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Original Article

Detection of SARS-CoV-2 antibodies among the pre-vaccine population in Bali

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the cause of coronavirus disease 2019 (COVID-19), has affected millions of people globally. It is a very contagious disease with various clinical manifestations. However, even in asymptomatic patients, it is believed that this virus exposure induces cryptic antibodies as in symptomatic patients. This current study aims to assess the prevalence of SARS-CoV-2 seropositivity by detecting the antibodies specific to the receptor-binding domain (SRBD) of SARS-CoV-2 in the pre-vaccine population in Bali. We assessed specific antibody titers against trimeric spike glycoprotein (S) of SARS-CoV-2 using Roche Elecsys Anti-SARS-CoV-2 S immunoassay in the serum of 510 pre-vaccine subjects without a previous documented history of SARS-CoV-2 infection. The average age was 35.53 years with 56.7% of the subjects being male. Among 510 subjects, 190 (37.3%) subjects were detected to have SARS-CoV-2 SRBD antibody or be seropositive. The range of the antibody titer was zero to 250 U/mL with the average being 44.3 U/mL. The number of subjects who had anti-SARS-Cov-2 SRBD titer above 132 U/mL was 76 (14.9%); it was the minimal antibody titer needed to donate plasma for plasma convalescent therapy. This study revealed a pre-vaccination population, without a history of COVID-19 infection, with seropositivity to SARS-CoV-2, which indicates the underdiagnosis of COVID-19, especially in asymptomatic individuals.

KEYWORDS: COVID-19, SRBD, anti-SARS-CoV-2, pre-vaccine.

INTRODUCTION

COVID-19, caused by SARS-CoV-2 infection, was first detected in Wuhan, China, in December 2019 [1]. It is a highly contagious disease and rapidly infects the population all around the world leading to a pandemic situation. The World Health Organization (WHO) reported more than 315 million confirmed cases with more than 5 million deaths [2]. The clinical manifestation of COVID-19 varies, from asymptomatic to severe and life-threatening cases. Over 80% of symptomatic cases have minor symptoms, whereas about 5% of cases, primarily older patients and those with co-existing diseases, develop serious symptoms such as severe respiratory distress syndrome and thrombosis [3-5]. Asymptomatic cases could be detected properly through contact tracing using any modalities available such as swab testing or antibody detection.

The SARS-CoV-2 attaches to the cell by binding the spike protein to the angiotensin-converting enzyme 2 receptors. The trimeric spike (S) protein of SARS-CoV-2 is a key component in vaccine

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development and a critical target for virus-neutralizing antibodies [6, 7]. Through the receptor-binding domain (RBD), the protein S interacts with its biological receptor on the host cells, human angiotensin-converting enzyme 2 (ACE) [7]. S protein is a 1273 amino acid polyprotein precursor synthesized on the rough endoplasmic reticulum [7]. The increase in the SARS-CoV-2 component count on the spike protein or the nucleocapsid protein induced the immune response to neutralize the virus [8, 9]. After recovery, neutralizing antibodies may provide protection for a few months, and in certain infections, they may even provide life-long protection. A study found that people infected with COVID-19 have protective immunity for at least six months [10]. This finding reveals that those people may not need any vaccine until the level of antibody decrease to the lowest protection level. Billions of vaccine doses are needed to prevent COVID-19 infection, however, some people with previous asymptomatic COVID-19 infections may not need it for some time. In the present study, we evaluated the anti-SARS-CoV-2 antibody titer to determine how high the seropositivity of COVID-19 is before vaccine administration in Bali.

METHODS

Study designs, ethical approval, and assay

We conducted a cross-sectional prospective study at several vaccination centers in Bali.

The study was approved by the institutional review board of the Faculty of Medicine and Health Sciences University of Warmadewa (document number:052/Unwar/FKIK/EC-KEPK/VI/2021).

Roche Elecsys Anti-SARS-CoV-2 S Electrochemiluminescence Immunoassay (ECLIA) was used in this study for the *in vitro* quantitative determination of antibodies (including IgG) to SRBD of SARS-CoV-2 from human serum (Roche Cobas e 601 modules). The correlation test between Roche Elecsys (units per mL) and WHO standards (BAU/mL) exhibited admirable correlation ($r^2 = 0.9992$, slope = 0.972, intercept = 0.0072). The titer ranged between 0.4 BAU/mL to 2500.0 BAU/mL. Values higher than 0.8 BAU/mL were considered positive.

Statistical analysis was performed using the STATA (SPSS Inc., Chicago, IL, USA), version 27.0.

RESULTS

The study was performed in March 2021 at two vaccination centers in Bali. The characteristics of the subjects are listed in Table 1.

A total of 510 subjects were tested using Roche Elecsys Anti-SARS-CoV-2 S ECLIA prior to vaccination. Among those, 190 (37.3%) of the subjects were seropositive for anti-SRBD of SARS-CoV-2 while others were seronegative (62.9%). As many as 64 (12.7%) had a titer of less than or equal to 50 U/mL and 48 (9.4%) subjects had a titer greater than 50 U/mL but less than 132 U/mL. The rest of the subjects (76 or 14.9%) had titer ≥ 132 U/mL.

Of all the participants in this study, only 97 (19.02%) subjects reported vaccine side effects. The five most common side effects reported were tiredness (20 (3.9%)), pain at the injection site (18 (3.5%)), headache (14 (2.7%)), myalgia (11 (2.1%)), and sleepiness (10 (1.9%)). We also evaluated the adverse events of the CoronaVac vaccine and the results are listed in Table 2.

DISCUSSION

The SARS-CoV-2 infection has spread to millions of people globally. The clinical manifestations of COVID-19 among the people infected with the virus vary from asymptomatic to critical symptoms. The predictors to detect more severe COVID-19 infections are dyspnea, anorexia, fatigue, higher respiratory rate, and higher systolic blood pressure [11]. Lower level of lymphocytes and hemoglobin, higher level of blood urea nitrogen, leukocytes, aspartate aminotransferase, alanine aminotransferase, blood creatinine, blood urea nitrogen, high-sensitivity troponin, creatine kinase, high-sensitivity C-reactive protein, interleukin 6, D-dimer, ferritin, lactate dehydrogenase, and procalcitonin are also associated with more severe COVID-19 [11]. Asymptomatic cases were found during contact tracing or surveys like the current study.

Table 1. Demographic data of the subjects.

Variable	N	Percentage (%)
Age, years old		
Mean (SD)	35.53 (13.77)	
Min-Max	16-77	
≤ 20	74	15.51
21-30	162	31.76
31-40	88	17.25
41-50	91	17.25
>50	95	18.63
Sex		
Male	289	56.67
Female	221	43.33
Body mass index (BMI)		
Underweight	56	10.98
Normal	169	33.14
Overweight	101	19.80
Obese class I	151	29.61
Obese class II	33	6.47
Titer anti-SRBD of SARS-CoV-2 pre-vaccination		
Mean (SD), U/mL	44.31 (81.98)	
Min-Max	0-250	
Patient status		
No previous COVID-19	320	62.75
Asymptomatic	188	36.86
Confirmed COVID-19	2	0.39
Side effect		
None	413	80.98
Yes	97	19.02

Note: maximum antibody titers that can be detected by the assay was 250 u/mL.

Since the documentation of SARS-COV2 antibody prior to vaccination is limited, the current study findings imply that asymptomatic infection can set off significant antibody production. This condition may be detected during research, contact tracing, or accidentally. These could be explained by the robust activity of SARS-CoV-2-specific T cell response observed in another study. A previous study of 100 asymptomatic and symptomatic post-COVID-19 patients who were

Table 2. The evidence of vaccine side effects.

Variable	Side effect		*p-value*
	None n (%)	Yes n (%)	
Age, years old			
≤ 20	57 (77.03)	17 (22.97)	0.703
21-30	133 (82.10)	29 (17.90)	
31-40	75 (85.23)	13 (14.77)	
41-50	72 (79.12)	19 (20.88)	
>50	76 (80.00)	19 (20.00)	
Sex			
Male	237 (82.01)	51 (17.99)	0.499
Female	176 (79.64)	45 (20.36)	
Body mass index (BMI)			
Underweight	51 (91.07)	5 (8.93)	0.270
Normal	134 (79.29)	35 (20.71)	
Overweight	78 (77.23)	23 (22.77)	
Obese class I	124 (82.12)	27 (17.88)	
Obese class II	26 (78.79)	7 (21.21)	
Titer pre-vaccination	1	OR 0.99 (0.99-1.00)	0.052[a]
Patient status			
No previous COVID-19	251 (78.44)	69 (21.56)	0.059[b]
Asymptomatic	161 (85.64)	27 (14.36)	
Confirmed COVID-19	1 (50.00)	1 (50.00)	

Note: [a]Simple logistic regression; [b]Fisher's exact test; $\alpha < 0.05$.

monitored for six months reported that the SARS-CoV-2-specific T cell response was still vigorous [12]. The study also assessed the magnitude of antibody level of spike glycoprotein that started to decline after two months of observation [12]. Another study found that anti-SRBD of SARS-CoV-2 was detected in the pre-vaccination group (<0.4 BAU/mL) using Roche Elecsys Anti-SRBD of SARS-CoV-2 ECLIA [13]. Fifty among 1,636 (3%) participants were found to have anti-SRBD-SARS-CoV-2 antibodies (IgA and IgG antibodies) using ELISA assay, while 22% were high (end-point titers ≥1:1,350), 42% were moderate (end-point titers 1:450), and 30% were low (end-point titers 1:150) antibody producers [14].

A SARS-CoV-2 sera-survey study among asymptomatic healthcare workers (HCWs) with no prior known exposure to SARS-CoV-2 reports that the seropositivity was 1.4% to 3.4% depending on the assay [15]. They used anti-nucleoprotein antibodies by CMIA (Abbott Diagnostic, USA) and anti-spike (S) antibodies (EuroImmun, Germany). Another sera-survey study was conducted among 115 asymptomatic HCWs as well. Only two (1.74%) were IgG seropositive for SARS-CoV-2 [16]. In the cohort study in Germany,

among 406 participants, 2.7% were found to be seropositive for SARS-CoV-2 [17]; meanwhile, the seropositivity was as high as 9.3% in Spain [18]. Public Health Ontario also revealed that 1.4% of the population were seropositive for COVID-19 [19]. A higher seropositive IgG anti-SARS-CoV-2 prevalence (11.4%) was reported in East Java [20]. The discrepancies may be related to the more sensitive assay utilized to detect the antibodies of the subjects in this study. The other reason may be associated with the time of the research, as our research was during the second year of the pandemic.

The average titer of SRBD antibody of SARS-CoV-2 in the current study was 44.31 U/mL which was found in 37.3% of participants. The protective level of neutralizing antibodies is not definitive yet. However, in the study wherein severe COVID-19 was treated using convalescence plasma, the antibody titer was more than 132 U/mL [21]. There are no other reports of the titer of SRBD of SARS-CoV-2 antibody in the population despite several studies reporting the measurement of neutralizing antibody titer with other assays such as c-Pass surrogate viral neutralization assay [22]. None of the participants in phases 1 and 2 of the CoronaVac vaccine trial were found to have neutralizing antibodies at the baseline [22].

This study reports primarily minor side effects of the COVID-19 vaccine administration. Adverse reactions in the phase 1 trial of an inactivated SARS-CoV-2 vaccine were reported to be as high as 29% in the 3 μg group, 38% in the 6 μg group, and 8% in the placebo group, with 14 days interval between the first and second vaccine shots [22]. A lower number of adverse reactions were reported in another cohort, with 28 days interval between the 2 vaccine shots, and account for 13%, 17%, and 13%, respectively. The most common adverse reactions reported in the study were minor adverse reactions such as injection-site pain. Just one hypersensitivity case with urticarial manifestation related to the vaccine was reported in the 6 μg group [22].

The limitation of the current study is the maximal anti-SRBD detection by the assay being 250 U/mL. The titer of the samples above those levels was not assessed using dilution or other methods. The current study results may not have captured the existent titer distribution in subjects with high antibody titer.

CONCLUSION

In conclusion, this study showed that the detection of anti-SARS-CoV-2 is not uncommon among the population since the pandemic. This result implies the high infectious rate of SARS-CoV-2 in the community and demonstrates the need to develop effective prevention methods.

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INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study. The study was approved by Warmadewa University Ethical Committee (Ethics approval number 47/Unwar/FKIK/KEPK/III/2021).

CONCEPTUALIZATION

SM; Methodology: SM; Validation: DS; Formal analysis: SM and DGWA; Investigation: RKD, MS; Resources: DGWA and SM; Data curation: RKD, MS; Writing-original draft preparation: SM, SPPL; Writing-review and editing: DS, Visualization: DS; Supervision: SM; Funding acquisition: DGWA and SM. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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