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Original Article

Serodiagnosis Profiling of Anti SARS-CoV-2 Among Blood Donor Surabaya Indonesia

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ABSTRACT

SARS-CoV-2 antibody detection is used as a response to natural infection and vaccination. Antibody response is very important in viral seroepidemiology and the potential role of antibodies in disease. This study was to determine serodiagnosis anti SARS-CoV-2 among blood donor Surabaya detect antibodies with protein molecular weight as serodiagnosis and immune response to SARS-CoV-2 in donors in Surabaya. The research used in this study was exploratory descriptive design. Samples from blood donors as many as 150 samples at UTD PMI Surabaya. The examination was carried out by detecting IgG SARS-CoV-2 antibodies ELISA method and followed by protein molecular weight examination SDS-PAGE method (semi-log The results of the ELISA method antibody examination showed that 67 people had IgG antibody titers against SARS-CoV2 (44.7%) and 83 people were negative (55.3%) (COI: 0.1535-0.1569). Furthermore, the results of the SDS- PAGE method examination obtained the average molecular weight in band 1: 49.73 kDA, band 2: 25.26 kDA; band 3: 19.63.4 kDA; band 4: 12.35 kDA and band 5: 7.33 kDA. There were IgG antibody of SARS CoV-2 in blood donor Surabaya meanwhile from protein screening results we could not find protein of spike SARS-CoV-2 in Blood donor Surabaya.

Keywords: *SARS-CoV-2, IgG, Antibody Protein, Blood Donor*

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INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2). The Covid 19 situation in Indonesia as of March 24, 2023, recorded 6,743,171 confirmed positive covid 19 cases and 160,985 deaths. The use of vaccines is used as a specific antiviral therapy for SARS-CoV-2 infection, so vaccines are one way to stop the pandemic [1]. One form of prevention that can be done is to carry out health protocols and vaccinations in accordance with government programs. The body's immune response that can occur after vaccination is a humoral response. The humoral response, namely antibodies, is the body's immune response that is most easily detected as a form of monitoring the immune system's resistance to infection, including infection by SARS-CoV-2. The humoral response is the body's immune response that is most easily detected as a form of monitoring the immune system's resistance to infection, including infection by SARS-CoV-2, namely the formation of antibodies.

Several antibody-level tests detect antibodies to the spike (S) protein or nucleocapsid protein. (N) SARS-CoV-2 virus. The interaction between the host and SARS-CoV-2 that causes infection involves a complex response from the immune system.

From Nidom et.al., 2022 showed that samples of people who were vaccinated against SARS-CoV-2 virus and survivors had a better IFN-γ. IFN-γ is a promoter of all immune system regulations when there are viral antigens that enter the body. IFN-γ not only plays an important role in innate immunity but also in adaptive immunity [2].

The SARS-CoV-2 antibody examination aims to detect antibodies produced by the human body in response to natural infection by SARS-CoV-2 and to vaccination. Detection of antibody responses, especially in the form of IgG, is very important in determining the seroepidemiology of the virus and the potential role of antibodies in disease[3]. In addition, monitoring of antibody levels in donors needs to be done as a form of protection, because the durability of the current vaccine protection unkhown and the nature of the antibody cycle which decreases over time [3,4]. Diagnostic methods to detect the presence of an immune response using serological approaches, namely using ELISA to measure antibodies and the SDS PAGE method to measure protein molecular weight. Monitoring of antibodies that have decreased over time is detected [5]. This study aims serodiagnosis anti SARS-CoV-2 among blood donor Surabaya and detect antibodies with protein molecular weight as serodiagnosis and immune response to SARS-CoV-2 in donors in Surabaya.

METHOD

The type of research used is exploratory descriptive research design. This research was conducted at Medical Laboratory Technology, Polytechnic Ministry of Health Surabaya,, UTD PMI Surabaya, Balai Besar Laboratorium Kesehatan Surabaya (BBLK), and Professor Nidom Foundation (PNF). This research has received Description of Ethical Exemption Number : .EA/1752/KEPK-Poltekkes_Sby/V/2023. The number of samples taken randomly was 150 blood donors. Donor blood samples have the criteria that the donor has done booster vaccination 2 until 3 times.

ELISA

Blood donor was examined using the ELISA method to measure SARS-CoV-2 IgG antibody levels and blood containing IgG antibodies using The SARS-CoV-2 Spike Protein IgG ELISA Kit (E-EL-E602; Elabscience, Houston, Texas, USA). The procedure was followed as in the kit.

SDS PAGE

Sera of blood donor then continued to analyze by SDS-PAGE to know the pattern of protein molecular weight. For Vertical electrophoresis was used Bio Rad MINI-PROTEAN with gel concentration 10% and dilution samples 30 times. Reading the gel result of SDS_PAGE was using the DocTM EZ Gel (Biorad) with Bio-Rad Precision Plus and Regression method Point to Point (semi-log) for determination of protein weight. Data analysis was presented descriptively in the form of IgG antibody titer results and protein molecular weight against SARS-CoV-2 virus in donor blood in Surabaya.

RESULTS

Collecting samples of blood donor was done from September 2022 until March 2023. From total 150 samples, 50 people have done the 2nd and 3rd vaccinations and 100 people just had done 2nd vaccinations. The distribution data of samples can be seen in table 1.

Table 1. Distribution of blood donor subject that had been vaccinated by SARS-CoV-2 vaccine

Samples Size	History Vaccination of SARS-CoV-2		Gender	
	2 time vaccination	3 time vaccination	Female	Male
150	100	50	38	112

Examination of antibody titer against SARS-CoV-2 by Enzyme Linked Immuno Sorbent Assay (ELISA) can be seen in Table 2. The total blood donors who had positive results for SARS-CoV-2 antibodies in 2022 and 2023 were 67 samples of the ELISA method, The range value of OD (optical density) from samples was explained in figure 1. The positive samples then continued examination of antibody spikes by the SDS-PAGE method.

Table 2. ELISA results of sera from blood donor

	Total Samples	Percentage
Number of Positive	67	44,7%
Number of Negative	83	55,3%
Total Quantity	150	100%

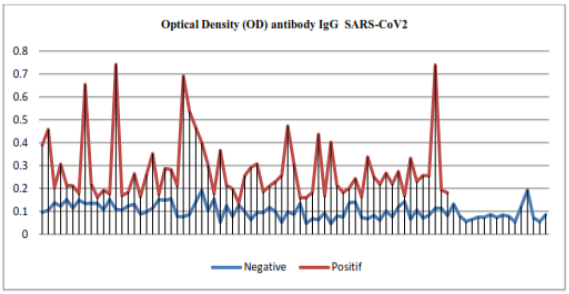
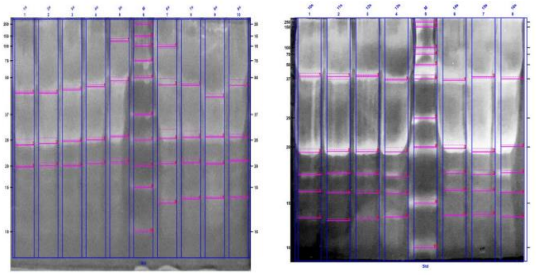


Figure 1. The optical density of antibody IgG of SARS-CoV-2. The cut off value was 0.1569, value below cut off was negative and value above the cut off determined to be positive

SDS-PAGE examination with the molecular weight of the protein bands that appear is calculated using the point to point method (semi-log). According to F. Hajizadeh et al. 2022 the weight of spike protein is 140kDa. The results of SDS-PAGE showed that out of 67 samples, 34 samples was no band in the area of 140KDa, the closest to the size of spike protein of SARS-CoV-2 was samples 5S with protein weight 123.4 kDa [6]. The results of SDS-PAGE can be seen in Table 3 and figure 2.

Table 3. Results of analysis of SDS-Page 1 protein expression dimensions of blood donor sera

	Molecular weight (kDa)				
Well samples	**Band 1**	**Band 2**	**Band 3**	**Band 4**	**Band 5**
1S	44,1	24,0	19,8		
2S	44,1	24,3	20,1		
3S	45,3	24,8	20,1		
4S	46,5	25,1	20,4		
5S	123,4	48,7	26,4	20,7	
6S	99,3	47,1	25,7	20,4	13,0
7S	47,1	26,1	20,6	13,5	
8S	42,8	26,3	20,4	13,6	
9S	47,0	26,3	21,0	13,7	
10S	39,1	19,5	17,4	15,9	13,1
11S	38,8	19,6	17,5	15,8	12,8
12S	39,1	19,5	17,4	15,8	13,0
13S	36,7	19,5	17,2	15,7	13,2
14S	36,5	19,9	17,5	16,0	13,4
15S	38,5	19,5	17,4	16,0	13,5
16S	37,0	20,1	17,5	15,8	13,2



(a) (b)

Figure 2. SDS-PAGE of Blood donor sera. (a) SDS-PAGE gel of samples code 1S-9S and (b) SDS-PAGE gel of samples 10S-16S

Furthermore, the results of the SDS-PAGE examination obtained the average molecular weight in band 1: 49.73 kDA, band 2: 25.26 kDA; band 3: 19.63.4 kDA; band 4: 12.35 kDA and band 5: 7.33 kDA.

DISCUSSION

Serodiagnosis of antibodies to SARS-CoV-2 in donor blood in Surabaya was conducted with Serologic testing in important populations for epidemiology and public health information in Surabaya. 150 donors who have been vaccinated with 2-3 booster. The results of antibody screening against SARS-CoV2 in donor blood were 67 people who had IgG antibodies and 83 negative donors. The presence or absence of antibodies to SARS-CoV2 indicates the level of immunity at a certain point in time as well as individual immune gaps [7]. Donors who have antibodies as an indicator of immunity in the population in Surabaya. Heterogeneity in susceptibility to infection or immunity acquired either through natural infection or through vaccination in the population, the presence of antibodies has the potential to prevent disease or disease severity sufficiently to prevent serious complications and the presence of seroprevalence in achieving herd immunity [5,7]. ELISA method testing is a serological examination protocol by analyzing the proteins IgG, IgM, IgA, spike, and RBD SARS-CoV2 antigen[8]. This examination provides very important information for the diagnosis, management, and recovery from COVID-19 infection and can help researchers evaluate how many people in the population have been infected, to plan infection control [9].

The results of antibody examination in donor blood using the ELISA method showed 67 people had IgG antibody titers against SARS-CoV2 (44.7%) and 83 people were negative (55.3%) (COI: 0.1535-0.1569). Proteins spike (S) viral induce neutralizing antibodies and immunization with vaccines encoding viral nucleocapsid (N) proteins can induce eosinophilic responses. To produce neutralizing antibodies, viral antigens are recognized by Antigen Presenting Cells (APC) which will stimulate the body's humoral immunity through virus-specific B cells and plasma. Antibody is one of the important humoral immune systems in the defense and protection of viral infections [1]. The antibody response will increase after being infected with SARS-CoV2. IgG concentrations remain high for approximately 4 to 5 months before decreasing and even disappearing slowly for 2 to 3 years later. IgG antibody formation may be important to provide a long-term protective role. A decline in antibody response over time is a natural occurrence in the life cycle of antibodies. Approximately 5-10% of individuals show a decrease in antibodies or IgG antibodies may not be detected due to post-infection or the response of humoral immunity, a component that can restart antibody production and coordinate the attack on the virus, the same mechanism that leads to immune memory after infection also forms the foundation for immunity after vaccination [1,10,11].

Donor blood with SARS-CoV-2 IgG antibodies was subjected to SDS-PAGE to detect antibody spikes. SDS-PAGE examination with the molecular weight of the protein bands that appear is calculated using the Point to Point (semi-log) method. All band samples are positive if they have protein spike S and if there is a band at 140 kDa. The results showed that out of 67 samples, 34 samples with bands less than 140 kDa were declared negative. The results showed the average molecular weight in band 1: 49.73 kDa, band 2: 25.26.9 kDa; band 3: 19.63.4 kDa; band 4: 12.35 kDA, and band 5: 7.33 kDA. This illustrates that the molecular weight produced is below 140 kDA which is the standard for the presence of protein spike (S) and nucleocapsid (N).

SARS-CoV-2 has proteins including spike (S) and nucleocapsid (N), which are considered the main immunogens and are widely used in immunoassays. It has been shown that anti-nucleocapsid antibodies appear earlier than spike antibodies. Therefore, the application of anti-nucleocapsid antibodies in ELISA assays can improve the clinical sensitivity of the assay. Nucleocapsids are small-sized proteins that are easily produced and purified in large quantities in prokaryotic or eukaryotic hosts. Homologous analysis shows that the SARS-CoV-2 nucleocapsid is 28- 49% amino acid identical to other alpha and beta coronaviruses The same level of similarity to other coronaviruses is also seen in the spike. The same level of similarity to other coronaviruses is also seen in the spike protein, which will increase on immunoassay examination[12]. The non-detection of antibodies in donors is due to antibody levels against anti-spike (S) and anti-nucleocapsid (N) proteins in SARS-CoV2 decreasing at 2-3 months and continuing up to 8 months after vaccination or after infection. Although the ability of the vaccine over time is still limited, it has been shown to limit the spread of SARS-CoV-2 [12].

Factors that cause antibodies not to form optimally are primary factors associated with immunization and history of infection. Secondary factors are associated with age, gender, nutritional status immune status, vaccine type, and comorbidities [13]. Increasing age theoretically leads to a decrease in naive T cells available to respond to vaccines. The normal ratio of CD4 cells to CD8 cells becomes much higher in older age, due to a significant decrease in CD8 T cells [14]. Production changes towards short-lived effector T cells rather than memory precursor cells, resulting in impaired helper T cell responses to vaccination. Reduced expression of Certain proteins with age leads to fewer antibodies being produced by B cells [15]. Qualitative changes include a shift in production towards short-lived effector T cells rather than memory precursor cells, resulting in impaired cellular response to vaccination. While B cell numbers tend to be consistent in old age, the expression of certain proteins in old age leads to fewer functional antibodies being produced, theoretically resulting in ineffective vaccination[16].

In a study conducted by Ross et al (2020), an increase in Body Mass Index (BMI) can affect the decline in immune function and

post-vaccination antibody titers. Obesity is associated with increased production of inflammatory cytokines, such as TNF-α, interleukins, and interferons that characterize chronic low-grade inflammation that impairs immune responses, both innate and adaptive. other studies have shown that higher body mass index (BMI) or obesity is associated with lower antibody titers as an immune response to the SARS-CoV-2 vaccine [17].

Our study has limitations. First, the donor population was of various ethnicities. In addition to differential polymorphisms that may explain susceptibility and even outcomes in different ethnic populations, the fact that ACE2 is localized on Xp22.2 may help explain male-related risks. Therefore, even in the absence of variation in this gene, the presence of the gene may affect the natural history and prognosis of COVID-19 in men. Comparative genetic analysis suggests that genomic variants of ACE2 may play an important role in susceptibility to COVID-19 and related cardiovascular conditions by altering the AGT-ACE2 pathway (i.e., p.Arg514Gly) [18].

CONCLUSION

Based on the research, this conclusions are : There were IgG antibody of SARS CoV-2 in blood donor Surabaya eventhough from protein screening results we could not find protein of spike SARS-CoV-2 in Blood donor Surabaya.

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