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Short Communication

Comparative study of anti-SARS-CoV-2 receptor-binding domain total antibody titer before and after heterologous booster with mRNA-based COVID-19 vaccine

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Abstract

The waning immunity following the COVID-19 vaccination become a significant concern and the immunological dynamics of vaccine-induced antibodies after vaccination need to be explored. The aim of this study was to compare anti-SARS-CoV-2 receptor-binding domain (RBD) antibody levels before and after a booster dose with heterologous COVID-19 vaccine and to identify factors influencing the levels after receiving the booster dose. A cross-sectional study was conducted in which individuals who received primary doses of CoronaVac and a booster dose with an mRNA-based vaccine were recruited using a purposive sampling technique. The titers of anti-SARS-CoV-2 RBD antibodies were measured using an enzyme-linked immunosorbent assay (ELISA), and plausible associated factors were collected using a questionnaire-assisted face-to-face interview. The Wilcoxon test was used to compare the titers before and after the booster dose, while the Kruskal-Wallis and Mann-Whitney tests, followed by multivariate linear regression, were used to assess the factors associated with RBD total antibody titers. The results showed that there was a significant increase of anti-SARS-CoV-2 RBD total antibody titers before and after receiving the booster dose (1,558.7 binding antibody units (BAU)/mL vs 140.6 BAU/mL, $p<0.001$). The analysis revealed that age ($p=0.555$), sex ($p=0.254$), type of vaccine ($p=0.914$), presence of hypertension ($p=0.541$), diabetes ($p=0.975$), chronic obstructive pulmonary disease (COPD, $p=0.620$), and gout ($p=0.364$) were not associated with anti-SARS-CoV-2 RBD total antibody titers. However, the titers of anti-SARS-CoV-2 RBD total antibody were significantly different between those with and without hyperlipidemia ($p=0.021$). This study suggests that a booster dose with a heterologous COVID-19 vaccine could significantly enhance immune responses against COVID-19, and therefore, this strategy may be recommended as part of preventive measures to strengthen immunity against COVID-19.

Keywords: Anti-SARS-CoV-2 receptor-binding domain total antibody, booster vaccine, heterologous vaccine, mRNA vaccine, COVID-19

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Introduction

Indonesia is one of the countries affected by the coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with a significant number of confirmed cases and deaths [1,2]. Following SARS-CoV-2 infection, the body responds by producing immunoglobulin M (IgM), which persists for 1–2 weeks, and immunoglobulin G (IgG), which persists for several weeks after the symptoms [3]. Seroprevalence studies reported that the titer of IgG to glycoprotein S increased in the first three weeks and then decreased in the following eight weeks [4,5]. This could increase the risk of reinfection.

One important preventive strategy for COVID-19 is the development of effective and safe vaccines [6] and multiple COVID-19 vaccines have efficacy above 50%, such as Sinovac vaccine (CoronaVac) 78.0%, the mRNA-1273 vaccine (Moderna) 94.1%, the BNT162b2 mRNA vaccine (Pfizer) 95.0%, and the ChAdOx1 nCoV-19/AZD1222 vaccine (AstraZeneca) 70.4% [7]. With the continuous emergence of the SARS-CoV-2 variant, there have been reports of a decrease in the effectiveness of these vaccines against SARS-CoV-2 infection [8,9].

As protection against SARS-CoV-2 infection continues to decrease after the second dose of vaccine, a policy of administering a third dose at regular intervals, known as a booster, is recommended [10]. A study showed that more than 20% of samples 5–6 months post-vaccination had anti-SARS-CoV-2 receptor-binding domain (RBD) total antibody less than the protective level, indicating a loss of protection from the second dose of COVID-19 vaccine after three months [11]. This significant reduction suggests the need for an additional booster dose to protect against COVID-19 [11]. The United Kingdom COMCOV trial showed that a heterologous booster vaccine (a different type of vaccine from the primary doses) was more immunogenic than a homologous vaccine [10]. Therefore, more studies comparing the immune responses between heterologous and homologous boosters, as well as assessing factors associated with reduced immune responses, are important. Therefore, the aim of this study was to evaluate the differences in immune responses in individuals receiving the second dose and after the heterologous booster dose of the COVID-19 vaccine.

Methods

Study design and samples

A cross-sectional study was conducted in Banda Aceh, Indonesia, in 2022 involving residents who had received the inactivated COVID-19 vaccine. Participants were aged between 18 and 65 years old, had no history of a COVID-19 diagnosis, and had received a booster dose of mRNA-based COVID-19 vaccine within 1 to 12 months prior to the recruitment period. The participants were recruited using a purposive sampling technique, a non-probability method where participants were selected based on predefined criteria. The minimum sample size was estimated using a sample size calculator [18], of which the alpha level of 0.05 and study power of 80% were set in the calculation. Based on a published recommendation reporting an average anti-SARS-CoV-2 RBD total antibody titer of 119.95±78.08 at 2–3 months [15], the required minimum sample size was calculated to be 148 respondents.

Study variables

The dependent variable, the anti-SARS-CoV-2 RBD total antibody titer, was assessed using the enzyme-linked immunosorbent assay (ELISA). The results were reported in binding antibody units per milliliter (BAU/mL) on a numeric scale. Some plausible independent variables such as demographic characteristics (age and sex), vaccination type (BNT162b2 and mRNA-1273), and comorbidities (hypertension, diabetes, hyperlipidemia, chronic obstructive pulmonary disease (COPD), asthma, and gout) were also collected. The plausible independent variables were collected through direct face-to-face interviews guided by a predetermined questionnaire.

Determination of anti-SARS-CoV-2 receptor-binding domain (RBD) total antibody titers

A total of 3 mL venous blood was collected, and the serum was separated and kept at -80°C until used. A total of 20 µL of the serum sample was used to measure the anti-SARS-CoV-2 RBD total

antibody titers using ELISA Elecsys Anti-SARS-CoV-2 S immunoassay using Roche cobas E411 (Roche Diagnostics, Basel, Switzerland) following the manufacturer's recommendation.

Data analysis

The Wilcoxon test was used to compare anti-SARS-CoV-2 RBD total antibody titers before and after the booster dose of the heterologous mRNA-based COVID-19 vaccine. In univariate, the Kruskal-Wallis test and Mann-Whitney test were used to determine factors associated with the titer of anti-SARS-CoV-2 RBD total antibody. In multivariate analysis, where all variables with $p<0.25$ in the univariate analysis were included, and multivariate linear regression was employed. All analyses were conducted using SPSS (IBM, New York, USA).

Results

Characteristics of research subjects

A total of 158 respondents who met the inclusion criteria were included of whom the anti-SARS-CoV-2 RBD total antibody titers were measured after receiving the heterologous mRNA-based booster dose. Characteristics of respondents included in this study are presented in **Table 1**. More than 50% of the total respondents were 20–30 years old, whereas the other 22.2% were 31–40 years old. Females accounted for 57.2% of the respondents. Among the participants, 3.2% had hypertension, 1.9% had hyperlipidemia, 7.0% had asthma or a history of asthma, and 1.3% had gout. Out of the total, 54.4% received Pfizer (BNT162b2) and 45.6% received Moderna (mRNA-1273) vaccines.

Table 1. Characteristics of respondents receiving primary doses of inactivated-based COVID-19 vaccine followed a booster dose of heterologous mRNA-based included in this study (n=158)

Characteristics	Frequency (%)
Age, years	
<20	18 (11.4)
20–30	85 (53.8)
31–40	35 (22.2)
>40	20 (12.7)
Sex	
Male	67 (42.1)
Female	91 (57.2)
Vaccine type	
Pfizer (BNT162b2)	86 (54.4)
Moderna (mRNA-1273)	72 (45.6)
Hypertension	
Yes	5 (3.2)
No	153 (96.8)
Diabetes	
Yes	1 (0.6)
No	157 (99.4)
Hyperlipidemia	
Yes	3 (1.9)
No	155 (98.1)
Chronic obstructive pulmonary disease (COPD)	
Yes	1 (0.6)
No	157 (99.4)
Asthma	
Yes	11 (7.0)
No	147 (93.0)
Gout	
Yes	2 (1.3)
No	156 (98.7)

Titer of anti-SARS-CoV-2 RBD total antibody before and after receiving heterologous mRNA-based booster

The titer of anti-SARS-CoV-2 RBD total antibody was determined in recipients of the mRNA-based booster dose vaccine and compared to levels prior to the booster dose. In this study, not all respondents had the titer of anti-SARS-CoV-2 RBD total antibody measured prior to booster

dose. The changes in anti-SARS-CoV-2 RBD total antibody titer among those who had both titers are presented in **Figure 1**. Data indicated that the titer of anti-SARS-CoV-2 RBD total antibody increased significantly following the booster dose. The mean titers of the total antibody before and after the vaccine booster dose were statistically significant (140.6 vs 1,558.7 BAU/mL, $p<0.001$).

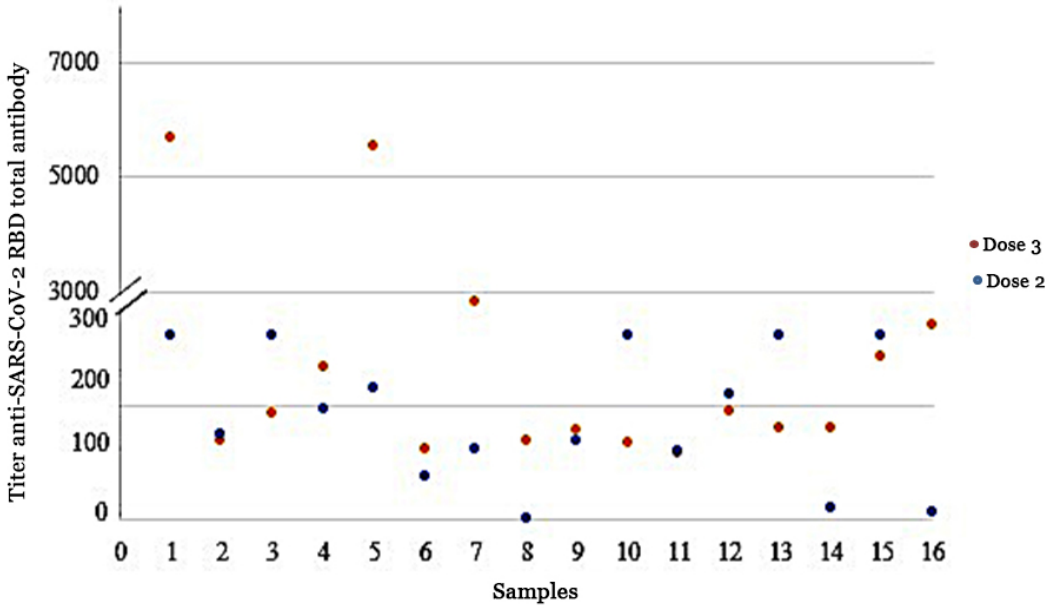

Figure 1. Changing in titers of anti-SARS-CoV-2 receptor-binding domain (RBD) total antibody of individuals receiving a booster dose of heterologous COVID-19 vaccine. The mean of titers after the booster dose was significantly higher at $p<0.001$.

Factors affecting titer of anti-SARS-CoV-2 RBD total antibody post booster dose of heterologous COVID-19 vaccine

Our data suggested that the mean titers of anti-SARS-CoV-2 RBD total antibody among respondents with hyperlipidemia were significantly different compared to those without hyperlipidemia (4,100.27 vs 1,494.97 BAU/mL, $p=0.021$). Other factors, including age ($p=0.555$), sex ($p=0.254$), type of mRNA vaccines ($p=0.914$), and comorbidities such as hypertension ($p=0.541$), diabetes ($p=0.975$), COPD ($p=0.620$), asthma ($p=0.239$), and gout ($p=0.364$) had no association with antibody titers (**Table 2**).

Table 2. Factors associated with the titer of anti-SARS-CoV-2 receptor-binding domain (RBD) total antibody after booster dose with heterologous mRNA-based COVID-19 vaccine (n=158)

Variable	Anti-SARS-CoV-2 RBD total antibody titer Mean±SD (BAU/mL)	*p*-value[a]
Age, years		0.555[b]
<20	1,736.9±1,775.1	
20–30	1,424.5±1,526.1	
31–40	1,455.0±1,340.7	
>40	2,037.1±1,950.1	
Sex		0.254
Male	1,401.5±1,489.9	
Female	1,649.6±1,635.5	
Vaccine type		0.914
Pfizer (BNT162b2)	1,638.1±1,753.0	
Moderna (mRNA-1273)	1,432.6±1,336.1	
Hypertension		0.541
Yes	2,137.4±2,180.2	
No	1,525.0±1,557.6	
Diabetes		0.975
Yes	0.0±0.0	
No	1.547.8±1,579.7	
Hyperlipidemia		0.021

Variable	Anti-SARS-CoV-2 RBD total antibody titer Mean±SD (BAU/mL)	*p*-value[a]
Yes	4,100.2±1,640.7	
No	1,494.9±1,537.9	
Chronic obstructive pulmonary disease (COPD)		0.620
Yes	0.0±0.0	
No	1,543.9±1,580.3	
Asthma		0.239
Yes	2,182.1±1,904.3	
No	1,496.7±1,904.3	
Gout		0.364
Yes	560.9±380.7	
No	1,557.1±1,581.1	

[a] Otherwise stated the *p*-value was obtained from Mann-Whitney test; otherwise, [b]Kruskal-Wallis test

Next, hyperlipidemia and asthma with $p<0.250$ were then included in multivariate linear regression analysis to assess their effect on anti-SARS-CoV-2 RBD total antibody titers compared to non-hyperlipidemia and non-asthma individuals. Analysis of the full model showed hyperlipidemia and asthma influenced the titer of anti-SARS-CoV-2 RBD total antibody ($R^2=0.066$; $F=5.442$; $p<0.01$) (**Table 3**). In the final model, where only a significant variable in the full model was included, hyperlipidemia was shown to have a significant effect on the titer of anti-SARS-CoV-2 RBD total antibody ($R^2=0.051$; $F=8.430$; $p<0.01$) (**Table 3**).

Table 3. Multivariate analysis showing factors associated with anti-SARS-CoV-2 receptor-binding domain (RBD) total antibody titers after a booster dose of heterogonous COVID-19 vaccine (n=158)

Variables	Unstandardized coefficient (B)	Standardized coefficient (Beta)	T	*p*-value
Full model ($R^2=0.066$; $F=5.442$; $p<0.01$)				
Hyperlipidemia (non-hyperlipidemia as reference group)	-2.657.78	893.99	-2.97	0.003
Asthma (non-asthma as reference group)	-739.58	479.41	-1.54	0.125
Reduced model ($R^2=0.051$; $F=8.430$; $p<0.01$)				
Hyperlipidemia (non-hyperlipidemia as reference group)	-2.605.29	897.29	-2.90	0.004

Discussion

In this study, anti-SARS-CoV-2 RBD total antibody titers were measured in 16 respondents before and after receiving the booster dose of mRNA-based COVID-19 vaccine, and statistical analysis indicated a significant increase in titers after the booster dose. Compared to the titers observed following the second CoronaVac dose, the booster dose produced antibody levels approximately ten times higher. This aligns with a previous study reporting that a third mRNA booster dose given at least five months after the second dose increased vaccine efficacy to 93% in preventing hospitalization [12]. Another study found that after the third dose of the COVID-19 mRNA vaccine, administered at least seven months after the second, titers rose to 92% and remained above 50% even 6–8 months later, suggesting that the third dose generated high and long-lasting antibody titers [13]. Additionally, studies found that an increase in anti-SARS-CoV-2 RBD total antibody titers provides protection from infection and disease severity [14,15]. Another study found that anti-SARS-CoV-2 RBD total antibody titers increased >90% after a booster dose of the BNT162b2 mRNA vaccine [16].

Vaccination is the most promising public policy to reduce the incidence of severe COVID-19 and death cases [17]. The expected result of COVID-19 vaccination is the production of increased titers of anti-SARS-CoV-2 RBD total antibodies, which are antibodies that have the effect of neutralizing the virus naturally by the host body, therefore, preventing the onset of COVID-19 infection and providing a duration of protection for a certain period of time [18]. Vaccines are able to induce the body's immune system and stimulate T lymphocytes and B lymphocytes to form a defense against disease-causing microorganisms [18]. Class II CD4+ T cells will activate B cells

which then proliferate and differentiate into plasma cells and memory B cells. The plasma cells will produce an anti-SARS-CoV-2 RBD total antibody that binds to spike proteins of SARS-CoV-2, while memory B cells could accelerate antibody formation in the event of reinfection or future exposure to the SARS-CoV-2 [19].

In this study, the only factor associated with anti-SARS-CoV-2 RBD total antibody titers after receiving the mRNA-based booster dose vaccine was hyperlipidemia, while other factors were not associated with total antibody titers. A study stated that the immune response due to vaccine administration was more strongly detected in children and adolescents aged 16–25 years against SARS-CoV-2 infection [20]. Another study that compared the immune response after BNT162b2 vaccination in two different age groups (>80 years; <60 years) found that ELISA-IgG and anti-SARS-CoV-2 RBD total antibody titers were lower in the >80 years age group [21]. The difference in antibody titer levels in each age category could be influenced by immunosenescence, which is described as the reduction of adaptive immune response in older age groups. In addition, older people have a high rate of morbidity due to infections and lack of responses to vaccination due to decreased cellular and humoral immunity [21]. However, our study found that the titers of anti-SARS-CoV-2 RBD total antibodies were not significant based on the age groups.

In the present study, there was also no significant effect of sex on the titer of the anti-SARS-CoV-2 RBD total antibody. However, a previous study found that women produced a higher humoral response than men after receiving the vaccine [22]. A study conducted in Northern Mexico found that women produced significantly higher titers of anti-SARS-CoV-2 RBD total antibody than men after BNT162b2 vaccination [23]. Based on a study, women have stronger adaptive cellular and humoral immune responses compared to men [24]. A study conducted to compare the estrogen and androgen hormones in women and men with the mortality rate of COVID-19 patients found that estrogen is more effective in regulating inflammation and immune response than androgen, making it a protective effect and could prevent death in COVID-19 patients [25]. Estrogen, a female hormone, could downregulate ACE2 receptors, which are SARS-CoV-2 receptors, regulating cell metabolism and improving their function, resulting in reduced incidence of SARS-CoV-2 infection [26,27]. The discrepancies observed between the findings of the present study and those of previous studies could be attributed to variations in sample size, demographic characteristics, vaccine types, or vaccination timelines, as well as differences in pre-existing immune responses or hormonal levels.

This study had some limitations that need to be addressed. Limited community interest in participating in the study led to an uneven sample distribution and reduced diversity in respondent characteristics. Data collection on previous COVID-19 infection and medical history relied on self-reported interviews, which could introduce recall bias and inaccuracies. In addition, although the sample size met the statistical requirement, this study had a limited number of respondents who had two titters available; therefore, a larger sample size might provide better generalizability and representation of the population.

Conclusion

The mRNA-based booster dose in individuals who previously received two doses of the CoronaVac vaccine resulted in a 10-fold increase in anti-SARS-CoV-2 RBD total antibody titers (1,558.7 BAU/mL vs 140.6 BAU/mL, $p<0.001$). A history of hyperlipidemia significantly influenced antibody titers, while other factors such as age, sex, vaccine type, and comorbidities like hypertension, diabetes, COPD, and gout showed no effect on antibody levels following the booster dose.

Ethics approval

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (No: 034/EA/FK/2022). All participants were informed about the study procedures and provided written consent before participation.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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