age, similar to our findings. Consequently, Muena et al[23] also indicated similar findings for neutralizing antibodies, showing that neutralizing antibody levels in naive subjects who received 2 doses of CoronaVac or 1 dose of BNT162b2 vaccine were similar to those of individuals with past coronavirus infection who were boosted with either CoronaVac or BNT162b2 vaccine.

In our study, we also investigated the fibrinogen and D-dimer concentrations following the first and second doses of the vaccine. In all groups, the fibrinogen concentrations decreased whereas D-dimer levels remained unchanged after the second vaccination in the 40–60 and ≥60 year age groups. Additionally, no statistically significant differences in fibrinogen and D-dimer concentrations were found across the age groups or between the subjects vaccinated and individuals with known past SARS-CoV-2 infections. Peyvandi et al[18] also detected no statistically significant changes in D-dimer concentration or other coagulation parameters other than mild thrombocytopenia after administration of the first and second doses of BioNTech.

When we evaluated the general and local adverse effects together with antibody responses, no differences in the IgG-RBD concentrations were obtained between the groups. Twelve subjects in the entire group had local reactions, including pain and swelling, whereas 15 subjects had systemic reactions after the second-dose vaccination. However, systemic reactions were observed in 20 of 91 subjects, and local adverse effects were seen in 12 of 91 subjects after the first dose. The most common adverse effects were pain at the injection site, making up 13% of local effects, and headache and vertigo, accounting for 10% of systemic effects. Systemic adverse effects were approximately 2 times more common than local effects for the 2 doses of vaccine. Zhang et al[26] reported that the most common adverse effect was local pain at injection site with a rate of 17% with CoronaVac, and the most severe adverse effect was observed as an acute hypersensitivity with urticaria with a rate of 4%. On the other hand, Tanriover et al[24] also reported that 0.1% of their study group had serious adverse effect including allergy and seizures. However, in our study no serious complications, including anaphylaxis or severe allergic reactions, were detected—except hypertensive attack, which was seen in 1 subject out of 91. Therefore, increased D-dimer concentrations were observed in subjects with systemic adverse effects compared with the noncomplicated subjects, while higher IgG and fibrinogen concentrations were obtained in noncomplicated subjects. In another study investigating the prevalence of side effects following CoronaVac, it was reported that the risk of experiencing side effects was higher in women than in men, in individuals younger than 30 years of age compared to older people, and that subjects with chronic diseases have a higher risk than those without chronic diseases.[30] Nevertheless, more severe complications, such as the development of immune thrombocytopenia, bleeding with no thrombosis, or thrombosis, were reported following the administration of mRNA vaccines.[31]

When we evaluated the variables affecting the IgG-RBD concentrations after the second dose of CoronaVac, age and hypertension in comorbidities were found associated with the IgG-RBD responses. Twelve percent of the entire study population had hypertension, and the IgG-RBD concentrations of the participants with hypertension were significantly lower compared with the normotensive subjects following the second vaccination. IgG concentration of 1.0 AU/mL and above was found in 63% of people with hypertension, while this rate was found to be 90.4% in normotensive individuals. However, no statistically significant associations were obtained among IgG responses with diabetes mellitus, asthma/COPD, or hypo/hyperthyroidism.

According to the recommendations of the Advisory Committee on Immunization Practices and WHO, the benefits of the COVID-19 vaccine are greater than the risk of progression to serious conditions or death when comparing healthy individuals with those with comorbidities.

In this study, we did not measure neutralizing antibody levels, which determines the ability to bind and inhibit the entrance of the host cells. Therefore, the limitations of the study are the lack of evaluation of neutralizing antibody levels and T cell responses following vaccines, the limited study group, and the lack of other validation studies such as carry-over and linearity but not precision.

Conclusion

Based on our results, the CoronaVac vaccine was safe and showed good efficacy against SARS-CoV-2 infection. However, the protective efficacy and duration of efficacy remain controversial for CoronaVac. Accordingly, it is essential for individuals to take personal protective measures, such as using masks and distancing, despite undergoing vaccination.

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