Evaluation of endothelial dysfunction in COVID-19 with flow-mediated dilatation

ABSTRACT

Aim: It is a well-known fact that inflammation plays a crucial role in many diseases including COVID-19. Using flow-mediated dilatation (FMD), we aimed to compare the effects of inflammation on endothelial dysfunction in patients with COVID-19 and the control group.

Materials and Methods: The present study was conducted on a total of 161 participants, of whom 80 were diagnosed with COVID-19 within the last 6 months (comprising 48 women and 32 men with a mean age of 32.10 ± 5.87 years) and 81 were healthy controls (comprising 45 women and 36 men with a mean age of 30.51 ± 7.33 years). We analyzed the findings of transthoracic echocardiography and FMD in all participants.

Results: Except for FMD, there was no statistically significant difference in echocardiographic parameters. (9.52 ± 5.98 vs. 10.53 ± 6.31, p=0.010). In multivariate analysis with the forward stepwise model, FMD was significantly different in the control group compared to the COVID group (1.086 (1.026 - 1.149), p=0.04). Spearman's correlation test indicated that FMD (r=0.27, p=0.006) had a significantly positive correlation with the presence of COVID. A receiver operating curve analysis revealed that an FMD value of <10.62% was capable of predicting the presence of COVID with a sensitivity and specificity of 64% and 59%, respectively (AUC=0.625, 95% CI, 0.538 - 0.711).

Conclusion: The value of FMD decreased significantly in COVID-19 patients compared to the healthy subjects, which may be an early marker for COVID-19 induced endothelial dysfunction.

KEYWORDS: COVID-19, endothelial dysfunction, flow-mediated dilatation (FMD).

INTRODUCTION

A new type of coronavirus disease emerged in December 2019 and called COVID-19 by WHO, primarily infects the respiratory tract and has spread rapidly around the world.[1]

As a member of RNA viruses capable of rapidly mutating and recombining, coronaviruses are known to primarily infect the respiratory tract or intestinal tract in humans and animals.[2] Coronaviruses enter the host cell by binding to the zinc peptidase angiotensin-converting enzyme 2, a surface molecule found in the endothelial cells of arteries and vessels, respiratory tract epithelium, arterial smooth muscle, small intestinal epithelium, and immune cells.[3-5]

Endothelial activation and dysfunction develop as a result of the infection of endothelial cells with COVID-19.[6] They lead to increased levels of pro-inflammatory cytokines (tumor necrosis factor-alpha, interleukin-1, and interleukin-6), chemokines (monocyte chemoattractant protein-1), von Willebrand factor (vWF) antigen and activity, anti-hemophilic factor (AHF), and acute-phase reactants (IL-6, C-reactive protein, and D-dimer).[6]

Although COVID-19 primarily affects the upper and lower respiratory tracts, vascular endothelium is the other known target. Endothelial dysfunction may be caused directly by the activity of the virus or by the resulting systemic inflammatory response. One of the different approaches for the evaluation of endothelial dysfunction is flow-mediated dilatation (FMD), a widely used and non-invasive method in recent years for its simplicity, cost efficiency, and lack of intervention.[7] Several studies have addressed the effect of FMD on various inflammatory diseases such as rheumatoid arthritis, peripheral vascular disease, coronary artery disease, diabetes mellitus, and hypertension.

In this study, we attempted to investigate the potential use of FMD in patients with COVID-19 and the control group.

METHODS

This single-centered study was carried out at Abant Izzet Baysal University Training and Research Hospital between October 2020 and February 2021. The study comprised 80 participants diagnosed with COVID-19 within the last 6 months and 81 control subjects, with an age distribution of > 18 and < 45 years.

Based on the Diagnosis and Treatment Plan of COVID-19 by the National Health Commission (7th edition), COVID-19 cases were classified into four clinical types: the mild (characterized by mild clinical symptoms without pneumonia on radiological imaging), common (characterized by fever, involvement of respiratory tract, and other symptoms with pneumonia on radiological imaging), severe (characterized by respiratory distress, a respiratory rate of ≥ 30 times/min, oxygen saturation of ≤ 93% at resting, PaO2/FiO2 of ≤ 300 mmHg), and the critical (characterized by a respiratory failure requiring mechanical ventilation, shock and another organ failure which necessitate monitoring and treatment at an intensive care unit).[8]

Lung involvement was classified using the "total severity score (TSS)" based on an assessment of chest computed tomography (CT) imaging. For this purpose, the percentages of involvement calculated for each of the five lobes were converted to one of the following score categories: none (0%) (Score 0), minimal (1-25%) (Score 1), mild (26-50%) (Score 2), moderate (51-75%) (Score 3), and severe (76-100%) (Score 4). Finally, the addition of all these scores yielded TSS value, ranging from 0 to 20.[9]

None of the COVID-19 patients had a serious infection that requires hospitalization. In our study, the patients belonged either to the mild type or the common type according to the clinical classification, with TSS scores ranging from 0 to 5.

We recorded demographic data and laboratory parameters of all participants. The exclusion criteria were as follows: age > 50 years, any presence of coronary artery disease, left ventricular heart failure (EF < 50%), moderate to severe valvular disease, congenital heart disease, atrioventricular conduction abnormality, moderate to severe kidney or liver disease, thyroid disease, electrolytic imbalance, systemic inflammatory disease or poor acoustic echocardiography window. The study protocol having been approved by the Local Ethics Committee, informed consent was obtained from each subject before participation.

Echocardiographic evaluation

We used a 4-Mhz transducer of Vivid S6 (GE Vingmed, N-3191 Horten-Norway) to perform the required echocardiographic procedures.

All echocardiographic images were obtained using continuous ECG monitoring by a single-blind cardiologist with the participants in the left lateral position. We took into account the

mean of three consecutive cardiac cycles and measured left ventricular end-diastolic and end-systolic diameters, left ventricular posterior wall thickness, left ventricular septum thickness, and left atrium diameters. A modified Simpson's rule was applied for measuring left ventricular ejection fraction. We performed two-dimensional and pulsed Doppler measurements based on the American Society of Echocardiography criteria.[10]

Ultrasonographic evaluation

Parameter measurements were carried out in a quiet, dark, and air-conditioned room (i.e. with an ambient temperature of 22 - 25°C) after a rest period of at least 15 minutes. In addition, subjects were asked to fast and to avoid exercising, smoking, consuming alcohol or caffeine for at least 8 hours before FMD measurements. We used a 7.5 MHz linear array transducer (GE Healthcare, M4S-RS, Tokyo, Hino-Shi, Japan) to measure the brachial artery diameter at the antecubital fossa. The skin was marked with a pencil, and thus all measurements were performed on the same line. We started with the basal diameter and flow rate of the brachial artery, and then increased the pressure up to 50 mmHg above systolic blood pressure, and waited for 5 minutes at this level; so the arm remained ischemic. Then cuff pressure was lowered, and the diameter and flow rate of the brachial artery were measured again at 1 minute after the pressure decrease.

FMD was calculated using the following equation:

FMD=100 x (maximum diameter at the 1st minute -baseline diameter)/baseline diameter.[11]

Statistical analysis

All statistical analyses were performed using SPSS 18.0 Statistical Package Software for Windows (SPSS Inc., Chicago, IL, USA). The data are presented as the mean ± standard deviation (SD) for quantitative variables and as numbers or percentages for qualitative variables. In order to analyze differences between independent groups, we used Student's t-test for normally distributed quantitative variables, Mann-Whitney's U-test for variables without normal distribution, and Chi-square test for qualitative variables. Spearman correlation analyses were conducted to evaluate correlations between COVID-19 and lymphocyte level, neutrophil/lymphocyte ratio, glucose and creatinine levels, and FMD. As independent predictors of COVID-19, the value of different baseline characteristics were

analyzed using multivariate logistic regression. For variables found to be significant in the univariate analysis, we employed multivariate logistic regression with the forward stepwise model in order to establish the independent prognostic factors of COVID-19. Receiver operating characteristic curves showed the ability of FMD to diagnose COVID-19. All results were considered statistically significant at the level of $p \leq 0.05$.

RESULTS

There was no significant difference between the two groups in terms of demographic data. We didn't find any statistically significant difference in laboratory parameters except the levels of glucose (93.50 (72 - 189) vs. 91 (71 - 112), p=0.038) and creatinine (0.78 (0.61 – 1.17) vs. 0.74 (0.57 – 0.96), p=0.042), lymphocyte counts (1.83 (0.48 – 5.83) vs. 2.24 (0.75 – 3.43), p=0.017), and neutrophil/lymphocyte ratio (2.05 (0.45 – 22.37) vs. 1.30 (0.75 – 8.72), p=0.044) (Table 1).

TABLE 1 Demographic and laboratory variables of the study population

Variables	COVID (n: 80)	Control Group (n:81)	P
Demographics			
Age (years)	32.10 ± 5.87	30.51 ± 7.33	0.407
Male/Female (n(%))	32/48 (40/60 %)	36/45 (44/56 %)	0.313
SBP (mmHg)	105 (80-145)	110 (80-140)	0.307
DBP (mmHg)	70 (50-95)	70 (40-100)	0.343
BMI (kg/m^2)	25.63 ± 3.74	25.00 ± 4.13	0.198
Laboratory parameters			
Fasting Plasma Glucose (mg/dL)	93.50 (72-189)	91 (71-112)	0.038
Creatinine (mg/dL)	0.78 (0.61-1.17)	0.74 (0.57-0.96)	0.042
Hemoglobin (g/dL)	14.20 (10.10-16.72)	14.40 (12.00-17.80)	0.875
Hematocrit (%)	42.30 (32.30-50.90)	42.90 (35.90-50.90)	0.851
Platelet counts (K/uL)	250 (134-459)	263 (126-372)	0.659
Lymphocyte counts (K/uL)	1.83 (0.48-5.83)	2.24 (0.75-3.43)	0.017
Neutrophil counts (K/uL)	4.15 (1.45-17.00)	3.82 (1.60-7.92)	0.291
Neutrophil/ lymphocyte ratio	2.05 (0.45-22.37)	1.30 (0.75-8.72)	0.044

Abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index.

Table 2 shows the symptoms of COVID-19 patients. Whereas the most common symptom was myalgia (65%), sweating (8%) was the least common symptom.

TABLE 2 Symptoms of COVID patients

Symptoms	Number	%
Myalgia	52/80	65
Loss of smell and/or taste	49/80	61
Weakness	33/80	41
Headache	32/80	40
Caugh	28/80	35
Fever	25/80	31
Dyspnea	19/80	24
Throat ache	15/80	19
Nausea	15/80	19
Diarrhea	13/80	16
Sweating	7/80	8

Table 3 shows the parameters of transthoracic echocardiography and FMD in participants. We found no statistically significant difference in echocardiographic parameters except FMD (9.52 ± 5.98 vs. 10.53 ± 6.31, $p=0.010$).

TABLE 3 Echocardiographic measurements of the study population

Variables	COVID (n: 80)	Control Group (n: 81)	P
Left atrium diameter (cm)	3.03 ± 0.5	2.92 ± 0.32	0.332
LVDD (cm)	4.48 ± 0.45	4.45 ± 0.42	0.281
LVSD (cm)	2.80 ± 0.30	2.81 0.29	0.711
PW (cm)	0.96 ± 0.14	0.96 ± 0.13	0.550
IVS (cm)	0.92 ± 0.16	0.90 ± 0.14	0.742
EF (%)	67.27 ± 5.02	65.90 ± 4.64	0.151
FMD (%)	9.52 ± 5.98	10.53 ± 6.31	0.010

Abbreviations: LVDD = left ventricular diastolic diameter; LVSD = Left ventricular systolic diameter; PW = posterior wall; IVS = interventrcular septum; EF = Ejection fraction; FMD = Flow- mediated dilatation.

We performed multivariate analysis for the parameters of glucose, creatinine, lymphocyte and FMD, and neutrophil/lymphocyte ratio, all of which were statistically significant in the univariate analysis. In multivariate analysis with the forward stepwise model, the FMD value was significantly different in the control group compared to the COVID-19 group (1.086 (1.026 – 1.149), p=0.04). (Table 4).

TABLE 4 Independent predictors of COVID by multiple logistic regression analysis

	OR 95%CI	p
Glucose	0.981 (0.957-1.005)	0.116
Lymphocyte	1.022 (0.646-1.616)	0.926
Neutrophil/lymphocyte ratio	0.895 (0.744-1.077)	0.240
Creatinine	0.093 (0.005–1.595)	0.101
FMD	1.086 (1.026-1.149)	0.004

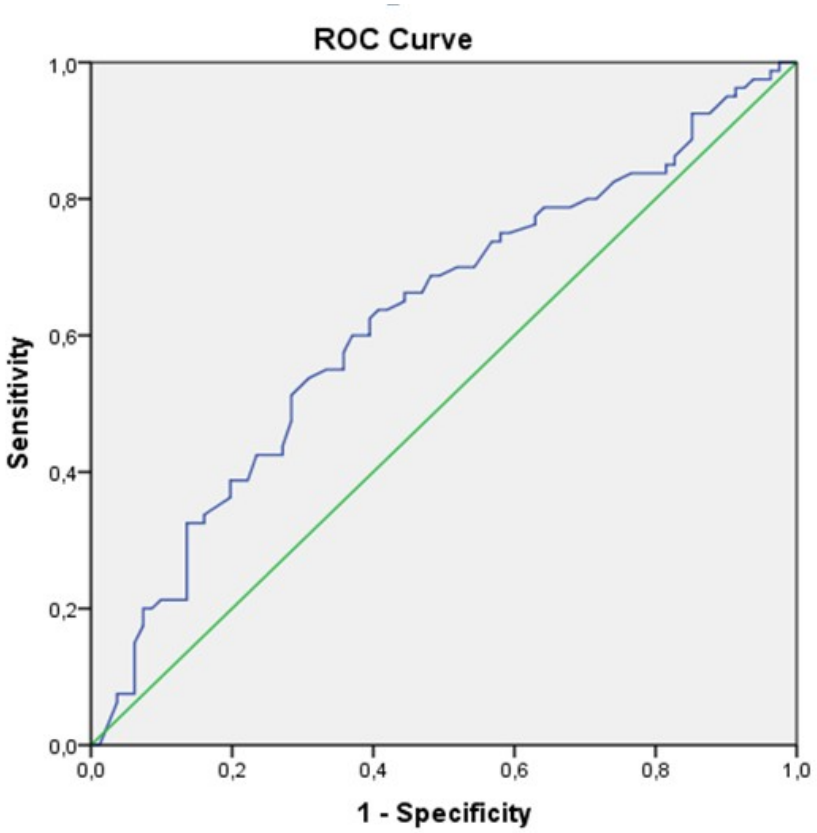
Abbreviations: FMD = flow- mediated dilatation; CI = Confidence interval; OR = Odds ratio.

Spearman's correlation test showed that FMD (r=0.27, p=0.006) had a significantly positive correlation with the presence of COVID-19.

The receiver operating curve (ROC) analysis revealed that an FMD value of <10.62% was able to predict the presence of COVID-9 with a sensitivity and specificity of 64% and 59%, respectively (AUC=0.625, 95% CI, 0.538 – 0.711) (Figure 1).

FIGURE 1 Receiver operating curve (ROC) analysis

FMD value of <10.62 % predicted the presence of COVID with a sensitivity of 64 % and specificity of 59 % (Area Under the Curve = 0.625, 95% CI, 0.538-0.711).



DISCUSSION

Our study demonstrated that the FMD value was lower in COVID-19 patients compared to the control group. Although all patients were mildly affected by COVID-19, this difference in FMD value may indicate that inflammation is, more or less, involved in every stage of the disease. Within the limits of our knowledge, this is the first study to show the possible use of impaired flow-mediated dilatation as an initial sign of endothelial dysfunction. Furthermore, the COVID-19 patients had significantly higher levels of blood glucose and creatinine than those of the control group. Blood glucose levels may rise abnormally in patients under COVID-19 stress, even if they are not diagnosed with diabetes mellitus. Studies show that high levels of blood glucose in COVID-19 patients can predict worse outcomes regardless of a DM history.[12]

It was found in a study that 14.4% of 701 patients had an increased serum creatinine level, 13.1% had a decreased glomerular filtration rate and approximately 5% had acute kidney injury. Kidney disease is associated with increased mortality from COVID-19.[13] Histopathological findings revealed acute tubular injuries, different impairments of the glomeruli, tubular necrosis, and glomerulosclerosis.[14]

Endothelial dysfunction, associated with oxidative stress, is known to be the earliest factor of many diseases.[15] Although inflammation is part of the body's normal repair response to healing and is essential in protecting our body from infections and dangerous environmental substances, it would be overly optimistic to say that it is completely beneficial. When it gets out of control, it can become detrimental and destructive to the body.[16] Likewise, it is known that systemically out-of-control inflammation is associated with adverse outcomes of COVID-19.[17]

The main purpose of our research is to investigate the potential use of FMD as an indirect marker of inflammation and endothelial dysfunction.

In a study where FMD was used in terms of predicting future cardiovascular events in patients who had undergone coronary bypass surgery, the lowest event rate was determined in patients with normal FMD (>8%), while a moderate event rate and the highest event rate were found in patients with a FMD value of 4 to 8% and of <4%, respectively.[18] In another study, patients with an FMD less than 6.2% had significantly lower ankle/brachial index than those with an FMD greater than 6.2%.[19] In addition, Maruhashi et al. showed that FMD had an inverse correlation with Framingham Risk Score, commonly used as a risk calculator and an index of cumulative cardiovascular risk for assessing the probability of a heart attack or death from heart disease within 10 years.[20]

A significant number of COVID-19 patients suffer from severe interstitial pneumonia, acute respiratory distress syndrome (ARDS), and systemic inflammatory response syndrome (SIRS). Anti-inflammatory and immunomodulatory agents typically used as therapeutics in ARDS and SIRS include glucocorticoids, IVIg, cyclosporine, IL-1, and IL-6 inhibition.[21]

Independent predictive factors of mortality from COVID-19 included advanced age, comorbidities such as DM, cardiovascular disease or cancer, and chronic obstructive pulmonary disease at presentation.[22] However, neither infants nor children had a significant increase in both morbidity and mortality during the COVID-19 pandemic.[23]

With the increase of age and age-related diseases, the chronic inflammatory state becomes dominated, and the anti-inflammatory response of the immune system becomes erratic and unable to suppress the inflammatory episode in a timely and effective manner.[24] In our study, we aimed to exclude the effects of such aging-related inflammation by including participants under the age of 45 years.

Endothelial activation and dysfunction result from an uncontrolled immune response in COVID-19. The present study demonstrates the reflection of this uncontrolled immune response using FMD in young patients with COVID-19.

The main limitations of the present study lie in the fact that it is single-centered and conducted on a relatively small number of patients. Another limitation is that the results are limited to a short period, and thus the absence of long-period follow-ups limits the interpretation of the results. Due to the exclusion criteria and age limit, the study population was strictly selected, and therefore the results can not represent all COVID-19 patients.

CONCLUSION

This study has shown that FMD decreased in young patients who were mildly affected by COVID-19 within the last 6 months. Therefore, this parameter may be used as an early marker for COVID-19 induced endothelial dysfunction. Undoubtedly, routine cardiovascular monitoring in patients with a history of COVID-19 may prevent future cardiovascular events. In order to better understand the possible cardiovascular effects in these patients, larger-scale studies including long-term follow-up should be considered.

CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTIONS

AKM, HS, IS, and YG participated in the study conception and design. AKM and HS organized the database. AKM, HS, IS, and YG contributed to the data acquisition and interpretation. IS performed the statistical analyses. AKM and IS drafted the manuscript. AKM, HS, IS, and YG performed a critical revision of the article. AKM, HS, IS, and YG approved the submitted and final version of the article.

DATA AVAILABILITY STATEMENT

Data are available on request from all authors.

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