Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2023;48(4):1199-1206
DOI: 10.17826/cumj.1316587

RESEARCH

Plasma kallistatin levels in patients with COVID-19

COVID-19 hastalarında plazma kallistatin düzeyleri

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Abstract

Purpose: The aim of this study was to evaluate kallistatin levels in patients diagnosed with COVID-19 and compare them with healthy controls.
Materials and Methods: A total of 40 patients diagnosed with COVID-19, and 45 healthy controls were included in the study. The patient group was divided into 2 groups: patients treated in the service (n:20) and patients treated in the intensive care unit (n:20). Kallistatin levels were measured using the ELISA method.
Results: There was a significant difference in kallistatin levels between the patient group (n:40) and the control group (n:45). There was no significant difference in kallistatin between COVID-19 patients treated in the service and those treated in the intensive care unit. We found that the AUC for kallistatin was 0.856 in the ROC analysis performed between the patient and control groups. When comparing service and ICU patients in terms of laboratory parameters, there was a significant difference between the groups due to elevated potassium, AST, creatinine, ferritin, HGB and LDH in ICU patients.
Conclusion: As a result, kallistatin levels were significantly higher in the patient group than in the control group. Comprehensive studies with more patients are needed to understand whether kallistatin is elevated in COVID-19 patients due to the effects of COVID-19 or to eliminate oxidative stress.
Keywords:. COVID-19, enzyme-linked immunosorbent assay, kallistatin

Öz

Amaç: Bu çalışmanın amacı, COVID-19 tanısı alan hastalarda kallistatin düzeylerini değerlendirmek ve sağlıklı kontrollerle karşılaştırmaktı.
Gereç ve Yöntem: Çalışmaya COVID-19 tanısı alan 40 hasta ve 45 sağlıklı kontrol dahil edildi. Hasta grubu serviste tedavi görenler (n:20) ve yoğun bakımda tedavi görenler (n:20) olmak üzere 2 gruba ayrıldı. Kallistatin seviyeleri ELISA yöntemi kullanılarak ölçüldü.
Bulgular: Hasta grubu (n:40) ile kontrol grubu (n:45) arasında kallistatin düzeyleri açısından anlamlı fark vardı. Serviste tedavi edilen COVID-19 hastaları ile yoğun bakımda tedavi edilenler arasında kallistatin açısından anlamlı fark yoktu. Hasta ve kontrol grubu arasında yapılan ROC analizinde kallistatinin EAA'sını 0.856 olarak bulduk. Servis ve yoğun bakım hastaları laboratuvar parametreleri açısından karşılaştırıldığında, yoğun bakım hastalarında potasyum, AST, kreatinin, ferritin, HGB ve LDH yükselişi nedeniyle gruplar arasında anlamlı fark vardı.
Sonuç: Sonuç olarak hasta grubunda kallistatin düzeyleri kontrol grubuna göre anlamlı olarak yüksekti. COVID-19 hastalarında kallistatinin COVID-19'un etkileri nedeniyle mi yoksa oksidatif stresi ortadan kaldırmak için mi yükseldiğini anlamak için daha fazla hasta ile yapılacak daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar kelimeler: COVID-19, enzim bağlı immünosorbent testi, kallistatin

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Received: 21.06.2023 Accepted: 11.10.2023

INTRODUCTION

COVID-19 disease, which affects people all over the world, has led to an increase in hospitalisation in intensive care unit (ICU) admissions and deaths. COVID-19 disease can be asymptomatic, moderate or critical depending on the age, body resistance and the presence of comorbidities (diabetes mellitus (DM), chronic respiratory disease, hypertension (HT), cancer, etc.) [1,2]. The effect of the novel coronavirus on the body is mainly the abnormal immune response caused by the viral invasion and the resulting imbalance in the ratio of cytokines produced by the body [1,3]. Inflammatory mediators and cytokine storm target lung epithelial cells and alveolar microvascular endothelial cells. As a result, lung epithelial cells and vascular endothelial cells are largely destroyed. In addition, tissue ischaemia and hypoxia exacerbate lung injury. As a result, patients with severe COVID-19 often present with progressive hypoxaemia. The hypoxic state not only causes lung tissue damage, but also increases inflammation, oxidative stress and free radical damage. In healthy people, free oxygen radicals produced by metabolism are removed by the antioxidant system, the body's defence mechanism. However, in COVID-19 patients, this radical damage is increased due to hypoxia, ischaemia, comorbidities, medical treatment and viral load [4-6]. Due to the widespread damage to cells and tissues caused by COVID-19 infection, it is difficult to identify of a specific laboratory parameter that can assess the severity of the disease and its impact on mortality.

Kallistatin was first identified by Chao et al. in 1986 as a serine proteinase inhibitor (serpin) specifically and covalently bound to tissue kallikrein in human serum. Kallistatin is mainly expressed in the liver, but is also widely distributed in the kidney, adrenal gland, vascular endothelial cells and vascular smooth muscle cells [7,8]. Kallistatin contains two structural elements, the active site and the heparin binding site. Through these domains, it provides vascular relaxation, regulates angiogenesis, inflammation, oxidative stress, cell apoptosis, the expression of several genes and controls the activation of several signalling pathways [7-9]. In this way, it protects against vascular and organ damage. Studies have reported that serum kallistatin levels are significantly reduced in patients with coronary artery disease and are negatively correlated with disease severity [10,11]. Kallistatin causes vasodilation, mainly by increasing nitric oxide (NO) formation, and prevents oxidative stress, inflammation and fibrosis. Kallistatin reduces the generation of reactive oxygen species and oxidative stress in endothelial cells, renal tubular cells and cardiomyocytes through NO generation [7,9,10]. Recently, kallistatin has been suggested to play a protective role in diabetic nephropathy by suppressing inflammation, fibrosis and lowering blood pressure [12,13]. In contrast to studies that highlighting the protective effect of high kallistatin levels in some diseases, there are also studies that associating high kallistatin levels with a worse prognosis in some diseases [14,15]. Given the increased inflammation and oxidative stress in COVID-19 patients, we hypothesised that kallistatin levels might be low in these patients.

In this study, we aimed to evaluate kallistatin levels in patients with COVID-19, compare them with plasma from healthy controls, and compare them with other laboratory data in the patients' files.

MATERIALS AND METHODS

Study population

The study was conducted with samples collected at the Van Training and Research Hospital between December 29 2021, and March 10 2022. The study was approved by the Ethics Committee. After receiving ethics committee approval, institutional permission was obtained from the hospital for the study. No sample size calculation was performed for the study. The number of participants that could be tested with one enzyme-linked immunosorbent assay (ELISA) kit was determined and samples were collected according to the requirement that the number of patients should be equal to or less than the number of controls. The study included 40 patients diagnosed with COVID-19 and 45 healthy controls. A total of 13 patients (5 patients >65 years of age, 4 patients diagnosed with DM, and 4 pregnant patients) were excluded from the study.

Exclusion criteria for the patient groupwere being <18 and >65 years of age, presence of chronic diseases (DM, HT, chronic obstructive pulmonary disease (COPD), chronic liver-renal failure, malignancy, etc.), pregnancy. Exclusion criteria for the control group were being <18 and 65> years of age, COVID-19 contact, any chronic disease (DM, HT, COPD, chronic liver-renal failure, malignancy, etc.), drug use, pregnancy.

Procedure

This study was approved by the KTO Karatay University, Faculty of Medicine, Ethics Committee for Pharmaceutical and Non-Medical Device Research, and research and publication ethics were followed in the article (No: 2021/029, date: 27 December, 2021). The study was conducted in accordance with the Declaration of Helsinki.

The patients and healthy controls were followed up and plasma samples were collected by a specialist in internal medicine. The study included healthy volunteers without health problems attending the internal medicine outpatient clinic for routine check-ups and patients with a confirmed diagnosis of COVID-19 by Real-Time polymerase chain reaction (RT-PCR). The patient group was divided into two groups: patients treated in the internal medicine service and patients treated in the general ICU. Kallistatin measurements were performed from the blood taken on the first moment of admission to the service and at the first moment of admission to the ICU. The age range was 18-65 years in both the patient and healthy control groups. No invasive procedures were performed during the study. Plasma samples from the patient and control groups were taken from the samples whose laboratory procedures had been completed. The remaining plasma samples were divided into 2 separate eppendorf tubes and stored at -80 °C until the ELISA test was performed. After freezing, no further freeze-thawing was performed until the kallistatin study. During the kallistatin study, the sample was removed from -80 °C and thawed. Kallistatin was measured in samples obtained from the patient and control groups. Age, gender, presence of comorbidities and other laboratory data of the patient and control groups were obtained from the hospital's automated system.

Biochemical analysis

Plasma kallistatin levels were measured by enzyme-linked immunosorbent assay method (ELISA). FineTest brand Human SERPINA4 (Kallistatin) ELISA Kit Catalog No: EH0209, Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China) was used and the ELISA study was performed according to the manufacturer's manual. ELISA Study was conducted at KTO Karatay University, Research Laboratory. ELISA analyses were performed by a faculty member of the Department of Medical Biochemistry and a medical student. A Multiskan Sky Thermo (A.B.D.) device was used to read the results in this study. A Combi Wash (Human) washer was used in the study. Results were calculated as using the percentage of the predicted calculated concentration. The intra and inter-assay coefficients of variation were <8% and <10%, respectively. The minimum detectable concentration of kallistatin was 9.375 ng/mL, and the diagnostic interval of the assay was 15.625-1000 ng/mL.

Statistical analysis

Statistical analysis of the data was performed using the SPSS 27.0 package (IBM SPSS, Chicago, IL, USA). Means±standard deviations were used to summarise numerical data, and numbers and percentages were used to summarise categorical data. Visual (histogram and probability plots) and analytical (Kolmogrorov-Smirnov) methods were used to analyse the conformity of the data to the normal distribution. The chi-square test ($\chi 2$) was used to compare categorical data. The relationship between non-normally distributed numerical data and categorical data was analysed using the Man-Whitney U test. The Kruskal-Wallis test was used when there were three or more non-normally distributed groups with numerical data. Post-hoc Man-Whitney U test with Bonferroni correction was used for pairwise comparisons between groups with significant Kruskal-Wallis test results. As the data were not normally distributed, comparisons between patient and control groups for age, laboratory parameters and kallistatin were made using the Mann-Whitney U test. The Kruskal-Wallis test was used to compare age and kallistatin levels between the three groups. Correlations of non-normally distributed numerical variables were analysed using Spearman's correlation coefficient. The correlation coefficient was in the range of 0.00-0.19 (very weak), 0.20-0.39 (weak), 0.40-0.69 (moderate),0.70-0.89 (strong), and 0.90-1.00 (very strong). The diagnostic decision-making properties of kallistatin levels in predicting disease were analysed by receiver operating characteristics (ROC) curve analysis. In the presence of significant cut-off values, the sensitivity, specificity, positive predictive value and negative predictive value of these cut-off values were calculated. The type 1 error level was accepted as 5% for statistical significance.

RESULTS

Forty (21F, 19M) patients diagnosed with COVID-19 and 45 (25F, 20M) healthy controls were included in this study. Of the group of patients diagnosed with

COVID-19, 20 were treated in the service (S) and 20 in the ICU. The mean age of all patients was 49±12.05 years. According to the medical records of the patients treated in the service and in the ICU, there were no additional diseases. When comparing service and ICU patients in terms of laboratory parameters, there was a significant difference between the groups due to elevated potassium, aspartate aminotransferase (AST), creatinine, ferritin, hemoglobin (HGB) and lactate dehydrogenase (LDH) in ICU patients ($p<0.05$). In addition, D-dimer levels were lover in COVID-19 ICU patients were lower than in service patients, but the difference was not statistically significant ($p>0.05$). Table 1 shows a comparison of the laboratory parameters of COVID-19 patients in the service and in the ICU.

Table 1. Comparison of age and laboratory parameters of patients in the service and in the ICU.

Variables	**Patients of service (n: 20) (median- IQR)**	**Patients of ICU (n: 20) (median - IQR)**	**p value**
Age	49.51 ± (41.75-58.00)	48.49 ± 11.73 (40.10-56.80)	0.78
Na (mmol/L)	140 (137-143)	138.50 (135-140)	0.10
K (mmol/L)	3.80 (3.12 - 4.00)	4.28 (3.95-4.45)	0.00**
AST (U/L)	15.40 (11.30 - 26.10)	32.55 (22.22 - 39.22)	0.00**
ALT (U/L)	16.30 (9.90 - 32.50)	26.25 (20.02 - 45.05)	0.12
Urea (mg/dL)	42 (32.80 - 62.50)	38.40 (27.62 - 48.07)	0.06
Creatinine (mg/dL)	0.74 (0.58 - 1.00)	1.03 (0.93 - 1.17)	0.00**
Ferritin (ng/ml)	317.25 (173.52 - 598.22)	556.10 (276.37 - 813.80)	0.03*
D-dimer (ng/ml)	456.50 (206.25 - 784.25)	390.50 (123.00 - 621.50)	0.06
WBC ($10^3/mm^3$)	8.93 (5.42 -11.03)	7.64 (4.59 - 10.37)	0.49
HGB (g/dL)	13.15 (9.20 - 14.40)	13.90 (12.90 -15.70)	0.03*
Albumin (g/dL)	3.15 (2.79 -3.39)	3.15 (2.73 - 3.61)	0.69
Lactate (mmol/L)	1.75 (1.50 - 2.67)	2.00 (1.70 - 2.90)	0.26
Lactate/ Albumin Ratio	0.54 (0.47 - 0.83)	0.62 (0.49 - 1.07)	0.44
LDH (U\L)	271.00 (224.00 - 313.25)	366.00 (334.00 - 491.00)	0.00**

The Mann-Whitney U test was used for the statistics, Na: Sodium; K: Potassium; AST: aspartate aminotransferase; ALT: alanine aminotransferase; WBC: white blood cell, HGB: hemoglobin; LDH: Lactate dehydrogenase, *p<0.05, **p<0.01

There was no difference in age and gender between the patients (S+ICU) and control group ($p>0.05$). There was a significant difference in kallistatin levels between the patient group (40) and the control group (45) ($p<0.01$). There was no significant difference in kallistatin between COVID-19 patients treated in the service and those treated in the ICU (p: 0.13). Data on kallistatin parameters in the patient and control groups are shown in Table 2.

Table 2. Comparison of the groups with regard to the kallistatin levels.

Laboratory findings	**Patient (S+ICU = n: 40)**	**Control (n: 45)**	**p value**
Kallistatin (ng/mL)	44.28 (43.68-44.67)	33.97 (23.53-43.24)	0.00**
	Patient (S, n: 20)	Patient (ICU, n: 20)	
Kallistatin (ng/mL)	44.18 (43.61-44.57)	44.39 (43.5-44.8)	0.13

Data are expressed as median-IQR, Mann-Whitney U was used for the statistics, *p<0.05, **p<0.01

When the correlations between the laboratory data of the patient group and kallistatin were analysed, there was a positive low correlation between kallistatin and ferritin (r: 0.290, p: 0.018) and a negative low correlation between kallistatin and D-dimer (r: -0.333, p: 0.036).

To investigate the predictive power of the kallistatin, we performed ROC curves and AUC analyses. We found that the AUC for kallistatin was 0.856 (p=0.00, CI=0.763- 0.949) in the ROC analysis performed between the patient and control groups. Figure 1 shows the ROC analysis plot.

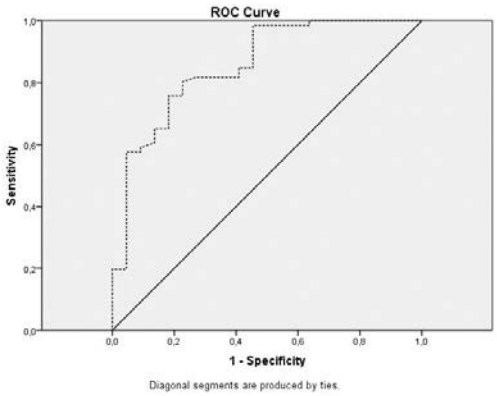

Figure 1. The ROC analysis for kallistatin (AUC: 0.856).

DISCUSSION

In this study, we compared kallistatin levels between the healthy control group and the patients diagnosed with COVID-19. We found that kallistatin levels were significantly higher in the patient group than in the control group.

COVID-19 is a serious respiratory disease that affects people of all ages. Patients requiring intensive care should be monitored closely, particularly liver and kidney function, because of the potential for multi-organ effects and drug side effects. In one study, a significant increase in AST levels in COVID-19 patients was associated with mortality [16]. In a study conducted in 2021 on patients with COVID-19 treated in the ICU (n: 205) and in the non-ICU (n: 809), the AST, ALT, LDH and ferritin levels of the patients treated in the ICU were significantly higher than those of the patients treated in the non-ICU ($p<0.0005$) [17]. In a study of 56 patients, of whom 35 were treated in the ICU and 29 in the service, procalcitonin, urea and creatinine levels were found to be significantly higher in the ICU patients than in service patients ($p < 0.05$) [18]. There are studies reporting that uncontrolled inflammation in COVID-19 increases ferritin levels and that elevated ferritin levels are associated with ICU admission and high mortality. The results of the study of 2523 adult patients hospitalised with COVID-19 pneumonia reported that elevated ferritin increased in-hospital mortality and the need for mechanical ventilation [19,20]. Consistent with studies in the literature, we found that the AST, creatinine, ferritin and potassium levels were significantly higher in patients treated in the ICU compared with those treated in the service.

In the pathogenesis of COVID-19, viral involvement, the excessive cytokine elevation, immobilization and comorbidities cause endothelial damage and activate of the coagulation system. Plasma ferritin, D-Dimer and troponin levels that are high at initial diagnosis, or increase during follow-up, are defined as "poor prognostic factors" associated with severe disease and mortality [1,21-23]. Zhou et al. [24] reported that elevated plasma ferritin levels were also associated with disease severity. In a study of a total of 458 patients diagnosed with COVID-19 who treated in the ICU (n: 405) and those who did not require ICU treatment (n: 53), CRP, ferritin and D-dimer levels were significantly higher in patients treated in the ICU than in the group who did not require ICU treatment ($p<0.001$) [25].

Progressive hypoxia is common in patients with COVID-19. Blood lactate and LDH levels are commonly used to assess tissue hypoxia [5,21,26]. In a study conducted involving 101 COVID-19 patients in the ICU, the effect of increasing LDH levels on 28-day mortality was reported to be significant (p: 0.003) [26]. In a study conducted by Ucciferri et al. [27] that included a COVID-19 group (n: 280) and healthy control group (n: 206), the LDH levels were significantly higher in the patient group ($p < 0.001$). In our study, we found that the D-dimer levels were lower in ICU patients, but the difference was not statistically significant. Ferritin and LDH levels were significantly higher in patients treated in the ICU compared with those treated in the service. The reason for the lower D-dimer levels in our ICU patient group may be due to differences in the patient numbers and methodological differences. In addition, they may have received anticoagulant therapy to lower their D-dimer levels while being transferred from the ward to the ICU. When selecting

patients for our study group, we excluded patients with additional chronic diseases. In all the other studies, the presence of chronic diseases is observed in the patient group. Although there are some differences between studies, our laboratory results for the patient group are compatible with the results of existing studies.

Kallistatin is a serine protease inhibitor with vasodilator, anti-angiogenic, antioxidant and anti-inflammatory effects. Most studies mention the protective effect of increasing kallistatin levels against disease. Decreased circulating levels of kallistatin have been described in many diseases, including several types of cancer, cardiovascular disease, septic syndrome, liver changes or inflammatory bowel disease [13,28,29]. In a study of patients with septic shock, survivors had significantly higher kallistatin levels than non-survivors [30]. A 2017 study found that kallistatin levels were significantly higher in patients with pre-diabetes compared to healthy controls [31]. The results of another study of 116 patients with type 1 diabetes reported that serum kallistatin levels were increased in type 1 diabetic patients with microvascular complications and hypertension, and this increase was associated with renal-vascular dysfunction [32].

Only one study has investigated kallistatin levels in COVID-19 patients. This study of patients with mild/moderate or severe/critical COVID-19 pneumonia measured the levels of five biomarkers associated with lung injury, namely SP-D, KL-6, presepsin, kallistatin and stratifin. The study found that kallistatin showed a significant reduction in severe and critical stages, but kallistatin was not recommended as a prognostic factor for COVID-19 [33]. In our study results, we found no difference in terms of kallistatin between the COVID-19 patient groups treated in the ICU unit and the service. However, there was a significant difference in terms of kallistatin between our patient group (S+ICU) and our healthy control group, and kallistatin levels were high in the patient group.

In most studies in the literature, kallistatin levels have been found to be low in various diseases and it has been suggested that increasing kallistatin levels is protective. In our study, kallistatin was high in the patient group. One study reported that they found a negative relationship between kallistatin levels and renal function, and they attributed the increase in kallistatin to a decrease in excretion due to impaired renal function [32]. There were no patients with chronic diseases in our patient group, but we obtained this information from the hospital automation system and we do not know how COVID-19 disease affects the kidneys. The creatinine levels of our patients hospitalized in the ICU patients were high. We can say that the increase in our study may be related to the decrease in renal excretion. Our main prediction is that it may have increased in order to alleviate and eliminate the endothelial dysfunction, inflammation and oxidative stress caused by COVID-19 and to play a protective role against these effects. However, to prove these hypotheses, new studies are needed with a larger number of patients and repeated measurements.

In this study, there was no significant difference in kallistatin between COVID-19 patients treated in the service and those treated in the ICU. There was a significant difference in kallistatin levels between the patient group and the control group. Most studies mention the protective effect of increasing kallistatin levels against diseases. Therefore, our study results need to be confirmed by prospective studies with more participants and taking into account conditions that may affect kallistatin levels, such as oxidative stress, atherosclerosis, hyperlipidaemia and obesity.

The small number of patients is a major limitation of this study. Kallistatin levels are also altered in conditions such as oxidative stress, atherosclerosis, hyperlipidemia and obesity. In our study, we obtained information about the presence of chronic diseases in COVID-19 patients from the automated system. For this reason, we were not able to obtain additional information about diseases that may affect kallistatin levels. The blood taken from the patients at the time of their first admission to the service and ICU was used. For this reason, the inability to make repeated measurements of kallistatin levels is among the important limitations of our study.

As a result, kallistatin levels were significantly higher in the patient group than in the control group. Comprehensive studies with more patients are needed to understand whether kallistatin is elevated in COVID-19 patients due to the effects of COVID-19 or to eliminate oxidative stress.

Author Contributions: Concept/Design : KY, SY; Data acquisition: AFG; Data analysis and interpretation: KY; Drafting manuscript: KY; Critical revision of manuscript: KY, AFG; Final approval and accountability: KY, SY, AFG; Technical or material support: KY, SY, AFG; Supervision: KY; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained from the Ethics Committee of Karatay University Faculty of Medicine, Non-Pharmaceutical and Non-Medical Device Research with the decision dated 20.12.2021 and numbered 10-2021/029.

Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: This research is financially supported by the Scientific and Technological Research Council of Türkiye (TUBITAK), Grant/Award Number: BIDEP-2209A Number: 1919B012109027.

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497-506.
2. Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): Current status and future perspectives. Int J Antimicrob Agents. 2020;55:105951.
3. Cecchini R, Cecchini AL. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. Med Hypotheses. 2020;143:110102.
4. Popadic V, Klasnja S, Milic N, Rajovic N, Aleksic A, Milenkovic M et al. Predictors of mortality in critically ill COVID-19 patients demanding high oxygen flow: A thin linebetween inflammation, cytokine storm, and coagulopathy. Oxid Med Cell Longev. 2021;20:6648199.
5. Lang M, Som A, Carey D, Reid N, Mendoza DP, Flores EJ et al. Pulmonary vascular manifestations of COVID-19 pneumonia. Radiol Cardiothorac Imaging. 2020;43:e200277.
6. Yucel K, Fuat Gurbuz A. Hypoxia-inducible factor-1α and ischemia-modified albumin levels in intensive care COVID-19 Patients. Horm Mol Biol Clin Investig. 2022;43:415-20.
7. Chao J, Li P, Chao L. Kallistatin: double-edged role in angiogenesis, apoptosis and oxidative stress. Biol Chem. 2017;398:1309-17.
8. Chao J, Guo Y, Chao L. Protective role of endogenous kallistatin in vascular injury and senescence by inhibiting oxidative stress and inflammation. Oxid Med Cell Longev. 2018;2018:4138560.
9. Liu Y, Bledsoe G, Hagiwara M, Shen B, Chao L, Chao J. Depletion of endogenous kallistatin exacerbates renal and cardiovascular oxidative stress, inflammation, and organ remodeling. Am J Physiol Renal Physiol. 2012;303:1230-8.
10. Wang G, Zou J, Yu X, Yin S, Tang C. The antiatherogenic function of kallistatin and its potential mechanism. Acta Biochim Biophys Sin (Shanghai). 2020;52:583-9.
11. Yao Y, Li B, Liu C, Fu C, Li P, Guo Y et al. Reduced plasma kallistatin is associated with the severity of coronary artery disease, and kallistatin treatment attenuates atherosclerotic plaque formation in mice. J Am Heart Assoc. 2018;7:e009562..
12. Yiu WH, Wong DW, Wu HJ, Li RX, Yam I, Chan LY et al. Kallistatin protects against diabetic nephropathy in db/db mice by suppressing AGE-RAGE-induced oxidative stress. Kidney Int. 2016;89:386-98.
13. Chao J, Bledsoe G, Chao L. Protective role of kallistatin in vascular and organ injury. Hypertension. 2016;68:533-41.
14. Gateva A, Assyov Y, Velikova T, Kamenov Z. Increased kallistatin levels in patients with obesity and prediabetes compared to normal glucose tolerance. Endocr Res. 2017;42:163-8.
15. Jenkins AJ, McBride JD, Januszewski AS, Karschimkus CS, Zhang B, O'Neal DN et al. Increased serum kallistatin levels in type 1 diabetes patients with vascular complications. J Angiogenes Res. 2010;22:19:1-8.
16. Ding ZY, Li GX, Chen L, Shu C, Song J, Wang W et al. Association of liver abnormalities with in-hospital mortality in patients with COVID-19. J Hepatol. 2021;74:1295-302.
17. Alhumaid S, Al Mutair A, Al Alawi Z, Al Salman K, Al Dossary N, Omar A et al. Clinical features and prognostic factors of intensive and non-intensive 1014 COVID-19 patients: an experience cohort from Alahsa, Saudi Arabia. Eur J Med Res. 2021;26:47.
18. Garrido P, Cueto P, Rovira C, Garcia E, Parra A, Enriquez R et al. Clinical value of procalcitonin in critically ill patients infected by SARS-CoV-2. Am J Emerg Med. 2021;46:525-31.
19. Cheng L, Li H, Li L, Liu C, Yan S, Chen H, et al. Ferritin in the coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. J Clin Lab Anal. 2020;34: e23618.
20. Ramonfaur D, Aguirre-García GM, Diaz-Garza CA, Torre-Amione G, Sanchez-Nava VM, Lara-Medrano R et al. Early increase of serum ferritin among COVID-19 patients is associated with need of invasive mechanical ventilation and with in-hospital death. Infect Dis (Lond). 2022;54:810-8.
21. Qiu F, Wu Y, Zhang A, Xie G, Cao H, Du M et al. Changes of coagulation function and risk of stroke in patients with COVID-19. Brain Behav. 2021;16:e02185.
22. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395:1054-62.
23. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395:507-13.
24. Zhou C, Chen Y, Ji Y, He X, Xue D. Increased serum levels of Hepcidin and ferritin are associated with severity of COVID-19. Med Sci Monit. 2020;26:e926178.
25. Bahadirli S, Kurt E. Predicting intensive care ünit admissions for COVID-19 patients in the emergency department. Disaster Med Public Health Prep. 2022;16:1594-8.

26. Singla K, Puri GD, Guha Niyogi S, Mahajan V, Kajal K, Bhalla A. Predictors of the outcomes following the tocilizumab treatment for severe COVID-19. Cureus. 2022;14:e28428..
27. Ucciferri C, Caiazzo L, Di Nicola M, Borrelli P, Pontolillo M, Auricchio A et al. Parameters associated with diagnosis of COVID-19 in emergency department. Immun Inflamm Dis. 2021;9:851-61.
28. Lin WC, Chen CW, Huang YW, Chao L, Chao J, Lin YS et al. Kallistatin protects against sepsis-related acute lung injury via inhibiting inflammation and apoptosis. Sci Rep. 2015;5:12463.
29. Sun HM, Mi YS, Yu FD, Han Y, Liu XS, Lu S et al. SERPINA4 is a novel independent prognostic indicator and a potential therapeutic target for colorectal cancer. Am J Cancer Res. 2016;6:1636-49.
30. Kim T, Suh GJ, Kwon WY, Kim KS, Jung YS, Shin SM. Lower serum kallistatin level is associated with 28-day mortality in patients with septic shock. J Crit Care. 2018;48:328-33.
31. Gateva A, Assyov Y, Velikova T, Kamenov Z. Increased kallistatin levels in patients with obesity and prediabetes compared to normal glucose tolerance. Endocr Res. 2017;42:163-8.
32. Jenkins AJ, McBride JD, Januszewski AS, Karschimkus CS, Zhang B, O'Neal DN et al. Increased serum kallistatin levels in type 1 diabetes patients with vascular complications. J Angiogenes Res. 2010;2:19.
33. Arakawa N, Matsuyama S, Matsuoka M, Kitamura I, Miyashita K, Kitagawa Y et al. Serum stratifin and presepsin as candidate biomarkers for early detection of COVID-19 disease progression. J Pharmacol Sci. 2022;150:21-30..