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Analysis of COVID-19 Antibodies in Patients Who Have Been Vaccinated With Non-Vaccinated Patients with Confirmed COVID-19 Life

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Abstract: COVID-19 immunization is a government initiative to prevent transmission and reduce the incidence of disease and death associated with the SARS-CoV-2 virus. Although the SARS-CoV-2 virus is no longer a significant threat, older adults with comorbidities or people who are immunosuppressed should remain vigilant by maintaining a distance of at least one meter from others, covering their mouths with their elbows when coughing or sneezing, washing their hands regularly, and wearing masks. However, many groups oppose vaccination for various reasons, including the perception that the COVID-19 vaccine is less effective and efficient in preventing SARS-CoV-2 virus infection. This study compared COVID-19 antibody titers in participants who had arrived, without mentioning the vaccine brand, with those who had not yet arrived but had a history of SARS-CoV-2 infection. This study design used a cross-sectional study; the number of samples in the study was 34, the location of this study was the Palembang Public Health Laboratory Center, and the statistical test in the study used the Independent T-test. The study results indicate that the mean antibody titer in the vaccinated group was 98.123. In contrast, in the unvaccinated group, it was 70.641, demonstrating a statistically significant difference with a p-value of 0.000. Further research suggests whether there is a relationship between antibody titers in subjects who have been vaccinated for the second and third time with a history of exposure to the COVID-19 virus.
Keywords: Antibodies; COVID-19; SARS-Cov-2; vaccines.

INTRODUCTION

Viruses are a type of microbe that can cause infectious diseases. Over the past two decades, various viral illnesses have triggered epidemics, including severe acute respiratory syndrome coronavirus (SARS-CoV) from 2002 to 2003, influenza H1N1 in 2009, and Middle East Respiratory Syndrome (MERS-CoV), initially detected in Saudi Arabia in 2012. The SARS-CoV-2 virus is a kind of coronavirus that leads to respiratory tract infections called COVID-19 (Wang, L., Wang, Y., Ye, D., & Liu 2020). The rapid global spread of this virus has caused an unparalleled health and economic crisis, leading to its classification as a pandemic by the World Health Organization (WHO) on March 11, 2020. The initial identification of SARS-CoV-2 infection occurred in Wuhan City, located in Hubei Province, China. The initial COVID-19 case in Indonesia was declared on March 2, 2020. As of October 11, 2021, Indonesia's total number of COVID-19 cases has reached approximately 4 million, with a daily increase of 51,000 new cases. In July 2021, the death toll reached 2000 cases per day (Zheng, 2020).

Laboratory tests such as quick antigen tests and polymerase chain reaction (PCR) swab tests are necessary to ascertain whether an individual is infected with COVID-19. Recent research findings support this, indicating that certain patients may

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exhibit consistently positive results despite the absence of symptoms. Research conducted in Korea indicates that while no virus can reproduce three weeks after the initial symptoms, SARS-CoV-2 RNA can still be found in examination specimens using RT-PCR for up to 12 weeks.

Recent research indicates that individuals who have recovered from COVID-19 may be susceptible to reinfection due to the gradual disappearance of COVID-19 antibodies during 3 to 12 months. Hence, even after being proclaimed free from COVID-19, individuals must adhere to health protocols (Gunardi, 2021) (Damo N. Y. et al., 2021).

Vaccines are a crucial component of the comprehensive approach to combatting COVID-19, with the implementation of health measures. In order to effectively minimize the occurrence of the sickness caused by SARS-CoV-2, approximately 60-70% of the population must develop immunity, thus achieving herd immunity. Eight distinct categories of vaccines have been circulating globally, employing diverse platforms (Bastian, B., K D. W. et al., 2022). Administering vaccines is a safe and effective measure to avoid problems or fatalities associated with COVID-19.

Community vaccination is a preventive measure employed to restrict the transmission of diseases. However, public opinions on COVID-19 vaccine inoculation vary, and some groups disregard the health precautions the government prescribes. Vaccine rejection groups can stem from a range of factors, including considerations related to health as well as religious convictions.

Agista's (2021) research reveals that a significant portion of individuals in the South Sumatra region still hold a pessimistic view towards the COVID-19 vaccine. Of the 440 respondents, 163 individuals (37%) exhibited a negative perspective, leading them to decline vaccination (Argista, 2021). Vaccination significantly reduces morbidity and mortality rates compared to those who are not vaccinated. Therefore, it is important to create a positive perception in the community regarding the vaccine. This perception should acknowledge that the COVID-19 vaccine is a government initiative to prevent and control the spread of COVID-19 (Tuloli et al., 2023).

An ongoing study conducted by multiple universities in Indonesia is investigating the efficacy of vaccines in inducing antibody production after COVID-19 vaccination. Researchers have not reported on the South Sumatra region (Argista, 2021). Furthermore, a subset of individuals are hesitant to receive immunization due to a limited comprehension of the advantages associated with vaccines.

Research continues at many universities to assess the efficacy of vaccination in generating antibodies following COVID-19 injection in Indonesia. Researchers acknowledge that data about the South Sumatra region remains unreported (Argista, 2021). Moreover, some individuals are reluctant to get vaccinated due to insufficient comprehension of its advantages. This study will offer detailed insights on antibody levels following COVID-19 vaccination in the South Sumatra region, a topic poorly documented in the current literature. Expanding the comprehension of vaccination efficacy in regions beyond the capital and other major cities in Indonesia is essential, yielding more representative statistics for locations outside Java.

MATERIALS AND METHODS

This research employed a descriptive methodology, utilizing analytical observations to examine the COVID-19 Antibody titer. The study was conducted using a cross-sectional methodology. The study was carried out at the Palembang Public Health Laboratory Center. The research subjects were blood serum samples from

individuals who had undergone examination. The subjects were divided into two groups: those who had never been vaccinated against COVID-19 and those who had been vaccinated without any information on the specific brand of COVID-19 vaccine used. The data collection commenced in February 2024, yielding 34 data points. Examining Sars COVID-19 antibodies using the IchromaxTM COVID-19 NAb tool with the Fluorescence Immunoassay method can detect the concentration of neutralizing antibodies in blood samples to a certain level (usually in antibody titer units).

The working procedure of this tool is as follows: Pipette 200 µL of detector diluent, then insert it into detector tube A. After that, pipette 50 µL of serum and insert it into detector tube A. Then homogenize 10 times. Incubate for 5 minutes. Then pipette 150 µL (Detector tube A + Sample mixing) and insert it into detector tube B. After that, homogenize again 10 times. Then, take a sample of 75 µL, then insert the sample into the test cartridge. Incubate for 15 minutes, then insert the test cartridge into the tool. Then, press the "Start" button and read the results on the screen. The number of samples is 17 data points obtained from the unvaccinated group and 17 from the vaccinated group. Statistical research used the Chi-square test to determine the correlation between these variables and the COVID-19 antibody titer. This study has received approval from the Ethics Commission of the Faculty of Medicine, Prima University of Medan, with reference number 021/KEPK/UNPRI/VIII/2024.

RESULTS AND DISCUSSION

The descriptive data were categorized into numerous groups, including gender, age, vaccination history, previous infection history, and the SARS-CoV-2 S-RBD antibody titer test findings. The antibody titer test results were then separated into three groups: high (>81 U/mL), intermediate (51-80 U/mL), and low (<50 U/mL).

Based on Patients Gender

Characteristics of the sample based on gender can be seen in the table below.

Table 1. Sample Characteristics Based on Gender

Variabel	Total Subject (n)	Sig
Gender		
Female	14	0,72
Male	20	

According to Table 1, the samples used consisted of 14 female participants (41.2%) and 20 male subjects (58.8%). This study reveals a disparity in serum collection between male and female patients, with more serum recovered from males. This discrepancy can be attributed to the fact that, throughout the study period, more men received COVID-19 antibody testing. The investigation revealed that the Sig value was more than 0.05 when examining the correlation between gender and antibody titer. Therefore, it can be concluded that there is no significant association between gender and COVID-19 antibody titer.

Age Patients

Table 2. shows the sample's age-based features. These characteristics are categorized according to the WHO's 2012 classification, which includes the following groups: early adulthood, late adulthood, middle age, elderly, and young old.

Tabel 2. Characteristics Based on Age

Age	n	(%)	Sig
26-35 Year	8	25	
36-45 Year	5	15	
46-55 Year	5	15	
56-65 Year	10	29	0,66
66-74 Year	6	18	
Total	**34**	**100**	

Table 2. shows that most individuals who undergo Ab-COVID examinations are older people (56-65 years), with a total of 10 people. This is followed by individuals in early adulthood (26-35 years) with eight people, then the elderly (66-74 years) with six people, late adulthood (36-45 years) with five people, and middle age (46-55 years) with five people. Individuals undergo Ab COVID-19 tests due to signs of COVID-19 infection to assess their antibody titers. The research data analysis shows that the Sig value> 0.05 indicates no statistical relationship between age and the S-RBD antibody titer (table 2). However, it is worth noting that in the table, subjects in the adult to middle age range (26-55 years) have the highest antibody titer compared to older people (> 56 years), where the antibody titer is <50 U/mL. The findings of this study are consistent with the research conducted by (Vassilaki et al., 2021). which demonstrated that younger age cohorts exhibit higher antibody levels than older cohorts. The population of naïve T cells in the body, which can recognize and react to antigens, tends to decline as a person ages. Furthermore, the process of ageing leads to a decline in the diversity of T cell receptors on CD8 and CD4 cells, which subsequently diminishes the longevity of T cells (El-far M, Halwani R, Said E, Trautmann L, Doroudchi M 2008). There will be a temporary rise in the quantity of B cells. The level of some proteins tends to remain stable as one ages. However, a decrease in the accumulation of specific proteins reduces the production of functional antibodies. In theory, older individuals may have a reduced response to vaccination (Abbas AK, Lichtman AH 2006). However, a study conducted by (Rotty I. et al., 2022). suggests that older individuals require more time to achieve the same level of antibodies as younger individuals, even though additional doses can result in higher antibody levels. In comparison to younger individuals. (Abbas AK, Lichtman AH 2006)

Immunization Status

The data gathered from the medical record search of research subjects reveals that individuals who have been administered the Pfizer or AstraZeneca vaccine brands for COVID-19 are included in the results.

Tabel 3. Vaccination Status of Study Subjects

Status	Antibody Titer Category			Sig	n
	Low	Medium	High		
No COVID-19 Vaccine	4	7	6		17
COVID-19 Vaccine	0	0	17	0,000	17
Total	**4**				**34**

The government's response to COVID-19 is to mandate vaccination for all members of the community in order to halt the virus's transmission and enhance herd immunity. Nevertheless, the majority of individuals decline to receive vaccinations. Certain groups refuse vaccination for various reasons, including health concerns, vaccine development, vaccination effects, and the efficacy of different vaccines. An

estimated 37% of the public still have a negative opinion of vaccination in early 2023, as per Ulfiyah and Hariyanto (2023). This negative perception is attributed to a lack of understanding and issues from the media, which have reported the negative impacts of the COVID-19 vaccine, including health impacts that can result in death, as per research conducted by (Bastiana B. et al., 2022)

The government has made an effort to mandate vaccination for all individuals while still considering each individual's health. For example, individuals must present a certificate of at least dose 1 when visiting public facilities, travelling long distances, and attending several schools and universities requiring vaccination. Nevertheless, the researchers in this study still discovered that some individuals did not receive vaccinations. Table 3 indicates that 17 subjects were not vaccinated, and the researchers could not determine the reason for their non-vaccination.

However, the table reveals that six individuals have not received vaccinations and have titers more significant than 80 U/mL. By examining the subject's medical records and referring to Table 4, it is evident that 5 of these individuals have a documented history of contracting the COVID-19 virus twice, while one individual was infected once within less than 6 months between post-infection and examination. The body's immune cells can generate defensive mechanisms such as antibodies and memory cells following an infection. This immune memory can last at least 6 months after the infection (Apriani & Dewi, 2022). According to (Jabal K. et al., 2021), individuals' immune response capacity varies based on age, endurance, and nutritional state. From the table provided, it is evident that six individuals were not vaccinated but had a COVID-19 antibody titer of more than 80 U/mL after contracting the SARS-CoV-2 virus. According to the antibody titer analysis, there is a significant association between the vaccine status and the S-RBD antibody titer, with a p-value of 0.000 <0.05.

Characteristics of COVID Exposure History with Vaccine Titer

According to the findings from the medical record search of the research participants, it is established that 15 individuals were exposed to COVID-19 once, and 19 individuals were exposed to COVID-19 twice. According to the data in Table 4, it is evident that six individuals had significant levels of antibodies (>80 U/mL) after being exposed once, whereas 17 individuals had been exposed twice. Vaccination of those who have previously had COVID-19 is expected to result in a stronger immune response, leading to higher levels of antibodies and immunoglobulins than individuals who have never been exposed to COVID-19. Once individuals infected with COVID-19 fully recover, their immune systems will develop a defence against the virus. Although the distance of infection in an individual can be significant, the vaccination procedure does not always result in loss of immunity despite decreasing nucleocapsid IgG antibodies following infection (Andrews, N. et al., 2022).

Table 4. Sample Characteristics Based on Exposure History

Status	**Antibody titer Category**			**Sig**	**n**
	Low	Medium	High		
Exposed 1 time	4	5	6		15
Exposed 2 times	0	2	17	0,006	19
Total	4	7	23		34

Statistical analysis of the data using the Chi-Square test reveals that there is no significant correlation between the history of exposure and the levels of antibodies, which are classified into three categories: low titer, medium titer, and high titer. The

resulting Sig value is 0.006, more significant than the critical threshold of 0.005. Therefore, there is no statistically significant link between the history of exposure to COVID-19 and the formation of antibody titers.

Hypothesis Analysis

In this investigation, the research data were determined to be normally distributed. Consequently, the mean was employed to describe the measure of data centralization, and the standard deviation (SD) was employed to describe the measure of data distribution. The Shapiro-Wilk Test was applied to the normality test results. The error rate was 5% (α: 0.05), and the confidence level in this study was 95%. The data is considered normal if the p-value is more significant than 0.005, while it is termed "not normally distributed" if the p-value is less than 0.005.

Table 5. Results of Descriptive Test and Normality Test

Variabel	N	Mean	SD	Shapiro wilk	Sig
Titer A*	17	98,123	1,568	0,089	> 0,05
Titer B**	17	70,641	21,105	0,057	

Note:
A* : Ab titer in samples vaccinated with COVID-19
B** : Ab titer in samples not vaccinated with COVID-19

Table 5. shows that the data tested by Shapiro-Wilk are normally distributed. The Sig value obtained from the Ab titer in vaccinated samples is p = 0.089, more significant than 0.05. Similarly, the Sig value obtained from the Ab titer in unvaccinated samples is p = 0.057, more significant than 0.05.

The Difference in Mean Ab Titers in The Vaccinated and Unvaccinated Groups

This hypothesis test aims to determine if there is a significant disparity between two groups: individuals who have received the COVID-19 vaccine and individuals who have not been vaccinated but have previously been exposed to COVID-19. The test utilizes statistical analysis to compare the COVID-19 antibody titer in samples from both groups. The data is measured on a ratio scale. The statistical analysis employed incorporates the Independent T-Test (Unpaired t-test). By employing this statistical test, it is possible to ascertain whether there exists a noteworthy disparity between these two groups.

Table 6. Independent Sample Test Output Results

		Levene's Tes Equality of Variance		**T-test for Equality of Means**		
		F	Sig.	t	Sig. (2-tailed)	Mean Difference
		25.221	0.000			
Antibody Titer	Equal variances assumed			-5.354	0.000	-27.482
	Equal variaes not assumed			-5.354	0.000	-27.482

According to table 6, the Sig. Levene's Test for Equality of Variance indicates that the F value is 25.221 and the Sig (p) value is 0.000. Given that p <0.005, there is a significant difference in variation across groups A and B. This means that the data

is not homogeneous, and the assumption of equal variance cannot be made. According to the data in Table 5.7, the significance (two-tailed) value is 0.000, less than 0.05. Therefore, based on this information, the null hypothesis (Ho) is rejected, and the alternative hypothesis (Ha) is accepted in the independent sample t-test. Hence, a substantial disparity exists in the mean Ab titer between the vaccinated and unvaccinated groups, despite both groups having a prior encounter with the SARS-CoV-2 virus.

A study has demonstrated that immune memory can endure for at least six months following infection, as research findings indicate. In a specific case, an individual exhibited an immunological response resulting in the persistence of antibodies for almost ten months after a PCR test. The Polymerase Chain Reaction (PCR) technique revealed that, despite not receiving vaccination, several participants exhibited Antibody titers >80 U/mL following infection with SARS-CoV-2, as seen in Table 5.5. Nevertheless, the antibodies generated upon infection do not confer complete immunity against subsequent infections (Vassilaki N. et al., 2021). Therefore, administering vaccines to those who have been exposed to SARS-CoV-2 can offer further safeguarding (Jabal, K. A. et al., 2021)

Vaccination facilitates the development of herd immunity within a community, which refers to the collective immunity within a population or group. The primary objective of vaccination is to enhance the human immune system's ability to combat illnesses and disorders caused by microorganisms, including the COVID-19 virus (Budiyanti, Isyawati, and Ganggi, 2023). In order to attain herd immunity, a minimum of 70% of the population must receive the COVID-19 vaccine. This threshold ensures that most of the population becomes immune to the virus, establishing widespread immunity (Apriani & Dewi, 2022). This collective immunity safeguards individuals with compromised immune systems or those who cannot receive the vaccine, a phenomenon known as indirect protection (Pratama et al., 2022).

Repeat vaccinations enhance antibody titers and broaden immunity, focusing on the elderly and individuals with compromised immune systems (Arumsari et al., 2021). A recent study indicates that delivering additional doses of the COVID-19 vaccine can shorten the duration of treatment and alleviate symptoms of recurring illnesses (Zheng, 2020). This study aligns with the findings of (Andrews N. et al., 2022), demonstrating that those who received the COVID-19 vaccine exhibited higher levels of COVID-19 serum antibody titers compared to those who were not vaccinated, despite both groups being exposed to the virus (Andrews, N. et al., 2022).

Halim (2021) states that in certain instances, individuals may require repeated doses of vaccination to achieve optimal immune system response, in addition to considering the specific type of vaccine administered (Eni Kusyati et al., 2022). Several vaccinations, like the COVID-19 vaccine, must be administered sequentially, either depending on the dosage or until the second dose, and so on (Hermiyanti, 2018). This is essential because individuals may not exhibit uniform reactions when administered a solitary dosage. Hence, to enhance herd immunity, it is imperative to administer boosters to the entire population or a targeted subset of individuals. Obtaining a double dosage of the COVID-19 vaccine is advisable to optimise community protection. Subsequently, a booster injection should be administered as a subsequent immunisation (Zhao et al., 2020).

This study has many limitations, namely, the number of samples is less than 30 and cannot trace the first vaccination data of the research subjects, so only data on vaccine titers after the second dose is obtained. This is because it requires large funds and a tracing time of more than 10 months for one subject.

CONCLUSION

The study found that people vaccinated against COVID-19 had an average antibody titer of 98.123 U/mL, while people who had not been vaccinated against COVID-19 had an average antibody titer of 70.641 U/mL. The statistical test showed a Sig value of 0.000, less than 0.005. This means there is a difference in antibody levels between people vaccinated against COVID-19 and people who had been exposed to the SARS-CoV-2 virus in the past but had not been vaccinated. Further research is recommended by looking at the relationship between antibody titers in subjects vaccinated for the second and third time with a history of exposure to the SARS-CoV-2 virus.

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CONFLICT OF INTEREST

There is no conflict of interest in this research.

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