Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2023;48(2):567-574
DOI: 10.17826/cumj.1279322

RESEARCH

Effect of serum adropin levels on circulating endothelial dysfunction biomarkers in COVID-19 patients

COVID-19 hastalarında serum adropin düzeylerinin dolaşımdaki endotel disfonksiyon biyobelirteçleri üzerine etkisi

Ramazan Güneşaçar[1], Durkadın Demir Ekşi[2], Ali Seydi Alpay[3], Ferhat Hanikoğlu[4], Haluk Erdoğan[3]

[1]Hatay Mustafa Kemal University, Tayfur Ata Sokmen Faculty of Medicine, Department of Medical Biology, Hatay, Turkey
[2]Alanya Alaaddin Keykubat University, Faculty of Medicine, Departments of Medical Biology, [3]Infectious Diseases and Clinical Microbiology, [4]Medical Biochemistry, Antalya, Turkey

Abstract

Purpose: Several studies show that the symptoms of severe COVID-19 infection reflect the clinical phenotype of endothelial dysfunction and share common pathophysiological mechanisms with endothelial dysfunction. Therefore, the aim of the study was to investigate the effect of serum adropin levels on endothelial dysfunction biomarkers and determine whether adropin could be a new biomarker for COVID-19.

Materials and Methods: The study included 40 patients with mild/moderate COVID-19, 48 patients with severe/critical COVID-19, and 37 controls. Serum adropin and circulating biomarkers of endothelial dysfunction including asymmetric dimethylarginine (ADMA), endothelin-1 (ET-1), endothelial nitric oxide synthase (eNOS), soluble intercellular adhesion molecule-1 (sICAM-1) and plasminogen activator inhibitor-1 (PAI-1) levels were determined by micro-ELISA.

Results: Serum adropin levels were found to be significantly higher in COVID-19 patients (165.2±11.49 pg/ml) than in controls (85.46±12.08 pg/ml). Serum adropin levels of patients with severe/critical symptoms (194±16.23 pg/ml) were significantly higher than the patients with mild/moderate symptoms (130.6 ±14.53). In addition, serum ADMA, eNOS, and, ET-1 levels were significantly higher in the COVID-19 subjects (150.5±8.67 ng/ml, 172.4±14.01 pg/ml, 159.3±10.19 pg/ml, respectively) than that those in the controls (104.5±9.182 ng/ml, 141.4±17.74 pg/ml, 100.1±11.37 pg/ml, respectively). Significant positive correlations were found between adropin and ADMA, eNOS, ET-1, sICAM-1, and PAI-1 levels in the patients.

Öz

Amaç: Çalışmalar, şiddetli COVID-19 enfeksiyonu semptomlarının endotel disfonksiyonunun klinik fenotipini yansıttığını ve endotel disfonksiyonuyla ortak patofizyolojik mekanizmalar paylaştığını göstermektedir. Bu nedenle çalışmamızda, serum adropin düzeylerinin endotelyal disfonksiyon biyobelirteçleri üzerindeki etkisini ve adropinin COVID-19 için yeni bir biyobelirteç olup olamayacağını araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmaya 40 hafif/orta semptomlu, 48 ağır/kritik semptomlu COVID-19 hastası ve 37 kontrol dahil edilmiştir. Serum adropin ve asimetrik dimetilarginin (ADMA), endotelin-1 (ET-1), endotelyal nitrik oksit sentaz (eNOS), çözünür hücrelerarası adezyon molekülü-1 (sICAM-1) ve plazminojen aktivatör inhibitörü-1 (PAI-1) gibi endotelyal disfonksiyon biyobelirteçlerinin serum seviyeleri mikro-ELISA yöntemiyle belirlendi.

Bulgular: COVID-19 hastalarında serum adropin düzeyleri (165.2±11.49 pg/ml), kontrol grubuna (85.46±12.08 pg/ml) göre anlamlı derecede yüksek bulundu. Şiddetli/kritik semptomları olan hastaların serum adropin düzeyleri (194±16.23 pg/ml), hafif/orta semptomları olan hastalardan (130.6 ±14.53) anlamlı derecede yüksekti. Ayrıca, COVID-19 olgularında serum ADMA, eNOS ve ET-1 düzeyleri (sırasıyla; 150.5±8.67 ng/ml, 172.4±14.01 pg/ml, 159.3±10.19 pg/ml) kontrollere göre (sırasıyla; 104.5±9.182 ng/ml, 141.4±17.74 pg/ml, 100.1±11.37 pg/ml) anlamlı derecede yüksek bulundu. Hastalarda, adropin ile ADMA, eNOS, ET-1, sICAM-1 ve PAI-1 düzeyleri arasında anlamlı pozitif korelasyonlar saptandı.

Sonuç: Bulgularımız, adropinin COVID-19 için yeni bir potansiyel biyobelirteç ve endotel hücre hasarının

Address for Correspondence: Ramazan Gunesacar, Hatay Mustafa Kemal University Tayfur Ata Sokmen Faculty of Medicine Department of Medical Biology, Antakya, Hatay, Turkey E-mail: rgunesacar@hotmail.com
Received: 09.04.2023 Accepted: 01.06.2023

Conclusion: We suggest that adropin may be a new potential biomarker for COVID-19 and an important molecule in restoring endothelial cell damage. Positive correlations between serum adropin levels and ADMA, eNOS, ET-1, sICAM-1 and PAI-1 levels in patients suggest that adropin may compensate for damage to endothelial cells.
Keywords:. COVID-19, adropin, endothelial dysfunction, biomarkers

onarımında önemli bir molekül olabileceğini göstermektedir. Hastalarda serum adropin düzeyleri ile ADMA, eNOS, ET-1, sICAM-1 ve PAI-1 seviyeleri arasındaki pozitif korelasyonlar, adropinin endotel hücrelerindeki hasarı telafi edebileceğini düşündürmektedir.

Anahtar kelimeler: COVID-19, adropin, endotelyal disfonksiyon, biyobelirteçler

INTRODUCTION

Adropin is a hormone encoded by the *ENHO* (energy homeostasis-associated) gene. This protein consists of 74 amino acids including a signal peptide sequence of 33 amino acids and a bioactive sequence of 41 amino acids. The adropin is primarily expressed in the liver, kidney, brain, pancreas, heart, coronary artery, and umbilical cord[1]. The studies on the association between adropin and cardiometabolic disorders have largely focused on the regulation and protection of endothelial cell functions. It was reported that the adropin might have potential protective effects on endothelial function and that the decreased adropin levels were related with endothelial dysfunction [2,3]. The low adropin level in cardiometabolic disorders is a risk factor for endothelial function. There are several reports indicating that the impairment of endothelial function could have a significant involvement in the onset and progression of cardiometabolic disorders[4-6].

Functional endothelial cells express factors that inhibit platelet aggregation and coagulation, increase blood flow, and promote fibrinolysis by stimulating vascular relaxation, while nonfunctional endothelial cells lead to vasoconstriction and thrombus formation[7].

Recent studies suggest that the symptoms and signs of COVID-19 resemble the clinical phenotype of endothelial dysfunction and share common pathophysiological mechanisms[8]. It has been proposed that endothelial dysfunction is the major pathophysiological process in various viral infections[9,10]. Information from previous coronavirus pandemics has triggered studies investigating the role of endothelial dysfunction in COVID-19 subjects. Recent studies indicated that endothelial dysfunction might contribute to severe pulmonary outcomes of COVID-19. Based on these studies, it is possible to say that endothelial dysfunction seems to be a common ground for many clinical aspects of severe COVID-19. Thus, in our study, it was aimed to investigate associations between serum adropin levels and biomarkers of endothelial dysfunction such as ADMA, eNOS, ET-1, sICAM-1 and PAI-1 levels, which have been extensively studied in COVID-19 in the literature and to evaluate whether the adropin could be a new biomarker for COVID-19.

MATERIALS AND METHODS

Subjects

This study was conducted at Alanya Alaaddin Keykubat University Faculty of Medicine between April 2021 and February 2022. The study included 88 patients with COVID-19 (42 female, 46 male; mean age ± SEM, 55.32±1.75 years) who presented to the Infectious Diseases and Clinical Microbiology Clinic of Alanya Education and Research Hospital. The inclusion and exclusion criteria for the patient group were implemented as follows: Adult patients (≥ 18 years) with confirmed COVID-19 diagnosis by RT-PCR test results for SARS-CoV-2. The patients with a clinical diagnosis of COVID-19 but negative RT-PCR test results were excluded from the study. The patients were classified into two groups based on the National Institutes of Health (NIH) criteria[11]: the patients with mild/moderate symptoms (40 patients) and those with severe/critical (48 patients) symptoms. In this case-control study, 37 healthy individuals (18 females, 19 males; mean age ± SEM, 54.84±1.33 years) were employed as controls. The inclusion criteria for the control group was the collection of serum samples from healthy individuals during the pre-pandemic period. The serum samples obtained during pandemic were excluded from the study. It is well-known that some individuals infected with SARS-CoV-2 may be asymptomatic and recruitment of such individuals could affect the results. For this reason, we used serum samples obtained in the pre-pandemic period to ensure that the individuals in the healthy control group were not

infected with the virus. Power analysis performed indicated that a sample size of 30 individuals per control and patient group were required with a 80% power, a 5% margin of error, and an effect size of 0.25. Ethical approval was obtained from the Clinical Research Ethics Committee of Alanya Alaaddin Keykubat University (01.03.2021/05-03) and informed consent was taken from the study participants.

Procedure

In all subjects, a blood sample of 5 mL was drawn into serum-separating tubes were left to clot at room temperature, which was centrifuged at 1500 g for 10 minutes in a refrigerated centrifuge thereafter. After centrifugation, serum samples were divided into aliquots of 1 ml and transferred to Eppendorf tubes. The serum samples were stored at -80 °C until assays. Serum samples were taken from the deep freezer on the working day and thawed at room temperature.

Serum adropin, ADMA, eNOS, ET-1, sICAM-1, and PAI-1 levels were measured using commercial micro-ELISA kits (SinoGeneClon Biotech Co, Ltd., China). The sandwich ELISA method was applied briefly as follows: Fifty µL of standards and diluted serum samples were placed in the wells of the ELISA plate and incubated at 37 °C for 30 minutes. After washing, 50 µL of HRP-conjugate reagent was added into all wells except blank well, and incubated at 37 °C for 30 minutes. Then, the ELISA plate was washed and 50 µL of chromogen A and B solutions were added to all wells. The ELISA plate was incubated at 37 °C for 15 minutes. After incubation, the reaction was stopped by adding 50 µL of stop solution to all wells. Within 15 minutes after the reaction was stopped, the absorbance was read at 450 nm on the ELISA plate reader. The concentration was calculated using the graph obtained according to the optical density values read against the concentrations of the standards.

Statistical analysis

All statistical analyses were performed using GraphPad Prism (v.6.0) software. The normal distribution of the data were tested using the Kolmogorov-Smirnov test. All statistical estimations were performed with the confidence interval (CI) of 95%. Mann-Whitney U test was used to compare continuous variables between two groups while Spearman's rank correlation analysis was used to determine correlations between the groups. The Kruskal-Wallis test was used to compare continuous variables in more than two groups. Receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic value of serum biomarker expression in the study groups. A p value <0.05 was considered as statistically significant.

RESULTS

Serum Adropin, ADMA, eNOS, ET-1, sICAM-1, and PAI-1 levels are shown in Table 1. Serum adropin levels were found to be higher in the patient group compared to controls ($P<0.0001$). Serum adropin levels of patients with severe/critical symptoms were significantly higher and statistically significant than that of patients with mild/moderate symptoms ($P= 0.0175$). Although serum ADMA, eNOS, and ET-1 levels were significantly higher in all patients with COVID-19 compared to controls ($P=0.0003$, $P=0.0137$, $P=0.0001$, respectively), there was no statistically significant difference between patients with mild/moderate and severe/critical symptoms ($P>0.05$, for all). Serum sICAM-1 and PAI-1 levels were found to be similar in all study groups ($P>0.05$, for all). In the correlation analyses (Table 2), positive correlations were found between adropin levels and ADMA, eNOS, ET-1, sICAM-1, and PAI-1 in the COVID-19 group ($P<0.05$ for all). Table 3 and Figure 1A, 1B and 1C show the ROC curve analysis results of serum adropin, ADMA, and ET-1 for all subjects including patients and controls while Figure 2 shows ROC curve analysis results of adropin for patients with severe/critical and mild/moderate symptoms. When COVID-19 patients were classified into two groups as those with and without at least one chronic disease such as diabetes or hypertension, no statistical difference was found between the groups for all serum parameters studied ($P >0.05$, for all).

Table 1. Serum Adropin, ADMA, eNOS, ET-1, sICAM-1 and PAI-1 levels in COVID-19 patients and controls

	Control group (n=37)	All patients with COVID-19 (n=88)	Mild/ Moderate (n=40)	Severe (n=48)	Overall	Control vs All patients with COVID-19	Severe vs Mild/moderate
Adropin (pg/ml)	85.46±12.08	165.2±11.49	130.6 ±14.53	194±16.23	**<0.0001**	**<0.0001**	**0.0175**
ADMA (ng/ml)	104.5±9.182	150.5±8.67	165.8±16.67	137.5±7.44	**0.0002**	**0.0003**	>0.9999
eNOS (pg/ml)	141.4±17.74	172.4±14.01	175.1±26.21	170.1±13.82	**0.0106**	**0.0137**	>0.9999
ET-1 (pg/ml)	100.1±11.37	159.3±10.19	158.7±18.59	159.8±10.64	**0.0001**	**<0.0001**	>0.9999
sICAM-1 (ng/ml)	853.2±136.2	884.6±78.33	928.8±140.5	847.8±84.32	0.2629	0.4197	>0.9999
PAI-1 (ng/ml)	168.3±11.45	167.5±10.97	173.1±21.55	162.9±9.29	0.7084	>0.9999	>0.9999

n: Sample size; X±SE: Mean±Standard error; ADMA: Asymmetric dimethylarginine; eNOS: Endothelial nitric oxide synthase; ET-1: Endothelin-1; sICAM-1: Soluble intercellular adhesion molecule-1, PAI-1: Plasminogen activator inhibitor-1.

Table 2. Correlations between serum Adropin levels and endothelial dysfunction biomarkers

	ADMA	eNOS	ET-1	sICAM-1	PAI-1
ADROPİN	r=0.3996 P=0.0001	r=0.3653 P=0.0005	r=0.2495 P=0.019	r=0.2403 P=0.0241	r=0.3464 P=0.0009

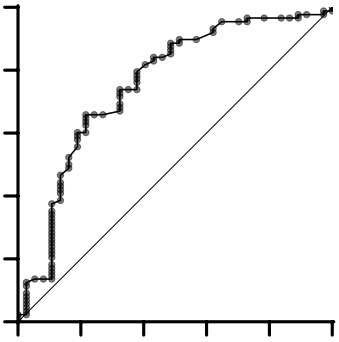
ADMA: Asymmetric dimethylarginine; eNOS: Endothelial nitric oxide synthase; ET-1: Endothelin-1; sICAM-1: Soluble intercellular adhesion molecule-1, PAI-1: Plasminogen activator inhibitor-1.

Table 3. Receiver operating characteristic (ROC) analysis results of serum adropin, ADMA, and ET-1 in all patients with COVID-19 and controls

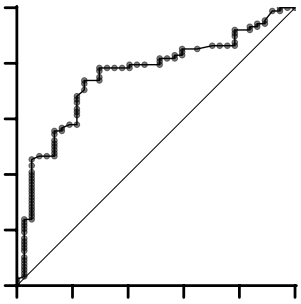
Under the ROC Curve	Adropin (pg/ml)	ADMA (ng/ml)	ET-1 (pg/ml)
Area	0.757	0.729	0.754
Standard error	0.045	0.052	0.050
95% confidence interval	0.667-0.846	0.626-0.833	0.654-0.853
Cut-off value	>94.50	>97.5	>107.00
Sensitivity	0.784	0.761	0.738
Specifity	0.702	0.648	0.675
P value	<0.0001	<0.0001	<0.0001

ROC: Receiver operating characteristic; ADMA: Asymmetric dimethylarginine; ET-1: Endothelin-1

A)



B)



C)

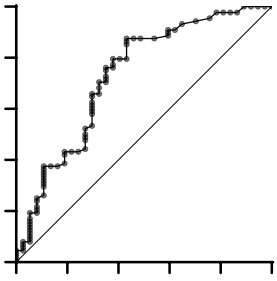


Figure 1. The area under the receiver operating characteristic curves (AUCs) for adropin (A), Asymmetric dimethylarginine (B), and Endothelin-1 (C) in all patients with COVID-19 and controls.

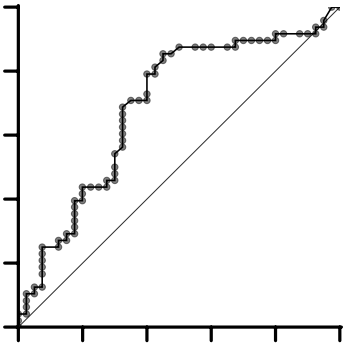


Figure 2. The area under the receiver operating characteristic curve (AUC) for Adropin in COVID-19 patients with severe/ critical and mild/moderate symptoms.

DISCUSSION

The adropin is an important molecule in the regulation of endothelial function, and studies have reported a potential association between low levels of adropin and endothelial dysfunction[2]. In our study, it was found that the serum adropin levels were significantly higher in all COVID-19 cases compared to the controls. When COVID-19 patients were compared after stratifying as mild/moderate or severe/critical based on clinical symptoms, it was found that serum adropin levels were higher in patients with severe/critical symptoms compared to those with mild/moderate symptoms. These findings suggest that high levels of serum adropin may be considered as a new potential biomarker for COVID-19 and its severity. Recent studies have suggested that various viral infections, including SARS-CoV-2, are associated with endothelial dysfunction[12]. In our study, serum adropin levels were found to be higher in all COVID-19 patients compared to the healthy controls as well as in patients with severe/critical symptoms compared to patients with mild/moderate symptoms, suggesting that it may also be an important molecule in the restoration of endothelial cell damage. It was shown that HIF-1α expression is significantly higher in COVID-19 patients than healthy controls due to the hypoxia. Stabilization of HIF-1α in SARS-CoV-2 infected macrophages could be a potential consequence of a secondary bacterial

infection in severe COVID-19[12]. On the other hand, Kurt et al[13]. found that the acute mesenteric ischemia group had significantly higher serum adropin concentrations compared to the healthy control group. Their correlation analysis showed a significant positive association between adropin and HIF-1α in all groups. It was suggested that inducing hypoxia may activate the cytoprotective signaling induced by HIF-1α and thereby alleviate the severity of illness and enhance the function of vital organs in COVID-19 patients[12]. In COVID-19 patients higher adropin levels may be associated with higher HIF-1α levels and may help restoration of cells. Further studies in larger COVID-19 patient groups and functional studies are needed to clarify the association between severity of COVID-19 and adropin. It was also found that there were positive correlations between serum adropin levels and serum ADMA, eNOS, ET-1, sICAM-1, and PAI-1 levels in the COVID-19 group, suggesting that adropin levels are increased to compensate for damage to endothelial cells. However, unlike our findings, in a recent work by Aydın et al., it was shown that serum adropin levels were lower in COVID-19 patients compared to the controls[15]. Aydın et al. included 25 controls and 25 COVID-19 subjects in their study, while 37 controls and 88 patients were included in our work. As can be seen, the number of patients and controls included in both studies is limited. Therefore, larger patient and controls should be studied to elucidate the relationship between adropin and COVID-19. It was reported that biomarkers for endothelial and platelet activation were increased in severe COVID-19 infection[16]. Cooke and et al. reported that ADMA, a potential biomarker for endothelial dysfunction, could lead to endothelial damage by inhibiting eNOS which catalyzes the production of nitric oxide (NO), an important molecule for endothelial function[17]. In our study, the findings that eNOS levels were increased and that there was a positive correlation between eNOS and ADMA ($r=0.446$; $P<0.0001$) indicate that endothelial dysfunction due to NO deficiency did not result from eNOS inhibition in COVID-19. It was suggested that increased serum ADMA level was associated with mortality in COVID-19 and ADMA could be a biomarker for assessing mortality risk[18]. It was also reported that the ADMA level was increased in COVID-19 subjects with pulmonary involvement when compared to those without and that the ADMA could be used as a laboratory parameter for a better understanding of COVID-19 pathophysiology[19]. Despite the lack of a significant difference in serum ADMA levels between patients with and without pneumonia in the present study, the finding of higher serum ADMA levels in COVID-19 subjects than in controls suggests its potential contribution to the elucidation of COVID-19 pathophysiology and highlights its potential as a biomarker for the disease.

ET-1, a molecule with a potent endogenous vasoconstrictor effect, enhances vasoconstriction and leads to fibrosis of vascular cells; in addition, it also stimulates reactive oxygen species production. It was suggested that high levels of blood ET-1 can be an important prognostic tool and biomarker to identify people at risk for severe COVID-19 infection[20]. It was reported that levels of ET-1 were increased in the acute phase of infection in hospitalized patients and ET-1 elevation was correlated with disease severity, suggesting that endothelin receptor antagonists may be beneficial in COVID-19 subjects[21]. Our study revealed that COVID-19 subjects exhibited higher levels of serum ET-1 compared to controls, indicating the potential involvement of ET-1 in the pathogenesis of the disease. These findings also suggest that ET-1 could serve as a potential biomarker for COVID-19. However, comparable levels of ET-1 among COVID-19 subgroups may be due to the insufficient sample size in the present study.

eNOS produces NO in the vascular endothelium, which has significant regulatory functions in cellular proliferation, leukocyte adhesion, vascular tone, and platelet aggregation[22]. The deficiency in eNOS-derived NO is an essential cause of endothelial dysfunction that may result in the disruption of vascular integrity[23]. The decreased NO level leads to unfavorable vascular events such as blood pressure disorders, prevention of vascular dilatation, loss of anti-thrombotic activity, fibrinolysis defects, and inhibition of platelet aggregation. It was reported that a reduction in serum NO levels would lead to oxidative stress in COVID-19[24]. It was reported that the adropin may regulate endothelial cell function by increasing NO release via stimulation of eNOS expression through VEGFR2-PI3K-Akt or VGEFR2-ERK1/2 signaling pathways; thus, it may be protective for the cardiovascular system by inducing neovascularization[2]. In consistent with these findings, in our study, the increased adropin level in COVID-19 subjects and positive correlation between adropin and eNOS suggest that adropin prevents reduction in eNOS level by eNOS stimulation; thus, maintains circulating NO level.

The sICAM-1, formed through proteolytic slicing of ICAM-1 molecule, plays a critical role in endothelial cell activation and damage. In previous studies, it was reported that elevated sICAM-1 level could be involved in the coagulopathy and was associated with disease severity and mortality in COVID-19[25,26]. The PAI-1 inhibits serine proteases such as urokinase and tissue plasminogen activator (tPA), which are involved in the resolution of coagulation, by binding these molecules. Normally, the plasminogen cannot be activated by tPA since the circulating PAI-1 activity is higher than tPA. The PAI-1 synthesis is stimulated by thrombin, cytokines, and lipoproteins, and 90% of PAI-1 is stored in platelets[27]. It was shown that elevated PAI-1 levels lead increased risk for atherothrombosis[28,29]. It was reported that there was a greater extent of increase in levels of PAI-1 and tPA in COVID-19[30-32]. In our study, on contrary to the literature, serum sICAM-1 and PAI-1 levels were found to be comparable between the COVID-19 group and controls or across COVID-19 subgroups.

Our study has several limitations. To establish a conclusive result regarding this matter, it is necessary to conduct studies with larger sample sizes from diverse populations, as the current study may have been limited by its insufficient number of patients. In addition, the inclusion of individuals who have asymptomatic COVID-19 in the study would be important for interpreting the effects of adropin on endothelial dysfunction biomarkers.

In conclusion, our findings showed that adropin may be a new potential biomarker for both COVID-19 and severe/critical symptoms of the disease. Our results also suggested that adropin may play role in the restoration of endothelial cell damage. In addition, ADMA and ET-1 may also be potential biomarkers for COVID-19. In COVID-19 patients, the increased adropin levels and positive correlation between adropin and eNOS suggest that adropin may maintain blood NO levels by preventing a reduction in eNOS levels through stimulation of eNOS. In addition, the positive correlations observed between levels of serum adropin and serum ADMA, eNOS, ET-1, sICAM-1, and PAI-1 in the COVID-19 group suggest that adropin may compensate for damage to endothelial cells.

Author Contributions: Concept/Design : RG; Data acquisition: RG, DDE, ASA, FH, HE; Data analysis and interpretation: RG, DDE, ASA, FH, HE; Drafting manuscript: RG; Critical revision of manuscript: RG, DDE; Final approval and accountability: RG, DDE, ASA, FH, HE; Technical or material support: ASA, FH, HE; Supervision: RG; Securing funding (if available): n/a.

Ethical Approval: Ethical approval was obtained from the Clinical Research Ethics Committee of Alanya Alaadin Keykubat University Faculty of Medicine by its decision dated 10.03.2021 and numbered 05-03.
Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors declare no potential conflict of interest.
Financial Disclosure: Authors declared no financial support
Acknowledgement: This study was supported by the Alanya Alaaddin Keykubat University Research Fund, Antalya, Türkiye (Project number: 2021-04-01-MAP13).

REFERENCES

1. Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA. Chouljenko VN et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. Cell Metab. 2008;8:468-81.
2. Lovren F, Pan Y, Quan A,. Singh KK, Shukla PC, Gupta M et al. Adropin is a novel regulator of endothelial function. Circulation. 2010;122:185-92.
3. Butler AA, Tam CS, Stanhope KL, Wolfe BM, Ali MR, O'Keeffe M et al. Low circulating adropin concentrations with obesity and aging correlate with risk factors for metabolic disease and increase after gastric bypass surgery in humans. J Clin Endocrinol Metab. 2012;97:3783-91.
4. Topuz M, Celik A, Aslantas T, Demir AK, Aydin S, Aydin S. Plasma adropin levels predict endothelial dysfunction like flow-mediated dilatation in patients with type 2 diabetes mellitus. J Investig Med. 2013;61:1161-4.
5. Celik A, Balin M, Kobat M.A, Erdem K, Baydas A, Bulut M et al. Deficiency of a new protein associated with cardiac syndrome X; called adropin. Cardiovasc Ther. 2013;31:174-8.
6. Gu X, Li H, Zhu X, Gu H, Chen J, Wang L et al. Inverse correlation between plasma adropin and ET-1 levels in essential hypertension: A cross-sectional study. Medicine (Baltimore). 2015;94:e1712.
7. Yau, JW, Teoh H, Verma S. Endothelial cell control of thrombosis. BMC Cardiovasc Disord. 2015;15:130.
8. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395:1417-8.
9. Steinberg BE, Goldenberg NM, Lee WL. Do viral infections mimic bacterial sepsis? The role of microvascular permeability: A review of mechanisms and methods, Antiviral Res. 2012;93:2-15.
10. Goeijenbier M, van Wissen M, van de Weg C, Jong E, Gerdes VE, Meijers JC et al. Review: Viral infections and mechanisms of thrombosis and bleeding. J Med Virol. 2012;84:1680-96.
11. National Institutes of Health. Clinical spectrum of SARS-CoV-2 infection. https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/ (accessed Jan 2022).

12. Serebrovska ZO, Chong EY, Serebrovska TV, Tumanovska LV, Xi L. Hypoxia, HIF-1α, and COVID-19: from pathogenic factors to potential therapeutic targets. Acta Pharmacol Sin. 2020;41:1539–46.
13. Kurt E, Tekin E, Kurt N, Bayramoglu A. The role of adropin, HIF-1α and apelin biomarkers in the diagnosis of acute mesentaric ischemia. Am J Emerg Med. 2022;51:223-27.
14. Prasad M, Leon M, Lerman LO, Lerman A. Viral endothelial dysfunction: A unifying mechanism for COVID-19. Mayo Clin Proc. 2021;96:3099-108.
15. Aydın P, Karabulut Uzunçakmak S ,Tör IH , Bilen A , Özden A. Comparison of serum adropin levels in patients with diabetes mellitus, COVID-19, and COVID-19 with diabetes mellitus. Eurasian J Med. 2022;54:197-201.
16. Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. Lancet Haematol. 2020;7:e575-82.
17. Cooke JP. Does ADMA cause endothelial dysfunction? Arterioscler Thromb Vasc Biol. 2000;20:2032-7.
18. Hannemann J, Balfanz P, Schwedhelm E, Hartmann B, Ule J, Müller-Wieland D et al. Elevated serum SDMA and ADMA at hospital admission predict in-hospital mortality of COVID-19 patients. Sci Rep. 2021;11:9895.
19. Emlek N, Yilmaz AS, Ergul E, Gundogdu H, Arpa M, Koc H, et al. The relationship of serum asymmetric dimethylarginine concentrations and lung involvement in patients with COVID-19 infection. Exp Biomed Res. 2021;4:314-21.
20. Farhangrazi, Shadi Z, Moghimi SM. Elevated circulating endothelin-1 as a potential biomarker for high-risk COVID-19 severity. Precis Nanomed. 2020;3:622-28.
21. Abraham GR, Kuc RE, Althage M, Greasley PJ, Ambery P, Maguire JJ et al. Endothelin-1 is increased in the plasma of patients hospitalized with Covid-19. J Mol Cell Cardiol. 2022;167:92-6.
22. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J. 2012:33:829-37.
23. Guan SP, Seet RCS, Kennedy BK. Does eNOS derived nitric oxide protect the young from severe COVID-19 complications? Ageing Res Rev. 2020;64:101201.
24. Green SJ. Covid-19 accelerates endothelial dysfunction and nitric oxide deficiency. Microbes Infect. 2020;22:149-50.
25. Tong M, Jiang Y, Xia D, Xiong Y, Zheng Q, Chen F et al. Elevated expression of serum endothelial cell adhesion molecules in COVID-19 patients. J Infect Dis. 2020;222:894–8.
26. Vassiliou AG, Keskinidou C, Jahaj E, Gallos P, Dimopoulou I, Kotanidou A et al. ICU admission levels of endothelial biomarkers as predictors of mortality in critically Ill COVID-19 patients. Cells. 2021;10:186.
27. Dellas C, Loskutoff DJ. Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. Thromb Haemost. 2005;93:631-40.
28. Vaughan DE. PAI-1 and atherothrombosis. J Thromb Haemost. 2005;3:1879-83.
29. Baluta MM, Vintila MM. PAI-1 inhibition - another therapeutic option for cardiovascular protection. Maedica (Bucur). 2015;10:147-52.
30. Ranucci M, Ballotta A, Di Dedda U, Bayshnikova E, Dei Poli M, Resta M et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. J Thromb Haemost. 2020;18:1747-51.
31. Masi P, Hekimian G, Lejeune M, Chommeloux J, Desnos C, De Chambrun MP et al. Systemic inflammatory response syndrome is a major contributor to COVID-19-associated coagulopathy: Insights from a prospective, single-center cohort study. Circulation. 2020;142:611-4.
32. Zuo Y, Warnock M, Harbaugh A, Yalavarthi S, Gockman K, Zuo M et al. Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients. Sci Rep. 2021;11:1580.