Original Research Report

LYMPHOCYTE CELL EXPRESSIONS AND SARS-COV-2 ANTIBODY TITERS IN HEALTHY DONORS’ BLOOD AT AN INDONESIAN BLOOD TRANSFUSION CENTER (UTD PMI SURABAYA)

Adelia Gita Prasasti*, Evy Diah Woelansari, Suhariyadi, Anita Dwi Anggraini

Medical Laboratory Technology Department, Poltekkes Kemenkes Surabaya, Surabaya, Indonesia

ABSTRACT

SARS-CoV-2 is a virus that infects the respiratory tract and attacks the respiratory mucosa and epithelial cells. Lymphocytes are a subset of agranulocyte leukocytes that have a role in the immune response to pathogenic microorganisms' attacks. The number of lymphocytes will increase if a virus causes an infection. An antibody is a protective protein produced by the body's immune system in response to the presence of an antigen. Antibodies bind and inactivate foreign substances so that the replication of pathogens can be prevented and destroyed. This mechanism can be expressed through the total number of lymphocyte cells. The objective of this study was to define the relationship between the number of lymphocytes and antibody titers against SARS-CoV-2. The type of research used was analytic observational with a cross-sectional approach using quantitative analysis methods and a purposive sampling method for sampling. The samples used were 30 donor blood samples from UTD PMI Surabaya. This research was conducted in May 2022 at the Hematology Laboratory, Department of Medical Laboratory Technology, Poltekkes, Ministry of Health, Surabaya, to examine the number of lymphocyte cells using the flowcytometry method and the Immunoserology Laboratory of the Surabaya Health Laboratory Center for antibody titer examination using the ELISA (Enzyme-Linked Immuno Sorbent Assay) method. The study showed an average lymphocyte level of 2.2633 x 10³/µl and an average antibody titer value expressed in OD of 0.2197. Data analysis using Spearman's rank correlation statistical test revealed p = 0.262 0.005. It showed no relationship between total lymphocyte count and antibody titers against SARS-CoV-2.

Keywords: SARS-CoV-2; Antibody; Lymphocyte; ELISA; Optical Density; Tropical Disease

*Correspondence: Evy Diah Woelansari, Medical Laboratory Technology Department, Poltekkes Kemenkes Surabaya, Surabaya, Indonesia. Email: evydiahws@gmail.com

Highlights:

1. There was no relationship between total lymphocyte count and antibody titers against SARS-CoV-2.
2. This original research report offers information on the several blood test result after getting COVID-19 vaccines and provides data that the public may use as scientific evidence to the further research.

INTRODUCTION

SARS-CoV-2 is a group of RNA viruses of animal origin that can be transmitted to humans. The virus can enter through the receptor Angiotensin Converting Enzyme 2 (ACE2), which can be recognized by TLR 3. The natural immune response mechanism triggered through the TLR initiates the formation of an adaptive immune response, including its involvement in stimulating B cells to become plasma cells and switching isotypes of the antibodies produced. TLR3 plays a role in (TLR 3-dependent) viral antigen presentation by dendritic cells to be presented to cytotoxic T lymphocytes (CD8+) and modulates and regulates T lymphocyte tolerance.

In the adaptive immune system, the antibodies that appear can function by binding to viral particles and blocking infection of host cells. T cells have a significant role in recognizing and destroying virus-infected cells. Previous viral infections can increase the ability of effector cells because there is a memory to activate recall responses. Several studies have shown that the immune response that elicits during the infection appears uncontrolled. The hyperactivation of monocytes and macrophages can cause an increased neutrophil, expression of IL-6, C Reactive Protein (CRP), and a decrease in the number of lymphocytes (Laili, 2020).

In SARS-CoV-2 infection, lymphocytes function as antigen-presenting cells (APCs) and effectors that can produce chemokines and cytokines. Lymphocytes can differentiate and proliferate into T helper cells, cytotoxic T cells, or B cells based on the stimuli they receive. B lymphocyte cells can produce IgM antibodies (antibodies that appear in the acute phase) and IgG antibodies (antibodies that represent a long-standing infection. After SARS-CoV-2 infection, IgM antibodies can be detected in the blood serum of survivors on days 3-6. In contrast, IgG can be detected after eight days of symptom onset. Seroconversion can be observed in the second week after symptom onset. Several previous studies have suggested that individuals who recovered from SARS-CoV-2 can achieve lasting immunity with high antibody titers (IgG).
Antibody titers in plasma can be found in reasonably stable amounts for at least 5 to 8 months post-infection (Ramanathan et al., 2020).

A meta-analysis study conducted by Lagunas-Rangel (2020) showed that the lymphocyte count could be used to monitor the treatment and diagnosis of infected patients. In addition, a study conducted by Shereen et al., (2020), also stated that a decrease in the number of lymphocytes was assessed as the main character in detecting the severity of the patient. Given the information provided earlier, we were interested to explore the relationship between antibody titers and the expression of the total number of lymphocytes from healthy donors at UTD PMI Surabaya City, Indonesia, who may have antibodies against SARS-CoV-2.

**MATERIALS AND METHODS**

The research design used was an analytical observational study with a cross-sectional approach using quantitative analysis methods, which aimed to define whether there was a relationship between the variables of antibody titer against SARS-CoV-2 and the number of lymphocytes in the blood of healthy donors at UTD PMI Surabaya. This research received an ethical clearance approval from Ethics Committee of Poltekkes Kemenkes Surabaya under decree EA/841/KEPK-Poltekkes_Sby/V/2022.

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 in the period from March to April 2022 at the PMI Blood Transfusion Unit, Surabaya City. The blood donors were donors who met the procedures and requirements for blood donation following the provisions of the UTD PMI Surabaya City and have been declared healthy and eligible to donate blood. Blood donors were native citizens or residing in the city of Surabaya. Those regarded as unqualified donors and not entitled to undergo blood donor procedures were those originating or residing outside the city of Surabaya.

Data collected were primary data with a ratio scale from the results of examinations in the Immunoserology Laboratory of the Surabaya Health Laboratory (BBLK) and the Hematology Laboratory, Department of Technology, Medical Laboratory, Poltekkes, Ministry of Health, Surabaya. Blood specimens from healthy donors that met the criteria were examined for antibody titers, and lymphocyte cell counts.

The data were obtained with a ratio scale, then analyzed with the Kolmogorov-Smirnov Normality test on the IBM Statistic SPSS 25 application. The data were found to be normally distributed, so that the test was continued with the Spearman correlation test. In contrast, the data that were not normally distributed were tested using the Spearman Rank correlation test and calculated using the SPSS statistical application program version 25.

**RESULTS**

This research was carried out by taking 3 ml of blood at PMI UTD Surabaya in the EDTA tube for checking the total number of lymphocytes and 3 ml in the plain tube for checking the antibody titer. Furthermore, the blood donors who had antibodies against SARS-CoV-2 were screened using a qualitative antibody test.

The results obtained revealed that all positive specimens had an IgG antibody response. In comparison, the IgM antibodies did not show a positive sign. This might be related to the fact that one of donor requirements was to have a minimum interval of 3-6 months after SARS-CoV-2 infection and after receiving the vaccination.

The examination results of the total number of lymphocyte cells in the blood of healthy donors were, on average, within normal limits. Physical activity, medication, and infection affect total lymphocyte levels in the body. (Tiara et al., 2016). In this study, most of the respondents had lymphocyte levels within normal limits. It was because the respondents were in good health and were not suffering from infectious diseases or not being exposed to infectious diseases.

Table 3 shows that there is no significant relationship between the two variables. The result of Spearman’s correlation test on the relationship between lymphocyte cell number and SARS-CoV-2 antibody titer revealed 0.211, while the negative sign indicated a negative relationship, where an increase in antibody titer was followed by a decrease in the number of lymphocytes.
Table 1. Qualitative antibody screening test. The specimens used in this study first passed screening using qualitative antibody testing.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample Codes</th>
<th>Examination Results</th>
<th>Ig G</th>
<th>Ig M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>X070401</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>X070402</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>X070403</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>X070405</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>X070406</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>X041101</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>X041102</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>X041103</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>X041104</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>X041105</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>X041106</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>X041107</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>X041108</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>X041109</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>X041110</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>X041301</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>X041302</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>X041303</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td>X041304</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>X041305</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td>X041306</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td>X041307</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>X041308</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td>X041309</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>25.</td>
<td>X041310</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>26.</td>
<td>X041311</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>27.</td>
<td>X041312</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>X041313</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td>X041314</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
<tr>
<td>30.</td>
<td>X041315</td>
<td>Reactive</td>
<td>Non Reactive</td>
<td></td>
</tr>
</tbody>
</table>

Tabel 2. Lymphocyte and SARS-CoV-2 antibody Test

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample Codes</th>
<th>Lymphocytes</th>
<th>Optical Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>X070401</td>
<td>2.3</td>
<td>0.2508</td>
</tr>
<tr>
<td>2.</td>
<td>X070402</td>
<td>4.3</td>
<td>0.2184</td>
</tr>
<tr>
<td>3.</td>
<td>X070403</td>
<td>2.7</td>
<td>0.2667</td>
</tr>
<tr>
<td>4.</td>
<td>X070405</td>
<td>3.8</td>
<td>0.1168</td>
</tr>
<tr>
<td>5.</td>
<td>X070406</td>
<td>2.3</td>
<td>0.2168</td>
</tr>
<tr>
<td>6.</td>
<td>X041101</td>
<td>1.2</td>
<td>0.769</td>
</tr>
<tr>
<td>7.</td>
<td>X041102</td>
<td>1.9</td>
<td>0.16</td>
</tr>
<tr>
<td>8.</td>
<td>X041103</td>
<td>1.8</td>
<td>0.0866</td>
</tr>
<tr>
<td>9.</td>
<td>X041104</td>
<td>1.6</td>
<td>0.1403</td>
</tr>
<tr>
<td>10.</td>
<td>X041105</td>
<td>1.6</td>
<td>0.1918</td>
</tr>
<tr>
<td>11.</td>
<td>X041106</td>
<td>1.4</td>
<td>0.183</td>
</tr>
<tr>
<td>12.</td>
<td>X041107</td>
<td>2.1</td>
<td>0.2012</td>
</tr>
<tr>
<td>13.</td>
<td>X041108</td>
<td>1.5</td>
<td>0.2436</td>
</tr>
<tr>
<td>14.</td>
<td>X041109</td>
<td>1.9</td>
<td>0.1637</td>
</tr>
<tr>
<td>15.</td>
<td>X041110</td>
<td>1.5</td>
<td>0.3373</td>
</tr>
<tr>
<td>16.</td>
<td>X041301</td>
<td>1.5</td>
<td>0.3028</td>
</tr>
<tr>
<td>17.</td>
<td>X041302</td>
<td>1.9</td>
<td>0.1777</td>
</tr>
<tr>
<td>18.</td>
<td>X041303</td>
<td>1.7</td>
<td>0.366</td>
</tr>
<tr>
<td>19.</td>
<td>X041304</td>
<td>2.2</td>
<td>0.2142</td>
</tr>
<tr>
<td>20.</td>
<td>X041305</td>
<td>2.6</td>
<td>0.1984</td>
</tr>
<tr>
<td>21.</td>
<td>X041306</td>
<td>2.7</td>
<td>0.1351</td>
</tr>
<tr>
<td>22.</td>
<td>X041307</td>
<td>2.5</td>
<td>0.2563</td>
</tr>
<tr>
<td>23.</td>
<td>X041308</td>
<td>2.6</td>
<td>0.292</td>
</tr>
<tr>
<td>24.</td>
<td>X041309</td>
<td>3.7</td>
<td>0.3075</td>
</tr>
<tr>
<td>25.</td>
<td>X041310</td>
<td>2.2</td>
<td>0.1348</td>
</tr>
<tr>
<td>26.</td>
<td>X041311</td>
<td>2.8</td>
<td>0.1066</td>
</tr>
<tr>
<td>27.</td>
<td>X041312</td>
<td>2.5</td>
<td>0.1861</td>
</tr>
<tr>
<td>28.</td>
<td>X041313</td>
<td>2.3</td>
<td>0.1519</td>
</tr>
<tr>
<td>29.</td>
<td>X041314</td>
<td>2.0</td>
<td>0.1091</td>
</tr>
<tr>
<td>30.</td>
<td>X041315</td>
<td>2.8</td>
<td>0.1054</td>
</tr>
</tbody>
</table>

Tabel 3. Correlation between lymphocytes cell and titer antibodies based on Spearmen’s test result

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Titer Antibodies (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coeff.</td>
<td>-0.211</td>
</tr>
<tr>
<td>Significance</td>
<td>0.262</td>
</tr>
</tbody>
</table>
DISCUSSION

Lymphocytes are a subset of agranulocyte leukocytes with several functional roles related to the immune response to the attack by microorganisms, foreign macromolecules, and cancer cells. In this study, no respondents had decreased lymphocyte levels—the percentage of lymphocytes below the normal range caused by the number of lymphocytes already in the tissue. Decreased lymphocyte levels can be caused by the migration of lymphocytes from the blood circulation to the tissues. The maximal load also causes a decrease in antibody production and a general decrease in lymphocyte function. In this study, one person (3.33%) had lymphocyte levels above normal. The total number of lymphocytes may increase due to lymphatic leukemia, mononuclear infection, or viral infection. Increased levels of lymphocytes can occur if there is damage to cells in tissues or organs that require a response to the destruction of damaged cells or apoptosis (Nisnawati et al., 2021).

In the humoral immune response to SARS-CoV-2, CD4+ T cells will interact with B cells. The binding of antigen to receptors on B cells’ surface causes B cells to be activated and differentiated into antibody-forming plasma cells (IgM and IgG). IgM and IgG will appear in the second week after exposure to the virus, followed by antibodies that can neutralize viral infection (neutralizing antibodies). IgM production begins declining in the fourth week and will disappear three weeks later. As a sign of the acute stage of infection, continued production of IgM for more than one month indicates the prolongation of SARS-CoV replication in exposed individuals (Ainu rohmah et al., 2020).

In contrast to IgM, IgG can persist for extended periods, 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 after infection. This indicates that IgG may have protective properties against re-infection (Morales- Núñez et al., 2021).

According to research by Banga Ndzouboukou et al., 2021, the decrease in IgG antibody titers in the body, which was observed approximately three months after exposure, was a natural thing that occurred in the antibody life cycle. Another study by Hutapea, 2021 revealed that antibodies in the body decreased after eight months. Although the levels varied considerably between individuals, the number of T-cells decreased slightly. The number of B-cells remained stable and sometimes increased even though it was difficult to measure. This suggests that despite a decrease in antibodies, components that can restart antibody production and coordinate attack against viruses persist at sufficiently high levels. The exact mechanisms leading to immune memory after infection also form the foundation for post-vaccination immunity.

The appearance of these antibodies can also lead to changes in blood components, especially those related to proteins (antibodies) and white blood cells. Some opinions, such as Roitt (1992), suggest that vaccination increases blood protein, leukocytes, and erythrocyte sedimentation rate. Meanwhile, Allan et al (1978) stated that protein and white blood cell levels in the blood would return to average three to five weeks after vaccination. Thus, other blood features will also return to their original state (Roitt, 1992).

Several factors that cause antibodies not to form optimally after the vaccine is grouped into two main factors: primary factors associated with the wrong immunization schedule and history of infection. At the same time, secondary factors are associated with age, gender, nutritional status, immune status, and comorbidities (Nisnawati et al., 2021).

Jackson et al., 2020 compared recovered patients with vaccinated healthy individuals. Previously infected patients had neutralizing antibodies, but not at the same level as those vaccinated, so previously infected patients would have higher levels of effective antibodies if they had similar vaccinations. Meanwhile, according to Ramanathan et al., 2020 Neutralizing Antibody titers were significantly lower in uninfected patients who received a second dose of vaccine than in previously infected subjects who received only one dose of vaccine. Increasing age theoretically causes a decrease in the naïve T cells available to respond to vaccines. The standard ratio of CD4 cells to CD8 cells becomes much higher at an older age due to a significant decrease in CD8 T cells. Aging also causes a loss of T-cell receptor diversity on CD8 and CD4 cells and overall reduces T-cell survival. Qualitative changes include a shift in the production of short-lived effector T cells rather than memory precursor cells, resulting in impaired follicular T-cell helper responses to vaccination. The number of B cells tends to be consistent in old age, but the reduced expression of specific proteins causes fewer functional antibodies (Cheng et al., 2021).

In women, cytotoxic T cells and lymphocytes showed higher activity than in men, including the expression of antiviral and proinflammatory genes that were upregulated in T cells. Several non-specific indicators of cell-mediated immunity were also increased in women, indicating that women had higher lymphocyte proliferation and increased immunological intolerance to foreign substances than men. In general, women show a more
significant antibody response than men. Both basal levels of immunoglobulins and antibody responses to vaccines were consistently higher in women than men (Kleina et al., 2014).

In a study conducted by (Ross et al., 2020) it was reported that there was a relationship between obesity and the immune response of the human body. Increased Body Mass Index (BMI) can affect immune function and antibody titers post-vaccination decline. Obesity is associated with increased production of inflammatory cytokines, such as TNF-, interleukins, and interferons that characterize low-grade chronic inflammation and impairs innate and adaptive immune responses. A study of healthcare workers in Italy showed that a higher body mass index (BMI) or obesity was associated with lower antibody titers in an immune response to the SARS-CoV-2 vaccine (Watanabe et al., 2022). Iqbal et al., 2021 found that patients with larger abdominal circumference were associated with lower antibody titers.

Concomitants include cardiovascular disease, diabetes, and obesity. Comorbid patients are not recommended to receive the vaccine unless under the supervision of the treating doctor. These conditions worsen the clinical outcome of 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 patients had their CD4+ and CD8+ T cells, B cells, and Killer T cells affected (Choi & Cheong, 2021).

In addition, the antibodies formed in the donor's blood are also affected by the administration of a different type of booster vaccine. Several research results reveal that the antibodies formed will decrease six months after the primary dose of vaccine is given, so further doses are needed to increase individual protection, especially in vulnerable communities. Based on the Circular of the Ministry of Health of the Republic Indonesia, the administration of further doses can be done by two mechanisms, the homologous (the type of booster vaccine is the same as the previously obtained primary vaccine) and heterological (the type of booster vaccine is different from the primary vaccine obtained previously) mechanisms (Kementerian Kesehatan Republik Indonesia, 2018). This can result in different levels of antibodies formed in each individual.

**Strength and limitations**

The samples in this study were collected in almost similar conditions, thereby reducing research bias. The results of the study illustrated the result of several blood test after COVID-19 vaccination. Therefore, the information in this study can provide a scientific picture of the importance of COVID-19 vaccination for the public. However, this was a unicentric study conducted in one hospital and covered only a small geographic area. This study also only discussed the relationship between the variables of the number of lymphocytes and antibody titers in healthy donors at UTD PMI Surabaya. Subjects used were less homogeneous, so no accurate data were obtained regarding the formation of antibodies based on the length of time the vaccine was administered and the variables studied were only limited to the absolute number of lymphocytes.

**CONCLUSION**

The antibody titer against SARS-CoV-2 and the number of lymphocyte cells in the blood of healthy donors were within normal limits. There was no relationship between the number of lymphocytes and antibody titers against SARS-CoV-2 in the blood of healthy donors.

**Acknowledgment**

We would like to thank the staff from UTD PMI Kota Surabaya for their hospitality and assistance during the data and specimens collecting phase.

**Conflict of interest**

None

**Ethical consideration**

This research has received permission from Ethics Commitee of Poltekkes Kemenkes Surabaya, Surabaya under decree number EA/841/KEPK-Poltekkes_Sby/V/2022

**Funding disclosure**

None

**Author contribution**

AGP and EDW contributed to the conceptualization study design and methodology. S were data collection, data analysis. ADA were contributed to the final revise.

**REFERENCES**


Indiarto et al.: Short-Term Maltodextrin in Rat Resistance Training