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Study of SARS-COV 2 Antibody Level Before and After the Third Covid-19 Vaccine Booster on Health Worker in Province of Nusa Tenggara Barat General Hospital

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ABSTRACT

Vaccination is one of the ways that countries around the world to manage the COVID-19. The effectiveness of a vaccine can be assessed from the degree of protective antibodies produced after the vaccination program. The purpose of this study was to determine the levels of SARS-CoV-2 antibodies in the sixth month after two doses of CoronaVac immunization and one month after MRNA-1273 vaccine immunization and to determine the relationship between age, sex, nutritional status, smoking habits and exercise habits on antibody levels in research subjects. Observational research with a cross sectional approach. The sample in this study were health workers who had received COVID-19 immunization who met the inclusion criteria and were taken using a purposive sampling technique. Then the data obtained will be analyzed descriptively using the SPSS program. A total of 29 subjects (51.72% women) participated in this study. The mean age of the research subjects was 35.71 years. The results of the antibody titer examination 6 months after the second dose of CoronaVac immunization (before MRNA-1273 vaccine immunization) showed the lowest antibody titer was 2.17 U/ml and the highest was > 250 U/ml, while the antibody titer 1 month after MRNA-1273 vaccine immunization showed > 250 U/ml in all subjects. The results of the correlation test showed that there were no significant results regarding the relationship between antibody levels 6 months after CoronaVac immunization with age (r= -0.282, p=0.139), gender (r= -0.095, p=0.623), nutritional status (r = 0.007, p = 0.973), smoking habits (r = 0.082, p = 0.672), and exercise habits (r = -0.243, p = 0.204). The levels of antibodies in the sixth month after the second dose of CoronaVac immunization were still detected in the range of 2.17 to > 250 U/ml, while the levels of SARS CoV-2 antibodies in the first month after the MRNA-1273 vaccine immunization all reached the upper limit of the examination. The levels of SARS-CoV-2 antibodies in the sixth month after the second dose of CoronaVac immunization were not significantly related to age, sex, nutritional status, smoking habits and exercise habits.

Keywords: *COVID-19, Vaccine, Antibody.*

1. INTRODUCTION

The COVID-19 pandemic started in December 2019 in Wuhan, China. The quantity of global COVID-19 cases as of 23 February 2021 was 112,319,446 cases with 2.21% deaths [1]. The first COVID-19 case in Indonesia was reported on 2 March 2020 or arround 4 months after the first case in China. Since it was first reported in Indonesia, the COVID-19 cases have expanded from time to time so that it requires attention [2]. In Indonesia, as of 23 February 2021, there were 1,298,608 cases with 35,014 deaths (2.7%) [3]. In West Nusa Tenggara Province there were 9103 cases with 380 deaths (4.17%) [4].

The COVID-19 pandemic which began at the end of 2019 had an impact not only on the health sector but also on all sectors including the economic, educational, social and cultural sectors. The impact caused by COVID-19 is so great that all countries in the world are working together to overcome the pandemic in various ways, one of which is through the COVID-19 vaccination program [3]. The aims of COVID-19 vaccination are to decrease the transmission of COVID-19 transmission, diminish morbidity and mortality due to COVID-19, accomplish group immunity in the community (herd immunity) and protect the community from COVID-19 in order to remain socially and economically useful [3,5]. Vaccines work by stimulating the formation of specific immunity against certain viruses/bacteria so that when exposed to someone who has been vaccinated, they will avoid transmission or serious illness due to the disease [5].

The COVID-19 immunization program in Indonesia is divided into 2 periods, the first period runs from January to April 2021 and the target of the first phase of the vaccination program is 1.3 million health workers and 17.4 million public officers, while the second period lasts April 2021 to March 2022 aimed at the general public in accordance with the criteria set by the government [5]. Based on president regulation number 99-year 2020 regarding the procurement of vaccines and the implementation of vaccinations in the context of dealing with the COVID-19 pandemic, the COVID-19 immunization program in Indonesia using the CoronaVac vaccine. The efficacy of this vaccine is 65.3% [6].

Based on a survey conducted by the Ministry of Health regarding vaccine acceptance in Indonesia to 115,000 respondents from 34 provinces in Indonesia, it was found that 65% were willing to receive the vaccine, 8% refused and 27% were still unsure. Further analysis of the reasons for respondents' refusal was largely due to concerns about vaccine safety (30%) and uncertainty about vaccine effectiveness (22%). Effectiveness affects the level of acceptance of the vaccine. The better the effectiveness of the vaccine, the easier it is accepted by the community. The effectiveness of a vaccine can be assessed from the level of protective antibodies produced after the vaccination program. Antibodies formed after vaccination provide a protective function against COVID-19 infection. Immunity begins to form 7 days after the first vaccination and reaches full immunity to the virus after 28 or 14 days after the second vaccination [6].

Currently there is no research that proves the levels of SARS-CoV-2 antibodies formed after immunization can last for how long and what factors affect the antibody levels. This study aims to examine the levels of SARS-CoV-2 antibodies in the sixth month after two doses of CoronaVac immunization and one month after MRNA-1273 vaccine immunization and to determine the relationship between age, sex, nutritional status, smoking habits and exercise habits on antibody levels in research subjects.

2. METHOD

2.1. Research Population

This study is an observational study with a cross sectional approach carried out at the Province of Nusa Tenggara Barat General Hospital on health workers who have received 2 doses of CoronaVac COVID-19 immunization and 1 dose of mRNA-1273 vaccine which has met the inclusion criteria measured antibody levels SARS-CoV-2 in the sixth month after CoronaVac vaccination and one month after mRNA-1273 vaccine vaccination. The research ethics committee of the Province of Nusa Tenggara Barat General Hospital approved this research.

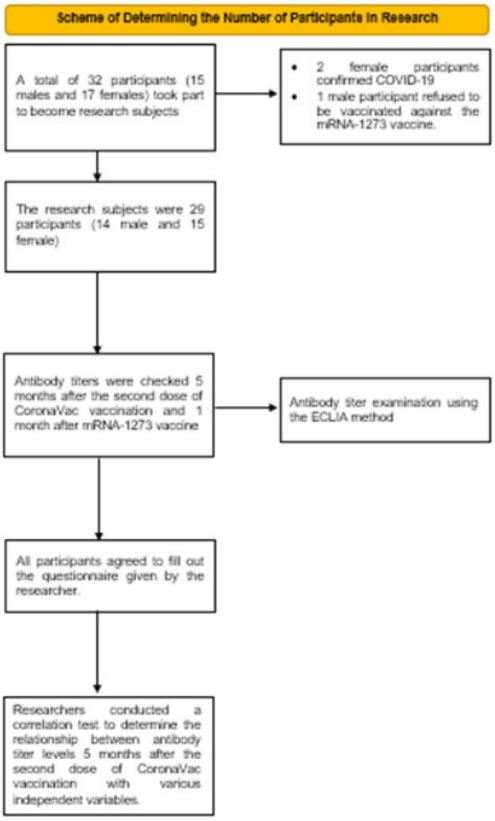

Figure 1 Research Population Scheme

This study involved 31 research subjects, then 2 people were excluded from the study (drop-out) because they were infected with COVID-19 and 1 person withdrew from the study so that in the end 29 research subjects were obtained (Figure 1). There is no history of COVID-19 infection of all participant based on participant's self-reported. the research subjects consisted of 14 men and 15 women. Blood samples for examination of SARS COV-2 antibody titer levels were taken 6 months after the second dose of CoronaVac vaccination and 1 month after mRNA-1273 vaccine vaccination. of these, 29 people completed a questionnaire given by the researcher containing clinical data (gender, age, height, weight, work unit, smoking habit, and exercise habit).

2.2. Antibody Test against SARS COV-2

Serum total antibody against SARS COV-2 S-RBD protein was calculated using the Electro-Chemiluminescence Immunoassay (ECLIA) method. ECLIA is a method that uses recombinantly engineered antigenic nucleocapsids to detect total immunoglobulins. The quantification range is between 0.4 and 250.0 U/mL.

2.3. Statistical Analysis

Statistical analysis was performed using SPSS version 23.0 (IBM Corporation). The suitability of the data with the normal distribution was determined by the Saphiro-Wilk test. The relationship between variables was calculated by the Pearson correlation test. A p value lower than 0.05 ($p < 0.05$) was considered to indicate a statistically significant result.

3. RESULTS

A total of 29 participants completed the questionnaire given by the researcher. The age of the vaccinated participants ranged from 21 to 45 years (median 35.71 years). The work units of each participant varied, but the majority were participants who worked in isolation rooms (69.95%). Most of the participants' nutritional status was normal (48.27%) and overweight (37.93%). Information about participants' exercise and smoking habits showed that 62.07% of participants did not regularly exercise and 86.20% of participants never smoked. The characteristics of the study participants are described in Table 1.

Table 1. Characteristics of Research Participants

Demographic Variable	Amount (n)	Percentage (%)
Gender		
• Male	14	48.28
• Female	15	51.72
Age (years old)		
• 21-25	2	6.90
• 26-30	9	31.04
• 31-35	3	10.34
• 36-40	12	41.38
• 41-45	3	10.34
Work Unit		
• Isolation Room	20	68.95
• Emergency Unit	4	13.80
• Ward	1	3.45
• Medical Record Room	1	3.45
• VCT	1	3.45
• Pulmonology Polyclinic	1	3.45
• Cardiology Polyclinic	1	3.45
Nutritional Status		
• Underweight	1	3.45
• Normal	14	48.27
• Overweight	11	37.93
• Obesity I	2	6.90
• Obesity II	1	3.45
Exercise Habits		
Routine	11	37.93
Not Routine	18	62.07

Demographic Variable	Amount (n)	Percentage (%)
Smoking Habits		
Active smokers or ex-smokers	4	13.80
Never smoking	25	86.20

The results of antibody titer examination 6 months after the second dose of CoronaVac vaccination using the ECLIA method (Table 2) showed varied results with the lowest antibody titer value being 2.17 U/ml and the highest being >250 U/ml. The results of antibody titer examination 1 month after MRNA-1273 vaccine vaccination using the ECLIA method (Table 2) showed that all participants had antibody titer values >250 U/ml. The results of the correlation test between antibody levels of SARS COV-2 6 months after the second dose of CoronaVac vaccination with several research variables are described in Table 3.

Table 2. The Results of the Antibody Titer Examination of Research Participants

Research Participant	6-months After CoronaVac Vaccination (U/ml)	1-month After MRNA-1273 vaccine Vaccination (U/ml)
Participant 1	166.3	>250
Participant 2	2.17	>250
Participant 3	72.05	>250
Participant 4	147.1	>250
Participant 5	53.48	>250
Participant 6	65.29	>250
Participant 7	250	>250
Participant 8	21.23	>250
Participant 9	34.35	>250
Participant 10	20.17	>250
Participant 11	64.11	>250
Participant 12	23.74	>250
Participant 13	>250	>250
Participant 14	>250	>250
Participant 15	116.7	>250
Participant 16	77.17	>250
Participant 17	>250	>250
Participant 18	>250	>250
Participant 19	21.72	>250
Participant 20	236.6	>250
Participant 21	18.24	>250
Participant 22	32.38	>250
Participant 23	175	>250
Participant 24	78.78	>250
Participant 25	63.16	>250
Participant 26	7.44	>250
Participant 27	218.1	>250
Participant 28	>250	>250
Participant 29	109.4	>250

Table 3. The results of the Correlation Test of Antibody Titer Six Months After CoronaVac Vaccination with Various Independent Variables of the Study.

Variable	Correlation (r)	*p-value* (p)
Age	-0.282	0.139
Gender	-0.095	0.623
Nutritional Status	0.007	0.973
Smoking Habits	0.082	0.672
Exercise Habits	-0.243	0.204

4. DISCUSSION

In spite of the fact that recognition of antibody responses to SARS COV-2 gives significant information for studying whether people have been recently infected with this infection, for diagnosing these infections, and for determining vaccine effectiveness, it is also essential to distinguish factors that impact the inconstancy of these antibody responses, comprehend the pathogenesis COVID-19, lead epidemiological research, and add to studies on vaccines. We led this research to acquire data on the relationship of postvaccine antibody levels with various demographic characteristics.

The results of antibody titer examination 6 months after the second dose of CoronaVac vaccination using the ECLIA method showed varied results with the lowest antibody titer value being 2.17 U/ml and the highest being >250 U/ml. Research conducted by Tekol, *et al.* stated that antibody titer levels after the second dose of CoronaVac vaccination usually decreased in the 4th or 5th month. The average antibody titer level in this study was 98.57 U/ml at the 4th month and 89.85 U/ml at the 5th month [7]. In this study, participants with antibody titers that exceeded the average, even 5 participants had antibody titers >250 U/ml. This could be due to the possibility that the antibody titer formed was very high, so that even at the sixth month it was still detected at the upper limit of the examination >250 U/ml. The second possibility is that the high antibody levels were caused by a history of asymptomatic COVID-19 infection experienced by the participants. This second possibility cannot be ruled out because health workers are individuals who are at high risk of contracting COVID-19 infection and at the Province of Nusa Tenggara Barat General Hospital there is no periodic SARS-CoV-2 PCR examination for health workers so that if someone is infected with COVID-19 asymptomatic will not be detected.

The results of antibody titer examination 1 month after MRNA-1273 vaccine vaccination using the ECLIA method showed that all participants had antibody titer values >250 U/ml. mRNA-1273 vaccine is an mRNA-1273 vaccine that encodes a stable version of the SARSCoV-2 glycoprotein trimer, S-2P. A study conducted by Self, *et al.* who examined the antibody response to three types of vaccines showed that participants who received the mRNA-1273 vaccine had

higher IgG antibody titers compared to the other two vaccines (Pfizer and Janssen) with an average IgG antibody titer level of 3059 BAU/ml [8]. These data suggest that the MRNA-1273 vaccine mRNA vaccine regimen provides more protection than other types of vaccines. In this study, researchers were only able to provide data on participants' antibody titer levels >250 U/ml at 1 month after MRNA-1273 vaccine vaccination due to the limitations of the ECLIA method which was only able to detect up to >250 U/ml.

The results of our study showed that the age of vaccinated participants had an insignificant negative correlation with antibody levels 6 months after vaccination (r= -0.282, p=0.139). Age is a significant factor impacting the vaccine response, and the older age have been announced to have a poor response to influenza, hepatitis A and B, and pneumococcal vaccines with lower formation levels of antibodies and vulnerable cell-mediated responses [9]. Notwithstanding a diminished post-vaccination response, the elderly individuals will generally have antibodies that decline quickly after vaccination. We here show a weaker vaccine response 6 months after the second dose of vaccination, confirming some of the results of the previous study but also showing that the results of this study were less significant after the second dose of vaccine [10,11].

The impact of gender on vaccine efficacy is inadequately perceived, in spite of the fact that it is broadly perceived as an issue of concern. Gender-related differences in vaccine efficacy and safety, in view of immunological, genetic, and hormonal backgrounds, have been recently announced to emphasize the possible influence of sex on COVID-19 vaccine results [12]. In our study, the sex of the vaccinated participants had an insignificant negative correlation with antibody levels 6 months after vaccination (r= -0.095, p=0.623). These results are different from the results of research conducted by Lo Sasso *et al.* which indicates that gender significantly affects vaccine-induced immunogenicity. In particular, male sex appears to be associated with less effective humoral responses. Lo Sasso *et al.* also found that antibody titers decreased significantly in the short time following the second dose of vaccine administration. Thus, it is important to understand whether men are more susceptible to COVID-19 infection. It is reasonable that the male sex ought to be firmly checked or monitored and may require earlier revaccination or/and increase dossage of vaccine to guarantee stronger and longer enduring immunity and protection from the infection [13].

One of the variables that impact the improvement of post-vaccine antibodies is body mass index (BMI). The explanation is that the responses of CD8 cytotoxic T cells, CD4 T-helper cells, and memory T cells have been demonstrated to be deficient and antibody levels vanish quicklyly after vaccination in obese individuals. Accordingly, nutritional status is significant in the level of post-vaccination immune response [14-16]. To compare the nutritional status of participants with antibody responses, the BMI values of all participants were calculated and grouped as underweight (BMI: <18.5), normal (BMI: 18.5-22.9), overweight (BMI: 23-24.9), obesity grade I (BMI: 25-29.9) and obesity grade II (BMI: 30-34.9). Our study showed that the nutritional status of vaccinated participants had an insignificant positive correlation with antibody levels 6 months after vaccination (r = 0.007, p = 0.973). The results of this study are similar to those conducted by Uysal, *et al.* also obtained insignificant results regarding the relationship of nutritional status calculated by BMI with postvaccine antibody response [17].

Smoking habits can cause a decreased antibody response to some vaccinations [14]. In our study, participants who smoked and vaccinated had an insignificant positive correlation with antibody levels 6 months after vaccination (r = 0.082, p = 0.672). This result is different from the research conducted by Nomura, *et al.* which shows that there is a significant negative correlation between smoking habits and antibody levels 3 months after the second dose of vaccination. [18] In addition, antibody titers were significantly lower in smokers compared to ex-smokers. The results of other studies also obtained significant results regarding the lower postvaccine antibody response in smokers compared to non-smokers [17]. These outcomes proposed that smoking cessation diminish the effect of lower antibody titers. Consequently, smoking itself is a major risk factor for low antibody titers, rather than smoking duration or number of cigarettes per day. Moreover, smoking cessation is relied to increase antibody titers more effective because these levels are essentially lower in current smokers compared to previous smokers [18].

Albeit explicit studies with COVID-19 vaccines have not been led, insight from the past vaccination programs (especially influenza) suggests that ordinary exercise might be an effective strategy for improving antibody responses after vaccination. For instances, young athletes after influenza vaccination have more articulated increases in T cells and antibodies than age-matched controls [19]. There is likewise cross-sectional study for a valuable impact of keeping up high levels of physical activity in elderly, with very active older Chinese ladies (>65 years) walking more than 18,509 steps/day in the weeks following vaccination showed a superior response of immunological reaction because of greater development of monocytes and plasma blasts in peripheral blood, and higher antibody reaction at 18-month follow-up than their less active partners that walking less than 10,927 steps/day [20]. In this study, we found that the exercise habits of vaccinated

participants had an insignificant negative correlation with antibody levels 6 months after vaccination (r= -0.243, p=0.204).

The researcher realizes that this research has several limitations. The small number of research samples (n=29) greatly affected the significance of the research results. This study did not measure antibody titers before the sixth month. In this study, there were still participants with high antibody titers 6 months after the second dose of CoronaVac vaccination, and there were even participants with antibody titers of 250 U/ml. The researchers suspected that this was caused by a history of asymptomatic infection experienced by the participants, but the researchers could not rule it out due to the lack of a screening program or routine examination of participants during the study.

According to the researcher, the ECLIA method used in this study has several weaknesses, namely not being able to detect specific antibodies, especially IgG (associated with post-vaccination antibodies) because this method only detects total immunoglobulins (IgM and IgG) so that researchers are unable to determine whether the increase in antibody titers these participants were caused by an acute infection with COVID-19 or caused by the vaccination itself. In addition, the ECLIA method was not able to detect the amount of antibody titer over its quantification range (>250 U/ml) which was evident in this study (Table 2). Another antibody titer examination method that can be considered to obtain a large range of quantification is the chemiluminescent microparticle immunoassay (CMIA) method. CMIA measures specific IgG antibody levels with a quantification range of 21.0 and 40,000.0 AU/mL [21,22].

5. CONCLUSION

This study involved 29 participants (51% women) with a median age of 35.71 years, most of whom worked in isolation rooms, had normal nutritional status, did not exercise regularly and never smoked. The level of SARS-CoV-2 antibodies in the sixth month after the second dose of CoronaVac immunization (before MRNA-1273 vaccine immunization) was still detected in the range of 2.17 to >250 U/ml. The levels of SARS-CoV-2 antibodies in the first month after MRNA-1273 vaccine immunization all reached the upper limit of the examination, which was >250 U/ml. The levels of SARS-CoV-2 antibodies in the sixth month after the second dose of CoronaVac immunization were not significantly related to age, sex, nutritional status, smoking habits and exercise habits.

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