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Evaluation of endothelial dysfunction and inflammation in recovered COVID-19 patients

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ABSTRACT

Aims: In our study, we aimed to evaluate the endothelial functions and hemogram parameters, which are considered as inflammation markers, in young people with a history of COVID-19 infection.

Methods: This prospective study included 109 recovered COVID-19 patients and 50 healthy controls. Demographic characteristics, laboratory values and flow-mediated vasodilation test (FMD) results of the groups were compared.

Results: Demographic and biochemistry parameters of the groups were similar. The calculated FMD values were significantly lower in the recovered COVID-19 patient group compared to the control group (8.66±3.31 vs 11.69±3.01; p=0.001). While there was no difference between the groups in terms of neutrophil/lymphocyte ratio (NLR) and Platelet/Lymphocyte ratio (PLR), systemic immune-inflammation index (SII) was found to be higher in the patient group with recovered COVID-19 patients (p=0.02). In correlation analysis, there was a low moderate negative correlation between FMD and SII (r=-0.35, p=0.002).

Conclusion: FMD measurement and SII are simple, easily accessible parameters that can be useful in the early period to evaluate cardiovascular risks in the long term after COVID-19. There is a need for larger and multicenter studies on this subject.

Keywords: Endothelial dysfunction, COVID-19, inflammation.

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a novel viral pneumonia that evolved into a pandemic within three months of the first confirmed cases. Although COVID-19 primarily affects the lungs, cardiovascular involvement has also been widely reported.[1] Direct myocardial cell damage, myocardial oxygen supply/demand mismatch, acute plaque ruptures leading to acute coronary syndrome as part of systemic inflammation, and catecholamine surges, and increased thrombosis have been reported as cardiac manifestations. Some of these are directly caused by the disease, while others are associated with potential side effects of drugs used in the treatment of COVID-19.[2,3]

Although endothelial dysfunction and inflammation play an important role in the initiation and progression of atherosclerotic disease, the biochemical and cellular events that initiate atherosclerosis and lead to its progression are not fully understood. During the acute phase of COVID-19 infection, a cytokine storm and subsequent endothelial damage and thrombosis are involved in the pathogenesis of cardiovascular complications.[4] However, few studies have focused on endothelial dysfunction in patients recovering from COVID-19.

Flow-mediated dilatation (FMD) is a non-invasive method that allows accurate assessment of the function of vascular endothelial cells. Decreased FMD, indicating endothelial dysfunction, is a predictive factor for major vascular complications, including cardiovascular disease.[5]

The systemic immune-inflammation index (SII), which is calculated by using platelet, neutrophil, and lymphocyte counts together, is a much more important marker of inflammation and immune response. Studies have shown that high SII values are associated with disease severity and poor prognosis in many diseases.[6,7]

Measuring endothelial function, in addition to markers of myocardial damage and pulmonary function in

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patients recovered from COVID-19, may be a possible tool for early detection of vascular sequelae after COVID-19. The aim of this study was to assess the risk of endothelial dysfunction and preatherosclerosis in young people with a history of COVID-19 infection by measuring FMD and investigating inflammation parameters.

METHODS

This prospective study was carried out with the permission of Kayseri City Hospital Clinical Researches Ethics Committee (Date: 10.02.2022, Decision No: 2022/578). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study included patients aged 18-40 years who presented to cardiology outpatient clinic of our hospital between 01.10.2022 and 05.05.2022 dates and had history of confirmed COVID-19 by laboratory data. Patients who have passed at least 3 months after COVID-19 infection were included. Healthy individuals aged 18-40 years without chronic disease and without COVID-19 infection were included as the control group. Exclusion criteria were known cardiovascular disease, hyperlipidemia, hypertension, diabetes mellitus, chronic renal failure, thyroid disease, rheumatologic disease, malignancy, obesity, active infection, smoking, and alcohol use.

Data on age, gender, body mass index (BMI), vital signs such as blood pressure (BP) and heart rate, biochemical parameters, and hemogram parameters were filled in detail in the relevant sections of the case report form. Blood samples were taken for hemogram and biochemistry parameters during admission. Venous blood draws were performed following vascular measurement. Neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and systemic immune-inflammation index score (platelet × neutrophil/lymphocyte counts) were calculated according to laboratory results.

Flow-mediated Vasodilation Test (FMD)

FMD measurement was performed by the ultrasonographic measurement technique of the brachial artery. The systolic and diastolic blood pressures of the patients were measured after 10 minutes of rest before the FMD measurement. Patients were placed in a comfortable supine position, and the brachial artery was palpated in the longitudinal plane just above the antecubital fossa. The transducer was placed over the right brachial artery tracing, and the brachial artery was visualized longitudinally in the area where there was no tortuosity and the best view was obtained. The diameter of the brachial artery was measured three times from intima to intima, and the average of these measurements was recorded as the basal diameter (BD). These measurements from the brachial artery were taken at the end of diastole, according to ECG monitoring. The cuff of the sphygmomanometer was placed on the upper part of the right antecubital fossa to create a brachial artery flow stimulus. After baseline measurements were recorded, the cuff pressure was increased to 50 mmHg above the patient's systolic blood pressure for complete cessation of arterial flow, and the cuff was held in this position for 5 minutes after antegrade blood flow was interrupted. Antegrade blood flow was interrupted, and ischemia was created. After the cuff was lowered, 2-dimensional images of the brachial artery in the longitudinal plane were taken until 60 seconds later. The mean of three different measurements was recorded as the post-flow brachial artery lumen diameter (endothelium-dependent vasodilator response-EBVR). FMD was expressed as a percentage increase relative to baseline vessel diameter (BC). Endothelium-dependent dilatation was calculated by the equation FMD="(EBVR-BC) \BC"X100.

Statistical Analysis

The categorical variables are expressed as percent while continuous variables are expressed as mean±standard deviation. The categorical variables were compared using the Chi-square test. The normal distribution of continuous variables were tested using Kolmogorov-Smirnov test and histograms. The variables with normal distribution was assessed using Student's t test while those with skewed variables were assessed using Mann Whitney U test. Associations between FMD and SII, Hs-CRP, NLR, PLR were analyzed using Pearson or Spearman correlation. All statistical analyses were performed using SPSS version 22.0 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL). A p value<0.05 was considered as statistically significant.

RESULTS

A total of 159 people were included in the study, including 109 patients who had recovered COVID-19 in the patient group (group 1) and 50 in the control group (group 2). The mean age of the group with COVID-19 infection was 30.86±6.55 years, and %48.3 of the patients were male. The groups had similar characteristics in terms of gender and mean age, and no statistically significant difference was observed. There was no difference in BMI between the groups. There was no significant difference between the 2 groups in terms of systolic blood pressure (SBP), diastolic blood pressure (DBP), and resting heart rate (**Table 1**).

Table 1. Demographic and laboratory variables of the study population

Variables	Recovered COVID-19 (n=109)	Control group (n=50)	P
Age (years)	30.86±6.55	30.02±7.69	0.54
Male/Female (n (%))	48.3	48.6	0.97
SBP (mmHg)	110 (90-150)	110(80-140)	0.48
DBP (mmHg)	70 (50-90)	70 (50-80)	0.23
Heart rate (BPM)	76.86±8.46	77.65±9.96	0.62
BMI (kg/m²)	24.15±2.90	23.91±2.68	0.130
Fasting plasma glucose (mg/dl)	90 (58-110)	86 (62-106)	0.108
Creatinine (mg/dl)	0.70 (0.30-1.20)	0.80 (0.30-1.10)	0.53
AST	19.38±5.92	19.20±6.26	0.86
ALT	20.21±5.37	20.14±5.77	0.91
LDL	96.12±31.21	95.26±29.19	0.487
HDL	32.04±20.84	34.93±21.21	0.109
Triglyceride (mg/dl)	239.92±51.82	192.7±60.43	0.422
Total cholesterol (mg/dl)	152.28±44.98	147.14±43.23	0.184
Hs-CRP (mg/dl)	1.73 (0.69-9.17)	1.67(1.18-7.2)	0.345
FMD (%)	8.66±3.31	11.69±3.01	0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; HDL, high density lipoprotein; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; FMD, Flow-Mediated Dilation

There were no differences between the groups in terms of glucose, creatine, AST/ALT, and lipid profiles (Table 1). Hs-CRP values were similar between the groups. The comparison of hematologic data for both groups is shown in Table 2. Hemoglobin, white blood cell count, platelet count, and mean platelet volume were similar in both groups. In addition, neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) did not differ between the groups, while systemic immune-inflammation index (SII) was higher in the patient group with COVID-19 infection (p=0.02) (Table 2).

Table 2. Hemogram variables of the study population

Variables	Recovered COVID-19 (n=89)	Control Group (n=50)	P
WBC (×10³/µl)	7417.04±1725.48	7203.42±1035.85	0.54
Neutrophil (K/ml)	4702.27±1429.73	4360.00±985.54	0.24
Lymphocyte (K/ml)	1950 (400-3800)	2100 (900-3700)	0.55
Hemoglobin (g/dl)	14.41±1.69	13.84±1.39	0.10
Platelet (K/ml)	233.50 (113.00-501.00)	243.00 (123.00-371.00)	0.46
MPV	10.05 (8.40-12.10)	10.00 (7.90-11.70)	0.56
NLR	2.40 (0.90-9.10)	2.10 (1.10-6.70)	0.13
PLR	143.25 (50.9-485.50)	116.80 (58.50-332.20)	0.09
SII	582.25 (58.60-2834.70)	440.30 (61.80-1699.30)	0.02

MPV, mean platelet volume; NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio; SII, systemic immune- inflammation index; WBC, white blood cell

The calculated FMD values were significantly lower in the patient group with COVID-19 infection compared to the control group (8.66±3.31 vs. 11.69±3.01; p=0.001). In correlation analysis, there was a low moderate negative correlation between FMD and SII (r=-0.35, p=0.002). There was a weak negative correlation between FMD and CRP (r=-0.251, p=0.036). There was a weak negative correlation between FMD and NLR and PLR (Table 3).

Table 3. Correlation analysis between FMD and other variables in the patient group

	FMD	
Variables	r	p
SII	-0.35	0.002
Hs-CRP	- 0.251	0.036
NLR	-0.238	0.045
PLR	-0.280	0.029

NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio; SII, systemic immune- inflammation index, hs-CRP high-sensitivity C-reactive protein, FMD, Flow-Mediated Dilation.

DISCUSSION

In our study, FMD values caused by endothelium-dependent reactive hyperemia were significantly lower in patients who recovered from COVID-19 infection compared to the control group. SII, which is predicted as a marker of inflammation, was found to be higher in patients who recovered from COVID-19 infection compared to the control group, and SII values were shown to be independently associated with decreased FMD.

Myocardial damage and heart failure due to both arrhythmic and ischemic complications have been reported in COVID-19 patients.[8,9] In addition, more severe forms have been shown to be associated with a high risk of stroke and thromboembolism.[10] However, cardiovascular risk may persist beyond the acute phase. Abnormalities were found by echocardiography and cardiac MRI in patients who recovered from COVID-19 but subsequently had a high rate of arterial and venous events.[11,12] However, despite the evidence, the mechanisms underlying these acute and post-acute manifestations of COVID-19 are still under investigation.

Recent epidemiological data show that the increased risk of arterial and venous thrombotic events persists for up to 12 months after recovery, even in non-hospitalized patients.[13] Several mechanisms have been proposed in the pathogenesis of this, including immune activation, persistent SARS-CoV-2 infection, reactivation of latent viruses, prolonged inflammation, and intense cardiopulmonary deconditioning.[14-18] Approximately 2.5% of recovered COVID-19 patients reported arterial and venous thrombosis events 30 days after discharge.[19]

However, it is not unreasonable to argue that COVID-19 has been an endothelial disease since the first moments of the pandemic.

FMD has been widely accepted as an accurate and non-invasive method for the clinical assessment of endothelial function, providing important prognostic data beyond traditional cardiovascular risk factors. Each 1% absolute increase in FMD has been shown to be associated with a 12% to 13% reduction in CV events.20,21 Endothelial dysfunction is a predictor of subclinical atherosclerosis and subsequent long-term cardiovascular events.[22,23] Therefore, early detection reduces the incidence of cardiovascular events, allowing for the combating of adverse events. Endothelial dysfunction has been shown in studies with low FMD values in COVID-19 patients in the acute phase compared to controls.[24] In the study conducted by Çiftel et al.[25] impaired endothelial function was detected with FMD in children diagnosed with COVID-19. While Oliveira et al.[24] showed endothelial vascular dysfunction with FMD at the early stage of the disease in patients hospitalized due to COVID-19, Ambrosino et al.[26] found improvement in FMD after discharge with pulmonary rehabilitation in patients recovering from COVID-19. In our study, we found decreased FMD values in patients without chronic disease and recovered from COVID-19.

Increased neutrophils indicate activation of inflammation, and lymphopenia is an indicator of physiologic stress. NLR indicates the balance between neutrophil and lymphocyte counts and can be considered a measure of systemic inflammation as well as the response to stress. In addition, neutrophils can cause hypercoagulability and are associated with reperfusion injury.[27] It is clear that COVID-19 infection causes severe stress, and it is plausible that it causes a decrease in the number of lymphocytes. Platelets play an important role in hemostasis, a physiological response that occurs to prevent extravasation of blood when vascular damage occurs. They also have both an inflammatory effect and activate the immune system by releasing chemokines and cytokines.[27] Xue et al.[1] showed a positive correlation between SII and COVID-19 severity in their study. Fois et al.[2] found that SII can be used as a biomarker for increased mortality. Salman et al.[3] showed that increased SII levels led to more marked progression and increased intubation and mortality rates. As a result of our study, we concluded that SII obtained from complete blood counts of COVID-19 patients at the time of hospital admission can predict disease progression, disease severity, and mortality in accordance with the literature.

The European Society of Cardiology (ESC) recommends noninvasive procedures, including FMD, for clinical assessment of endothelial function to monitor the risk of long-term cardiovascular complications in the follow-up of recovered COVID-19 patients.[28] The limitations of our study are that it is not multicenter and the number of patients participating is not large.

In the light of the results of our study, we think that FMD, which is a reliable and cost-effective procedure, can be used for cardiovascular system evaluation after COVID-19 in accordance with this recommendation.

CONCLUSION

In our study, we showed that FMD, a marker of endothelial dysfunction, decreased as an indicator of early atherosclerosis in patients who had COVID-19 and recovered. While COVID-19 causes widespread cardiovascular involvement in the acute disease picture, evidence now suggests that patients recovering from COVID-19 also have an increased cardiovascular risk. However, the long-term consequences for healthy survivors are still unclear. Detecting changes at an early stage with easily accessible parameters and determining treatment follow-up protocols accordingly may be useful to reduce cardiovascular disease in the future. Larger-scale, long-term studies are needed for this.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kayseri City Hospital Clinical Researches Ethics Committee (Date:10.02.2022, Decision No: 2022/578).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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