Comparison of Antibody Persistence up to 6 Months after Additional Booster Vaccination with ChAdOx1 nCoV-19 Vaccine

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Abstract: Vaccines are crucial for controlling the COVID-19 pandemic, and booster doses are becoming increasingly important. This study aimed to assess the efficacy of the ChAdOx1 nCoV-19 vaccine from AstraZeneca as a third dose in healthcare workers at different time intervals (one, three, and six months). Two methods to measure immune response—ELISA (EUROIMMUN Medizinische Labordiagnostika AG, Luebeck, Germany) and ELISpot (Mabtech AB, Macka Strand, Sweden)—were used. A total of 170 participants were included in the study. The results showed that while IgG levels decreased at six months compared to levels at one and three months, they were still significantly higher than the baseline. Furthermore, neutralizing levels at three and six months and after the third dose were not significantly different. These findings suggest that the immune response induced by the vaccine was robust and effective for several months. These results have significant implications for public health policymakers, as they provide strong support for booster vaccinations. The ChAdOx1 nCoV-19 vaccine appears to be a reliable option for preventing the spread of COVID-19, and this study provides valuable information for healthcare workers and policymakers in managing the pandemic.

Keywords: ChAdOx1 nCoV-19; immunogenicity; SARS-CoV-2; COVID-19; neutralizing antibodies; durability; booster

1. Introduction

As of the 28 March 2023, more than 761 million people have been affected by SARS-CoV-2, and more than 6.8 million people have died worldwide. While vaccines play a critical role in public health preventive measures to prevent coronavirus from infecting our cells [1], the emergence of mutations in this virus has led to concerns about vaccine efficacy, particularly among frontline healthcare workers as they interact with COVID-19 patients, leading to a higher risk of infection and further spread [2].

During the initial phase of the COVID-19 outbreak in Thailand, access to vaccines and the gold-standard test was limited [3,4]. In response, the inactivated CoronaVac vaccine from Sinovac Biotech was introduced as a mass vaccination for both healthcare workers and the general population. However, antibody levels to SARS-CoV-2 were found to decrease three months after two doses of CoronaVac were given [5], necessitating a third booster dose. While the BNT162b2 demonstrated higher immunogenicity compared to the CoronaVac booster [6], Thailand did not have access to this vaccine until the end of 2021. Instead, the viral vector vaccine ChAdOx1 nCoV-19 from AstraZeneca was a promising option for use as a third booster dose in the general population who had previously received two doses of...
ChAdOx1 nCoV-19, showing promising immunogenicity for both humoral and cellular immune responses [7,8].

Given the limited evidence regarding the immunogenicity of ChAdOx1 nCoV-19 as a third booster dose following two doses of CoronaVac, we previously conducted a study on healthcare workers that demonstrated adequate but declining antibody levels to protect against SARS-CoV-2 at three months post-vaccination [9]. With the recommendation for the fourth booster vaccination in Thailand four months after the third booster vaccination [10], there was concern about the sustainability of SARS-CoV-2 protection after three months, given the significant decrease in antibody levels. As such, this study represents an extended prospective cohort study that is aimed at evaluating the long-term sustainability of ChAdOx1 nCoV-19 immunogenicity as the third vaccine dose in healthcare workers, specifically six months after vaccination.

2. Materials and Methods

This study was an observational, prospective cohort study that included 170 healthcare workers who received two doses of CoronaVac between February to March 2021 and subsequently received a third dose of the ChAdOx1 nCoV-19 vaccine. Proof of the CoronaVac vaccination was obtained through the use of case record forms (CRFs).

2.1. Antibody Measurement

To determine the levels of antibodies to the SARS-CoV-2 spike protein, blood samples were collected from all participants before (day 0) and after the administration of the third vaccine dose (at 1, 3, and 6 months). The levels of both the immunoglobulin G (IgG) antibody and neutralizing antibody were measured using an enzyme immunoassay (Euroimmun, Lübeck, Germany) ELISA. The IgG antibody assay worked on the principle that the test kit (Euroimmun, Lübeck, Germany) contained microplate strips with eight break-off reagent wells that were coated with the recombinant S1 domain of the spike protein of SARS-CoV-2 (Euroimmun, Lübeck, Germany). In the first reaction step, the diluted samples are incubated in the wells. If these samples were positive, specific IgG (also IgA and IgM) antibodies could bind to the antigens. To detect these bound antibodies, a second incubation was performed with an enzyme-labeled anti-human IgG (enzyme conjugate, Euroimmun, Lübeck, Germany), which catalyzed a color reaction. The neutralizing antibody assay used in this study involved microplate strips with 8 reagent wells that were coated with a recombinant S1/RBD domain of the SARS-CoV-2 spike protein. In the first step, both the controls and samples were diluted with a sample buffer containing soluble biotinylated ACE2 before being incubated in the wells. If neutralizing antibodies were present in the sample, they competed with receptor ACE2 for the binding sites of SARS-CoV-2 S1/RBD proteins. Any unbound ACE2 was removed in a subsequent wash step. To detect the bound ACE2, a second incubation step was performed with peroxidase-labeled streptavidin, which catalyzed a color reaction in the third reaction step. The intensity of the color formed was inversely proportional to the concentration of the neutralizing antibody in the sample.

Participants were categorized into quartiles based on their antibody levels prior to receiving their third dose. A random selection of five participants from each quartile was conducted to measure their IgG antibody levels. SARS-CoV-2-specific T-cell responses were measured using an Interferon-gamma ELISpot before and after vaccination with ChAdOx1 nCoV-19. Subgroup analysis was conducted to determine the effect of antibody level on each IgG group, with the Negative group defined as IgG < 32 BAU/mL and the Positive group defined as IgG ≥ 32 BAU/mL [11]. The pseudovirus-based neutralization assay (PVNT) was utilized to evaluate the neutralization activity against SARS-CoV-2 variants, including Wuhan, Delta, and Omicron (BA.1, BA.2 and BA.4/BA.5). Serum samples were incubated at 37 °C for 30 min to inactivate the complement factors, and then a two-fold serial dilution of the serum samples starting at 1:40 was mixed with 50 µL of a SARS-CoV-2 pseudovirus in a 96-well culture plate. This mixture was incubated for 1 h at 37 °C, 5% CO2,
then HEK293T/17-hACE2-TMPRSS2 was transfected to cell suspension ($4 \times 10^6$ cells/well), which were seeded into each well of the tissue culture plates and incubated for 48 h at 37 °C, 5% CO₂.

2.2. Statistical Analysis

The SARS-CoV-2 pseudovirus titer was measured based on luciferase activity using a microplate reader, and the IC50 was calculated using GraphPad Prism8 software. Statistical analyses were conducted using STATA software, version 15. Descriptive statistics were used to report the mean and a 95% confidence interval for normally distributed quantitative data and the median and interquartile range for non-normally distributed quantitative data. Paired t-tests were performed to compare anti-spike IgG levels before vaccination to levels at 1, 3, and 6 months after vaccination for all the participants. Statistical significance was defined as $p < 0.05$.

2.3. Research Ethics and Trial Registration

This study was approved by the Ethics Committee for Research related to COVID-19 Disease or Public Health Emergency, Department of Disease Control, Ministry of Public Health (Ref. No. 64064, IRB. No. FWA 00013622). The participants provided written informed consent. The protocol was registered at the Thai Clinical Trial Registry (TCTR20211005001). The study received financial support from the National Research Council of Thailand (N5B640133).

3. Results

3.1. Characteristics of Participants

The study included 170 healthcare workers who received ChAdOx1 nCoV-19 as a third dose following two shots of CoronaVac. None of the participants had a history of COVID-19 infection. The majority of participants were female (81.8%), and 42.35% had comorbidities. The mean age of the participants was 45 (IQR, 35–52) years. Before the administration of the third dose of ChAdOx1 nCoV-19, a subgroup of individuals underwent a seroneutralization test, and their results were analyzed based on their IgG levels. The test considered concentrations below 32 BAU/mL to be negative. Of the 170 participants, 25 (14.7%) had negative anti-spike IgG levels at the baseline. A table summarizing the characteristics of the participants has been previously published [9].

3.2. Humoral Immunogenicity

This study investigated the levels of anti-spike IgG in 170 participants before and after receiving their third dose of ChAdOx1 nCoV-19 and following two shots of CoronaVac. The mean anti-spike IgG level before vaccination was 86.02 BAU/mL (95% CI, 73.10–98.93). After the third dose of ChAdOx1 nCoV-19, the mean anti-spike IgG level significantly increased to 2476.69 BAU/mL (95% CI, 2198.41–2754.97) one month after vaccination, and these levels remained elevated at 833.77 BAU/mL (95% CI, 742.79–924.75) three months after vaccination and 582.51 BAU/mL (95% CI, 498.37–666.65) six months after vaccination (Figure 1). Furthermore, it was observed that SARS-CoV-2-specific IgG levels peaked one month after vaccination and remained higher compared to pre-vaccination levels until 6 months after vaccination. Although there was a decrease in IgG levels at 6 months post-vaccination compared to 1 and 3 months, these levels were significantly higher than the baseline.

The neutralizing antibody levels of all 170 participants were tested using the aforementioned method (Figure 2). %IH is the percentage of inhibition of RBD-ACE2 binding when measured at different time points. After one month of vaccination, the mean neutralizing antibody level showed a significant increase from 19.55% to 98.43% when compared to the baseline. At the 6-month mark, the mean neutralizing antibody level remained high at 86.42% ($p < 0.001$). The data also indicated that there was no significant difference between the neutralizing levels at 3 and 6 months after the third dose of ChAdOx1 nCoV-19, indicat-
ing that the immune response elicited by the vaccine was robust and could be sustained over several months.

![Figure 1](image1.png)

**Figure 1.** Anti-spike level after ChAdOx1 nCoV-19 vaccination among healthcare workers at months 0, 1, 3, and 6.

![Figure 2](image2.png)

**Figure 2.** Neutralization antibody response in ChAdOx1 nCoV-19-vaccinated healthcare workers at months 0, 1, 3, and 6.

### 3.3. Comparisons of an Antibody Level between Positive vs. Negative IgG Subgroups

The mean IgG levels decreased significantly from 635.27 BAU/mL (95% CI, 435.31–814.23) at 3 months to 529.92 BAU/ml (95% CI, 222.95–836.88) at 6 months after an additional shot
In the positive IgG group, the mean IgG level also decreased significantly at 6 months from 866.40 BAU/mL (95% CI, 764.94–96 to 7.86) at 3 months to 590.40 BAU/mL (95% CI, 502.17–678.62) after additional vaccination ($p < 0.001$) (Figure 3). Although the mean anti-spike IgG level was significantly higher in the positive group at 3 months, there was no significant difference in the mean anti-spike IgG levels between the positive and negative groups at 6 months after vaccination.

Neutralizing antibody levels decreased rapidly in both the positive and negative groups during the first 3 months after vaccination and then decreased at a relatively slower rate after 6 months. The level of neutralizing antibodies in the positive group decreased from 94.45% (95% CI, 93.17–95.73) at 3 months to 86.79% (95% CI, 93.53–90.06) at 6 months, while the level in the negative group decreased from 90.36% (95% CI, 81.99–98.72) at 3 months to 83.92% (95% CI, 75.66–92.17) at 6 months. Both the positive and negative groups experienced a significant decrease when neutralizing antibody levels during the 6 months after vaccination ($p < 0.001$ and $p = 0.002$, respectively). There was no significant difference in the neutralizing antibody levels between the positive and negative groups at 6 months after vaccination ($p = 0.397$) (Figure 4).

### 3.4. Neutralizing Antibody Responses to SARS-CoV-2 Variants 6 Months after Boosting

The 50% pseudovirus neutralization titer (PVNT$_{50}$) was used to evaluate neutralization activity against SARS-CoV-2 variants among the participants 6 months after boosting. The results showed a high neutralization activity against the Wuhan variant, with a mean PVNT$_{50}$ level of 177.55 (95% CI, 134.94–220.16). However, for the other variants, the mean PVNT$_{50}$ levels were lower, with 91.92 (95% CI, 56.53–127.31) for Delta, 72.22 (95% CI, 17.87–126.57) for BA.4/BA.5, 53.97 (95% CI, 22.88–85.07) for BA.2, and 49.92 (95% CI, 9.85–90.00) for BA.1 (Figure 5).
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4. Discussion

In this prospective observational study, 170 healthcare workers were enrolled, among whom 146 had positive anti-spike antibody levels at the baseline, three months after receiving two doses of CoronaVac, and these antibody levels decreased dramatically over time. The administration of ChAdOx1 nCoV-19 as a third booster dose resulted in a significant improvement in both the anti-spike and neutralizing antibody levels. Although antibody levels declined at 3- and 6-month time points after vaccination, they remained...
above the protective threshold, thereby providing adequate protection against severe
disease. Additionally, our findings demonstrated a notable neutralizing activity against the
five most common variants after 6 months of ChAdOx1 nCoV-19 as the third dose.

In a recent study, we investigated the antibody levels of healthcare workers who
received two doses of CoronaVac followed by a third dose of the ChAdOx1 nCoV-19
vaccine [9]. Our findings revealed a significant decline in antibody levels to SARS-CoV-
2 after 3 months, raising concerns about the potential inadequacy of antibody levels to
protect against the virus and prompting questions about the optimal timing for a fourth
booster dose.

However, we also observed that the decline in antibody levels was slower in the 3- to
6-month period after vaccination compared to the 1- to 3-month, and adequate antibody
protection was maintained 6 months after the third dose of ChAdOx1 nCoV-19. This
provides evidence to support the sustainability of the vaccine effect and allays concerns
about the need for a fourth booster dose.

The current recommendation in Thailand is to administer a fourth booster dose
4 months after the third dose, with a 2-month window of protection in case the schedule
cannot be met [10]. Our study suggests that a longer interval between the third and fourth
booster doses may be possible since there is evidence of protection against SARS-CoV-2
6 months after a third booster dose of ChAdOx1 nCoV-19. However, it is important to note
that the current recommendation for a fourth booster dose is based on previous evidence
that lacked studies on long-term protection.

Six months after administering the ChAdOx1 nCoV-19 booster dose, neutralizing
activity against the most common SARS-CoV-2 variants, including the Wuhan, Delta, and
Omicron subtypes BA.1, BA.2, and BA.4/5 was sufficient. The highest protection was
observed against the Wuhan variant, followed by the Delta variant, which caused more
severe COVID-19 than the Omicron variant [12,13]. These findings were consistent with
the previously published short-term results for the ChAdOx1 nCoV-19 vaccine as the third
dose in participants who had received two doses of ChAdOx1 nCoV-19 [14,15]. Notably,
we observed high neutralization activity against the highly infectious Omicron subtype
BA.4/5, which is currently prevalent in Thailand [16]. Although neutralization activity
against the Omicron subtypes BA.1 and BA.2 was lower, it was still sufficient to protect
against severe disease caused by these variants. This was demonstrated in a vaccine efficacy
study where participants received two doses of CoronaVac followed by a third dose, which
was a booster of ChAdOx1 nCoV-19 [17].

While the findings of this study are significant, it is important to note that the study
population consisted solely of healthcare workers and may not be fully generalizable to the
broader population. Therefore, any extrapolation of these results to other groups should
be conducted with caution. Nevertheless, these results provide valuable insights into
the durability of immune responses following a ChAdOx1 nCoV-19 booster vaccination in
individuals who previously received two doses of CoronaVac, suggesting that the vaccine
provides sustained protection against SARS-CoV-2 for up to 6 months.

5. Conclusions

Our study provides evidence that the ChAdOx1 nCoV-19 vaccine as a third booster
dose can induce a sustained antibody response against SARS-CoV-2 for at least 6 months,
including protection against commonly circulating variants such as Delta and Omicron.
However, the duration and extent of protection against emerging variants of concern
remain for evaluation in future studies. It is important to note that the findings of this
study may not be generalizable to the population, as our sample was limited to healthcare
workers. Despite these limitations, our study highlights the importance of the continued
monitoring of vaccine effectiveness and the potential need for additional booster doses to
maintain immunity against SARS-CoV-2.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in this study.

**Data Availability Statement:** The supporting data for the findings of this study are available from the corresponding authors upon reasonable request.

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