Original article

CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Antibody response to COVID-19 vaccination in patients on chronic hemodialysis

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Purpose: Since patients on hemodialysis (HD) are known to be vulnerable to coronavirus disease 2019 (COVID-19), many studies were conducted regarding the effectiveness of the COVID-19 vaccine in HD patients in Western countries. Here, we assessed antibody response of HD patients for 6 months post-vaccination to identify the duration and effectiveness of the COVID-19 vaccine in the Asian population.

Materials and Methods: We compared antibody response of the COVID-19 vaccine in HD patients with healthy volunteers. Patient and control groups had two doses of ChAdOx1 nCoV-19 and mRNA-1273, respectively. Immunoglobulin G (IgG) was measured before vaccination, 2 weeks after the first dose, 2 and 4 weeks, 3 and 6 months after the second dose. Neutralizing antibody was measured before vaccination and at 2 weeks, 3 and 6 months after second dose. Since the third dose was started in the middle of the study, we analyzed the effect of the third dose as well.

Results: Although antibody production was weaker than the control group (n=22), the patient group (n=39) showed an increase in IgG and neutralizing antibody after two doses. And, 21/39 patients and 14/22 participants had a third dose (BNT162b2 or mRNA-1273 in the patient group, mRNA-1273 in the control group), and it did not affect antibody response in both group. Trend analysis showed IgG and neutralizing antibody did not decrease over time. Age, sex, and HD vintage did not affect antibody production in HD patients. Patients with higher body mass index displayed better seroresponse, while those on immunosuppressants showed poor seroresponse.

Conclusion: Two doses of vaccination led to significant antibody response in HD patients, and the antibody did not wane until 6 months.

Keywords: Antibody formation, COVID-19, COVID-19 vaccines, Neutralizing antibodies, Renal dialysis

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, which began in 2019, has infected more than 29,955,000 people and caused more than 33,100 deaths in South Korea by 20 January 2023 [1]. Vaccines were developed rapidly to provide protection from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In South Korea, COVID-19 vaccination was initiated in February 2021.

Patients who require chronic hemodialysis (HD) regularly are known to be vulnerable to COVID-19, with higher rates of hospitalization and mortality than the general

population [2]. According to one Korean study, HD status was an independent risk factor for in-hospital death, leading to a two-fold higher risk for death compared to that of the non-chronic kidney disease population [3]. Thus, when the vaccination against SARS-CoV-2 was introduced, priority was given to patients with chronic HD. Since these patients are known to display a weak response to other vaccines like hepatitis B virus [4], there were certain concerns regarding the antibody production against SARS-CoV-2 after vaccination, which might be poor. However, the SARS-CoV-2 vaccine administered to HD patients reportedly showed an antibody production rate of 80%–95% or more, although it was slightly lower and the response was slower than that for the general population [5-7]. Moreover, vaccination lowered the hospitalization rate of HD patients, suggesting that the SARS-CoV-2 infection did not progress to a more serious illness [8,9]. Recent studies have focused on assessing the duration of the effect of vaccination, where vaccine-induced antibodies have been shown to wane over time in HD patients, highlighting the need for an additional vaccination dose [10].

Studies regarding the effectiveness and duration of SARS-CoV-2 vaccination in HD patients have been carried out in Western countries. Therefore, we aimed to determine whether a similar trend is observed in South Korea, East Asia by measuring immunoglobulin G (IgG) levels in HD patients up to 6 months post-vaccination. Moreover, this study included the estimation of neutralizing antibody, a key factor in preventing viral infection, to assess the protective effect of vaccination. Thus, by determining antibody response to SARS-CoV-2 vaccination in patients with chronic HD, we believe that this study would be helpful for future vaccine administration strategies in HD patients.

Materials and Methods

Study population and design

This prospective observational study included adult patients with chronic HD at Ajou University Hospital, the tertiary medical center in South Korea. Patients who underwent HD at least twice a week, which started before SARS-CoV-2 vaccination were included in this study. To compare the seroresponse of end-stage renal disease patients with that of a healthy population, we recruited healthy adults without renal disease from February to September 2021. Individuals with previous SARS-CoV-2 infection or those known to have acquired the infection during this study period were excluded.

At the start of the vaccination, two doses were planned. According to the vaccination schedule of each participant, blood samples were collected before vaccination, 2 weeks after the first dose, 2 and 4 weeks, and 3 and 6 months after the second dose. Titers of IgG were measured in all blood samples. Further, the neutralizing antibody levels were estimated before vaccination and 2 weeks, 3 months, and 6 months after the second dose. The process of this study is described in (Fig. 1).

During the study period, researchers or patients could not choose the vaccine administration interval and vaccine type in Korea, with the government's policy being followed thoroughly. Due to the unavailability of enough vaccines worldwide, those purchased by the government differed from time to time. Therefore, the vaccine type administered differed according to the time of vaccine administration and the patient's area of residence. In the midst of these conditions, this study selected the study subjects with as much homogeneity as possible. Therefore, we included participants who received the highest number of vaccines twice in each group.

When the vaccination was underway, two doses of vaccine administration were expected; so, we designed the experimental timeline to measure antibodies at 3 and 6 months after the second dose. However, since many variants of SARS-CoV-2 emerged and the pandemic continues to infect people, a third dose of vaccination was recommended. Consequently, many of the study participants received a third dose within 6 months of the second dose. The time interval between the third dose and blood sampling 6 months after the second dose varied among participants, ranging from 1 week to over 2 months. Considering the effect of the third dose on antibody response at 6 months after the second dose, we divided the patients into groups 1 and 2 based on those who did not

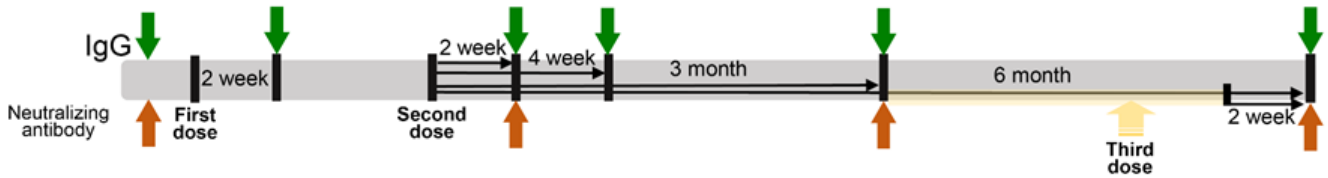

Fig. 1. The study timeline. IgG, immunoglobulin G.

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receive and those who received the third dose. Since it has been known that it takes 1–2 weeks following COVID-19 vaccination to build immunity [11], the third dose of vaccination which was given within 2 weeks before 6 months after the second dose was considered as not to have been received. Likewise, the control group was divided into groups 3 and 4, where participants who did not receive the third dose were included in group 3 and those who received were in group 4.

Demographic and clinical data of each patient such as sex, age, body mass index (BMI), duration of HD, comorbidities, and course of any immunosuppressive drug was obtained through health records. Subgroup analyses were carried out to determine whether each variable has a relationship with seroresponse of SARS-CoV-2 vaccination.

IgG response to SARS-CoV-2 spike-receptor-binding domain protein

The antibody titer was estimated using enzyme-linked immunosorbent assay (ELISA) according to a previous paper [12]. Briefly, 100 μL of SARS-CoV-2 spike-receptor-binding domain (RBD) protein (adjusted as 0.1 μg/mL; purchased from AIVD Biotech Inc., Shenzhen, China) was added to a 96-well immune plate (Thermo Fisher Scientific, Roskilde, Denmark). The sera of HD patients, infected patients, and healthy, unvaccinated individuals (each diluted 1:100 with phosphate-buffered saline) were applied as test samples, a positive, and negative control, respectively. After final incubation, the goat anti-human whole IgG and immunoglobulin M (1:5,000 dilution) conjugated with alkaline phosphate in a substrate buffer containing 20 mg of p-nitrophenyl phosphate tablet (Sigma-Aldrich, St. Louis, MO, USA) was added. The absorbance was measured at 405 nm using an ELISA reader (EPOCH2; BioTek, Santa Clara, CA, USA).

Neutralizing ability of SARS-CoV-2 spike-RBD antibodies assessed using the neutralizing assay

The binding inhibition capacity of patient serum samples was detected using a fluorescence-based competitive SARS-CoV-2 neutralizing assay (GenBody FIA COVID-19 NAb; GenBody, Cheonan, Korea), which has been proven to show consistent results with plaque reduction neutralization tests and SARS-CoV-2 Surrogate Virus Neutralization Test Kit (GenScript, Piscataway, NJ, USA; US Food and Drug Administration approved) [13]. To confirm the accuracy of the SARS-CoV-2 neutralizing assay (GenBody) in this study, we also used the SARS-CoV-2 surrogate virus neutralization test kit (GenScript) to detect neutralizing antibody at 2 weeks after the second dose. No difference was observed in the neutralizing antibody between the two assays, hence only the neutralizing assay from GenBody was used for further experiments.

First, the recombinant human angiotensin-converting enzyme-2 (hACE-2) protein was immobilized on the test line of the device, and the recombinant spike protein of SARS-CoV-2 that could bind to hACE was conjugated with a fluorescent dye. When the fluorescent conjugates reacted with the sample, the mixture migrated in the membrane by capillary motion. If the neutralizing antibodies were not present, they could not interfere with the reaction of the recombinant spike-RBD protein and the recombinant hACE-2, so they were bound to the test line, and the fluorescence was detected. In contrast, when the neutralizing antibodies were present, the neutralizing antibodies reacted with the recombinant spike-RBD protein ("blocked") and the fluorescent conjugate could not bind to the test line, thus the signal was reduced or not detected. The reduced signal and neutralizing ability were analyzed using a special analyzer (Confiscope F20; GenBody). The sera of HD patients that were examined using ELISA were applied on the GenBody FIA COVID-19 NAb assay according to the manufacturer's instructions, where neutralizing antibody higher than 30% has a protective effect against SARS-CoV-2.

Statistical analysis

A generalized estimating equation model was used to compare antibody responses between the control and the patient groups. For the patient group, antibody responses were further categorized and compared according to their age, sex, BMI, HD vintage, as well as consumption of immunosuppressive agents. Moreover, a trend analysis was performed to test whether the antibody response has a linear trend over time. For all results, a p-value <0.05 was considered statistically significant. Missing data was ignored, and all the statistical analyses were performed using R software ver. 4.1.2 (The R Foundation for Statistical Computing, Vienna, Austria; 2021).

Ethical statement

This study followed the Declaration of Helsinki, and written informed consent was obtained from all participants. Approval was obtained by the Institutional Review Board of Ajou University Hospital (IRB approval no., AJIRB-BMR-SMP-21-156).

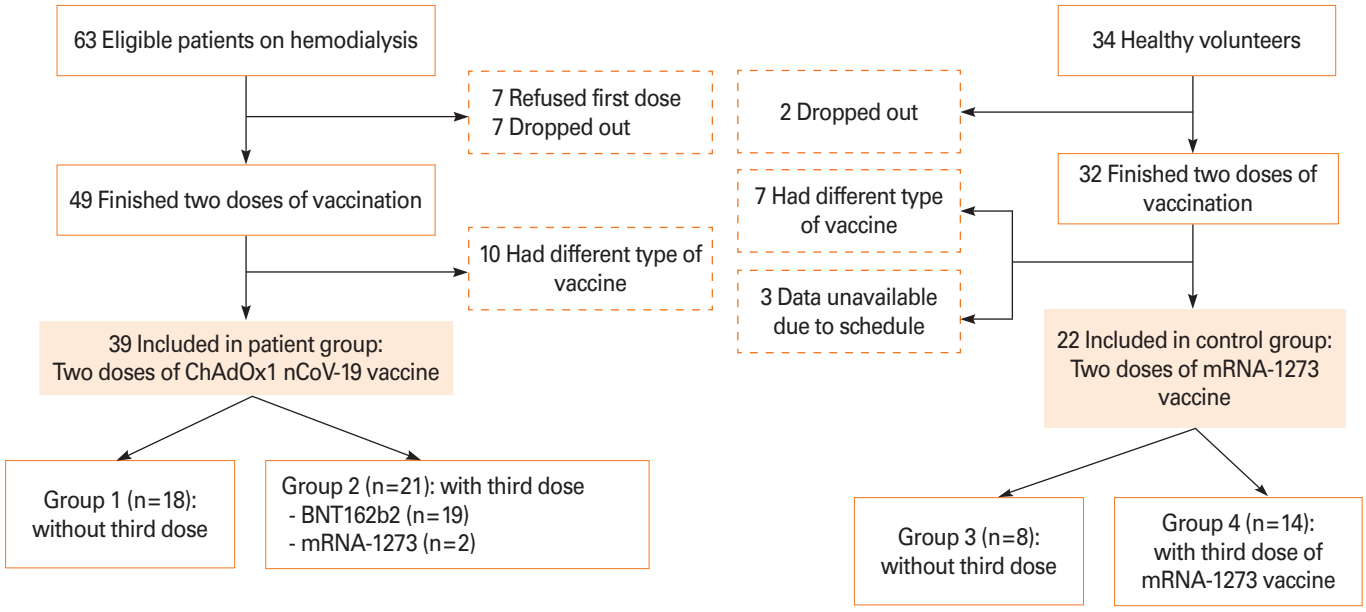

Fig. 2. Selection of study participants.

Results

Study population

Sixty-three patients with chronic HD in Ajou University Hospital were eligible for this study (Fig. 2), of which seven patients refused vaccination due to concerns about adverse effects even though they initially agreed to enroll in this study. Seven other patients were dropped out in the middle of the study period because of SARS-CoV-2 infection (n=1), kidney transplantation (n=1), death for reasons unrelated to SARS-CoV-2 (n=1), transfer (n=1), and refusal of the second dose of vaccination (n=3). Patients had various types of vaccines, and for homogeneity, we included only those vaccinated with the highest number of patients twice. The vaccine type was the ChAdOx1 nCoV-19 vaccine (AstraZeneca, Cambridge, UK). As a result, 39 patients with chronic HD were enrolled.

For the control group, 34 healthy individuals volunteered to participate in this study, of which two volunteers were excluded for personal reasons, and data of three volunteers were not available yet due to their vaccination schedule. Likewise, those who received the highest number of volunteers twice were included. In the control group, the vaccine type was mRNA-1273 (Moderna, Cambridge, MA, USA) and 22 healthy volunteers were finally enrolled.

In the patient group, 18 participants refused to take a third dose of vaccination and they were included in group 1. The remaining 21 patients were classified as group 2 with vaccination of the third dose. Similar to the first and second doses, researchers and patients could not choose the vaccine type. Thus, 19/21 (90.5%) of group 2 had BNT162b2 (Pfizer, New York, NY, USA) and 2/21 (9.5%) had mRNA-1273 vaccine as third dose. In the control group, eight participants were included in group 3 without a third dose, and 14 participants were included in group 4. All group 4 participants had the mRNA-1273 vaccine as the third dose.

The baseline characteristics of the patient and control group are shown in Table 1. Age of the patient group ranged from 35–71 years, with a mean±standard deviation (SD) of 56.4±9.6 years, where 53.8% of them were women with a mean BMI 22.5±3.7 kg/m^2 and 38.5% of participants were overweight (≥23 kg/m^2). The mean duration of HD in the patient group was 70±62.2 months. In the control group, the mean age was 23.9±1.4 years, much younger than the patient group, where 36.4% were women. Mean BMI was 21.0±3.0 kg/m^2 and 40.9% of the group was overweight. While HD patients had many comorbidities, such as diabetes mellitus, hypertension, coronary artery occlusive disease, and cancer, no comorbidities were observed in the control group. Four patients (10.35%) in the patient group were on immunosuppressive drugs like steroids or tacrolimus due to solid organ transplantation.

In the patient group, one patient did not have a blood sample before vaccination, and the other patient did not have a blood sample at 6 months after the second dose, both of them due to personal reasons. In the control group, two participants did not take blood samples at 2 weeks after the second dose, because they were isolated due to contact with

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Table 1. Baseline characteristics of patient and control group

Characteristic	Patient group (n=39)	Control group (n=22)
Age (yr)		
<60	21 (53.8)	22 (100.0)
≥60	18 (46.2)	0
Sex		
Female	21 (53.8)	8 (36.4)
Male	18 (46.2)	14 (63.6)
Body mass index (kg/m^2)		
<23	24 (61.5)	13 (59.1)
≥23	15 (38.5)	9 (40.9)
Comorbidity		
Diabetes mellitus	17 (43.6)	0
Hypertension	28 (71.8)	0
Solid organ transplantation transplantation	8 (20.5)	0
Coronary artery occlusive disease	7 (17.9)	0
Cancer	6 (15.4)	0
Hemodialysis duration (yr)		
<5	19 (48.1)	0
≥5	20 (51.3)	0
Immunosuppressant		
Yes	4 (10.3)	0
No	35 (89.7)	22 (100.0)
Third dose of vaccination		
No	18 (46.2)	8 (36.4)
Yes	21 (53.8)	14 (63.6)

Values are presented as number (%).

confirmed COVID-19 cases.

IgG response to SARS-CoV-2 spike-RBD protein

In the patient group, baseline IgG was 0.412±0.499 optical density (OD), and it increased after the first dose of vaccination to 0.493±0.572 OD, but the change was marginal compared to that in the control group. IgG increased to 1.993±0.95 OD at 3 months after second dose of vaccination (1.596±0.770 OD at 2 weeks and 1.738±0.697 OD at 4 weeks after second dose). At 6 months after second dose, both group 1 and 2 patients showed a decrease in IgG levels, and the difference between groups 1 and 2 was not statistically significant (p=0.2973) (Fig. 3A).

In the control group, IgG against SARS-CoV-2 spike-RBD protein increased after the first dose of vaccination (from 0.705±0.345 OD before vaccination to 1.896±0.283 OD at 2 weeks after first dose), and further increased to 2.297±0.503 OD at 2 weeks after the second dose. However, IgG declined at 4 weeks after second dose of vaccination (1.846±0.562 OD). It recovered at 3 months after second dose to 3.176±0.347 OD, but both group 3 and group 4 showed decrease of IgG again at 6 months after second dose. As with group 1 and 2, the difference between group 3 and 4 was not statistically significant (p=0.6642). Overall, IgG of control group was higher than that of patient group at each time point, with this difference between patient group and control group being statistically significant (p<0.001).

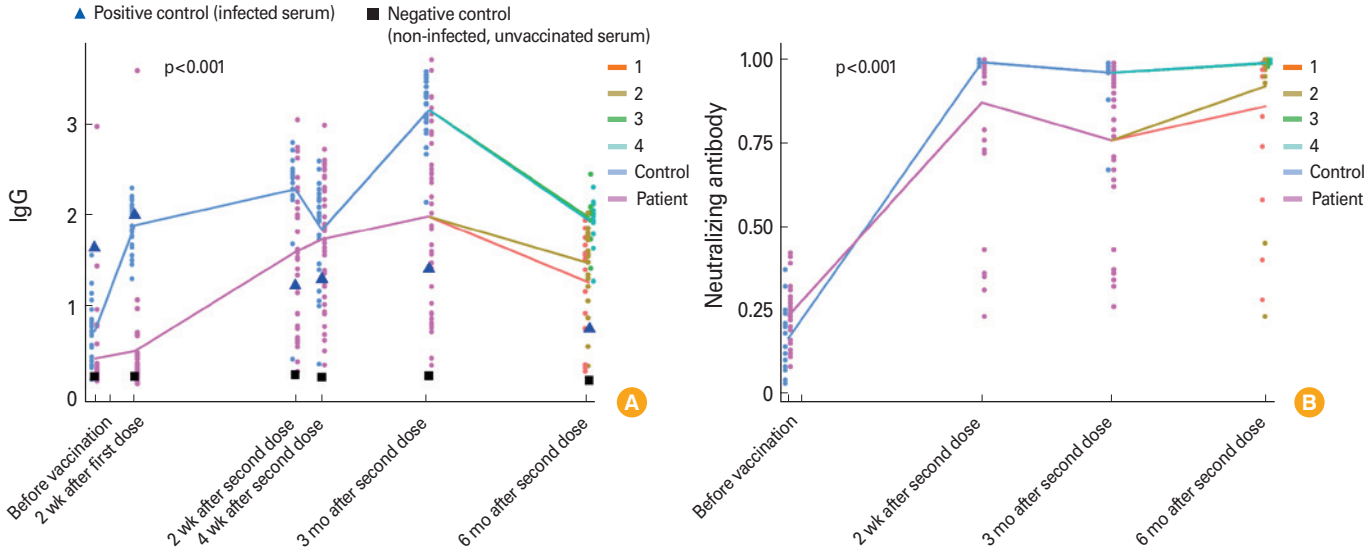

Fig. 3. Changes in the immunoglobulin G (IgG) and neutralizing antibody after vaccination in the patient and the control group. Group 1 (red), patient group without third dose; Group 2 (yellow), patient group with third dose; Group 3 (green), control group without third dose; Group 4 (turquoise), control group with third dose; negative control (black square, unvaccinated and non-infected serum); positive control (blue arrowhead, serum of coronavirus disease 2019 infected patients). **(A)** Trend of IgG after vaccination and **(B)** trend of neutralizing antibody after vaccination.

Even though the control group showed decrease in IgG at 4 weeks after second dose and all four groups showed decrease in IgG at 6 months after second dose, the IgG levels showed a positive linear trend with time in both the patient and control group ($p<0.0001$).

Compared to the negative control (non-infected, unvaccinated serum) and positive control (SARS-CoV-2 infected serum), IgG in both the patient and control group were higher than that in the negative control at each time point, with some participants showing even higher IgG levels than those in the positive control after vaccination.

Neutralizing ability of SARS-CoV-2 spike-RBD antibodies by neutralizing assay

Neutralizing antibody against SARS-CoV2 spike-RBD protein showed similar pattern in both patient and control groups. It increased rapidly at 2 weeks after two vaccination doses, but decreased at 3 months after the second dose (Fig. 3B), and increased again at 6 months after second dose.

In the patient group, mean value±SD of neutralizing antibody before vaccination was 23%±8%, and it increased to 87%±22% at 2 weeks after second dose. Mean value of neutralizing antibody was 76%±25% at 3 months after second dose. At 6 months after second dose, mean value of the neutralizing antibody in group 1 was 86%, and that in group 2 was 92%, but the difference between them was not significant ($p=0.2953$). One patient had neutralizing antibody lower than 30% throughout the whole study period, while that in another patient dropped below 30% at 6 months after second dose. Rest of the group showed neutralizing antibody greater than 30% throughout the study period, which means they have gained protective effect from the vaccine.

In the control group, mean value of neutralizing antibody before vaccination was 16%±9%, and it increased to 99%±1% at 2 weeks after second dose. All of the samples displayed 99%–100% results for neutralizing antibody at 2 weeks after the second dose. At 3 months after the second dose, the mean value was 96%±7%, and it increased to 99%±0% at 6 months after the second dose in both groups 3 and 4. These groups showed no significant difference in neutralizing antibody at 6 months after the second dose ($p=0.8158$). None of the participants in the control group showed less than 30% of neutralizing antibody after two doses of vaccination, which means all of the control group have gained protective effects from vaccination. Neutralizing antibody was lower in the patient group than in the control group at all times ($p<0.001$).

Both the patient and control group showed a decrease in the neutralizing antibody at 3 months after the second dose. However, the trend analysis of neutralizing antibodies in both groups showed a positive linear trend with time ($p<0.0001$).

The HD patient whose neutralizing antibody did not increase over 30% during the whole study period (29% before vaccination, 23% at 2 weeks after the second dose, 26% at 3 months after the second dose, 23% at 6 months after the second dose, with the third dose of vaccination) was taking immunosuppressive drugs (tacrolimus 8 mg, prednisolone 5 mg daily) because of pancreas and kidney transplantation. We think that this might have affected the antibody production post-vaccination. Another patient whose neutralizing antibody dropped below 30% at 6 months after the second dose (20% before vaccination, 93% at 2 weeks after the second dose, 36% at 3 months after the second dose, 28% at 6 months after the second dose, without a third dose of vaccination) was not taking immunosuppressive drugs. We could not find the reason for the rapid decrease in the neutralizing antibody in this case.

Factors associated with seroresponse to SARS-CoV-2 vaccine in chronic HD patients

To investigate the factors affecting the antibody production after vaccine administration in HD patients, we divided patient group based on age, sex, BMI, HD vintage, and consumption of immunosuppressive agents. Because of small population size, we did not divide them based on whether patients had third dose of vaccination or not.

There was no difference in both IgG levels and neutralizing antibody formation after vaccination according to age and sex, and this result correlates with previous findings (Fig. 4A–D) [14]. Considering BMI, patients with high BMI ($\geq 23\ kg/m^2$) showed higher IgG and neutralizing antibody than that in normal or underweight patients (Fig. 4E, F). There was no difference in seroresponse according to the duration of HD when we divided the patient group with the cutoff of 60 months of HD vintage (Fig. 4G, H). Since HD vintage is known to be related with antibody response of SARS-CoV-2 vaccination in previous study [15-17], we tried to assess whether the cutoff duration of HD led to any difference of antibody production. However, there was no difference in antibody production according to duration of HD even though we compared patients by changing the cutoff duration multiple times. Use of immunosuppressive drugs led to a difference in antibody production post-vaccination in HD patients (Fig. 4I, J). Both IgG and neutralizing antibody were produced less in patients consum-

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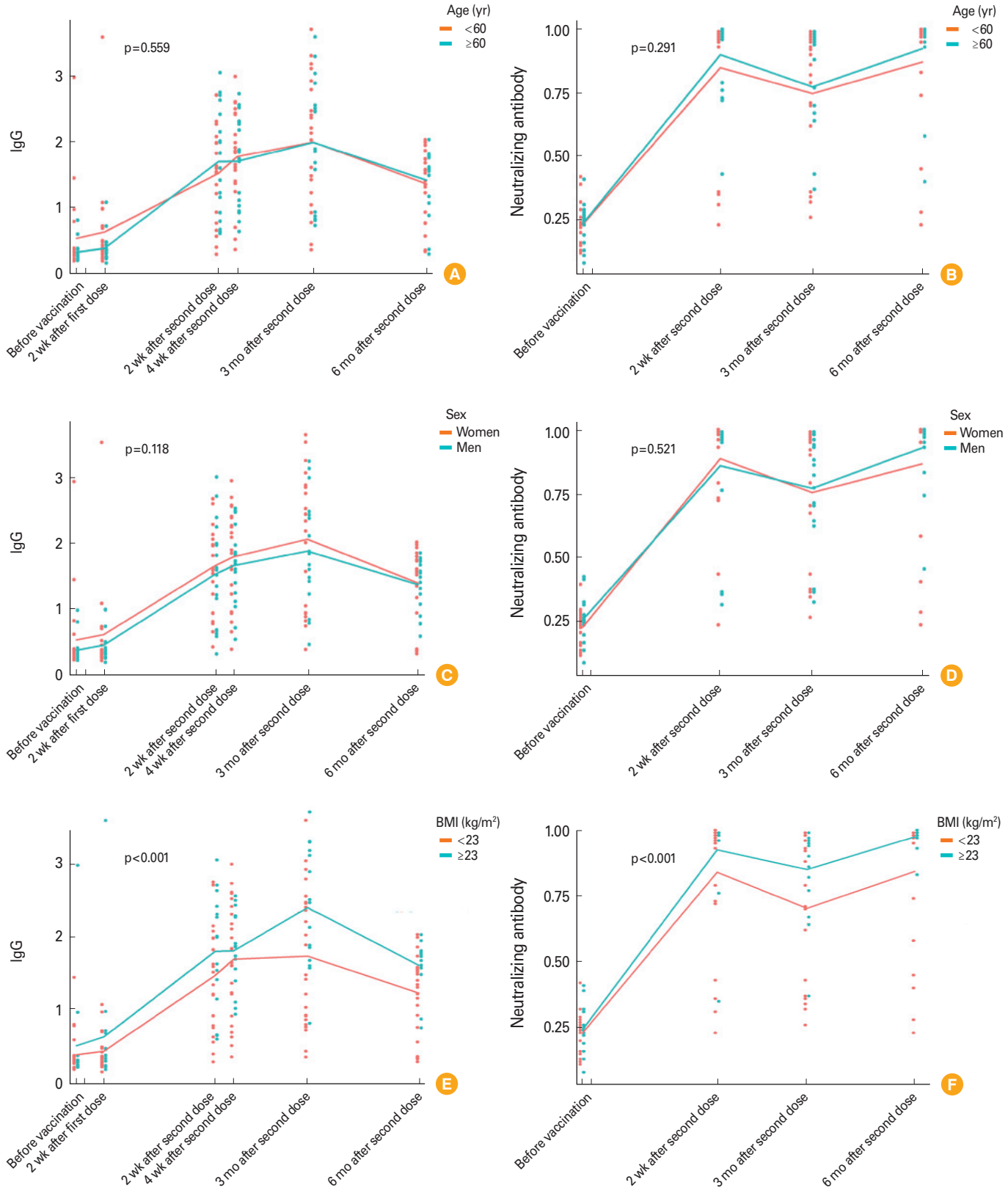

Fig. 4. Immunoglobulin G (IgG) and neutralizing antibody according to single variable. IgG and neutralizing antibody according to age **(A, B)**, sex **(C, D)**, body mass index (BMI) **(E, F)**, and hemodialysis (HD) vintage **(G, H)** in patient group. **(I, J)** IgG and neutralizing antibody in presence/absence of immunosuppressive agents. (Continued on next page.)

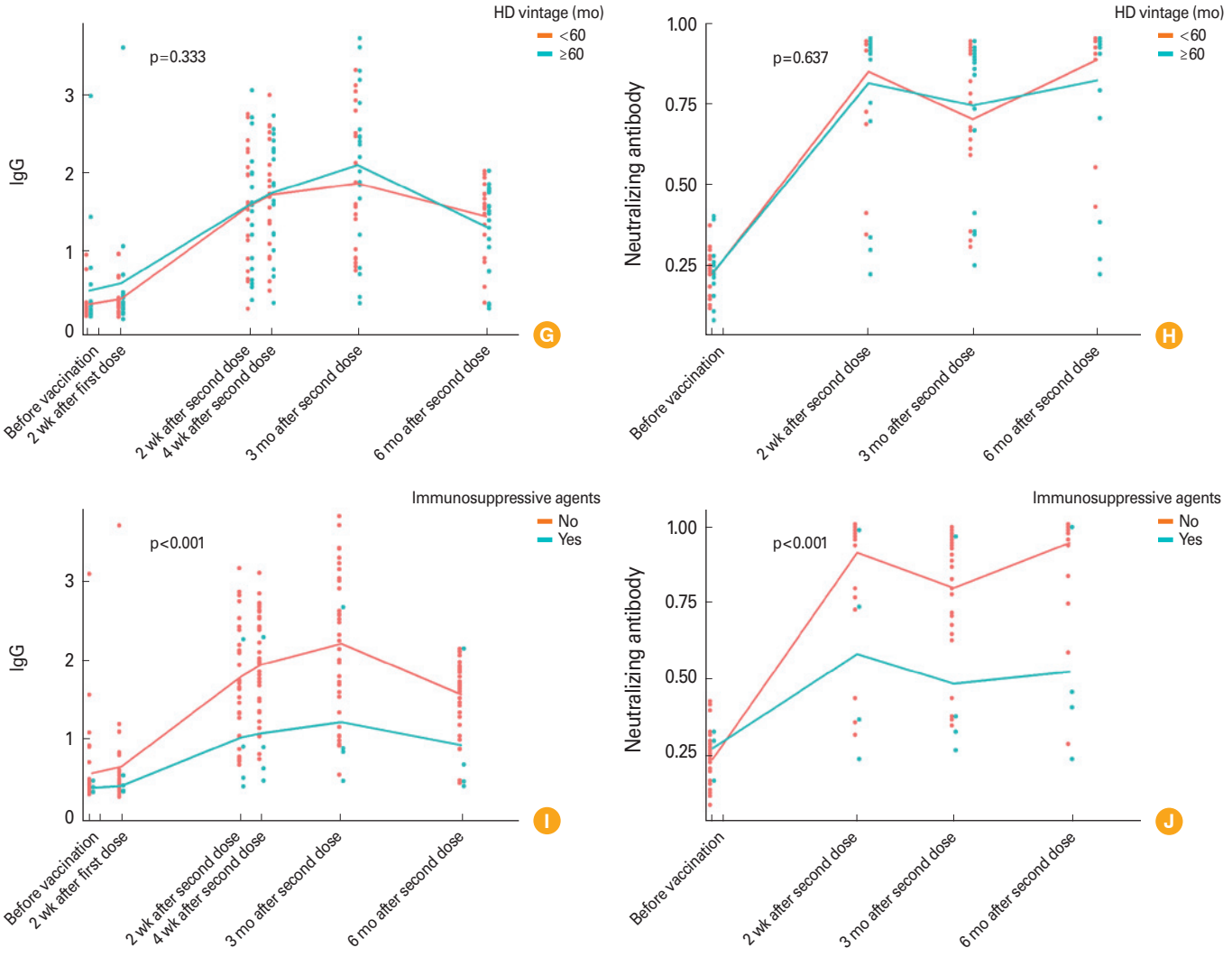

Fig. 4. (Continued; caption shown on previous page).

ing immunosuppressive drugs at 2 weeks after second dose, and the difference between the two groups was maintained over time. This indicated that immunosuppressive drugs attenuate the effect of vaccination. However, since the number of patients taking immunosuppressants was too small in this study, study with a large number of participants is required.

In the trend analysis, all subgroups showed positive trend of IgG and neutralizing antibody with time, except patients who took immunosuppressive agents. The correlation coefficient value of patients on immunosuppressive drugs was positive, but the p-value of the analysis of neutralizing antibody was insignificant (p=0.0221 in IgG, p=0.1714 in neutralizing antibody). These findings indicated that the neutralizing antibody made after vaccination can wane in patients taking immunosuppressive agents over time.

Discussion

In this prospective observational study, we measured IgG and neutralizing antibody against SARS-CoV-2 sequentially after the first and second dose of ChAdOx1 nCoV-19 vaccine in patients on chronic HD, and compared them with that of healthy control group who received two doses of mRNA-1273 vaccine. Furthermore, we assessed whether different contributing factors like administration of third dose of vaccine, age, sex, BMI, HD vintage, and use of immunosuppressive drugs are associated with antibody formation of SARS-CoV-2 vaccine. Here, patients on HD showed significant increase of IgG and neutralizing antibody, but the response was weaker than that of healthy control group. The third dose of vaccine did not lead to a significant difference in IgG and neutralizing antibody. According to trend analysis, both IgG and neutralizing anti-

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body showed positive linear trend with time, indicating that IgG and neutralizing antibody do not decline until 6 months after the second dose. In the HD patients, there was no difference in antibody response according to age, sex, and HD vintage, but patients with higher BMI and those who did not consume immunosuppressive drugs showed a better response.

Patients on HD had lower IgG and neutralizing antibody than the control group, and this result correlates with previous studies [17-19]. However, in this study, HD patients took the ChAdOx1 nCoV-19 vaccine and healthy patients had the mRNA-1273 vaccine, which is known to have a superior effect than other vaccines [15,20]. Moreover, participants in the control group were much younger than that in the patient group and had no comorbidities. These differences in vaccine type, age, and comorbidities may have led to the difference in antibody titer between the two groups bigger. Even though the response to vaccination in the patient group was weaker than that in the control group, it caused an increase in IgG and neutralizing antibody in HD patients.

To verify the effect of the third dose of vaccination, we divided the patient and control group according to based on the administration of the third dose. For IgG levels, the differences between groups 1 and 2, and groups 3 and 4 were not statistically significant. Likewise, neutralizing antibody was not significantly different according to whether participants had a third dose or not. With this result, we can estimate that the third dose of BNT162b2 or mRNA-1273 vaccine does not induce more antibody production in HD patients who had taken two doses of ChAdOx1 nCoV-19 vaccines previously, and the third dose of mRNA-1273 does not contribute to more antibody production in the healthy population who had taken two doses of mRNA-1273 vaccines previously.

With regard to the duration of antibody produced by vaccination, we analyzed the trend between time and antibody. In both the patient and control group, IgG and neutralizing antibody levels had a positive linear relationship with time, meaning that antibody developed by vaccination does exist until 6 months in both HD patient and healthy population. Moreover, when we analyzed the trend between time and IgG, neutralizing antibody in group 1 and group 2, both showed a positive linear trend, which means antibody is preserved even without a third dose in HD patients.

We obtained different results from previous studies in terms of the effectiveness of the third dose and the duration of the vaccine effect. Bensouna et al. [21] reported that the third dose of the BNT162b2 vaccine substantially increased antibody levels in patients receiving maintenance dialysis. Hsu et al. [10] have shown a decline in antibody made post-vaccination in HD patients over time. This might be due to the different races of participants and types of vaccines. Patients included in the study by Bensouna et al. [21] received three doses of the BNT162b2 vaccine while our patients had two doses of ChAdOx1 nCoV-19 vaccines and BNT162b2 or mRNA-1273 vaccine as the third dose. Also, Hsu et al. [10] included patients who administered BNT162b2, Ad26.COV2.S, and mRNA-1273 unlike this study. Differences in the type of vaccine and the combination of vaccines may have made the difference in study results. However, since the number of participants is so small, a large-scale study is needed.

There was no difference in antibody production according to age and sex in HD patients, and this result correlates with the previous study that was carried out in South Korea with the ChAdOx1 nCoV-19 vaccine in a healthy population [14]. HD patients with higher BMI ($\geq$23 kg/m^2) showed better responses to vaccination than normal or underweight patients did in this study. This is different from the findings in the previous study [14], which reported that obesity is not related to antibody responses to the ChAdOx1 nCoV-19 vaccine in a healthy population. This might be due to the difference in participants' underlying diseases. Higher BMI in HD patients can represent better nutritional status [22], so antibody production might be better. Even though previous studies proved that high HD vintage was identified as an independent predictor of a poor serological response [15-17], this study did not show a relationship between HD vintage and antibody formation or duration. This might be due to small population size. Previous studies have shown that immunosuppressive drugs had a negative impact on SARS-CoV-2 vaccination in HD patients [15]. The formation of antibody was significantly lower in patients taking immunosuppressive agents in this study. Moreover, trend analysis showed that neutralizing antibody could not be preserved over time in them. Thus, additional doses of vaccine should be considered in HD patients on immunosuppressive drugs.

There are some limitations in this study. First, it was carried out in a single center with a small population. However, we compared the seroresponse of the SARS-CoV-2 vaccine in HD patients with healthy participants and measured both IgG and neutralizing antibody to estimate the protective effect of the vaccine. Second, we could not adjust the vaccine type. Since the type of vaccine and the duration of administration were determined by the government policy, the effec-

tiveness of the same vaccine could not be known. However, we tried to achieve maximum homogeneity within the patient and control groups. Moreover, although this study has a limitation in adjusting vaccine type, it reflects the situation of Korea receiving the vaccine according to the government policy, and it is the first time in Korea to observe seroresponse of SARS-CoV-2 vaccine in HD patients. Third, because the third dose was not planned at the time of study design, the time interval between the third dose and blood sampling of 6 months after the second dose varied among participants. This time interval could make a difference in antibody response in each participant. Moreover, we divided participants under the assumption that the vaccination effect would start 2 weeks after the third dose, but there may have been a slight increase in antibodies within 2 weeks. However, only two patients were classified as group 1 despite receiving the third dose for reason that blood sampling of 6 months after the second dose was done within 2 weeks of the third dose. In the control group, there were five participants who had a third dose but were classified as group 3 for the same reason. Fourth, we did not consider the effect of the third dose when analyzing the effects of age, sex, BMI, HD vintage, and consumption of immunosuppressive drugs because of the small population. A study with a large number of participants would be needed. Fifth, neutralizing antibody was not measured as often as IgG due to high cost, so we could not know the rate of change of neutralizing antibody as efficiently as IgG. Finally, we did not consider the T-cell immune response. However, although there is increasing evidence that neutralizing antibody and anti-S1 IgG titers correlate with protection, it is known that "SARS-CoV-2-specific T cell responses are not associated with protection against reinfection in hemodialysis patients [23]."

We are still paying attention to the spread of COVID-19. Considering their susceptibility to COVID-19, getting a vaccination and maintaining proper antibody levels would be important in HD patients. In this study, we found out that two doses of vaccination led to a significant antibody response in chronic HD patients and this effect exists until 6 months. Longer follow-up is required to know the duration of vaccination and the need for additional doses.

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