Assessment on anti-SARS-CoV-2 receptor-binding domain antibodies among CoronaVac-vaccinated Indonesian adults

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The immunogenicity of CoronaVac among Indonesian adults at the academic premises was investigated. Two doses of CoronaVac vaccine induced a complete seroconversion on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) naïve adults with titers of anti-SARS-CoV-2 receptor-binding domain (RBD) antibodies ranging from 9.1 to 151.9 U/mL. The median value was lower than the one observed in recovered adults with mild coronavirus disease 2019 (38.7 vs. 114.5 U/mL). Nonetheless, 93.6% of the vaccinated adults, in contrast to 76.5% of the recovered adults, displayed inhibition rates above the cut-off to block RBD-angiotensin-converting enzyme 2 binding. This suggests that two doses of CoronaVac were immunogenic and likely to be protective among Indonesian adults.

Keywords: COVID-19, CoronaVac vaccine, Anti-SARS-CoV-2 RBD antibody

The CoronaVac is an aluminum hydroxide-adjuvanted, inactivated whole virus vaccine against coronavirus disease 2019 (COVID-19) [1], which has been approved by Indonesia's food and drugs authority for emergency use since early 2021. However, scientific data regarding the efficacy or even immunogenicity of the CoronaVac among Indonesian is limited. It was predicted that neutralizing antibody levels against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) served as a reliable indicator of immune protection from symptomatic COVID-19 [2]. An important subset of those neutralizing antibodies binds the angiotensin-converting enzyme 2 (ACE2)-binding site on the viral receptor-binding domain (RBD), i.e., anti-SARS-CoV-2 RBD antibodies, thus blocking the viral entry [3]. We therefore assessed anti-SARS-CoV-2 RBD antibodies, both quantitatively and qualitatively, among the CoronaVac-vaccinated adults at the academic premises.

This cross-sectional observational study was conducted by recruiting eligible adults of 18–55 years old at Universitas Pelita Harapan and Mochtar Riady Institute for Nanotechnology at Banten, Indonesia. The study was approved by the Mochtar Riady Institute for Nanotechnology Ethics Committee (2103008-05). Total participants were 89 subjects, classified into three groups. The first group consisted of 31 CoronaVac-vaccinated adults without any prior history of COVID-19 infection (i.e., SARS-CoV-2 naïve subjects). The time interval between the first and second dose was 14 days. Their blood samples were obtained 28 until 71 days after the 2nd dose. A minimum of 28 days after the 2nd dose was imposed because the publication on safety and immunogenicity of CoronaVac vaccination in healthy adults had analyzed the humoral responses on 28

Table 1. Descriptive data of gender, age, and time interval until blood collection (for the vaccinated and recovered groups)

Identity	Gender	Age (yr)	Interval between blood collection and second dose of vaccination (day)	Interval between blood collection and confirmation of diagnosis (day)
Vaccinated-1	F	23	53	NA
Vaccinated-2	F	53	54	NA
Vaccinated-3	F	28	52	NA
Vaccinated-4	F	32	53	NA
Vaccinated-5	F	26	53	NA
Vaccinated-6	M	25	53	NA
Vaccinated-7	M	23	54	NA
Vaccinated-8	F	36	54	NA
Vaccinated-9	F	28	54	NA
Vaccinated-10	M	33	54	NA
Vaccinated-11	M	55	54	NA
Vaccinated-12	M	33	53	NA
Vaccinated-13	M	26	55	NA
Vaccinated-14	F	24	56	NA
Vaccinated-15	F	33	55	NA
Vaccinated-16	F	34	55	NA
Vaccinated-17	F	37	55	NA
Vaccinated-18	F	31	56	NA
Vaccinated-19	F	31	61	NA
Vaccinated-20	F	22	61	NA
Vaccinated-21	M	24	61	NA
Vaccinated-22	M	27	61	NA
Vaccinated-23	M	39	33	NA
Vaccinated-24	F	37	66	NA
Vaccinated-25	F	23	61	NA
Vaccinated-26	M	41	28	NA
Vaccinated-27	M	35	31	NA
Vaccinated-28	F	23	31	NA
Vaccinated-29	M	31	31	NA
Vaccinated-30	M	26	32	NA
Vaccinated-31	M	31	71	NA
Recovered-1	F	20	NA	86
Recovered-2	F	21	NA	86
Recovered-3	F	19	NA	84
Recovered-4	F	20	NA	147
Recovered-5	F	20	NA	72
Recovered-6	F	20	NA	151
Recovered-7	F	20	NA	72
Recovered-8	F	21	NA	102
Recovered-9	F	20	NA	97
Recovered-10	F	21	NA	76
Recovered-11	F	20	NA	71
Recovered-12	F	20	NA	102
Recovered-13	F	21	NA	98

Table 1. Continued

Identity	Gender	Age (yr)	Interval between blood collection and second dose of vaccination (day)	Interval between blood collection and confirmation of diagnosis (day)
Recovered-14	F	21	NA	90
Recovered-15	F	21	NA	96
Recovered-16	F	20	NA	101
Recovered-17	F	20	NA	95
Recovered-18	F	20	NA	89
Recovered-19	M	20	NA	83
Recovered-20	M	20	NA	164
Recovered-21	M	20	NA	70
Recovered-22	M	20	NA	70
Recovered-23	M	18	NA	86
Recovered-24	F	20	NA	78
Recovered-25	F	20	NA	34
Recovered-26	F	19	NA	106
Recovered-27	F	20	NA	34
Recovered-28	F	20	NA	39
Recovered-29	M	19	NA	76
Recovered-30	M	20	NA	54
Recovered-31	M	19	NA	76
Recovered-32	F	19	NA	62
Recovered-33	F	22	NA	107
Recovered-34	M	29	NA	99
Control-1	M	36	NA	NA
Control-2	F	21	NA	NA
Control-3	F	26	NA	NA
Control-4	M	34	NA	NA
Control-5	F	22	NA	NA
Control-6	M	24	NA	NA
Control-7	F	29	NA	NA
Control-8	M	39	NA	NA
Control-9	M	43	NA	NA
Control-10	F	21	NA	NA
Control-11	F	21	NA	NA
Control-12	F	21	NA	NA
Control-13	M	37	NA	NA
Control-14	F	22	NA	NA
Control-15	F	23	NA	NA
Control-16	F	21	NA	NA
Control-17	F	22	NA	NA
Control-18	F	22	NA	NA
Control-19	M	23	NA	NA
Control-20	M	21	NA	NA
Control-21	M	20	NA	NA
Control-22	M	23	NA	NA
Control-23	F	22	NA	NA
Control-24	M	22	NA	NA

M, male; F, female; NA, not available.

days after the second dose [1]. The second group consisted of 34 recovered patients with mild COVID-19 cases. All subjects served self-isolation at their houses during the infection. None of the recovered adults had been vaccinated prior to the blood collection. Their blood samples were collected 34 until 164 days after being diagnosed with COVID-19, i.e., during the first 6 months after the diagnosis. The final group consisted of 24 adults without any prior history of COVID-19 infection and have not received any COVID-19 vaccination (i.e., the control group). No history of COVID-19 infection was supported by negative/non-reactive results upon prior screening using antigen- or antibody-detecting rapid diagnostic test. Subjects from all groups were bled once in April 2021 to obtain sera samples (Table 1). The sera samples were subsequently aliquoted into multiple tubes and frozen at -80°C until tested.

Two commercially available assays were used in this study. First, the Elecsys Anti-SARS-CoV-2 S assay (Roche, Basel, Schweiz), an electrochemiluminescence immunoassay, was used to measure titer of anti-SARS-CoV-2 RBD antibodies (including immunoglobulin G). Briefly, this assay was performed by an independent laboratory, according to the manufacturer's instruction by using the Cobas e 411 analyzer (Roche). The cut-off was at 0.8 U/mL, in which value below 0.8 U/mL was considered as non-reactive for anti-SARS-CoV-2 RBD antibody. Second, the GenScript SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) assay (GenScript, Singapore), an enzyme-linked immunosorbent assay (ELISA), was used to measure functionality of anti-SARS-CoV-2 RBD antibodies to block any interaction between RBD of the virus and ACE2 human cell-surface receptor. Briefly, the ELISA was performed according to the manufacturer's instruction by comparing the sera samples to the provided positive and negative controls. The percentage of inhibition was calculated by measuring the difference in the amount of labelled RBD between test versus control samples. The cut-off ratio for inhibition rate was at 20%, in which value below 20% was considered as no inhibition. Data analyses and visualization were performed using GraphPad Prism ver. 9.1.2 (GraphPad Software, San Diego, CA, USA).

By using the Elecsys assay to quantify anti-SARS-CoV-2 RBD antibodies in serum (Fig. 1A), both vaccinated (31 out of 31; 100%) and recovered (28 out of 34; 82.4%) groups were observed to have the antibodies, in contrast to the control group as baseline (0 out of 24; 0%). Pertaining to the vaccinated group, the titers ranged from 9.1 to 151.9 U/mL. In contrast, titers in the recovered group were more varied, ranging from 0.4 (consid-

ered non-reactive as below the cut-off) to 512 U/mL. This suggested that in most subjects, two doses of CoronaVac vaccine induced lower titers of anti-SARS-CoV-2 RBD antibodies than mild cases of COVID-19 (p=0.0107). Nonetheless, a higher rate of seroconversion was achieved by CoronaVac vaccination than by SARS-CoV-2 infection (100% versus 82.4%). In addition, 28 out of those 31 vaccinated adults were routinely monitored for titer of anti-SARS-CoV-2 RBD antibodies (14, 42, and 70 days after the 2nd dose) as they served in the COVID-19 reverse transcription-quantitative polymerase chain reaction (RT-qPCR) team (unrelated to this study). The finding was reassuring as the vaccination-induced antibodies persisted during the routine screening (Fig. 1B).

The sVNT assay was used to assess functionality of anti-SARS-CoV-2 RBD antibodies in serum to block the interaction between RBD and ACE2 (Fig. 1C). By using the cut-off at 20%, majority sera samples from the vaccinated (29 out of 31; 93.6%) and recovered (26 out of 34; 76.5%) groups were able to inhibit the RBD-ACE2 interaction. In contrast, almost no sera sample from the control group demonstrated an inhibitory activity (one out of 24; 0.04%). Despite the median value of inhibition rate in the recovered group was higher than the one in the vaccinated group, the difference was not statistically significant (p=0.4664). Fourteen out of 31 vaccinated adults displayed inhibition rates above 50%, hence their sera samples were further analyzed through serial dilution (final dilution at 1:40, 1:80, and 1:160). As shown in Fig. 1D, at 1:40 dilution, 14 out of 14 samples (100%) still had inhibition rates above 20%. However, there were only six out 14 samples (42.9%) and two out of 14 samples (14.3%) had inhibition rates above 20% at 1:80 and 1:160 dilution rates, respectively. This profound declining trend was likely due to lower titers of anti-SARS-CoV-2 RBD antibodies post-vaccination, as the Spearman's correlation indicated a strong correlation between titer and functionality of anti-SARS-CoV-2 RBD antibodies (Spearman's rho=0.9103, p<0.0001) (Fig. 1E). However, a caution must be given as the R^2 value was only 0.5470, suggesting that 45% of the variability in inhibition rates could not be explained by the titers of anti-SARS-CoV-2 RBD antibodies.

As prior mentioned, the vaccinated adults in this study were SARS-CoV-2 naïve subjects, hence could explained the low titers of induced antibodies. This finding was in line with a preprint result from Chile [4], reporting that after the CoronaVac vaccination, the SARS-CoV-2 naïve subjects produced lower neutralizing antibody response than the previously seropositive adults. In addition, our finding was supported by
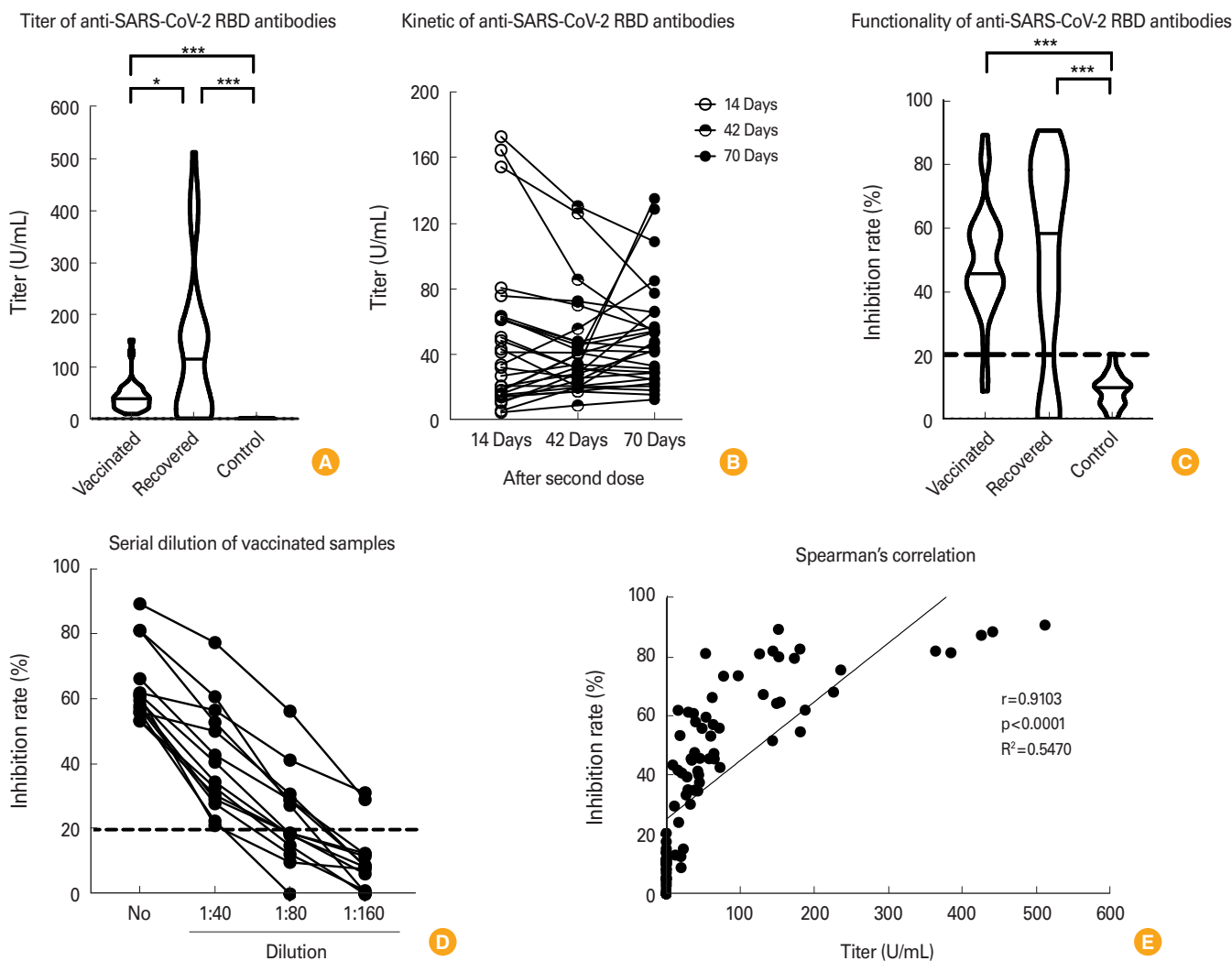
Fig. 1. Titer and functionality of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor-binding domain (RBD) antibodies. Sera samples from 89 adults, divided into the vaccinated group (n=31), the recovered with mild COVID-19 group (n=34), and the control group (n=24), were assessed for anti-SARS-CoV-2 RBD antibodies. (A) Titer of anti-SARS-CoV-2 RBD antibodies are depicted per group. Solid horizontal line within each violin plot refers to the median value. Mann-Whitney tests were performed to determine any statistically significant difference between two groups, in which * and *** signs refer to p=0.0107 and p<0.0001, respectively. (B) Titers of anti-SARS-CoV-2 RBD antibodies in 28 vaccinated samples were monitored on 14, 42, and 70 days after the second dose of vaccination as a part of the routine surveillance. (C) Functionality of anti-SARS-CoV-2 RBD antibodies are depicted per group. Solid horizontal line within each violin plot refers to the median value. Cut-off at 20% is represented by the broken horizontal line. Mann-Whitney tests were performed to determine any statistically significant difference between two groups, in which *** sign refers to p<0.0001. (D) Top 14 vaccinated samples with inhibition rates above 50% were subjected for serial dilution (1:40, 1:80, and 1:160). "No" refers to undiluted samples. Cut-off at 20% is represented by the broken horizontal line. (E) Spearman's correlation analysis on titers and inhibition rates of anti-SARS-CoV-2 RBD antibodies across 89 samples. Values of Spearman rho, p, and R^2 are depicted inside the box.

other studies [1,4], reporting that average levels of neutralizing antibodies in the recovered adults were higher than those in CoronaVac-vaccinated healthy adults. Nonetheless, it is important to mention that the neutralizing antibody responses in previously seropositive individuals could be further boosted by two doses of CoronaVac vaccines [4], providing a merit of using CoronaVac vaccine to protect against symptomatic

COVID-19.

There are limitations to our study. First, it was a small observational study as total analyzed subjects were 89. This was partly due to a limited vaccine's supply and the national prioritization strategy on selecting subjects to be vaccinated. Before April 2021, the vaccination was prioritized for elderlies and health care personnel, including those worked in the CO-

VID-19 RT-qPCR laboratory services. In addition, the blood collection was only performed if the vaccinated individuals already reached 28 days or more after the 2nd dose. Hence, it was difficult to find eligible vaccinated adults to participate in this study. Second, we could not exclude a possibility of asymptomatic cases in the control group. We screened eligible adults by performing a short interview with each candidate and checking their COVID-19 screening results with antigen- or antibody-detecting rapid diagnostic test in the past month. Only subjects with no history of COVID-19 and negative/non-reactive results would be recruited into the control group. Nonetheless, our results on anti-SARS-CoV-2 RBD antibodies demonstrated that no seroconversion observed in the control group. Thus, titers and inhibition rates observed in the control group could be used as the baseline values. Third, it is elusive whether our findings could be applied against more infectious variants of SARS-CoV-2, in particular the variant Delta (B.1.617.2). It has been reported that the variant Delta was more resistant to neutralization by some monoclonal antibodies toward RBD and N-terminal domain of SARS-CoV-2 as well as by two doses of Pfizer or AstraZeneca vaccine [5]. A preprint result from Thailand suggested a similar finding, in which CoronaVac-vaccinated subjects had a much reduced neutralizing capacity against the variant Delta [6]. As two doses of CoronaVac only induced relatively low titers of anti-SARS-CoV-2 RBD antibodies, it is likely that CoronaVac-vaccinated individuals will require a booster. It is important to note, however, that T-cell responses could be equally important and protective upon SARS-CoV-2 infection and COVID-19 vaccination [7,8]. Thus, the neutralizing levels of humoral immune response should not be interpreted as the only parameter of a successful COVID-19 vaccination.

In conclusion, we observed that the CoronaVac vaccine induced anti-SARS-CoV-2 RBD antibodies in healthy adults. Despite two doses of CoronaVac vaccine generated relatively low titers of anti-SARS-CoV-2 RBD antibodies, serum from majority of vaccinated adults could block the RBD-ACE2 binding. This suggests that the CoronaVac vaccination was immunogenic and likely to be protective among Indonesian adults.

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