Comparison of Waning Neutralizing Antibody Responses Against the Omicron Variant 6 Months After Natural SARS-CoV-2 Infection (With/Without subsequent COVID-19 Vaccination) Versus 2-dose COVID-19 Vaccination

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Running title: Antibody Response of Hybrid Immunity

Abstract

Following SARS-CoV-2 infection, subsequent ChAdOx1 nCoV-19 induced similar neutralizing antibody levels against the original strain but significantly higher levels against the Omicron variant compared to those who were not vaccinated. Prior SARS-CoV-2 infection exhibited higher neutralization antibody titers than vaccination alone for both original strains and the Omicron variant.

Keywords: Hybrid immunity, SARS-CoV-2, COVID-19, vaccine, antibody response

Introduction

Virus exposure, either by natural infection or vaccination, elicits protective immunity. Third booster shot of COVID-19 vaccination has shown protective effects against the SARS-CoV-2 Omicron variant [1], but there are limited data on immune responses against SARS-CoV-2 and its Omicron variant by hybrid exposure to the virus or viral protein—that is, natural infection followed by 2-dose vaccination—in terms of long-term immunity. In this prospective cohort study, we compared the antibody response induced by 2-dose vaccination between natural infection–convalescent and infection-naïve individuals over 6 months.

Methods

Study Participants and Specimen Collection

We enrolled healthcare workers (HCWs), from March 2021, who received ChAdOx1 nCoV-19 (ChAdOx1) or BNT162b2 with or without a history of SARS-CoV-2 infection (confirmed by S1-specific IgG test) before vaccination. The interval between the first and second dose was 12 weeks for the ChAdOx1 vaccine and 3 weeks for the BNT162b2 vaccine. The ChAdOx1 vaccine was assigned to those without direct contact with COVID-19, and the BNT162b2 was assigned to high-risk HCWs involved in the care of COVID-19 patients according to the South Korean government policy.

We recruited participants with natural SARS-CoV-2 infection who were admitted to our hospital between June and September 2020 when the vaccines were not available until at least 6 months after these individuals were diagnosed with COVID-19, and we also enrolled patients with SARS-CoV-2 infection between October 2020 and February 2021 who refused to be vaccinated for up to 6 months. This study was reviewed and approved by the institutional review board of Asan Medical Center (IRB No. 2020-0297 and 2021-0170), and

all participants gave written informed consent.

We measured serum neutralizing antibody levels 6 months after SARS-CoV-2 infection for those who were not vaccinated after infection and 6 months after the first dose vaccination for those who underwent subsequent ChAdOx1 vaccination after natural infection. Also, serum neutralizing antibody was measured 6 months after the first dose vaccination for the study participants who underwent two-dose vaccination alone without natural infection. . The severity of illness during COVID-19 infection was classified according to the National Institutes of Health classification as follows: asymptomatic or presymptomatic infection=1, mild illness=2, moderate illness=3, severe illness=4, critical illness=5.

Measurement of Immune Responses

Neutralizing antibodies were measured using a microneutralization assay. The SARS-CoV-2 live virus used in this study was the lineage A (an early SARS-CoV-2 isolate) (BetaCoV-19/Korea/KCDC03/2020) and the "Omicron variant" is the lineage B1.1.529 (hCoV-19/Korea/KDCA447321/2021) provided by the Korea Disease Control and Prevention Agency (KDCA). Briefly, 100 tissue culture infective dose 50% (100 TCID50) of SARS-CoV-2 was mixed with diluted plasma specimen in equal volume, incubated at 37°C for 30 minutes, and added to Vero cells. Vero cells obtained from ATCC (CCL-81) were used and were negative for TMPRSS2. After 96 hours, the cytopathic effect of SARS-CoV-2 on the infected cells was measured. The neutralizing antibody titer was presented as the reciprocal of the highest test plasma dilution factor at which 50% neutralization was attained (ID_{50}). The Microneutralization assay was performed in a Bio Safety Level (BSL)-3 laboratory at the Institut Pasteur Korea (Seongnam, Republic of Korea).

Statistical Analysis

We used the chi-squared test or Fisher's exact test to analyze categorical variables. Student's *t* test or the Mann-Whitney *U* test was used for continuous variables depending on the normality of the data. Two-tailed *P* values <.05 were considered statistically significant. R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA) were used for the analysis and graphical presentation of the results.

Results

A total of 49 infection-naïve HCWs who received 2 doses of ChAdOx1 (n = 25 [51%], ChAdOx1 group) or BNT162b2 (n = 24 [49%], BNT162b2 group) and 22 participants with previous SARS-CoV-2 infection were enrolled in this study. Among the 22 patients with previous SARS-CoV-2 infection, 9 (37%) were HCWs who received 2 doses of the ChAdOx1 vaccine within the 12-week interval after natural infection. The median interval (interquartile range) between natural infection and vaccination was 103 (92-147) days. The remaining 13 patients (63%) with SARS-CoV-2 infection did not undergo COVID-19 vaccination until 6 months after SARS-CoV-2 infection either because the vaccine was not available in South Korea until this time, or they refused to be vaccinated. Among patients with SARS-CoV-2 infection in our study, there was a significant difference in COVID-19 severity between vaccinated and unvaccinated patients after infection ($P < 0.001$, Supplementary Table 1). The baseline characteristics of the study participants are shown in Supplementary Table 1.

Levels of neutralizing antibodies against the original strain 6 months after SARS-CoV-2 infection in patients without subsequent vaccination were similar to levels 6 months after infection in patients who subsequently received 2 vaccine doses ($P = .78$, Figure 1). However, compared with patients with previous SARS-CoV-2 infection without subsequent

vaccination, those with previous SARS-CoV-2 infection followed by vaccination had significantly higher neutralizing antibody levels against the Omicron variant 6 months after infection ($P < 0.001$, Figure 1). Compared with participants who received 2 doses of BNT162b2 or ChAdOx1 alone without natural infection, subsequent ChAdOx1 vaccination following natural infection induced significantly higher neutralizing antibody levels against both the original strain ($P <.001$) and the Omicron variant ($P <.001$) 6 months after the vaccination. Furthermore, natural SARS-CoV-2 infection without subsequent vaccination also exhibited significantly higher neutralizing antibody levels against both the original strain ($P <.001$) and the Omicron variant ($P <.001$) compared with vaccination alone without natural infection. Among infection-naïve participants, the BNT162b2 group had higher neutralizing antibody levels than the ChAdOx1 group against the original strain at 6 months after the vaccination ($P =.006$), while the 2 groups were comparable in terms of levels of neutralizing antibody levels against the Omicron variant at 6 months after the vaccination ($P =.05$).

Discussion

Previous studies have shown that 2 doses of mRNA-based vaccines elicit poor neutralizing antibody responses against the Omicron variant, while 3 doses of mRNA-based vaccines elicit potent responses against the Omicron variant [2-3]. Additionally, patients infected with the Alpha, Beta, or Delta variants have been shown to have poor neutralizing antibody responses against the Omicron variant, while hybrid immunity (natural infection followed by COVID-19 vaccination) has been associated with potent neutralizing antibody responses against the Omicron variant [4]. However, there are limited published data comparing the long-term immunity against the Omicron variant elicited by natural infection (with or without COVID-19 vaccination) with that elicited by 2-dose COVID-19 vaccination. We found that

natural SARS-CoV-2 infection, itself, yielded higher neutralizing antibody levels against Omicron, as well as against the original strain, 6 months after infection, than the levels induced by 2-dose COVID-19 vaccination 6 months after vaccination. More importantly, hybrid immunity (natural infection followed by 2-dose vaccination) was more durable than natural SARS-CoV-2 infection alone or 2-dose COVID-19 vaccination alone, while, compared with natural infection alone, hybrid immunity was associated with similar neutralizing antibody levels against the original strain. Only one recent study (a longitudinal study over 7 months) reported that 1- or 2-dose vaccination after natural SARS-CoV-2 infection elicited more potent neutralizing activity compared with 2-dose vaccination in infection-naïve individuals against all variants of concern [5].

Our study was limited by the small number of subjects with prior infection and the lack of those with prior infection who received mRNA vaccines. Moreover, the inconsistent COVID-19 severity among participants with previous SARS-CoV-2 infection might be a confounder. Some may argue that the elderly itself or underlying conditions in patients with SARS-CoV-2 infection who did not receive the COVID-19 vaccines would affect more selectively rapid declining neutralizing antibody levels against the Omicron variant than the young HCWs with SARS-CoV-2 infection who received ChAdOx1 vaccines. However, the recent studies reporting that COVID-19 vaccination in persons with previous SARS-CoV-2 infection offers the highest level of protection against the omicron variant than unvaccinated individuals with prior SARS-CoV-2 infection [6-7] are consistent with our findings. Therefore, given that the neutralizing antibody levels against the original strain in the patients with SARS-CoV-2 infection who did not receive the COVID-19 vaccines were similar to those in HCWs with SARS-CoV-2 infection who received ChAdOx1, the age difference did not substantially affect our main findings on the waning neutralizing antibody levels against the Omicron variant.

Despite these limitations, our data suggest that natural infection followed by COVID-19 vaccination induces a more durable antibody response against the Omicron variant than natural SARS-CoV-2 infection or 2-dose COVID-19 vaccination alone.

Conflict of Interest

There are no conflicts of interest for any of the authors.

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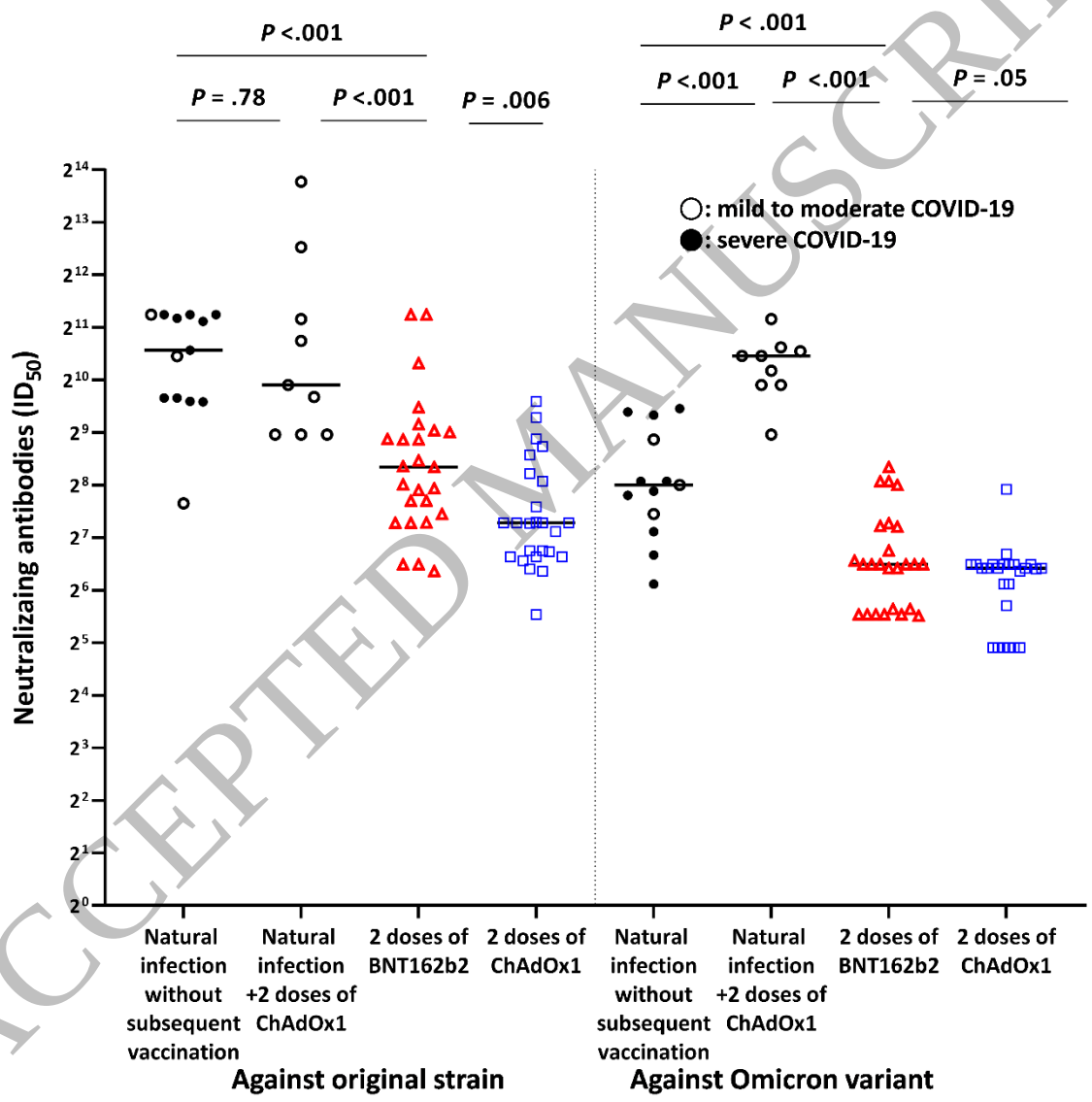
Figure legends

Figure 1. Comparison of neutralizing antibody against the original strain and Omicron variant at 6 months after natural SARS-CoV-2 Infection with/without subsequent COVID-19 vaccination versus 2-dose COVID-19 vaccination



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