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Could Asymmetric Dimethylarginine Have a Role in COVID-19 Cases?

Asimetrik Dimetilarjininin COVID-19'daki Rolü

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Abstract

Objective: Coronavirus disease-2019 (COVID-19) is a disease with respiratory involvement and the virus-induced endothelial dysfunction is pronounced in the clinical course. In COVID-19 patients, to analyze the laboratory findings and the associations may help to better understand the pathophysiology of this disease.

Method: We analyzed the laboratory markers including white blood cell, platelet, mean platelet volume, red cell distribution width (RDW), lactate dehydrogenase, procalcitonin, arterial blood gas values and asymmetric dimethylarginine (ADMA) levels of 83 hospitalized COVID-19 patients with pneumonia. Thirty healthy individuals were enrolled as a control group and their laboratory findings were compared.

Results: No significant difference of ADMA levels (median value 225.6 μg/L vs 225.6 μg/L) was noted between COVID-19 patients and the control group (p=0.771). ADMA had an inverse correlation with RDW (r=-0.391, p<0.001). C-reactive protein (CRP) was the significant variable when patients were compared to the healthy group (p=0.002).

Conclusion: ADMA levels do not increase at the beginning of COVID-19 clinical course. CRP, as an established inflammation marker, has an imperative role in the clinical spectrum of this coronavirus infection.

Keywords: ADMA, COVID-19, infection

Öz

Amaç: Koronavirüs hastalığı-2019 (COVID-19) solunum tutulumu olan bir hastalıktır ve virüs kaynaklı endotelyal disfonksiyon klinik seyirde belirgindir. COVID-19 hastalarında laboratuvar bulgularının ve birlikteliklerin analiz edilmesi bu hastalığın patofizyolojisinin daha iyi anlaşılmasına yardımcı olabilir.

Yöntem: Hastaneye yatırılan 83 pnömonili COVID-19 hastasının beyaz kan hücresi, trombosit, ortalama trombosit hacmi, kırmızı hücre dağıtım genişliği (RDW), laktat dehidrogenaz, prokalsitonin, arter kan gazı değerleri ve asimetrik dimetilarginin (ADMA) düzeylerini içeren laboratuvar belirteçleri incelendi. Kontrol grubu olarak 30 sağlıklı kişi alındı ve laboratuvar bulguları karşılaştırıldı.

Bulgular: COVID-19 hastaları ve kontrol grubu arasında ADMA seviyelerinde (ortanca değer 225,6 μg/L'ye karşı 225,6 μg/L'ye karşı) anlamlı bir fark görülmedi (p=0,771). ADMA, RDW ile ters bir korelasyona sahipti (r=-0,391, p<0,001). Hastalar sağlıklı grupla karşılaştırıldığında C-reaktif protein (CRP) anlamlı bir değişkendi (p=0,002).

Sonuç: ADMA seviyeleri COVID-19 klinik seyrinin başlangıcında artmamaktadır. Yerleşik bir enflamasyon belirteci olarak CRP, bu koronavirüs enfeksiyonunun klinik spektrumunda zorunlu bir role sahiptir.

Anahtar kelimeler: ADMA, COVID-19, enfeksiyon

Introduction

Coronavirus disease-2019 (COVID-19) has been a global challenge since 2019. The World Health Organization (WHO) declared it as a pandemic in early 2020 (1). It is caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and it has a variable clinical course from asymptomatic patients or mild respiratory symptoms to critical illness and death. Despite the extensive vaccination campaigns, third and fourth waves of the disease are challenging for the World. SARS-CoV-2 infects the host

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cells via binding angiotensin-converting enzyme 2 (ACE_2) receptors, which are expressed in the lungs, heart, kidney, gut and endothelium (2). Several studies including autopsy series show viral inclusion bodies which are found in endothelial cells and directly infected by SARS-CoV-2 (3,4). ACE_2 receptor also regulates the vascular functions via nitric oxide (NO) release, the molecule which plays a key role in endothelial inflammation and dysfunction (5,6). NO is involved in a broad spectrum of processes: It stimulates vasodilatory and bronchodilator effects in the lungs, and may help preventing pulmonary infections by producing antimicrobial effects (7). During the SARS outbreak in 2002-2003, also caused by another coronavirus infection, some studies demonstrated NO's antiviral effect (8). It is achieved by inhibiting viral replication of SARS-CoV or by reduction of expression of the spike (S) protein and the fusion of S protein-ACE_2 receptor (8). Inhaled NO was tested in some SARS patients and some beneficial effects, such as the improvement of arterial oxygenation and reduced spread of lung infiltrates, were noted (9). Stemming from these preliminary studies, some scientists recommend using inhaled NO in the management of severe hypoxia due to COVID-19 (10). NO is synthesized from its precursor L-arginine by endothelial nitric oxide synthetase (NOS) (11). Asymmetric dimethylarginine (ADMA) is a competitive inhibitor of NOS and accumulation of ADMA reduces NO levels. ADMA levels are increased in cardiovascular disease, renal failure, and diabetes (12,13). The role of ADMA, as a predictor of cardiovascular events or death in critically ill patients with sepsis, has been reported previously (14,15). Rising data suggest that COVID-19 is a vascular and thrombotic disease with the phenomenon of endothelial dysfunction, which particularly effects the patients with cardiometabolic disorders like hypertension, diabetes, and obesity (3). However, the underlying mechanisms are still not completely understood (16). C-reactive protein (CRP) is shown to be discriminative marker for the severity of COVID-19 from the early days of the pandemic (17). We need novel biomarkers which will help to understand these viral pathogenetic mechanisms and to find appropriate clinical approach for COVID-19 treatment. In this study, we analyze the plasma concentration of ADMA, an indirect marker of endothelial dysfunction (18), and its association with other inflammation markers like white blood cell (WBC), red cell distribution width (RDW), platelet count, mean platelet volume (MPV), CRP, lactate dehydrogenase (LDH), procalcitonin, and parameters of arterial blood gases [partial oxygen pressure ($P0_2$), partial carbon dioxide pressure (PCO_2), oxygen saturation percentage (SO_2%)].

Materials and Methods

The study was conducted in a tertiary care center after Institutional Ethics Committee approved the research plan on April 22, 2020 (no: 2737) and performed in accordance with the principles of Declaration of Helsinki. Written informed consents were taken from the participants. This study included 83 consecutive, laboratory-confirmed COVID-19 patients (48 male, 35 female; age 65±16.3 years) followed in pandemic wards between May and July 2020. COVID-19 patients who had pneumonia based on chest computed tomography findings and respiratory symptoms, without hypoxia, categorized as "moderate COVID-19 cases'' according to WHO case definitions were included in this study (1). Patients under the age of 18 years, those who were pregnant and who were transferred to intensive care unit or who passed away during follow-up were excluded from the study. Thirty gender-matched healthy individuals (15 male, 15 female; age 58.2±11.0 years) acted as the control group. Basic demographic data and comorbidities were recorded. Serum ADMA levels were measured together with other laboratory markers [WBC, RDW, platelet, MPV, CRP, LDH, procalcitonin, arterial blood gas analysis: ($P0_2$), PCO_2 SO_2% oxygen saturation percentage]. Patients were included only if the blood samples were obtained on the first day of admission, biomarker analysis was available and samples were stored under standardized conditions. For measuring ADMA, fasting blood samples were taken into vacuum tubes (BD, Plymouth, England). After keeping the samples at room temperature for 2 hours, they were centrifuged at + 4 °C at 1.000 x g for separating serum. Separated serums were kept at -80 °C until the analysis. Serum ADMA levels were analyzed by enzyme linked immunosorbent assay method using Elabscience Pharmaceuticals kit, (Houston, Texas, USA). The measurable range was 15.63-1.000 ng/mL, sensitivity was <9.38 ng/mL, and coefficient of variation was <10%.

Statistical Analysis

Descriptive statistics were given as mean ± standard deviation and median with minimum-maximum for continuous variables depending on their distribution. Numbers and percentages were used for categorical variables. Normality of the numerical variables was analyzed by the Kolmogorov-Smirnov test and checked by Q-Q plots and histograms.

Based on the distribution of sex and comorbid diseases, the Mann-Whitney U test was applied for variables without normal distribution. The Kruskal-Wallis test was

used to compare the platelet count and ADMA values. The Spearman Rho correlation coefficient was used to analyze the associations between ADMA and age and other numerical variables.

Demographic variables differing between the groups were controlled using the analysis of co-variance. The sm.ancova package in R software was used for non-parametric tests.

For statistical analysis and figures, Jamovi project (2020), Jamovi version 1.6.3 [Computer Software] (Retrieved from https://www.jamovi.org) and JASP (version 0.13.1) (Retrieved from https://jasp-stats.org) were used. The significance level (p-value) was set at 0.05 in all statistical analyses.

Results

Plasma ADMA concentrations, and laboratory findings of 83 hospitalized COVID-19 patients were analyzed. In Table 1, demographic and clinical characteristics and laboratory features of the study group are summarized. The enrollees consisted of 48 (57.8%) male and 35 female (42.2%) patients with a mean age of 65±16.3 years. Forty-seven patients (56.6%) of all patients had at least one comorbid disease. Hypertension was the most common comorbidity seen in 29 patients (34.9%).

Among the inflammation markers, the mean WBC count was 8.4±4.0 (x109/L), whereas the mean values of CRP and procalcitonin were measured as 51.8±57.9 mg/L and 1.5±6.1 ng/mL, respectively. In the analysis of arterial blood gas, PO_2 and SO_2 were detected as 65.8±15.1 mm Hg and 90.5±5.2%, while PCO_2 was 37.01±4.3. The mean ADMA value was found as 360±420.8 µg/L with the minimum and maximum values of 58.6 and 2684.5 µg/L.

The median ADMA value was 196.9 µg/L in males, whereas 292.22 µg/L in females. There was no significant difference between male and female patients (p=0.166). There was also no significant difference found in serum ADMA concentrations of the patients with or without comorbidities (p=0.869) (Table 2).

We detected a significant inverse correlation between ADMA and RDW (r=-0.391, p<0.001) (Figure 1).

However, there were no significant correlations found between ADMA and age, WBC, platelet count, CRP, LDH, procalcitonin, PO_2, PCO_2 and SO_2% (p>0.05 for all) (Table 3).

In Table 4, the association of ADMA with the groupings based on upper and lower laboratory ranges of platelet count, CRP and procalcitonin is given. Although the median ADMA levels in patients with CRP <5 mg/L were higher than the levels in patients with CRP ≥5 mg/L, the difference was statistically insignificant (p=0.061). There was no significant association in the serum ADMA levels of the patients when grouped based on the higher and lower values of platelet count and procalcitonin (p=0.739 and p=0.942).

Table 1. Demographic and clinical characteristics and laboratory features of the study group

	Value	Min-max
Age (year)†	65±16.3	64.0 (26.0-94.0)
Sex‡		
Male	48 (57.8%)	
Female	35 (42.2%)	
Comorbid disease, yes‡	47 (56.6%)	
Hypertension	29 (34.9%)	
Diabetes mellitus	18 (21.7%)	
Chronic renal failure	8 (9.6%)	
Cerebrovascular accident	5 (6.0%)	
Atrial fibrillation	1 (1.2%)	
Malignancy	2 (2.4%)	
WBC count (x10⁹/L)†	8.4±4.0	7.3 (2.2-19.0)
RDW (%)†	14.3±1.7	14.0 (12.0-21.1)
Platelet count (x10³/L)†	219.8±76.2	212.0 (41.0-410.0)
MPV (fL)†	9.4±1.1	9.3 (7.4-13.4)
CRP (mg/L)†	51.8±57.9	27.8 (0.6-246.7)
LDH (U/L)†	278.6±98.4	254.0 (141.0-594.0)
Procalcitonin (ng/mL)†	1.5±6.1	0.2 (0.1-53.0)
PO_2 (mmHg)†	65.8±15.1	63.9 (41.9-99.7)
PCO_2 (mmHg)	37.01±4.3	36.7 (30.0-47.0)
SO_2 (%)†	90.5±5.2	90.8 (79.1-98.8)
ADMA (µg/L)†	360±420.8	225.6 (58.6-2684.5)

†: Mean ± SD, ‡: n (%). SD: Standard deviation, WBC: White blood cell, RDW: Red cell distribution width, MPV: Mean platelet volume, CRP: C-reactive protein, LDH: Lactate dehydrogenase, PO_2: Partial pressure of oxygen, PCO_2: Partial pressure of carbon dioxide, $SO_{2\,\%}$: Oxygen saturation percentage, ADMA: Asymmetric dimethylarginine

Table 2. Distribution of serum ADMA according to sex and presence of comorbid diseases

	ADMA (µg/L)β	p
Sex		
Male (n=48)	196.9 (58.64-2684.5)	0.166
Female (n=35)	292.22 (64.4-2096)	
Comorbid disease		
Absent (n=36)	221.5 (64.4-1445.2)	0.869
Present (n=47)	237.7 (58.64-2684.5)	

β: Median (min-max), Mann-Whitney U test, ADMA: Asymmetric dimethylarginine

Table 3. Correlation of ADMA with numerical variables

			Spearman's rho	p
ADMA	-	**Age**	0.095	0.416
ADMA	-	**WBC count**	-0.132	0.261
ADMA	-	**RDW**	-0.391	**<0.001**
ADMA	-	**Platelet count**	-0.178	0.127
ADMA	-	**MPV**	0.182	0.121
ADMA	-	**CRP**	-0.133	0.257
ADMA	-	**LDH**	-0.196	0.092
ADMA	-	**Procalcitonin**	-0.172	0.141
ADMA	-	**PO_2**	-0.007	0.952
ADMA	-	**PCO_2**	0.028	0.809
ADMA	-	**SO_2%**	0.045	0.703

Spearman Rho correlation coefficient, ADMA: Asymmetric dimethylarginine, WBC: White blood cell, RDW: Red cell distribution width, MPV: Mean platelet volume, CRP: C-reactive protein, LDH: Lactate dehydrogenase, PO_2: Partial pressure of oxygen, PCO_2: Partial pressure of carbon dioxide, SO_2 %: Oxygen saturation percentage

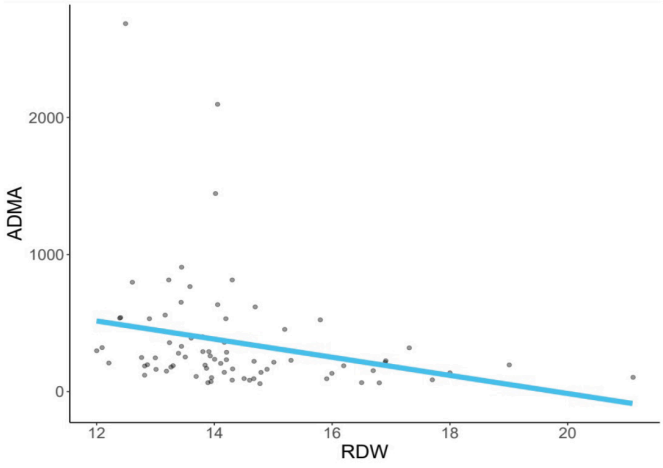

Figure 1. Correlation of ADMA with RDW (p<0.001)
ADMA: Asymmetric dimethylarginine, RDW: Red cell distribution width

Table 5 summarizes the comparison of the two groups as patients (n=83) and controls (n=30). Gender distribution was similar in both groups (p=0.599). The mean age of the patients was 65.0±16.3 years, whereas the age of the participants in the control group was significantly lower (58.2±11.0 years, p=0.013).

There were significant differences between the two groups in terms of erythrocyte distribution width (RDW), platelet count, CRP, LDH, procalcitonin, and O_2% (p<0.001 for all). When age was analyzed as a co-variance factor, CRP remained the only significant variable between the two groups (p=0.002).

Table 4. Association of ADMA with the groups according to upper and lower laboratory ranges of platelet count, CRP and procalcitonin

	ADMA	p
Platelet count (x10³/L)		
<4.5	305.86 (94.46- 652.06)	0.739
4.5-10.5	225.6 (58.64- 2684.5)	
>10.5	207.57 (65.62- 2096)	
CRP (mg/L)		
<5	509.7 (94.9- 907.9)	0.061
≥5	214.68 (58.64-2684.5)	
Procalcitonin (ng/mL)		
<0.12	204.78 (84.48-2684.5)	0.942
≥0.12	225.6 (58.64-2096)	

ADMA: Asymmetric dimethylarginine, CRP: C-reactive protein

The median value of ADMA was 225.6 µg/L in the patient group and 225.6 µg/L in the control group. There was no significant difference noted between the patient and control groups considering the ADMA values (p=0.771). WBC, MPV, PO_2 and PCO_2 were similar between the patients and controls (p>0.05).

Discussion

ADMA is a methylated product of L-arginine and an endogenous inhibitor of NOS, which is a known risk marker for cardiovascular disorders, end-stage renal disease, liver insufficiency and sepsis survival (19-21). ADMA is formed through protein arginine methyltransferase enzyme reaction (PRMT), which occurs in response to hypoxia in the airways (22). Previous studies suggested that elevated levels of ADMA were due to the upregulation of PRMT expression under chronic hypoxia in different clinical issues (23).

The aim of our study was to determine whether plasma concentrations of ADMA were elevated in moderate COVID-19 patients who were followed up in the pandemic wards and whether it could be useful markers in means of helping new treatment approaches. We found no difference in serum ADMA levels between COVID-19 patients and healthy control group. There was no statistically significant correlation observed between ADMA levels and basic inflammation markers (WBC, CRP, LDH, procalcitonin) or arterial blood oxygenation also.

NO maintains endothelial integrity by mediating host defense, contributing to pathogen elimination and vascular tone, which is formed in the endothelium by the activity of NOS. NO is a reactive free radical in the body and is involved in the respiratory disease pathophysiology (6). ADMA levels

Table 5. Comparison of demographic characteristics and laboratory parameters between the groups

	Groups			Covariants age
	Patient (n=83)	**Control (n=30)**	**p**	**p**
Sex‡				
Male	48 (57.8)	15 (50.0)	0.599	-
Female	35 (42.2)	15 (50.0)		
Age (year)†	65.0±16.3	58.2±11.0	**0.013**	-
WBC (x10^9/L)$^\beta$	7.3 (5.4-10.5)	7.8 (5.9-8.7)	0.528	-
RDW (%)†	14.3±1.7	12.8±1.2	**<0.001**	0.739
Platelet count (x10^3/L)†	219.8±76.2	327.8±92.6	**<0.001**	0.545
MPV (fL)†	9.4±1.1	9.2±1.5	0.693	-
CRP (mg/L)$^\beta$	27.8 (12.4-70.8)	3.5 (2.1-4.7)	**<0.001**	**0.002**
LDH†	278.6±98.4	181.4±40.1	**<0.001**	0.091
Procalcitonin (ng/mL)$^\beta$	0.2 (0.1-0.3)	0.0 (0.0-0.1)	**<0.001**	0.444
PO_2 (mmHg)†	65.8±15.1	65.8±6.8	0.976	-
PCO_2 (mmHg)†	37.0±4.3	36.9±3.3	0.884	-
SO_2 (%)†	90.5±5.2	96.0±1.7	**<0.001**	0.137
ADMA (μg/L)$^\beta$	225.6 (145.5-396.0)	243.4 (191.6-352.4)	0.771	-

Independent samples t-test or Mann-Whitney U test. Co-variate analysis for age, †: Mean ± SD, ‡: n (%), $^\beta$: Median (min-max), SD: Standard deviation, WBC: White blood cell, RDW: Red cell distribution width, MPV: Mean platelet volume, CRP: C-reactive protein, LDH: Lactate dehydrogenase, PO_2: Partial pressure of oxygen, PCO_2: Partial pressure of carbon dioxide, SO_2 %: Oxygen saturation percentage, ADMA: Asymmetric dimethylarginine

have been shown to impair NO generation and to induce endothelial dysfunction (12). SARS-CoV-2 infection may be regarded as a vascular disease targeting endothelial cells throughout the body (3). The cardiovascular disorders, hypertension, obesity, and diabetes are the most common pre-existing morbidities in COVID-19 in which the main driver is endothelial dysfunction (3). Endothelial damage makes some COVID-19 patients prone to worse clinical outcomes characterized by thrombotic episodes (24). CRP, as a prototypic marker of inflammation, has been shown to cause downregulation of NOS activity and NO bioavailability in endothelial cells in previous studies (25). As ADMA, CRP has an important role in endothelial dysfunction (26). During the course of bacterial infection, procalcitonin, as a cytokine, amplifies NO production via the expression of inducible NOS (27). In earlier studies conducted by the administration of *Escherichia coli* endotoxin in healthy men, it was hypothesized that ADMA was essential in limiting cytokine-stimulated NO synthesis by iNOS, but it was not proven (28).

To date, less is known about the pathophysiology of SARS-CoV-2 infection, but it resembles that of SARS-CoV infection, with inflammatory responses damaging the airways (29). Although most of the patients do not progress beyond the phase of upper respiratory tract infection, COVID-19 has two major complications: Pneumonia and acute respiratory distress syndrome. The severity of the disease is both due to viral infection itself and host response. Initially, the patients presenting to emergency departments are categorized as “mild”, “moderate”, and “severe” according to symptoms and laboratory findings. During the SARS outbreak in 2003, improvement in lung function was reported in limited patients for whom inhaled NO was used in a pilot study (8). Martel et al. (30), inspired by this experience, provided a comprehensive study to increase airway NO for the prevention and treatment of COVID-19. Their study has stemmed from the hypothesis that NO deficiency is the cornerstone which results from endothelial dysfunction and contributes to thrombus formation, because of the endothelial cell tropism of SARS-CoV-2 virus (31). However, the role of NO in acute infection and inflammation still remains controversial. Nowadays, although many SARS-CoV-2 vaccines have been carried out worldwide, ongoing viral evolution and the substantial mortality due to COVID-19 have caused concern. This prompted an increased interest of scientists for an effective biomarker research. Hannemann et al. (32) analyzed ADMA in hospitalized, critically-ill COVID-19 patients to predict in-hospital mortality. In principal, their study group differed from ours. They included patients who had respiratory failure and global hypoxemia, and they had

shown high ADMA serum concentrations as a predictive biomarker for COVID-19 survival in these patients. They marked this finding in line with aforementioned studies reporting successfully administered inhaled NO in severe COVID-19 treatment.

Increased levels of ADMA were observed in previous studies of patients with sepsis, who were under treatment in intensive care unit (20,21). During this current research, which was performed to identify COVID-19 patients with high mortality risk, elevated levels of ADMA were also reported similarly (32).

They suggest that ADMA accumulation and improper function of endothelial NO synthase and multi-organ failure in critically-ill COVID-19 patients may explain the underlying mechanisms. It is noteworthy that our study setting is different from severe septicemic or critically-ill patients as they have organ-failure, and ADMA rises due to decreased hepatic turnover. The infection resolved in our patient group and no in-hospital death was registered.

Another group, different from our study, found higher ADMA concentrations in COVID-19 patients with lung involvement than in those who had not and in the healthy control group (33).

ADMA response in acute infectious diseases is a subject of debate. NOS is known to produce peroxynitrite, a strong oxidant, which causes organ damage in response to acute infections (34). The role of NO in underlying biochemical mechanisms of infection/inflammation is uncertain. On the one hand it has anti-inflammatory effects on host defense, but on the other hand it may have negative haemodynamic effects.

Zoccali et al. (18) coincidentally found increased serum concentrations of ADMA during the resolution of inflammation when the inflammation markers like CRP declined, but not changed in acute phase of bacterial infection. They suggested that the suppression of ADMA served to raise NO synthesis rather than a decrease, as NO's defensive, anti-microbial protective properties influenced acute phase of infections. Furthermore, cytokines like CRP stimulates the enzyme dimethylarginine dimethylaminohydrolase activity, which metabolizes ADMA, ensuing in suppression of ADMA levels. These findings are in line with our observations. We found no elevated levels of ADMA but found increased levels of CRP as a discriminative predictor in early days of COVID-19 patients. We may speculate that ADMA modulates NO/NOS production in favor of host defense actions versus peroxynitrite-dependent oxidative-stress.

Priorly, the increase of CRP levels was attributed to mostly bacterial infections but high levels were also reported in H1N1 influenza and SARS-CoV-2 infections (35,36). Elevated levels of CRP, as an indicator of deterioration, in hospitalized COVID-19 patients have been associated with cardiovascular complications, which are demonstrated in several studies from the early days of global pandemic (17).

Study Limitations

The study has several limitations. Firstly, it was a single-center study with a small number of patients. The individuals in the healthy control group we selected were younger than the COVID-19 patients, the main reason for which was the curfew for >65 year-old individuals. As we performed statistical analysis of covariance for age, no difference was found in ADMA levels according to age for the groups. We could have measured ADMA levels after infection subsided and note the interchange of this molecule. RDW, platelet count, CRP, LDH, procalcitonin and oxygen saturation % levels of the patient group were higher than those of the control group ($p<0.001$). However, only CRP was found to be the significant variable for COVID-19 patients after the analysis of covariance ($p=0.002$). This accentuates the value of CRP in SARS-CoV-2 infection in line with the literature. No association was shown between ADMA and commonly used parameters, except an inverse correlation with RDW ($p<0.001$).

Conclusion

Our data imply that ADMA concentrations do not rise in the acute setting of moderate COVID-19 patients. This may be attributed to its effect on NO synthesis, as NO has antimicrobial properties in host defense. CRP reflects the inflammatory response in patients with COVID-19, the disease in which pathogenesis is still incompletely understood. Future observations may shed light on the novel biomarkers which could help possible treatments for this lethal disease.

Ethics

Ethics Committee Approval: The study was conducted in a tertiary care center after Institutional Ethics Committee approved the research plan on April 22, 2020 (no: 2737) and performed in accordance with the principles of Declaration of Helsinki.

Informed Consent: Written informed consents were taken from the participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: M.A.Ö., Design: M.A.Ö., Data Collection or Processing: M.A.Ö., Z.M.Y., Analysis or Interpretation: M.A.Ö., M.İ.B., I.K.A., Drafting Manuscript: M.A.Ö., Z.M.Y., I.K.A., Critical Revisation of Manuscript: M.A.Ö., M.İ.B., Z.M.Y., Technical or Material Support: M.A.Ö., M.İ.B., I.K.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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