Observational study of antibody levels after second and third SARS-CoV-2 vaccinations in medical workers

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Abstract

Background: Countries around the world are actively promoting vaccination against COVID-19. We observed the changes in serum neutralizing antibody titers in medical workers vaccinated with inactivated COVID-19 vaccine, in order to explore the necessity of a third dose of vaccination.

Methods: A total of 62 medical workers in our hospital were observed. Novel coronavirus neutralizing antibody titers in serum were detected by ELISA (enzyme-linked immunoassay). Neutralizing antibody tests followed in four batches according to the different time periods after three vaccinations. Sixty-two observers participated in the first batch of testing for neutralizing antibody, and 18 of them participated in all four batches. Fasting venous blood was taken from all the participants in the morning to detect serum neutralizing antibody titers.

Results: Sixty-two medical workers were divided into age groups of 21–30, 31–40, and >40 years, and the antibody titer in the oldest group was significantly lower than that in youngest group (p = 0.0137). There was a gradual decrease in antibody titers over time at around 1, 3, and 6 months after the second dose of vaccine (p < 0.0001). The antibody positive rate also decreased gradually (p = 0.0003). The neutralizing antibody titer around 1 month after the third dose was significantly increased (p < 0.0001). Unexpectedly, three participants with negative neutralizing antibody after the first and second dose produced neutralizing antibody with a measurable titer after the third dose.

Conclusions: The neutralizing antibody titer in serum increased significantly after the third dose of vaccine. A third immunization even produced neutralizing antibody in previously negative individuals.

Keywords
COVID-19, medical worker, neutralizing antibodies, strengthening immunization, vaccine

1 | INTRODUCTION

Since December 2019, SARS-CoV-2 has spread globally, resulting in over 500 million confirmed infections and over 6 million deaths. Vaccination can prevent viral infection; however, the immune protection produced by vaccines may weaken over time. Inactivated vaccines, mRNA vaccines, recombinant protein vaccines, and adenovirus vector vaccines will gradually weaken the immunity of the human
body after the completion of immunization, and there is therefore a need to strengthen the immunization after around 6 months. Many countries are using inactivated vaccines, and a Chinese study found that booster injections at intervals of 6–8 months were most effective at increasing the neutralizing antibody titer, possibly by more than 3–5 times. Boosters can use either homologous boosts or heterologous boosts. Homologous boosting means that the booster immunization uses a vaccine of the same technical type as the basic immunization; heterologous boosting means that booster immunization uses a vaccine with a different technical type from that of the basic immunization. At present, China uses the original vaccine that has been given for booster immunization, but researchers in many countries are exploring the protective effect of using heterologous booster immunization. It has been reported that the use of heterologous booster was better. However, the use of homologous intensifiers from different manufacturers is rarely reported. In this study, it was found that the antibody titer increased significantly after the third dose, and three individuals with negative neutralizing antibody produced a measurable titer of neutralizing antibody after the third dose.

2 | MATERIALS AND METHODS

2.1 | Participants and study design

Sixty-two medical workers who received inactivated COVID-19 vaccine were included in this observational study, including 11 doctors (17.74%), 11 nurses (17.74%), 14 clinical laboratory technicians (22.58%), and 26 other workers in the hospital (41.94%). Seventeen participants who worked a 12–15-hour night shift per week were identified as night shift workers; 45 participants who did not work night shifts were identified as daytime workers. The Chinese body mass index (BMI) judges normal as 18.5 ≤ BMI ≤ 24, overweight as 24 < BMI ≤ 27.9, and obese as 28 ≤ BMI ≤ 32. Among these medical workers, 14 had BMI > 24 and 48 workers had BMI ≤ 24. Only two of the 62 participants smoked. By neutralizing antibody titer analysis, we found no statistically significant differences between different work type groups, the day and night shift groups, BMI > 24 and BMI ≤ 24 groups. The titers are shown in Figure 1A–C.

The study was approved by the Ethics Committee of Shenzhen Hospital, Southern Medical University (SZYYEC2021R030). The changes in serum neutralizing antibody titer after the second dose of vaccine were dynamically observed. Blood samples were collected at around 1 month after the second dose for the first batch of testing, at around 3 months for the second batch and at around 6 months for the third batch. The fourth batch of blood was drawn around 1 month after the third dose. Sixty-two medical workers comprising 22 males and 40 females, aged 22–59 years with an average age of 37.3 years, were enrolled. The age distribution of the 62 participants according to gender was not statistically significant at the first batch of testing, as shown in Figure 2. The age distribution according to gender of the 18 individuals who participated in the whole four batches of testing was also not statistically significant, as shown in Figure 3. Participation in each batch of testing is shown in Figure 4. Venous blood was collected in the morning on an empty stomach from all the participants. The serum was separated after centrifugation at 1760 g for 10 min and stored in a refrigerator at −20°C.

2.2 | Laboratory examination

The enzyme-linked immunoassay (ELISA) reagent kit for neutralizing antibody titers is produced by Shandong Kanghua Biomedical Technology. The protein of the host cell receptor ACE2 is pre-coated in the wells of the microplate, and the purified receptor-binding domain (RBD) is labeled with horseradish peroxidase (HRP). The anti-2019-nCoV neutralizing antibody in the sample interacts with HRP-RBD, blocking the binding of RBD to ACE2. When coupled with the enzyme substrate, the OD value is read by a microplate reader and the antibody titer is calculated. The instructions for batch testing were followed. Result judgment: according to the results of the calibrator, a four-parameter fitting method was used to establish a standard curve to calculate the anti-SARS-CoV-2 neutralizing antibody titer of each sample (positive result: >1 U/ml, negative

![Image](image_url)
LIU et al. result: ≤1 U/ml). The model of microplate reader used was a Huisong MB-580.

2.3 | Statistical analysis of data

Statistical analyses were performed using Statistical Analysis System software SPSS 19.0 (IBM) and GraphPad Prism 7.0 (GraphPad). The Shapiro–Wilk normality test was used to evaluate whether the data were normally distributed. Normally and non-normally distributed data were presented as mean ± SD or median (25th percentile and 75th percentile), as appropriate. Analysis of differences between groups were carried out using one-way ANOVA, t test, Mann–Whitney test, or Kruskal–Wallis test, as appropriate. Two-sided chi-squared tests were used to compare the proportions of different groups. A value of $p < 0.05$ was defined as statistically significant.

3 | RESULTS

3.1 | Neutralizing antibody titers around 1 month after the second dose of vaccination

There was no statistically significant difference in neutralizing antibody titers between male and female participants ($p = 0.1785$), and the results are shown in Figure 5A. When participants grouped according to age (21–30, 31–40, and >40 years), the difference was statistically significant ($p = 0.0137$), and the antibody titer was shown to be lower in older individuals (Figure 5B).

3.2 | Repeated monitoring after the second dose of vaccination

After inoculation, the titer of antibodies gradually decreased over time, and the difference was statistically significant ($p < 0.0001$). Different individuals have different immune characteristics, and the antibody titer of some individuals was increased at 3 months when compared to 1 month (Figure 6A). Over time, the antibody positivity rate also decreased gradually, and the difference in the triple positive rate was statistically significant ($p = 0.0003$; Figure 6B).

3.3 | Comparative analysis of the effects of vaccination around 1 month after the second dose and around 1 month after the third dose

The difference between the neutralizing antibody titer after the third dose and that after the second dose was statistically significant ($p < 0.0001$; Figure 7A). With regard to the analysis of the positive rate, the number of people involved in the neutralizing antibody test around 1 month after the third dose was very small compared with the sample size around 1 month after the second dose, and the difference in the positive rate between the two test batches was therefore not statistically significant (Figure 7B).

3.4 | Repeated monitoring and analysis of the effect of the third dose

After the third dose, the antibody titer was significantly increased. Three participants who had negative neutralizing antibody titers at
around 1 month and around 3 months after the second dose produced a measurable titer of neutralizing antibody after the third dose (Figure 8).

4 | DISCUSSION

The protective factors assessed for clinically licensed vaccines are basically neutralizing antibodies, as is the case for the current SARS-CoV-2 vaccines. If antibody titers are high enough, neutralizing antibody responses are quite effective in blocking infection. The spike protein (S protein) on the surface of SARS-CoV-2 is the target of neutralizing antibodies, and all currently licensed vaccines are based on this antigen. Human infection with SARS-CoV-2 causes immune responses, including the production of antibodies (IgE, IgM, IgG, IgA, and IgD) in the blood that bind rapidly and strongly to the pathogen, providing protection against future infection with the virus, known as neutralizing antibodies. The neutralizing antibody kit used in this study utilized a total antibody to detect neutralizing antibodies. The historical method of producing vaccines based on whole inactivated virus has also been used for SARS-CoV-2 vaccines, and several such vaccines have been approved for clinical use. All participants in this study received inactivated vaccines and had not been infected with SARS-CoV-2.

Medical workers are in the front line of medical treatment, and their risk of infection is very high. The evaluation of COVID-19 vaccine effectiveness for medical staff is of far-reaching significance. In this study, participants were grouped according to different types of work, whether they undertook rotating night shifts,
and their BMI; there was no statistically significant difference in the level of neutralizing antibodies among the groups. The results of a recent study show that, in their univariate analysis of data, night shift work was associated with a reduced rate of decrease in the anti-SARS-CoV-2 antibody titer. However, this association was not significant upon subsequent multivariate analysis after controlling for study covariates. It has also been reported in the literature that night work and BMI > 30 were associated with a markedly greater risk of COVID-19 diagnosis. Different types of work, rotating night shifts, BMI, and smoking may affect the titer of neutralizing antibodies, but these hypotheses need further study with a larger sample size.

Our study found that there was no statistically significant difference in neutralizing antibody titers between male and female participants around 1 month after the second dose of the vaccine, but there was a statistically significant difference among different age groups. The antibody titer of the older group was low, which is consistent with the traditional notion that immune function declines in the middle-aged and the elderly. Another study confirmed that the average antibody level is significantly correlated with the age of health care workers, with older individuals having significantly lower anti-SARS-CoV-2 antibody levels than younger ones. Using repeated monitoring after the second dose of vaccine, at around 1, 3, and 6 months, it was found that the antibody titer and positive rate gradually decreased with the passage of time. This is consistent with the results of other studies: in Israel, the protection rate of the Pfizer mRNA vaccine was 90% initially, and decreased to 39% after half a year; in Chile, the estimated protection rate of the Sinovac inactivated vaccine decreased from 66% initially to 56% after 5 months.

In addition, the virus continues to mutate, especially the recent pandemic SARS-CoV-2 Omicron variant, which has as many as 59 mutations in the entire genome, with up to 36 in the spike protein which is the main target of neutralizing antibodies. Studies of previous SARS-CoV-2 variants have shown that mutations within the receptor-binding domain (RBD) mediate escape from vaccine-induced neutralizing antibodies. Sinovac’s inactivated vaccine is widely used worldwide and has been approved in 48 countries, with 85% and 80% efficacy against hospitalization and death, respectively. However, with the emergence of new variants of SARS-CoV-2 and the weakening of vaccine immunity over time, several countries have begun to use booster doses. Considering the immunogenicity, safety and other factors related to the vaccines, China currently uses homologous inactivated vaccines from Sinovac, Beijing Bio, Chengdu Bio, and Wuhan Bio for enhanced immunization. People receiving two doses of the same novel coronavirus inactivated vaccine are, in principle, being immunized with one dose of the original inactivated vaccine. Follow-up studies have found that homologous vaccine boosters provide good immunogenicity and immune safety. Studies have shown a 7.7-fold increase in neutralizing antibodies against the Omicron variant in the high-dose group when homologous immunization with Sinovac vaccines was boosted 5–9 months after completion of the regular two-dose regimen. These results indicate that homologous booster immunization could significantly increase the level of neutralizing antibodies and reduce the incidence of infection with 2019-NCoV variants. However, other studies have also reported the better protective effect of heterologous enhanced immunity. In this study, use of a homologous vaccine booster showed that the third dose of vaccine significantly increased the serum titer of neutralizing antibody, and three participants with negative neutralizing antibody 1 and 3 months after the second dose also produced neutralizing antibody with a measurable titer. The three negative participants received their first and second doses of vaccine by inoculation with the Beijing Bio vaccine, and the third dose was provided by Chengdu Bio to two negative participants, and by Beijing Kexing Zhongwei to one negative participant. Possible reasons for these observations may be as follows: (1) the immune response requires stimulation from multiple antigens; (2) changes in vaccine manufacturers or batches can improve the immunogenicity; and (3) individual differences.

Some limitations are evident in our study. First, although the results are basically consistent with those of other related studies, the sample size of this study is relatively small owing to its long duration and design as a longitudinal study. Second, only the titers and positive rates of neutralizing antibodies after the second and third doses of vaccine were compared and analyzed. The detection of antibody levels after SARS-CoV-2 vaccinations should be followed up in a future study.

5 | CONCLUSION

The titer of neutralizing antibodies decreased gradually with time after the second dose of vaccine. The third dose of vaccine significantly increased the titer of neutralizing antibody in serum, and a third immunization can even produce neutralizing antibody in previously negative individuals.

AUTHOR CONTRIBUTIONS

Xingwang Jia, Qing Liu, and Wency Zhou contributed to the study design. Qing Liu contributed to the clinical specimens collection and experiments. Xingwang Jia, Qing Liu, and Wencyan Jiang contributed
to the data analysis. Xingwang Jia and Qing Liu contributed to the manuscript preparation. Qing Liu, Wenyan Zhou, Wencan Jiang, and Xingwang Jia reached an agreement on the final approval of the version to be submitted.

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DATA AVAILABILITY STATEMENT
All data analyzed during this study are available from the corresponding author upon reasonable request.

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