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Serodiagnosis of Severe Acute Respiratory Syndrome Corona Virus (ANTI-SARS-CoV-2) Antibody in Blood Donors in Surabaya, Indonesia

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2). So far, the incidence of direct blood-borne transmission is not widely known. This is because the viremia of SARS-CoV-2 virus infection is very small, between 2-3 days after infection. Immunoglobulin M (IgM) is an antibody that is formed in the early days of a person being infected with the virus, which is around the third day and can persist in the blood for up to 3-4 months after infection. The purpose of this study was to perform serodiagnosis of SARS-CoV-2 antibodies in blood donors in Surabaya. The type of research used is an experimental laboratory with an exploratory research design. The research sample consisted of 100 donor blood samples from UTD PMI Surabaya. Rapid diagnostic test examination at the Immunology Laboratory, Medical Laboratory Technology Department, and the Immunoserology Laboratory of BBLK Surabaya, Indonesia for antibody titer examination using the ELISA method in May 2022. Results of antibody analysis against SARS-CoV- 2 in blood donors in Surabaya by 58% (Cut Off Index = 0.1569), so further research is needed on factors that can affect the formation of antibodies.

Keywords: SARS-CoV-2 IgG; SARS-CoV-2 IgM; blood donor

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2). SARS CoV-2 is a new type of coronavirus that has never previously been identified in humans. There are two types of corona viruses that cause diseases with severe symptoms, such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). By the International Committee of Taxonomy OF Viruses (ICTV) the pneumonia cluster was named SARS CoV-2, while the disease caused was named COVID-19.(1) In January 2020, this outbreak was later declared as Coronavirus Disease 2019 (COVID-19) pandemic by WHO because it had spread to 18 countries with 4 countries having reported human-to-human transmission. The SARS-CoV-2 virus is a member of the Coronaviridae family. This family of viruses is known as the cause of diseases related to the respiratory tract. This virus can penetrate the defense of human body and cause illnesses from mild ones such as common cold to severe ones such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS).(2)

SARS CoV-2 manifested particularly in the respiratory tract of infected humans is transmitted through the air that contain the virus or through infectious droplets. Transmission of infection can also be through saliva, nasal secretions, feces, blood and direct contact with body fluids contaminated by SARS CoV-2.(3) So far, the incidence of direct blood-borne transmission is not widely known. This is because the viremia of SARS-CoV-2 virus infection is very short, between 2-3 days after infection. In addition, the virus in the blood is rarely detected during the presence of the symptoms of SARS-CoV-2 infection. However, viremia can be symptomatic or asymptomatic. Immunoglobulin M (IgM) is an antibody that is formed in the early days of a person infected with the virus around the third day and can persist in blood for up to 3-4 months after the infection. This indicates that a person has been infected with the early SARS CoV-2 virus. The person is called an asymptomatic person.(3) Immunoglobulin G (IgG) is an antibody formed through the switching of the formed IgM. IgG appears later than IgM, about 7-10 days after infection, and can persist for a longer time than IgM. The results of SARS CoV-2 IgG and IgM examination were interpreted as non-reactive, IgM reactive, IgG reactive, and IgG and IgM reactive. A positive result indicates that the body is actively forming antibodies against SARS CoV-2 virus, while a negative result

indicates that antibodies in the body have not yet been formed.[4] While immune responses to SARS-CoV-2 could start as soon as the first week following the symptom onset, in most infected people, seroconversion changes usually start within 10–12 days for IgM and 12–15 days for IgG. Serum IgM levels peak in two to three weeks, whereas IgG antibodies peak in three to four weeks following the symptom onset.[5]

So far, no studies have detected the presence of specific antibodies in donor blood. The SARS CoV-2 virus is not transmitted through blood donation and is not a blood-borne disease. However, this serodiagnostic identification in donors can provide predictions of SARS CoV-2 virus circulation between healthy individuals and the likelihood of a distinct new cluster occurring in a population.[6]

The detection of the presence of SARS-CoV-2 virus antibodies in donor blood at the Indonesian Red Cross in Surabaya had never been carried out, so early monitoring or screening was needed to limit transmission to humans through blood. Antibody screening with known serological tests will easily detect donors who have been infected with the SARS CoV-2 virus, so serodiagnostic evaluation of donor blood is very important. [5]. Determining the prevalence of SARS-CoV-2 in blood donors enables the control of virus circulation in healthy individuals and helps implement strategies to reduce transmission, especially in the absence of seroprevalence surveys. Therefore, research on the serodiagnosis of SARS CoV-2 antibodies in donor blood in Surabaya, Indonesia, was needed.

This study aimed to prove the presence of antibodies against SARS-CoV-2 in blood donors in Surabaya, Indonesia. In addition, this study also analyzed the presence of antibody against SARS-CoV-2 in blood donors in Surabaya.

METHODS

This research was an experimental laboratory research with an exploratory design, a design used to find new phenomena in order to develop science. This research was conducted at the Immunology Laboratory Department of Technology, Medical Laboratory, Health Polytechnic, Ministry of Health. The population in this study were healthy donors who had passed a series of blood donor screenings and were declared eligible to donate their blood from March to April 2022 at the PMI Blood Transfusion Unit, Surabaya, Indonesia. Blood specimens from healthy donors that have met the criteria were subjected to antibody titer examination. The data collected is primary data from the results of examinations in the Immunoserology Laboratory, Central for Health Laboratory (*Balai Besar Laboratorium Kesehatan*, BBLK), Surabaya, Indonesia. Data were presented descriptively in the form of IgG and IgM antibody titers against SARS-CoV-2 virus in blood donors in Surabaya, Indonesia. Data obtained were the data with a ratio scale, which were then analyzed by the Kolmgorov-Smirnov test on the IBM Statistic SPSS 25 application to test the normality of the data.

RESULTS

Analysis of the results of the study regarding the Serodiagnosis of Anti Severe Acute Respiratory Syndrome Coronavirus (Anti-SARS-CoV-2) in blood donors in Surabaya was carried out through qualitative antibody examination. All 100 donors were found to have positive IgG SARS-CoV-2 antibodies (Figure 1).

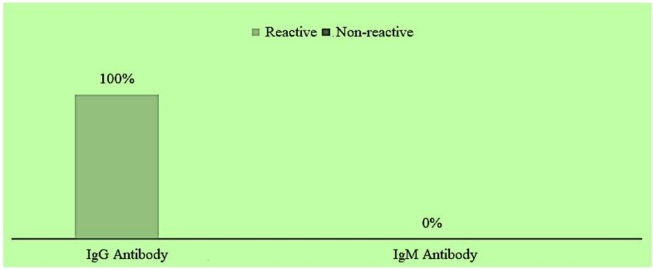

Figure 1. Results of a qualitative SARS-CoV-2 antibody screening test on blood donors at PMI Surabaya

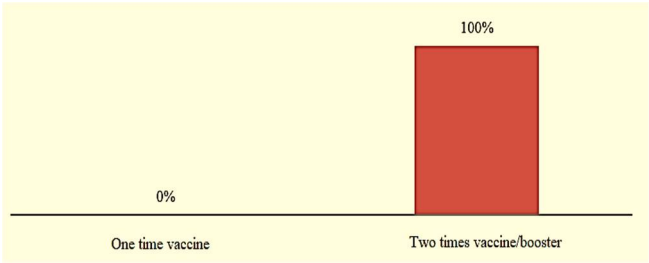

Figure 2. Data on the vaccinated donors

Examination showed that all positive donors had SARS-CoV-2 IgG antibodies. This was because most of the donors already had antibodies from Covid-19 infection or from vaccinations. Figure 2 shows the results of PMI Surabaya questionnaire which showed that 100 donors had already been vaccinated against SARS-CoV-2.

Then, the examination was continued on the antibody titer against SARS-CoV-2 using Enzyme Linked Immunosorbent Assay (ELISA) method. The results of the positive and negative optical density (OD) examination in upper, middle, and lower limits are presented in Figure 4.

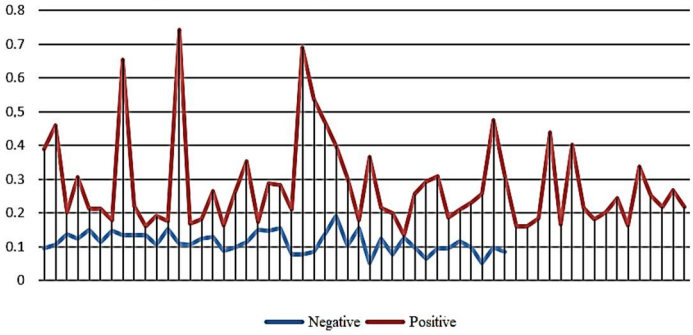

Figure 3. Optical density (OD) value of SARS-CoV2 antibodies in the donors

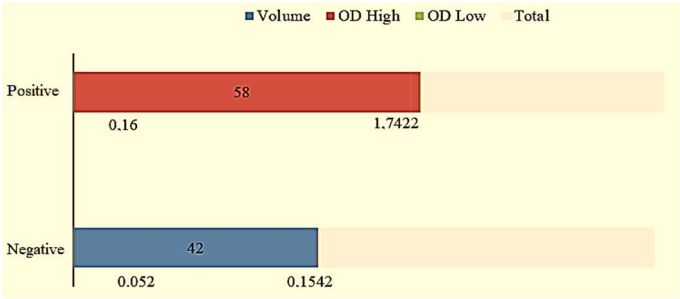

Figure 4. Optical density (OD) value of SARS-CoV2 antibodies in the donors

Data normality test intended to determine whether the research data regarding the Serodiagnosis of Anti-Severe Acute Respiratory Syndrome Coronavirus (Anti-SARS-CoV-2) in Blood Donors in Surabaya had a normal distribution was performed by using One Sample Kolmogorov-Smirnov test (Table 1).

One-Sample Kolmogorov-Smirnov test					
Mean ± SD (U/mL)	Maximum (U/mL)	Minimum (U/mL)	Positive	Negative	p-value
0.2171 ± 0.14412	1.7422	0.16	0.192	-0.133	0.000

The Kolmogorov-Smirnov normality test showed that the optical density examination showed a p-value of 0.000. The p-value of the data was less than 0.05, then the data was normally distributed.

DISCUSSION

Serodiagnosis of anti-SARS-CoV-2 in donors' blood at UTD PMI Surabaya obtained from antibody screening qualitatively showed that all donor blood was reactive to IgG antibodies. This was in accordance with the requirements for donors at the PMI, namely, among others, having a minimum interval of 3-6 months after SARS-CoV-2 infection and after receiving vaccination. Individuals who had previously been exposed or vaccinated may take two to three weeks to develop an antibody response. The administration of antibodies has the potential to prevent disease or shorten the duration or severity of disease sufficiently to prevent serious complications.[2]

Antibodies produced by B cells after vaccination allow the donor to prevent infection, although the immune system response differs from person to person. Post-vaccination antibody responses begin to occur in an average of 1-3 weeks. Anti-N antibodies are antibodies to nucleocapsid proteins in the SARS-CoV-2 virus. Antibodies that function to provide viral inhibition are called neutralizing antibodies which are mostly formed against spike (S) protein.[7] Seroconversion that results in the emergence of IgG, instead of IgM, generally occurs in the second or third week, after rapid IgM decline and prolonged IgG persistence.[8]

IgG is the main class of immunoglobulins found in blood, comprises 75% of the total serum immunoglobulins and has long-term immunity and immunological memory. Therefore, viral antigen-specific measurements of IgM and IgG in combination have been used in various serological tests to detect SARS-CoV-2 infection as previously used for SARS and other coronaviruses.[9] IgM production, which indicates the presence

of acute infection episodes of up to more than one month, suggests prolonged SARS-CoV replication in exposed individuals. In contrast to IgM, IgG can persist for a longer period of time, such as IgG from SARS-CoV which can still be detected up to week 24. Other studies have shown that IgG and neutralizing antibodies can persist for up to two years post-infection. This indicates that IgG may have protective properties against recurrent infection or be significantly higher than IgM levels after the second vaccination dose.[10,11]

Mitani (2020)[12] in his research shows a significant decrease in IgM titers due to several factors, including sex (male), age, smoking, and comorbidities of hypertension, cardiovascular, and diabetes mellitus, as well as a history of COVID-19 in the past, which may have influenced the clinical course of the disease. IgG titers are negatively correlated with cardiovascular comorbidities, and positive with COVID-19. Interestingly, all of these, with the exception of COVID-19, have been reported as risk factors for severe illness from COVID-19. It has also been reported that overall IgM decreases with age which will influence the trend of IgM titers.

Antibody testing using rapid method is very commonly used, which is done by detecting the presence of antibodies in an individual's blood. This test plays an important role in assisting the discovery of a vaccine, but not for clinical diagnosis because it cannot be determined whether the infection in the patient being examined is ongoing. WHO recommends that such tests can assist disease surveillance and epidemiological research. Detection of these antibodies can also reveal cross-reactions with other pathogens, such as other types of human-infecting coronaviruses, resulting in false-positive results.[13]

Another antibody test that can be performed is a virus-induced antibody test. This assay has unique advantages in clinical diagnostics, particularly in identifying persons who acquire immunity to pathogens without overt symptoms.[9] ELISA examination is a confirmation examination of the results of antibody examination using the rapid method. This test provides critical information on the diagnosis, management and recovery of COVID-19 infection and can help researchers evaluate how many people in the population have been infected in order to plan infection control.[14]

Antibody examination by ELISA method showed 58% positive and 42% negative (Cut Off Index = 0.1569) (COI < negative; COI > positive). Positive results indicate the presence of SARS CoV2 antibodies. Antibody is one of the important humoral immune systems in defense and protection against viral infections. When the body is infected by a virus, antibodies are in charge of connecting the infected cells with effector cells. IgG antibodies are divided into two domains, Fab and Fc. Fab domains play a role in recognition and binding to specific antigens and Fc plays a role in linking bound antigens through cellular receptors called Fc gamma receptors (FcγRs). In general, the role of IgG antibodies is to trigger the expression of FcγRs which will cause phagocytosis, antibody-dependent cell-mediated cytotoxicity, and the activation of the complement system.[15,16]

This study obtained a negative result (COI < 0.1569) of 42%, indicating a decrease in IgG antibodies in individuals who received inactivated vaccine. Another study also demonstrated a decrease in IgG antibody response observed three months after two doses of vaccination with the inactivated vaccine. A decrease in antibody response over time is a natural occurrence in the antibody life cycle.[16] This shows that, despite the decrease in antibody, the components that can restart antibody production and coordinate attack against the virus persist at a fairly high level. The same mechanism leading to immune memory after infection also forms the foundation for immunity after vaccination.

Several factors that cause antibodies are not formed maximally after the vaccine are grouped into 2 main factors, primary factors that are related to the wrong immunization schedule and history of infection, and secondary factors that are related to age, sex, nutritional status, immune status, and comorbidities.[17]

According to Haft (2020)[18] theoretically, increasing age causes a decrease in naive T cells available to respond to the vaccines. The normal ratio of CD4 cells to CD8 cells becomes much higher in older age because of the significant decrease in CD8 T cells. Aged donors may also cause a loss of T-cell receptor diversity on CD8 and CD4 cells. Changes in the production of effector T cells, which are shorter-lived than memory precursor cells, result in impaired response of T helper cells to vaccination. The number of B cells tends to be consistent in old age, but the reduced expression of certain proteins in old age causes fewer functional antibodies to be produced.[19]

A research by Ross et al (2020) showed that an increase in Body Mass Index (BMI) could affect the decline in post-vaccination immune function and antibody titers. Obesity is associated with increased production of inflammatory cytokines, such as TNF-α, interleukins, and interferons, which characterize low-grade chronic inflammation that impairs both innate and adaptive immune responses. Other studies have shown that a higher body mass index (BMI) or obesity is associated with lower antibody titers in an immune response to the SARS-CoV-2 vaccine.[20]

Comorbid patients are not recommended to receive the vaccine unless under the supervision of the treating doctor. This is because such condition predisposes to poorer clinical outcomes in COVID-19 infection. One of the salient features of SARS-CoV-2 infection is lymphopenia which is associated with the severity of the disease. In several studies, it was found that lymphopenia in patients affected CD4+ and CD8+ T cells, B cells and T killer cells.[21]

In current situation, with the increase in SARS-CoV-2 variants, important of serosurveys of blood donors in monitoring the transmission pattern and epidemiological trends of SARS-CoV-2 transmission. the results of our study suggest that blood donation centers could be incorporated into COVID-19 surveillance systems with the role of regularly providing quantitative estimates of SARS-CoV-2 seroprevalence in the population. The addition of new variants of the virus (Omicron) has been predicted since the beginning of the pandemic, which is feared to affect the pathogenicity, nature of transmission, and response to the body's immune system. Examination of IgG antibody levels after vaccination can be a way of monitoring antibody responses in individuals, especially in someone who has a high risk of being exposed to SARS-CoV-2, so that it requires reconsideration related to revaccination or boosters. [17,22]

CONCLUSION

Based on the results, the presence of IgG SARS-CoV-2 antibodies in blood donors in Surabaya, Indonesia was 58%.

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