

ORIGINAL ARTICLE

SARS-CoV-2 IgG antibody status in unvaccinated and 2-dose vaccinated Indonesians by AstraZeneca

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DOI: 10.4081/jphia.2023.2697

Abstract. Indonesia began deploying a COVID-19 vaccine in January 2021, prioritising vaccination for high-risk groups such as healthcare workers, the elderly and those with comorbidities, and ending with the general public due to limited vaccine availability. Our study aimed to evaluate antibody response in Indonesians who had received two doses of the vaccine vs. those who had not. The study design was a cohort study involving 46 unvaccinated people and 23 people who had received the second dose of the AstraZeneca vaccine in three months. Methods used for the qualitative and quantitative detection of IgG antibodies included rapid RI-GHA and ELISA tests. Findings showed that positive IgG antibodies qualitatively detected by the rapid RI-GHA test were significantly higher in those vaccinated (60.9%) than in unvaccinated people (26.1%). Using the ELISA assay, all vaccinated individuals qualitatively showed positive antibodies (cut-off ≥ 4.33 BAU/ml), and the average quantitative titer of anti-SARS-CoV-2 s-RBD IgG was significantly higher in vaccinated (157.06 ± 238.68 BAU/ml) than in unvaccinated (51.90 ± 87.60 BAU/ml) individuals. Some unvaccinated individuals with no history of infection were found to have anti-SARS-CoV-2 antibodies that may have been previously asymptomatic, although their mean antibody titers were certainly lower than those in the 2-dose group. Approximately 56% of vaccinated individuals had antibody titers above 60 BAU/ml as a cut-off for protective threshold, a significantly higher proportion than unvaccinated individuals. In conclusion, vaccination with two doses AstraZeneca increased anti-SARS-CoV-2 antibodies which resulted in enhanced immunity against symptomatic COVID-19.

Introduction

The Coronavirus Disease 2019 (COVID-19) outbreak was a global emergency as its rapid spread and high mortality rate caused severe disruptions (1). The COVID-19 outbreak was first reported in Wuhan, China, in late 2019 and spread to 216 countries and territories worldwide (2). The number of people infected with COVID-19 increased rapidly around the world. In Indonesia, the first two cases of COVID-19 were confirmed on March 2, 2020, with symptoms of fever, cough and shortness of breath (3,4). As of April 2, 2020, the number of positive confirmed cases of COVID-19 was 1,790 cases, with 113 new cases, 170 deaths and 112 recovered patients (5,6).

The Indonesian government took many steps and adopted policies to overcome the pandemic problem. One of the initial steps taken by the government was to introduce social and physical distancing and restrict movement among communities. This step aimed to break the chain of transmission of the COVID-19 disease by requiring people to maintain a safe distance of at least two metres from other humans, not to make direct contact with other people and to avoid mass gatherings. Moreover, people had to wear a mask and wash their hands properly according to the health protocols for avoiding COVID-19 infection (7).

Healthcare workers, the elderly and those with comorbidities are particularly high-risk (8). To expedite the end of COVID-19 transmission and protect high-risk populations, and of course also the general public, implementation of health protocols was not enough, and other effective interventions such as vaccination were also required (9). In the past, vaccination has been shown to be an efficient way to control a variety of infectious diseases (10-14). COVID-19 vaccines were successfully developed and released by several companies and research institutions after clinical trials to verify their effectiveness and safety. However, vaccines approved by the U.S. Food and Drug Administration (FDA) or Authorised for Emergency Use (EUA) were distributed to the Indonesian public.

Indonesia began vaccination against COVID-19 on January 13, 2021. COVID-19 vaccination in Indonesia has covered more than 75% of the population, with around 208

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Key words: SARS-CoV-2, vaccine, antibodies, ELISA, rapid test

million people being fully vaccinated (first and second doses). The program was carried out in four stages, starting from the most prioritised recipients to less prioritised categories. The Indonesian government obtained more than 426 million doses of vaccine from Sinovac Biotech, Novavax, COVAX/GAVI, Pfizer, and AstraZeneca (15). This enabled the government to administer sufficient doses to achieve herd immunity. In Indonesia, the vaccine acceleration strategy was implemented by expanding vaccine acceptance.

Vaccination induces antibodies that can neutralise the virus, which may be an affordable health strategy to prevent COVID-19 infection and minimise negative effects (16-18). The aim of this study was to evaluate the antibody response after vaccination in groups that received two doses of the vaccine vs. those that were not vaccinated, using serological tests.

Materials and methods

Sample collection. Our research was a cohort study conducted in September 2021. We recruited 69 healthy volunteers, including 46 unvaccinated people with no history of infection previously as controls and 23 people who had received a second dose of the AstraZeneca vaccine in around three months. Blood samples (5 ml of peripheral blood) were collected from each individual and then transported to the Institute of Tropical Diseases, Universitas Airlangga in 24 h. The samples were left at room temperature for 30 min to coagulate, then centrifuged at 1,300 relative centrifugal force (RFC) for 1 min in a swinging bucket rotor. The serum was then separated and transferred into clean tubes and frozen at -80°C until further use. Informed consent was obtained from the participants prior to the study. The design of this study was reviewed and approved by the Ethics Committee of Universitas Airlangga Hospital (approval number 163/KEP/2020).

Serological assays to detect SARS-CoV-2 antibodies

Qualitative detection of IgG antibodies to SARS-CoV-2 by Rapid RI-GHA test. The rapid antibody tests, namely RI-GHA (Republic of Indonesia-Gadjah Mada-Hepatika-Airlangga, Indonesia) kits developed by researchers in Indonesia, were used in this study and performed according to the manufacturer's instructions, for the COVID-19 IgG rapid tests. The results were read after 10 min (max 15 min) with the naked eye. The test was considered positive if a line was observed on the control and test (IgG positive) areas. The intensity of the colour was not relevant.

Qualitative and Quantification detection of anti-SARS-CoV-2 Receptor Binding Domain (RBD) IgG by ELISA assays. IgG antibodies were measured with an ELISA method, a two-step chemiluminescent microparticle immunoassay SARS-CoV-2 IgG anti-RBD (SNIBE, Shenzhen, China). The assays were performed according to the manufacturer's instructions. Results ≥ 1 AU/ml were considered positive. The cut-off value in arbitrary units (AU)/ml, the conversion factor to obtain binding antibody unit (BAU)/ml, the cut-off value in BAU/ml and the linearity range in AU/ml respectively are: 1, 4.33, 4.33 and 0.18-100, as declared by the manufacturer. Binding antibody units per millilitre (BAU/ml) as proposed by the World Health Organization (WHO) to standardise any

device to the WHO-International Standard (WHO-IS) were calculated by applying the conversion factors suggested by the manufacturers, whenever possible.

Statistical analysis. Categorical data were presented in numbers and percentages and continuous data were presented in mean, Standard Deviation (SD) and range. Differences according to demographic variables in antibody test results were tested by independent samples t-test and χ^2 test for continuous variable and categorical variable, respectively. The mean antibody concentration was further compared according to gender and age. Two-sided P-values were reported, and a P-value < 0.05 was considered statistically significant. The analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

Results

Study participants were 32 men and 37 women with an average age of 45.52 years. The mean age of participants in the unvaccinated and 2-dose vaccinated groups was 47.24 and 40.35 years, respectively. In the vaccinated group in particular the average duration after administering the second dose of the vaccine was 2.9 months. Among the findings, the qualitative detection of SARS-CoV-2 antibodies by the rapid test RI-GHA showed significantly higher positive results in the 2-dose vaccinated group (14/23, 60.9%) than in the unvaccinated group (12/46, 26.1%). However, antibody response in unvaccinated individuals may result from natural infection, whether current or previously asymptomatic or symptomatic. Even so, the study includes unvaccinated individuals without a history of COVID-19 infection previously and for the current infection, we did not testing for the SARS-CoV-2 virus.

In our study, antibodies were also detected by ELISA assay for a qualitative and quantitative anti s-RBD SARS-CoV-2 IgG. There was no significant difference in the frequency of antibody-reactive results between the unvaccinated and vaccinated groups. For ELISA tests in particular, the result is classified as reactive or positive if it exceeds a threshold of 1 AU/ml (converted to BAU/ml this becomes 4.33 BAU/ml). Overall, the frequency of non-reactive results with antibody titers below 4.33 BAU/ml was seen in only six unvaccinated individuals, with the rest of the unvaccinated (40/46, 86.96%) and all vaccinated individuals (23/23, 100%) showing high antibody titers equal to or greater than 4.33 BAU/ml (Table I).

Anti-SARS-CoV-2 IgG s-RBD antibody titers determined by ELISA assays were grouped according to vaccination status, and IgG reactivity results were measured by the rapid assay RI-GHA. The results showed that the mean titer of the unvaccinated group was 51.90 BAU/ml, which was significantly lower than that of the vaccinated group (157.06 BAU/ml) (Fig. 1A). There were also significant differences when comparing mean titers between groups with IgG reactivity and non-reactivity detected by the rapid assay RI-GHA. In the IgG result group, the average titer of the IgG reactive group as detected by the rapid test RI-GHA (196.83 BAU/ml) was significantly higher than that of the non-reactive group (20.52 BAU/ml) (Fig. 1B).

In our study, the proportion of participants with antibody response was determined by cut-off between protective

Table I. Characteristics of Unvaccinated and Vaccinated groups.

Characteristics	Vaccination status		P-value ^a
	Unvaccinated (N=46)	Vaccinated two doses (N=23)	
Age (average years \pm STD)	47.24 \pm 19.97	40.35 \pm 9.85	0.059
<30 years old	7 (15.2%)	3 (13%)	
\geq 30 years old	39 (84.8%)	20 (87%)	1.000
Gender, n/N (%)			
Male	28/46 (61%)	4/23 (17%)	0.002
Female	18/46 (39%)	19/23 (83%)	
Duration after vaccinated 2-dose (average months)	-	2.9	-
Antibody detected by Rapid Test R-GHA, n/N (%)			
IgG reactive (positive)	12/46 (26.1%)	14/23 (60.9%)	
IgG non-reactive (negative)	34/46 (73.9%)	9/23 (39.1)	0.011
Anti-SARS-CoV-2 IgG s-RBD detected by ELISA, n/N (%)			
Reactive (\geq 4.33 BAU/ml)	40/46 (86.96%)	23/23 (100%)	
Non-reactive ($<$ 4.33 BAU/ml)	6/46 (13.04%)	0/23 (0%)	0.070

^aP-value less than 0.05 indicates significant difference between the two groups and was marked by bold in table.

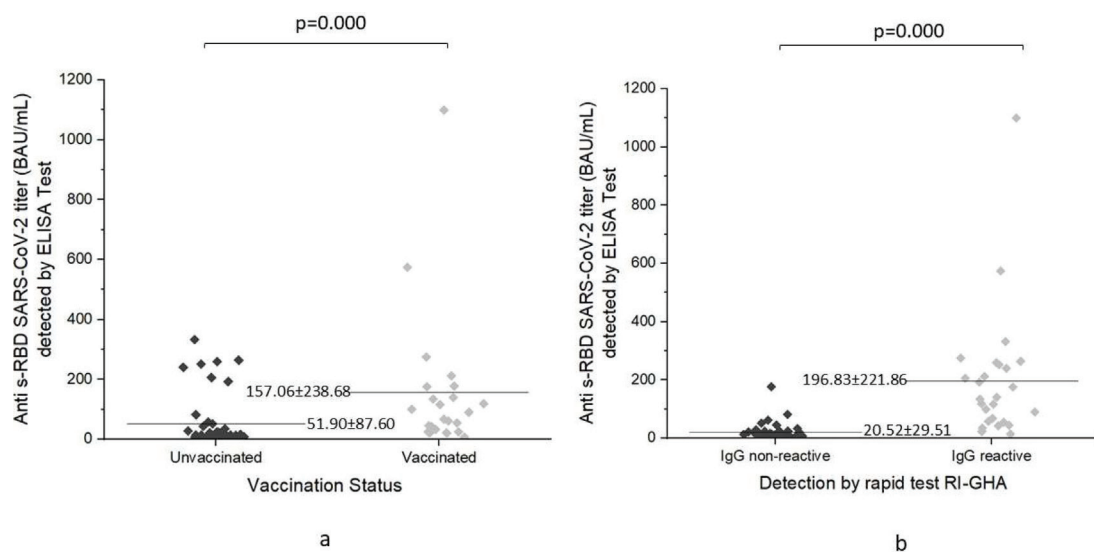


Figure 1. Comparisons of anti-SARS-CoV-2 s-RBD mean titer (BAU/ml) (A) between: unvaccinated and vaccinated (after 3 months by 2-dose) participants and (B) between IgG reactivity test results detected by rapid test RI-GHA. Horizontal bars indicate are the mean of antibodies titers. Statistical significance was determined using two-tailed t-tests. Significant differences are $P<0.05$.

and non-protective antibody levels; *i.e.* that all individuals, whether immunised or controls, with antibody above the threshold are protected and those with antibody below it are at risk. The protective level of SARS-CoV-2 antibodies titer used was 60 BAU/ml and 154 BAU/ml, according to a previous study (19). The data shows that among those who received vaccine, a considerable number had antibodies above 60 BAU/ml, a relatively high proportion, even 3 months after the second dose, and the difference was significant compared with those who had not been vaccinated (Fig. 2). However, as to the protective threshold of 154 BAU/ml there was no significant difference between the two groups (Fig. 2).

Discussion

During the COVID-19 pandemic, vaccination was a strategy to achieve herd immunity and combat spread of the disease. Indeed, the high burden of COVID-19 prompted the development and distribution of effective vaccines to slow the spread of the virus, reduce disease severity and reduce mortality. While vaccine development can typically take up to 10-15 years, a COVID-19 vaccine could be developed less than a year after COVID-19 was discovered. Different platforms of approved vaccines are currently being administered all over the world: mRNA vaccines (Pfizer-BioNTech, Moderna); recombinant

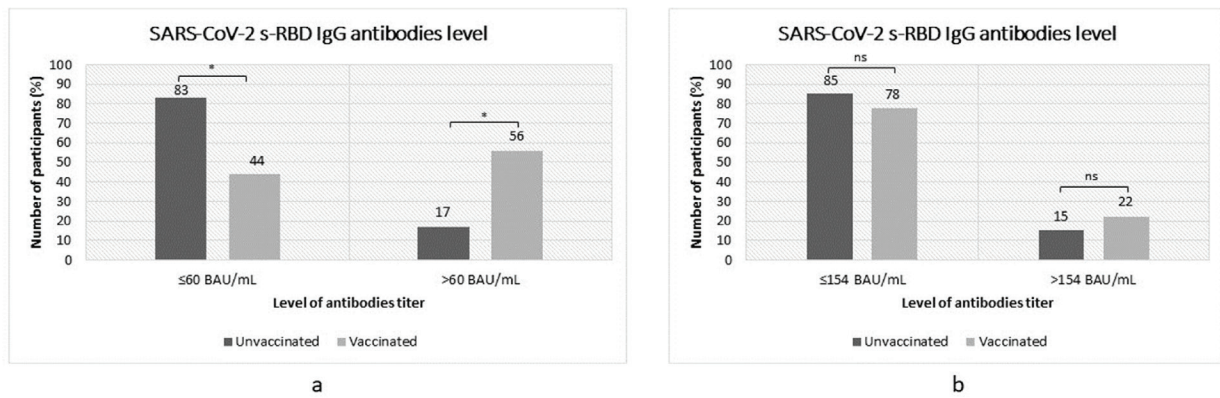


Figure 2. Proportion of individuals unvaccinated and 2-dose vaccinated over 3 months according to anti-SARS-CoV-2 s-RBD titer (BAU/ml) level (A) less and greater than 60 BAU/ml (B) less and greater than 154 BAU/ml. Statistical significance was determined using Chi-Square tests. Significant differences are indicated by asterisk (*) if P-value<0.05.

adenovirus vectored vaccines (AstraZeneca, Cansino, Gamaleya, Johnson Pharm); inactivated vaccines (Sinopharm, Sinovac) (20-23).

Indonesia's COVID-19 vaccination target was to cover at least 75% of the Indonesian population, or about 208 million people. Due to the limited availability of a COVID-19 vaccine, the vaccination program was carried out in four stages, starting from the most prioritised to the less prioritised (24). In the beginning Indonesia procured 400 million doses of vaccines. The vaccines were from Sinovac, Novavax, Pfizer, and AstraZeneca (25). The AstraZeneca vaccine in particular was authorised for use by the National Agency of Drug and Food Control (BPOM) in March (26). COVID-19 vaccination for people aged 18 and over began in early May, with two mandatory doses, including of the AstraZeneca vaccine (27). Vaccines against COVID-19 did not reach many people in Indonesia at first. Therefore, our study aimed at qualitative and quantitative assessments of Immunoglobulin G (IgG) antibody response in people who had received the second dose of the AstraZeneca vaccine over three months ago, compared with unvaccinated people. In this study, IgG antibodies were qualitatively detected using rapid assays as well as ELISA assays. In addition, ELISA assays were also used to quantify antibodies.

Rapid antibody test used was the rapid RI-GHA (which stands for the Republic of Indonesia-Gajah Mada Hepatika Airlangga) test developed by Indonesian researchers, through collaboration between Universitas Gajah Mada, Hepatika Laboratorium and Universitas Airlangga (28). The results showed that the positive rate of IgG antibodies qualitatively detected by the rapid RI-GHA test was significantly higher in vaccinated individuals (60.9%) than in unvaccinated individuals (26.1%). Rapid RI-GHA tests did not detect SARS-CoV-2 antibodies in all vaccinated individuals, suggesting that negative results of rapid tests may be due to antibody titers below the detection threshold (LOD) of such tests. No data were yet available on the LOD of the rapid RI-GHA assay, but all negative results in our study were found to be below 50 BAU/ml by ELISA assay in vaccinated individuals. When compared with the results of antibodies titer detected by ELISA test, antibodies in the reactive group (196.83 ± 221.86 BAU/ml) by rapid RI-GHA test were significantly higher on average

than in the non-reactive group (20.52 ± 29.51 BAU/ml). The rapid assay kits might only be beneficial for detecting higher IgG titers (29).

All vaccinated individuals tested positive for qualitative antibody reactivity by ELISA, as determined using an ELISA with a cut-off anti-SARS-CoV-2 s-RBD titer of 4.33 BAU/ml, although not significantly compared to unvaccinated individuals. However, the quantitative values for the average of anti-SARS-CoV-2 s-RBD titers detected by ELISA assay in the vaccinated group (157.06 ± 87.60 BAU/ml) were significantly higher than those for the unvaccinated group (51.90 ± 87.60 BAU/ml). The amount of antibody titers can be used to indicate vaccinated persons who have protective immunity. In previous studies, anti-spike IgG antibody concentrations in 154 BAU/ml (for mRNA vaccine) and 60 BAU/ml (for vaccines other than mRNA vaccines, including AstraZeneca) after vaccination have been suggested as a potential threshold for protective immunity against symptomatic COVID-19 (19,30). In our data, 13/23 (56%) of those who received a second dose had an antibody titer above 60 BAU/ml after 3 months, whereas only 8/46 (17%) of unvaccinated people had more than 60 BAU/ml. The purpose of the protective threshold (sometimes referred to as the minimum antibody protective level) is to define the boundary between antibody levels considered sufficient to provide protection in the population and antibody levels considered not protective (19). Our results assume that vaccinated individuals with antibody levels above the threshold of 60 BAU/ml are expected to be protected. However, some vaccinated people still had low titers that were expected to rise after 3 months. The immune response varies from person to person (31).

The detection of COVID-19 antibodies in some unvaccinated individuals suggests that they may have asymptomatic or symptomatic infection without a history of COVID-19, although their average of antibody titers was certainly lower than in the 2-dose group. Some vaccines require multiple doses, such as two or more doses, weeks or months apart, to generate enough immune antibodies. Multiple doses are sometimes needed to allow for the production of long-lived antibodies and the development of memory cells (32). Over time the antibodies will gradually disappear, but the memory B cells will remain dormant in your body for many years (33,34).

However, previous systematic reviews pooled sensitivity stratified by test type and immunoglobulin class and reported lower sensitivities with the rapid or Lateral Flow Immunochromatographic Assay (LFIA) tests compared with ELISA and Chemiluminescent Immunoassay (CLIA) (35). The qualitative performance of the developed ELISA gave 96% sensitivity, 97.5% specificity and 0.968 accuracy (36). In addition, it is important to evaluate the SARS-CoV-2 IgG antibodies level using ELISA for quantity detection, as a measure of immunity against COVID-19. Nevertheless, the rapid (minutes vs. hours) and instrument-free detection of SARS-CoV-2 and low-cost tests such as the rapid RI-GHA test are required to detect antibody response as an alternative solution for mass screening of large populations in surveillance of vaccine studies.

Conclusions

In conclusion, vaccination was shown to increase antibodies against SARS-CoV-2 as detected by rapid RI-GHA test and ELISA assay. Most 2-dose vaccinated individuals by AstraZeneca had anti-SARS-CoV-2 titers to protect against later infection and disease severity.

Acknowledgments

Samples were collected from Universitas Airlangga staff, in particular those of the Faculty of Public Health and the community surrounding the Universitas Airlangga campus. We thank them for the supporting the samples collection.

Funding

This work is supported by the Internal Funding of Universitas Airlangga 2021.

Availability of data and materials

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Authors' contributions

LNy, J, NLAM, conceptualization; LNy, J, NS, ASP, SMD, NLAM, sample collection, laboratory experiment, literature search and data analysis; LNy, J, NLAM, TU, SM, MAI, MIL, original draft preparation; LNy, design of figures; LNy, J, NLAM, TU, SM, MAI, MIL, review and editing; CYL, TU, SM, MAI, MIL, supervision. All the authors have read and approved the final version of the manuscript and agreed to be held accountable for all aspects of the work.

Ethics approval and consent to participate

This study protocol was reviewed and approved by the Ethics Committee of Universitas Airlangga Hospital, approval number 163/KEP/2020. Written informed consent was received from the participants to participate in the study before collecting data and samples.

Conflict of interest

The authors declare no potential conflict of interest.

Accepted: 18, November 2023; received: 13, May 2023.

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