Volume 2, No. 11 November 2023 - (1346-1354)
p-ISSN 2980-4868 | e-ISSN 2980-4841
https://ajesh.ph/index.php/gp

ASSOCIATION BETWEEN ERYTHROCYTE INDICES AND COVID-19 SEVERITY

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ABSTRACT:
Rapid oxygen desaturation has been widely reported in patients with severe and critical Coronavirus Disease 19 (COVID-19). Recent scientific evidence indicates that this virus can interact with erythrocytes and interfere with the function of hemoglobin as an oxygen carrier. This study aims to analyze the association between the erythrocyte indices and the severity of COVID-19 patients. The current study is a cross-sectional analysis of 120 confirmed COVID-19 cases at the PHC Surabaya hospital. This research was conducted from January 2021 to July 2021. The research data was collected from electronic medical records at the Primasatya Husada Citra Hospital (PHC) Surabaya. Patients in this study were classified as mild-moderate, severe-critical. The Spearman rank correlation test was used for statistical analysis. The erythrocyte indices value decreased in patients with severe-critical grades. According to the Spearman rank correlation test, MCV (r = -468, p=0.000), MCH (r = -337, p=0.000), and MCHC (r = -203, p=0.026) all had a significant correlation with a weak correlation strength. This study demonstrates that there is a significant correlation but with a weak correlation strength, indicating that this parameter cannot be used to predict patient prognosis and severity. Further research is needed for better knowledge regarding COVID-19 and erythrocytes.

Keywords: COVID-19, Erythrocyte Indices, Severity.

INTRODUCTION

At the end of 2019, China reported to the World Health Organization (WHO) that a type of pneumonia had been found which is now known as coronavirus disease 2019 (COVID-19) (Sohrabi et al., 2020). This disease is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) (Zhou et al., 2020). On January 2, 2022, the WHO reported that confirmed cases had reached 288,867,634 worldwide (COVID, 2021) . The first case of COVID-19 was reported in Indonesia on March 2, 2020. Until December 15, 2021, Indonesia had a total of 4,259,644 confirmed cases and 143,969 confirmed deaths (Cho et al., 2021). Based on the severity of COVID-19 can be classified into asymptomatic, mild, moderate, severe, and critical. Hypoxemia and severe respiratory distress are frequently present in patients with more severe conditions (Anggraini, 2021).

According to World Health Organization (WHO) research, 80% of patients have mild to moderate symptoms, 13.8% have severe symptoms, and 6,1% have critical symptoms. However, patients with severe and critical degrees show a higher mortality rate (Organization, 2022). This virus is composed of proteins called spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid (N), which is the innermost layer. The virus's protein S binds to the ACE2 receptor, allowing the virus to enter host cells. Although ACE2 is expressed in nearly all human organs, it is most abundant in type II alveolar epithelial cells, making the lungs the primary target of SARS-CoV-2. Non-structural proteins such as ORF1ab, ORF3a, ORF6, ORF7a, ORF10, and ORF8 also contribute to the formation of this virus (Belmehdi et al., 2021), (Hamming et al., 2004).

In silico analysis revealed that the proteins ORF1ab, ORF10, and ORF3a are capable of directly attacking heme and releasing iron ions from porphyrins. Additionally, protein Si and nonstructural protein ORF8 can form a complex with porphyrins that inhibits iron ion binding (Wenzhong & Hualan, 2020) . Other studies have demonstrated that infection with SARS-CoV-2 at high levels in bone marrow can result in cellular apoptosis and decreased hematopoiesis.

This demonstrates that SARS-CoV-2 can impair erythrocytes' function as oxygen carriers, resulting in oxygen desaturation in patients (Xu et al., 2020), (Amgalan & Othman, 2020). Conditions that interfere with hemoglobin in erythrocytes can alter the volume and concentration of hemoglobin in erythrocytes, thereby affecting the value of the erythrocyte indices. The erythrocyte indices is a hematological parameter that is frequently used to diagnose anemia. These are the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) (Brugnara & Mohandas, 2013). By examining the association between the erythrocyte indices and the severity of COVID-19 patients, this research aims to provide a clinical

perspective on the effect of SARS-CoV-2 on erythrocytes.

RESEARCH METHODS

Study design and data source

This is a cross-sectional observational analytic study. The data for this study were collected from the electronic medical records of the Primasatya Husada Citra Hospital (PHC) in Surabaya. PHC Hospital is one of the referral hospitals for COVID-19 patients in Surabaya, appointed by the Indonesian Ministry of Health. PHC Hospital has a capacity of 235 inpatient beds, nearly half of which were used to treat COVID-19 patients during the pandemic period.

Study population and data collection

The population in this study were all confirmed COVID-19 patients using real-time polymerase chain reaction (RT-PCR) who were hospitalized at the PHC hospital in the January-July period 2021. This study uses a non-probability sampling technique, specifically consecutive sampling. A total of 120 patients' samples were obtained. This study excluded patients with a history of hematological disorders, pack red cell (PRC) or whole blood transfusions, as well as those with Human Immunodeficiency Virus/Acquired Immuno Deficiency Syndrome (HIV/AIDS). This study collected data on age, gender, clinical manifestations, and laboratory findings. Patients were classified according to their ages as follows: 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, and 81-90. Male and female are the two genders. Severity was classified as mild-moderate, severe-critical. According to guidelines published by the Ministry of Health of the Republic of Indonesia, severity is classified based on clinical manifestations, respiration rate, and peripheral oxygen saturation of patients (Kemenkes, 2011).

The erythrocyte indices reference value used in this study was derived from the PHC laboratory's Sysmex XN 1000 hematology analyzer. The MCV reference range is 79-97 fl. 27- 31 pg is the MCH reference value. MCHC has a reference range of 32-36 g/dl.

Statistical analysis

The data for categorical variables are presented in the form of numbers (N) and percentages (%), whereas continuous variables are represented by the mean standard deviation (SD). Due to the non-parametric nature of the variables in this study, the correlation analysis relies on the Spearman correlation test, which is considered significant when the p value is less than 0.05. All statistical analyses were conducted using SPSS version 25.0 software statistical product and service solutions.

Ethical considerations

This research has been approved by the Health Research Ethics Committee, Widya Mandala Catholic University, Surabaya, Indonesia, with reference 159/WM12/KEPK/MHSW/T/202.

RESULTS AND DISCUSSION

A total of 120 patients with COVID-19 were used as subjects in this study. Most of the research subjects were male (55%). The mean age of the patients in this study was 52.18 ± 13.5 years. The most reported patients were in the age range of 51-60 years, as many as 32 patients (26.7%). Patients with the most severe-critical severity were reported as many as 62 patients (51.7%) (Table 1).

Table 1
Demographic Characteristics of Research Subjects

Variable	N	%
Characteristics		
Age	52.18 ± 13.51	3.3
21-30	4	20
31-40	24	20.8
41-50	25	26.7
51-60	32	17.5
61-70	21	10.8
71-80	13	0.8
81-90	1	
Gender		
Male	66	55
Female	54	45
Severity		
Mild-moderate	58	48.3
Severe-critical	62	51.7
Total	456	100.0

Patients with mild-moderate severity were mostly reported to be between the ages of 31-40 years, with as many as 16 patients (27.6 %). Males were the most frequently reported in both the mild-moderate and severe-critical categories (Table 2).

Table 2
Age and Gender Distribution of Patients With Mild-Moderate and Severe-Critical COVID-19

Variable	Severity			
	Mild-moderate		Severe-critical	
	N	%	N	%
Age				
21-30	2	3,4	2	3,2
31-40	16	27,6	8	12,9
41-50	14	24,1	11	17,7
51-60	9	15,5	23	37,1
61-70	10	17,2	11	17,7
71-80	7	12,1	6	9,7
81-90	0	0	1	1,6
Gender				
Male	34	58,6	32	51,6
Female	24	41,4	30	48,4
Total	58	100	62	100

Details of the erythrocyte indices profile in this study are shown in table 3 and table 4. There is a difference in the mean of MCV, MCH, and MCHC variables between the mild-moderate and severe-critical groups as shown in Table 3. MCV decreased the most in the severe-critical group. critically as many as 25 patients (40.3%) while in the mild-moderate group only 1 patient (1.7%) experienced a decrease.

Table 3
The Mean Erythrocyte Indices Of Patients With Mild-Moderate and Severe-Critical Grades Of COVID-19

Erythrocyte Indices	Mild-moderate	Severe-critical
MCV	84.23 ± 2.99	79.62 ± 7.43
MCH	28.82 ± 1.32	26.76 ± 3.12
MCHC	34.19 ± 1.02	33.51± 1.49

Table 4
Distribution Of Erythrocyte Indices Values In Patients With Mild-Moderate and Severe Critical Grades Of COVID-19

Variables	Mild-Moderate		Severe-Critical	
	N	%	N	%
MCV				
Decreased	1	1,7	25	40,3
Normal	57	98,3	37	59,7
Increases	0	0	0	0
MCH				
Decreased	5	8,6	27	43,5
Normal	50	86,2	31	50
Increases	3	5,2	4	6,5
MCHC				
Decreased	0	0	3	4,9
Normal	56	96,5	59	95,1
Increases	2	3,5	0	0
TOTAL	58	100	62	100

MCH also showed the greatest decrease in the severe-critical group, as many as 27 patients (43.5%) while in the mild-moderate group only 5 patients (8.6%) experienced a decrease. MCHC showed the greatest decrease in the severe-critical group, as many as 3 patients (4.9%) and no decrease in MCHC values was found in the mild-moderate group (Table 4).

A weak negative correlation between MCV and the severity of the patient was discovered using the Spearman rank correlation test (r = -0.468; p = 0.000). Correlation analysis between MCH and severity revealed significant results with a weak negative correlation (r = -0.337; p = 0.000) in this study. As with MCV and MCH, the results of this study's analysis of the relationship between MCHC and severity revealed significant results with a weak negative correlation (p = 0.026; r = -0.203). (Table 5).

Table 5
Spearman Rank correlation test between erythrocyte indices and severity

Erythrocyte Indices	r	p-value
MCV	-0.468	0.000
MCH	-0.337	0.000
MCHC	-0.203	0.026

This study showed that there were differences in the erythrocyte indices value between patients with mild-moderate and severe-critical severity. Descriptively, the average value of the erythrocyte indices decreased in patients with severe-critical severity when compared with patients with mild-moderate severity. A decrease in the value of the erythrocyte indices was also more commonly found in patients with severe-critical severity compared to patients with mild to moderate degrees. Based on the Spearman rank correlation test in this study, it was found that there was a weak negative correlation between MCV, MCH, and MCHC with the severity of the patient.

In silico research conducted in China demonstrated that the proteins contained in this virus, specifically ORF1ab, ORF3a, and ORF10, have the potential to bind to hemoglobin chains and release iron ions from porphyrins. Additionally, the same study reported that protein S and ORF8 may bind to porphyrins and inhibit heme synthesis (Wenzhong & Hualan, 2020). Conditions that interfere with hemoglobin can alter the volume and hemoglobin content of the erythrocyte, resulting in a decrease in the MCV, MCH, and MCHC values (Brugnara & Mohandas, 2013).

A study conducted in the United States of America discovered a strong correlation between the amount of virus (viral load) in the blood plasma and the patient's severity (Fajnzylber et al., 2020). While in the blood plasma, this virus has the potential to bind to erythrocytes via the ACE2 receptor and cause hemoglobin disturbances, increasing the risk of erythrocyte indices value decrease in patients with a high degree of severe-critical severity. Iron ions released as a result of the virus's interaction with

hemoglobin can also cause inflammation and toxic effects on cells, thereby deteriorating the patient's condition (Liu & Li, 2020).

The findings in this study are not in line with the research conducted by Waris et al. of 101 patients in Pakistan. Research conducted by Waris showed that there was no correlation between MCV, MCH, and MCHC with the severity of patients with 14-year-old (Waris et al., 2021). In contrast to the study in Pakistan, a study of 100 COVID-19 patients in Bangladesh showed results that were in line with the MCV and MCH indicators in the study. However, the MCHC indicator is not different from the research conducted in Pakistan (Layla et al., 2021).

CONCLUSION

There was a significant correlation between erythrocyte indices and severity in this study, but the correlation strength was low. The low correlation coefficient indicates that there is minimal association between the variables, implying that the erythrocyte indices parameter has limited potential for use as a predictor of severity. Our study did not collect blood samples at various stages of the disease's progression so further research is needed to gain a better knowledge regarding SARS-CoV-2 and erythrocytes which can help clinicians in making a structured treatment approach.

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First publication right:
Asian Journal of Engineering, Social and Health (AJESH)