Correlation between Lymphocyte Count and RT-PCR Cycle Threshold Value in Patients with Corona Virus Disease-19 at RS Pelamonia Makassar

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ABSTRACT

Background: COVID-19 caused by Coronavirus (CoV) attacks the human respiratory system. COVID-19 as an outbreak at the end of 2019 caused deaths all over the world. Diagnosis of COVID-19 through RT-PCR and laboratory test such as lymphocyte was count. **Purposes:** The aims of the research is the relationship between CT values and lymphocytes. **Methods:** This type of observational research with a cross sectional study. The sample technique in this study is purposive sampling. The data used is secondary during the July-December 2021 period at the TK II Pelamonia Makassar Hospital. The population in this study was all Covid-19 patients at TK II Pelamonia Makassar Hospital with inclusion criteria of positive RT PCR results, aged 25-50 years and without history of autoimmune disease. The results of lymphocyte and CT values used medical record data. The sample size was 130 subjects accordance theinclussion criteria. **Results:** The research subjects were 130 subjects consisting of 71 (54.6%) male and 59 (45.4%) female. The mean age of the research was 37.88 years. The average of lymphocyte count is 27.95 and average of CT values is 32.30. **Conclusion:** There is no relationship between lymphocytes and CT values with gender and there is no significant relationship between CT values with the lymphocytes counts.
Keywords: covid-19; cycle threshold values; lymphociyte

INTRODUCTION

December 2019 there was an outbreak of pneumonia with unknown causes. World Health Organization (WHO) named the outbreak is Corona Virus Disease 2019 (COVID-19). COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) (Yuliana, 2020). SARS-CoV-2 attack the respiratory system and cause flu to more severe diseases. COVID-19 is a new type of outbreak that is known to have never been identified as attacking humans (Mona, 2020; Rauf et al., 2020; Shereen et al., 2020; Ysrafil et al., 2020, 2021).

On January 24, 2020, it was reported that at least 830 cases had been diagnosed in nine countries like China, Japan, Thailand, Vietnam, Singapore, South Korea, Taiwan, Nepal and the United States. (Mus et al., 2021). In Indonesia, on January 28, 2021 it was reported that a record number of daily

deaths reached 476 people. Until January 31, 2021, the death rate reached 36.18%. This shows that the death rate is more than 7 times of the safe limit set by WHO (5%)(Suni, 2021). The age range of 26-35 years is more susceptible to exposure to COVID-19 and the most COVID -19 patients are aged 31-45 years (Elviani, Anwar and Sitorus, 2021; Hikmawati and Setiyabudi, 2021)

Positive patients with the infection of SARS-CoV-2 and COVID-19 show if the RT-PCR results of the Cycle Threshold (CT) value is below the threshold value. The CT value will be proportionally inversely proportional to the amount of target nucleic acid in the sample, it means that the lower of the CT value, the greater the amount of nucleic acid is detected. RT-PCR test results with a CT value is below the threshold value of the patient at diagnostic positive (Candrawati and Cassidy, 2021).

RT-PCR results with a CT value <29 showed results with a strong positive reaction. This means that there are nucleic acids in large quantities. CT values between 30-39 are positive, there is a moderate amount of nucleic acid targets and CT values > 40 are weak positive, there are small amounts of nucleic acid targets ((PAMKI), 2020).

The immune response to SARS-CoV-2 infection will be stimulated and will cause an excessive increase in cytokines and a decrease in lymphocytes (Rosyanti dan Hadi, 2020). The results of the lymphocyte test are used to support laboratory tests for COVID-19 disease and have prognostic value in determining severe cases. A decrease in the number of lymphocytes or a decrease in the immune system that occurs in people with COVID-19 disease will have a dangerous impact and cause death if the number of lymphocytes continues to decrease along with the increasing severity of the COVID-19(Permana et al., 2021).

Based on research by Permana, Yari and Aditya (2021), lymphocyte test in COVID-19 patients with lymphocytopenia was found in 8 males (26.7%) and 5 in females (16.7%). Study by Laksmi and Sari (2020) about lymphocytopenia occurs in 63% of patients with a lymphocyte count of less than $1x10^9$/L. According to the research by Wijono (2020) show that the results of lymphocytopenia were found in male (68%) and female (32%). Research by Ayu and Yustian (2021) showed the lymphocytopenia value was obtained with a lower percentage compared to other studies, it found 11.8%.

Based on the description above, a study was carried out related to the relationship between lymphocyte count and CT values of RT-PCR in COVID-19 sufferers at Pelamonia Makassar hospital. The purpose of this study is to find out the relationship between lymphocyte count and CT values of RT PCR in COVID-19 patients at Pelamonia Makassar hospital.

METHOD

This research uses observational research with a cross-sectional study. The sample technique in this study was purposive sampling which is according to the criteria of research. The data used is secondary during the July-December

2021 at Pelamonia Makassar hospital. The population in this study was all Covid-19 patients with the inclusion criteria of positive RT PCR results with a CT value <40, aged 25-50 years and had no history of autoimmune disease. The results of lymphocyte test and CT values used medical record data. The sample size of this study was 130 subjects who met the inclusion criteria. Sample collection based on patients tested positive for COVID and conducting leukocyte test. The data obtained was carried out statistical tests. Data is tested for normality, abnormal data is transformed and tested again for normality. The data did not show normally distributed, so the Mann Whitneyand Fisher chi-squarenonparametric tests were used. Nonparametric statistics with Mann Whitney for comparing 2 independent samples between the normal and lymphocytopenic groups based on sex and Fisher's test was used to see the correlation between CT and lymphocyte values due to ordinal scale in both groups.

RESULT
A. Characteristics of Subject
Based on the table 1 of subject characteristics, there were 130 research subjects consisting of 71 (54.6%) male and 59 (45.4%) female subjects. The mean age of the research subjects was 37.88 years. Lymphocyte test results and CT values of RT PCR showed an average of 27.95% lymphocyte values and 32.30 CT values.

B. Correlation of gender to lymphocytes and CT values
Table 2 shows the relationship between gender and lymphocyte results and CT values. The results in table 2 show that there is no relationship between gender and lymphocytes and CT RT PCR values.

C. Correlation of lymphocyte with CT values
Based on table 3 it showed the relationship between lymphocytes and CT values. The results of data analysis showed that there was no relationship between lymphocytes and CT values.

DISCUSSION
The results showed that the average aged of COVID-19 patients was > 35 years. Previous studies have reported that the incidence of COVID at the age of 20-39 in various countries such as Spain, Canada, China, Italy and Germany is quite high (Bulut dan Kato, 2020). These results are supported by research conducted by Hikmawati and Setiyabudi (2021) that most of the COVID -19 patients are aged 31-45 years as 29.9% and the smallest group aged 0-5 years as 1.9%. The highest incidence of COVID-19 was reported with aged 31-45 years as 29% and the least affected were patients aged 0-5 years as 1.7%.

Based on the gender, lymphocytes count increased more in the male. This is because male are more susceptible to COVID-19 than female due to chromosomal and hormonal factors. Females have an X chromosome and the hormone progesterone. The X chromosome has links with many genes involved in the innate and adaptive

immune system as well as several effectors that can control the activation of receptors of cytokines that function as mediators and regulators of the immune system, so that female have a dominant immune system compared to male(Seftiya and Kosala, 2020). In addition, according to research that the age range of 26-35 years is more susceptible to exposure to COVID-19, this shows that this age is a productive age. At productive age the possibility of getting COVID-19 is greater due to high mobility and activity outside the home and the frequency and social interaction of productive groups is also higher(Elviani, Anwar and Sitorus, 2021).

Based on the CT value, the CT result was obtained with an average of 32.30. its show that the average arepositive and there is a moderate amount of nucleic acid targets. CT values at the first infection are usually 20-30 or less, and tend to increase thereafter, reflecting the reduced amount of viral RNA due to the role of the body's immune system. It is important to note that CT values do not reflect the true viral load, which requires standardization with a reference curve (Candrawati and Cassidy, 2021). Based on Research by (Manurung and Sukohar, 2021)that a lower CT value is associated with a higher probability of viral culture. There is study report that, infectivity was defined as growth in cell culture significantly reduced when the CT RT-PCR value was greater than 24. This decrease in lymphocytes can lead to a cytokine storm, the mechanism by which the virus invades the respiratory

tract via ACE2 receptors and then attacks target cells through the CD147 protein, then it causes a decrease in lymphocyte levels (Rahman et al., 2021).

Based on gender, there was no significant difference between normal lymphocytes and lymphocytopenia. This shows that lymphocytopenia is not affected by gender. Although based on a study by Seftiya and Kosala, (2020)show that there are differences in hormones and the immune system between male and female. In male, it is found normal lymphocytes of 46 (64.8%) and lymphocytopenia 25 (35.2%). Whereas in female, normal lymphocytes were 43 (72.9%) and lymphocytopenia were 16 (27.1%). The decrease in the total number of lymphocytes shows that the corona virus affects many immune cells and inhibits the function of the cellular immune system. T cell response was severely disrupted during SARS CoV-2 infection due to the activation of macrophage cells, B cells lymphocyte and T cells lymphocytes responding by producing proinflammatory cytokines and chemokines. However, the release of cytokines can lead to depletion and exhaustion of T cells. The weakening of T cell response is also influenced by mature detritic cells (DC) migrating to lymphoid organs because they are important for activating T cells, thus causing lymphocytopenia(Mus et al., 2021).

Based on gender, there was no significant different between CT values ≤ 29 as 24 (33.8%) and CT values > 29 as 47 (66.2%) in male. In female, CT values ≤ 29 as 16 (27.1%) and CT values > 29 as 43 (7.9%). In daily practice, CT

values of RT-PCR are used as surrogate markers for the amount of virus in a sample and more importantly, for inferring patient infectivity. However, it is not yet figured out how many SARS-CoV-2 virions are needed to cause infection, how long infective virus persists in patients through different stages of infection, or whether CT values correlate with the number of infectious viral particles. A higher CT value indicates a lower amount of viral RNA in a sample, this value is often used as a surrogate parameter for the number of infectious particles but the relevance of the CT value threshold as a measure of infectivity is unclear(Platten et al., 2021).

In this study, no significant relationship was found between the CT value and the number of lymphocytes. This is probably due to the results of the study showing that most of the CT values were > 29. In Another study shown thatlower CT values were significantly associated with: higher lower lymphocyte counts and/or percentages (Liu et al., 2020; Chen et al., 2021). Lymphopenia indicates damage to the immune system during SARS-CoV-2 infection. Lymphopenia is caused by viral attachment or indirectly by immune injury from inflammatory mediators. In addition, exudation of circulating lymphocytes to the inflamed lung tissue can also cause lymphopenia(Rahmadiyanti, Adhia and Yudistira, 2022).

This study assesses the relationship between CT and lymphocytes in COVID-19 patients. besides that, the study also looked at the comparison between CT values and lymphocyte counts by sex. This is based on the fact that there are differences in hormones and the immune system between males and females.

In this study there were a number of limitations including the small samples size due to time constraints in data collection, medical records for CT values and lymphocytes count limited in subject.

CONCLUSION

The average lymphocyte count was 27.95 and the CT value was 32.30 with lymphocytopenia mostly occurring in males. The results showed that there was no significant difference between the number of lymphocytes and CT values based on gender. The study also found no relationship between CT values and lymphocytes.

SUGGESTIONS

It is necessary to conduct research with a larger population size and with various age categories.

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Table 1. Subject characteristics

Characteristics	N=130
Aged (Years)	37.88 (25.00-50.00)
Gender	
Male	71 (54.6%)
Female	59 (45.4%)
Limfosit (%)	27.95 (3.00-40.00)
CT Values	32.30 (20.19-39.79)

Percent or median data (min-max)

Table 2. Correlation of gender to lymphocytes and CT values

Characteristics	Gender		P value
	Male	Female	
NormalLimfosit (%)	46 (64.8)	43 (72.9)	0.325
Lymphocytopenia	25 (35.2%)	16 (27.1%)	
CT Values			
≤29.00	24(33.8%)	16 (27.1%)	0.413
>29.01	47(66.2%)	43 (72.9%)	

P value with Mann Whitney nonparametric test

Table 3. Correlation of lymphocyte with CT values

Parameter	Lymphocyte		P value
	Normal	Lymphocytopenia	
CT Values			
≤29.00	26(29.2%)	14 (34.1%)	0.683
>29.01	63(70.8%)	27 (65.9%)	

P value with fisher chi-square test