

How to Cite:

Maghfirah, A. I., Esa, T., Widaningsih, Y., & Bahrin, U. (2022). Correlation of serum ferritin levels and COVID-19 severity in Makassar. *International Journal of Health Sciences*, 6(S2), 9971–9979. <https://doi.org/10.53730/ijhs.v6nS2.7536>

Correlation of serum ferritin levels and COVID-19 severity in Makassar

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Abstract---Background: Covid-19 pandemic has become a global problem. One of parameters to assessed the severity of Covid-19 disease was ferritin. Limited researches of ferritin with Covid-19 in Makassar. Aim: Determine relationship between serum ferritin levels and severity of Covid-19. Method: A cross-sectional by taking primary data of Covid-19 patients aged ≥ 18 years in 67 samples at Unhas Hospital Makassar in September 2020-May 2021. Pregnant women and have a history of chronic infection were excluded. Serum ferritin levels(ng/mL) was measured by the Enzyme Linked Fluorescent Assay(ELFA) method, mini VIDAS Biomerieux. Kruskal Wallis difference test and Spearman correlation test were performed. ROC curve to determine the cut-off point. Results: Total of 67 confirmed Covid-19 patients aged 18-63 years, with highest number being women(53.7%). Total of 27(40.3%) had comorbidities. Covid-19 patients at mild(53.7%), moderate(23.9%), and severe(22.4%). There is significant difference between serum ferritin levels and severity of Covid-19($p < 0.001$). Ferritin levels were also strongly positively correlated with severity of Covid-19($r = 0.754$, $p < 0.001$). Cut-off ferritin at mild-moderate patients=153.89 ng/mL, and cut-off ferritin at mortality=1145.54 ng/mL.Discussion: Ferritin is a major mediator of

immune dysregulation. Ferritin contributes cytokine storms. So serum ferritin levels can be used as a predictor severity of Covid-19. Conclusion And Suggestion: Serum ferritin levels correlated with severity of Covid-19. Ferritin cut-off at mild to moderate severity of Covid-19=153.89 ng/mL and ferritin cut-off at mortality=1145.54 ng/mL. Ferritin levels in Covid-19 patients who have comorbidities shows varying values at mild severity.

Keywords---serum ferritin, COVID-19, severity COVID-19.

Introduction

Coronavirus Disease-19 (Covid-19) is a disease caused by a strain of the new coronavirus. This disease is named Severe Acute Coronavirus-2 (SARS CoV-2) and was first reported in the city of Wuhan, China in December 2019. The increased number of Covid-19 cases took place quite quickly and has spread between countries. SARS-CoV-2 has affected more than 212 countries, and as of October 12, 2021. The cumulative number of confirmed COVID-19 cases that have been reported globally has reached 237,655,302 confirmed cases and the death victim reached 4,846,981 cases.^{1,2}

COVID-19 can cause mild, moderate or severe symptoms. The main clinical symptoms that appear are fever (temperature > 38oC), cough and difficulty breathing. In addition, it can be accompanied by severe shortness of breath, fatigue, myalgia, gastrointestinal symptoms such as diarrhea and other respiratory symptoms. In severe cases the worsening rapidly and progressively, such as Acute Respiratory Distress Syndrome (ARDS), septic shock, metabolic acidosis that is difficult to correct and bleeding or coagulation system dysfunction within a few days. Some patients, the symptoms appear mild, not even accompanied by fever.³⁻⁶

Some of the laboratory parameters that assess severity of Covid-19 disease, one of which is ferritin, a major iron storage protein found in human body tissues. Ferritin consists of 24 subunits in variable ratios of heavy and light chain ferritin which are encoded by Ferritin Heavy Chain (FTH) and Ferritin Light Chain (FTL), respectively. The function of ferritin is to store iron, especially in the liver, spleen and bone marrow. Excess iron will be stored and when needed can be remobilized.⁷⁻⁹

Ferritin is the main mediator of immune dysregulation, especially if there is extreme hyperferritinemia. Through its direct immune suppressive and proinflammatory effects, ferritin contributes to the cytokine storm. Inflammation caused by SARS CoV-2 can trigger an increase in ferritin production to prevent the pathogenic effect of iron. Production of active ferritin by macrophages and cytokines can cause hyperferritinemia, which will result in increased production of proinflammatory cytokines (IL-1 β) and immune suppression (IL-10). Excess ferritin also contributes to the formation of reactive oxygen species (ROS) which can lead to tissue damage or fibrosis. On this basis it supports the hypothesis that ferritin may be a factor influencing the severity of the disease Covid-19.¹⁰⁻¹⁴

Mehta et al, have reported an increase in serum ferritin levels in patients with severe clinical severity of Covid-19 and serum ferritin levels can also be used as a predictor of the severity of Covid-19 disease and are associated with the presence of cytokine storms in these patients, in line with research conducted by Fei Zhou et al at Jinyintan Hospital Wuhan China in December 2019, increased ferritin was associated with death in Covid-19 patients ($p < 0.001$). Liu et al, also reported that, as patients recovered, ferritin and interleukin-6 (IL-6) concentrations decreased. This can confirm that hyperferritinemia is associated with the inflammatory state in SARS-CoV-2 infection.¹³⁻¹⁷ Research on ferritin parameters in Covid-19 patients has been established in several international journals, but the lack of research data conducted in Indonesia, especially Makassar. The purpose of this study was to determine the relationship between ferritin levels and the severity of Covid-19 patients in Makassar.

Method

A cross sectional study by taking primary data from Covid-19 patients aged 18 years who were treated at the Unhas Makassar hospital from September 2020 to May 2021. Patients were pregnant and had a history of chronic infections, namely tuberculosis (TB), human infection virus (HIV), and systemic lupus erythematosus (SLE) were excluded from this study. The diagnosis of Covid-19 was confirmed through a real time Reverse Transcriptase-Polymerase Chain Reaction (rtRT-PCR) examination using a nasopharyngeal swab sample. The severity of Covid-19 patients is divided into three groups based on the World Health Organization (WHO), namely: (1) Mild illness, namely Covid-19 patients with non-specific symptoms such as fever, cough, sore throat, nasal congestion, malaise, sore throat, headaches, and muscle aches. (2) Moderate illness, namely Covid-19 patients with clinical symptoms of fever, cough, and dyspnea (frequency 20-30 times/minute) and no signs of severe pneumonia. (3) Severe illness, i.e. Covid-19 patient with fever or under surveillance of respiratory tract infection, with: tachypnea (respiratory rate > 30 breaths/minute), severe respiratory distress or patient oxygen saturation $< 90\%$.

Serum ferritin levels were measured using the Enzyme Linked Fluorescent Assay (ELFA) method using the VIDAS Biomerieux mini-tool, expressed in units of nanograms per milliliter (ng/mL). The data were statistically analyzed by using SPSS and presented in the form of tables and narratives. Approval of ethical feasibility was obtained from the Health Research Ethics Commission, Faculty of Medicine, Hasanuddin University, Hasanuddin University Hospital and Central General Hospital Dr. Wahidin Sudirohisodo Makassar, with Number: 646/UN4.6.5.31/PP36/2020.

Results

A total of 67 confirmed Covid-19 patients aged 18-63 years, the most in the 41-60 years age range (62.7%) and most of them were women (53.7%). A total of 27 patients (40.3%) had comorbidities, most of them were hypertension (19.4%). (Table 1).

Table 1.Characteristics of Covid 19 Patients

Characteristics		n = 67	%
Gender			
-	Man	31	46.3
-	Woman	36	53.7
Age (Years)			
-	18 – 40	24	35.8
-	41 – 60	42	62.7
-	>60	1	1.5
Comorbid			
-	There is	27	40.3
-	There is no	40	59.7
Comorbid Type			
-	Diabetes	8	11.9
mellitus		13	19.4
-	Hypertension	3	4.5
-	Chronic	1	1.5
Kidney Disease(CKD)		1	1.5
-	Sepsis	1	1.5
-	Stroke		
-	Diabetes		
Mellitus + Hypertension			
Severity			
-	Mild	36	53.7
-	Moderate	16	23.9
-	Severe	15	22.4
Outcome			
-	Get well	55	82.1
-	Died	12	17.9
Ferritin (ng/mL)			
-	0 – 300	33	49.3
-	301 – 800	19	28.4
-	801 – 1200	15	22.4

Source: Primary Data

Based on the severity of the disease, most of the subjects studied were mild (53.7%), and based on the outcome, 55 patients (82.1%) who were studied improved (Table 2).

Table. 2. Comparison of serum ferritin levels against the severity of Covid-19

Severity	Ferritin (ng/mL)						*p
	n=67	Mean	SD	median	Min	Max	
Mild	36	199.76	209.93	103.34	2.92	797.5	<0.001
Moderate	16	371.77	219.56	345,74	67.59	785.81	
Severe	15	1164.47	101.34	>1200	805.17	>1200	

*Kruskall Wallis Test

Table 2 shows a significant difference between serum ferritin levels and severity, with $p < 0.001$.

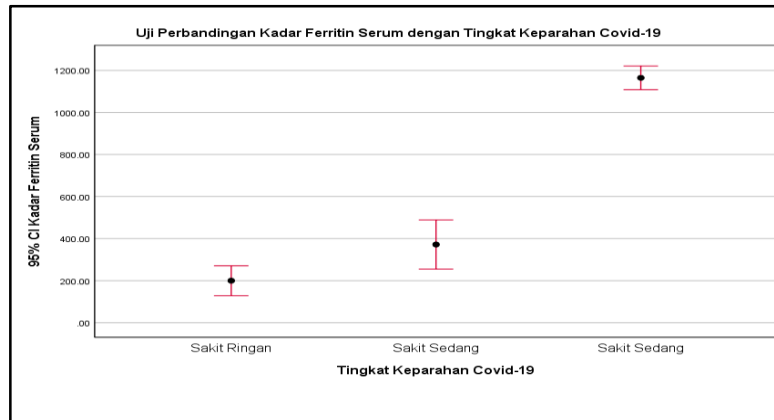


Figure 1. Comparison of serum ferritin levels by severity of Covid-19

The correlation test was carried out to assess the relationship between serum ferritin levels and the severity of Covid-19. The results of the Spearman correlation test (Table 3) showed that serum ferritin levels were strongly positively correlated with the severity of Covid-19 ($r=0.754$, $p < 0.001$). This shows that the higher the serum ferritin level, the more severe the severity of Covid-19 disease.

Table 3. Serum Ferritin Level Correlation Test based on Covid-19 Severity Level

Severity	Ferritin		*p
	n=67	r value	
Mild	36	0.754	<0.001
Moderate	16		
Severe	15		

*Spearman Correlation Test

Determination of the cut-off value of serum ferritin was carried out using Receiver Operator Characteristic (ROC) curve analyses to determine whether ferritin could be used as a predictor of severity. Based on the Area Under Curve (AUC) value of ferritin is 0.767 ($p < 0.001$) indicating that ferritin can be used as a predictor of the severity of Covid-19.

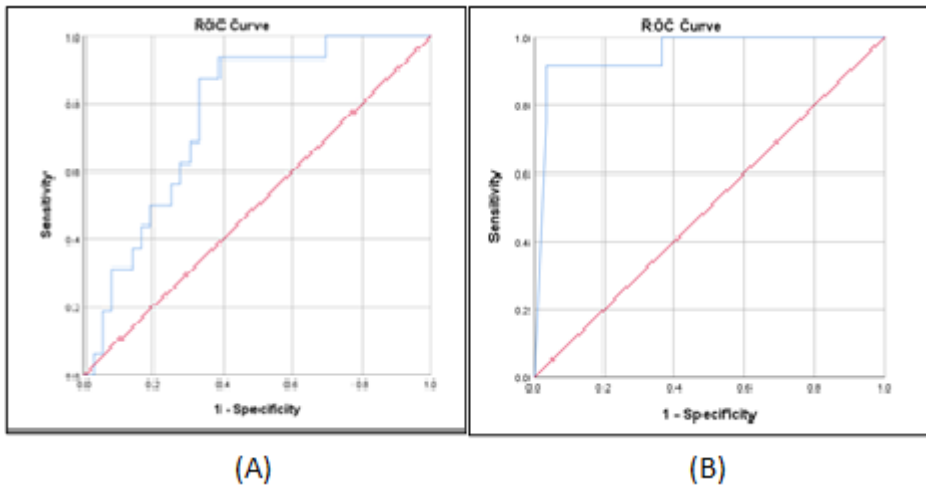


Figure 2. (A) ROC curve of serum ferritin levels between mild and moderate severity of Covid-19. (B) ROC curve of serum ferritin level on mortality

The ROC curve (A) shows the ferritin cut-off value from mild to moderate severity of Covid-19 is 153.89 ng/mL which is considered to have a sensitivity value of 87.5%, specificity 66.7%, positive predictive value (PPV) 53.8%, negative predictive value (NPV) 92.3%. ROC curve (B) shows the cut-off value of ferritin on mortality is 1145.54 ng/mL with an AUC value of 0.950 ($p < 0.001$) considered to have a sensitivity value of 91.7%, specificity 96.4%, PPV 84.6% , NPV 98.1%.

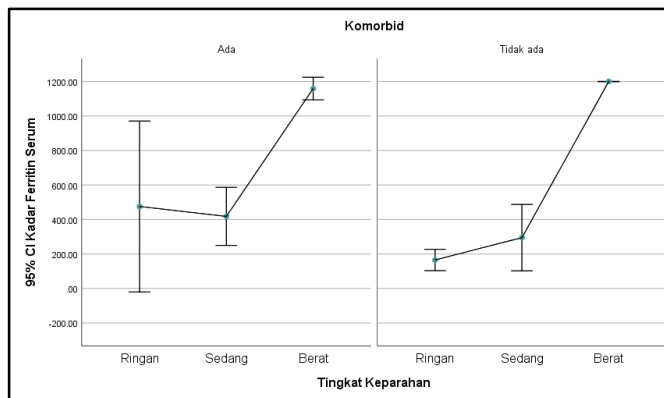


Figure 3. Differences in serum ferritin levels with the severity of Covid-19 with comorbid (left) and without comorbid (right)

The graph above (Figure 3) shows that the serum ferritin level with comorbidities varies in mild illness (0-1000 ng/mL), while the serum ferritin levels with no comorbidities range from (20-210 ng/mL).

Discussion

The basic characteristics of this study showed that there were more female patients than male patients, as many as 36 patients (53.7%). This is different from Hashem's research et al who reported that the incidence of Covid-19 was

more found in men in all age groups (52.1%). It is generally known that women's biological characteristics are better at producing a stronger immune response in dealing with infections including viral infections compared to men. However, according to population data in Makassar City in 2020, data on the number of women as many as 4.56 million residents and men 4.50 million residents, so that in this study the number of female patients was more than male.¹⁸⁻²⁰

Most of the research subjects were patients aged 41-60 years, according to a study conducted by Hashem et al who reported the average age of the Covid-19 group was 45-57 years. This is because with age, it will be followed by a decrease in immunity and organ function. More patients with no history of comorbidities than patients with comorbidities, more patients with mild severity than patients with severe severity, and more patients who recovered than patients who died. This is in accordance with the research of Kangdra et al who found that as many as 55.2% of patients had no history of comorbidities and 44.8% had comorbidities, as well as 45.5% of patients with mild symptoms, 42.7% with moderate symptoms and 11.8% with severe symptoms.

The results of this study indicate that there is a significant relationship with a strong positive correlation ($r=0.754$, $p<0.001$) between serum ferritin levels and the severity of Covid-19, which means that the higher the serum ferritin level, the more severe the symptoms of Covid-19 patients. This is in line with research conducted by Dahan S et al, in 2020, that an increase in serum ferritin levels is positively correlated with the severity of Covid 19 with a p value <0.001 .¹³

Around 36 mildly ill Covid-19 patients, 16 moderately ill Covid-19 patients, and 15 seriously ill Covid-19 patients, with their respective mean serum ferritin levels of 199.76 ng/mL, 371.77 ng /mL, and 1164.57 ng/mL which were statistically significant using the Kruskal Wallis comparison test ($p<0.001$). This is in line with the study conducted by Sonweber et al in Austria in 2020 that serum ferritin levels had a significant difference in the severity (mild, moderate, and severe) of Covid-19 with a p value of <0.001 . Fei Zhou et al at Jinyintan Hospital Wuhan China in December 2019 reported that increased ferritin was associated with death in Covid-19 patients ($p<0.001$).

Ferritin is a major mediator of immune dysregulation. Inflammation caused by SARS CoV-2 can trigger an increase in ferritin production to prevent the pathogenic effect of iron. Production of active ferritin by macrophages and cytokines can cause hyperferritinemia, which will result in increased production of proinflammatory cytokines (IL-1 β) and immune suppression (IL-10). Excess ferritin also contributes to the formation of reactive oxygen species (ROS) which can lead to tissue damage or fibrosis.⁹⁻¹⁶

This study shows that ferritin can be a predictor of severity in Covid-19 patients, ferritin cut-off value was 153.89 ng/mL, AUC 0.767 ($p<0.001$) was considered to have a sensitivity value of 87.5%, specificity 66.7%, PPV 53.8%, and NPV 92.3%. This study can also predict mortality with the cut off value of ferritin on mortality was 1145.54 ng/mL, AUC 0.959 ($p<0.001$) obtained sensitivity 91.7%, specificity 96.4%, positive predictive value (PPV) 84.6%, negative predictive value (NPV) 98.1%. This is in line with the research of Patora J et al that with a ferritin cut-off

of 400 ng/mL, AUC 0.660 ($p < 0.001$) can be a predictor of severity and with a ferritin cut-off value of 1400 ng/mL, AUC 0.690 ($p < 0.001$) can be a predictor of mortality in Covid-19 patients

This study showed that serum ferritin levels in comorbid Covid-19 patients varied in mild severity. The lack of data specifically examines serum ferritin levels that have comorbidities on the severity of Covid-19 compared to Covid-19 patients who do not have comorbidities. However, Rahman A et al, June 2021 reported that comorbid Covid-19 patients had higher serum ferritin levels at severe than non-severe levels with $p < 0.001$.²⁰

Conclusion

1. Serum ferritin levels are associated with the severity of COVID-19. The higher the serum ferritin level, the more severe the severity of COVID-19 disease.
2. The cut-off value of serum ferritin for mild-moderate severity of Covid-19 is 153.89 ng/mL and the cut-off value of serum ferritin on mortality is 1145.54 ng/mL.
3. Serum ferritin levels in Covid-19 patients who have comorbidities show varying values at mild severity.

Suggestion

Serum ferritin level with cut-off value of 153.89 ng/mL can be used as a predictor of mild to moderate severity, and serum ferritin level with cut-off value of 1145.54 ng/mL can be used as a predictor of mortality.

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