Assessment of Endothelial Dysfunction With Flow-Mediated Dilatation in Myeloproliferative Disorders

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Abstract
Background: Thrombosis is the most important cardiovascular complication of classical myeloproliferative disorders (MPDs). Endothelial dysfunction (ED) is known to play a major role in the mechanism of thrombophilia in MPDs. Methods: Endothelial dysfunction and its associations with other parameters were investigated. A total of 18 patients with polycythemia vera (PV), 24 with essential thrombocytosis (ET), 7 with primary myelofibrosis (PMF), and 30 healthy patients as a control group were included in the study. To assess the ED, flow-mediated dilatation (FMD) measurements were used. Results: The FMD (%) result showing ED was determined as 9.9 (0.0-21.6) in the patients with PV, 7.3 (0.0-30.5) in patients with ET, 7.5 (0.0-18.0) in patients with PMF, and 13.9 (6.2-26.7) in the control group. The FMD (%) was markedly impaired in all patients with MPD compared to the control patients (7.8 [0.0-30.5] vs 13.9 [6.15-26.8], \( P = .02 \)). According to the disease subtypes, FMD (%) was significantly lower in the ET group than in the control group (\( P = .01 \)). Conclusion: Endothelial function was assessed in patients with MPD having FMD and was determined to demonstrate ED. Lower FMD was associated with older age, leukocytosis, thrombocytosis, and thrombosis history.

Keywords
flow-mediated dilatation, endothelial dysfunction, myeloproliferative disorders, risk factors, thrombosis

Introduction
Myeloproliferative disorders (MPDs) are clonal disorders that are characterized by excess proliferation of 1 or more myeloid lineage cells.¹ According to the World Health Organization, classic Philadelphia negative MPDs are classified as polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF).²,³ It has recently been shown that overall survival in patients with PV and ET is affected by advanced age, leukocytosis, and thrombosis.⁴ The incidence of all thrombosis has been reported to be 12% to 19% in patients with PV and 11% to 25% in patients with ET. In PMF, the incidence of fatal and nonfatal thrombosis has been found to be 7.2%. The incidence of nonfatal thrombosis has been reported as 3.8% in patients with PV and 3.3% in patients with ET.⁵-⁸ Thrombosis may be seen both in arteries and in veins and in some cases may involve unexpected regions such as the hepatic, mesenteric, and portal system.⁹ Arterial thrombosis occurs more frequently than venous thrombosis in MPDs.¹⁰

The pathogenesis of thrombosis in MPDs is considered to be multifactorial. However, authors have indicated 2 main mechanisms. One is the prothrombotic phenotype expression of blood cells, and the other is the inflammatory response of host vascular cells to release cytokines and mediators from malignant cells. In other words, overproduction of blood cells and endothelial dysfunction (ED) play a major role in this process. Activated and damaged endothelial cells overexpress adhesion molecules on their surfaces, and these result in the adhesion of thrombocytes and leukocytes on the endothelium.¹¹

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These then cause secretion of thrombogenic and angiogenic peptides from local inflammatory cells. During all these processes, excess production of procoagulant microparticles from activated thrombocytes and endothelial cells is one of the main mechanisms that causes induction of hypercoagulation and thrombosis in patients with PV and ET. Moreover, it has been previously shown that ED may begin and predispose to thrombosis in the preclinical phases of these patients.10

Flow-mediated dilatation (FMD) is endothelial-dependent arterial dilatation caused by increased intravascular blood flow leading to shear stress. Celermajer et al first developed this noninvasive detection of ED by FMD measurement with ultrasound (USG) in 1992.11 Subsequently, Anderson et al showed a correlation of FMD with coronary artery functions detected by invasive methods.12 Later, many studies used FMD for the detection of ED, which is known to be a risk factor for cardiovascular diseases.13 In the first study of patients with MPD in 2001, it was detected that the FMD of patients with PV was significantly impaired compared to the healthy control group.10

Using the FMD method in this study, an assessment was made of the ED of patients with newly diagnosed MPD and its relationship with thrombosis. It was also analyzed whether or not there was any relationship with hematological parameters.

Materials and Methods

Patients

A total of 49 patients who presented at the Outpatients Clinic of the Hematology Department and were newly diagnosed with MPDs between November 2013 and November 2015 were enrolled in this study. A control group was formed of 30 healthy patients who presented at the Outpatients Clinic of the Internal Medicine Department. The patients were classified as 18 cases of PV, 24 cases of ET, and 7 cases of PMF according to the World Health Organization 2008 diagnostic criteria. Thrombosis risk stratification in PV and ET was classified as 2 risk categories: high risk (age >60 years or thrombosis history) and low risk (absence of both risk factors). The control group was formed of 30 healthy patients who did not have diabetes mellitus, hypertension, hyperlipidemia, chronic renal failure, or a history of any cardiovascular disease (CVD). Informed consent was obtained from all participants. The patients included in the study were volunteers. Patients younger than 18 years or older than 75 years were excluded from the study. The demographic and clinical characteristics of the patients were recorded. Comorbidities such as diabetes mellitus, hypertension, hyperlipidemia, CVDs, cerebrovascular events, and smoking status were questioned and recorded. The CVD risk factors are defined as hyperlipidemia, diabetes mellitus, smoking, and hypertension.

The blood pressure (BP) of all the patients was measured in the sitting position after at least 10 minutes rest, and patients with BP >140/90 mm Hg and/or who were using antihypertensive drugs at the beginning of study were accepted as hypertensive. Serum total cholesterol level >200 mg/dL, serum triglyceride level >150 mg/dL, and/or lipid-reducing drug usage of patients was accepted as hyperlipidemia. All patients underwent a detailed physical examination.

Laboratory Investigations

Hemoglobin (Hb) levels, hematocrit (Hct) levels, leukocyte (WBC) count, platelet (Plt) count, lactate dehydrogenase (LDH) levels, JAK-2 mutation status, peripheral blood CD34 count, and bone marrow biopsy results of all patients were obtained from the patient medical records and hospital data.

Flow-Mediated Dilatation Measurements

The ED was assessed by measuring the FMD of the right brachial artery. Ultrasound imaging of the brachial artery was performed using a 5- to 13-MHZ linear transducer with a VIVID 7 (Vingmed-General Electric, Horten, Norway) medical USG machine. After at least 6 hours fasting, all the measurements were taken by the same radiologist with the same USG machine in the same quiet room which was kept at a temperature of 22°C to 25°C. Patients were forbidden from consuming alcohol or caffeine before the measurements. With the patient in a relaxed supine position, the transducer was placed on the right brachial artery trace at 4 to 5 cm above the right elbow, which is the location known to provide the best images. Three consecutive measurements were taken of the brachial artery diameter and the average of those measurements was recorded as the baseline brachial artery diameter. After recording the baseline diameter, the sphygmomanometer cuff was put around the forearm, inflated to 50 mm Hg higher than the baseline systolic BP, and kept at that pressure for 5 minutes. After 5 minutes, the sphygmomanometer cuff was deflated suddenly, and measurements were taken of the brachial artery diameter at 15 seconds, 1 minute, and then 2, 3, 4, and 5 minutes. The average of the highest 3 measurements was recorded. The FMD due to reactive hyperemia induced by inflation and then deflation of a sphygmomanometer cuff around the forearm was measured using the following formula:

\[
\text{FMD} (\%) = \frac{\text{average brachial artery diameter after reactive hyperemia} - \text{baseline brachial artery diameter}}{\text{baseline brachial artery diameter}} \times 100
\]

Statistical Analysis

Statistical analyses were made using IBM SPSS Statistics version 20 software. The conformity of quantitative variables to normal distribution was evaluated using the Kolmogorov-Smirnov or Shapiro-Wilk test, histogram, and P-P plot according to the number of patients. Quantitative variables were given as average (standard deviation) and median (min-max). Qualitative variables were given as frequency and percentile distribution. A value of \( P < .05 \) was accepted as statistically significant.

The comparison of 2 dependent variables of quantitative data was analyzed using the \( t \) test or Wilcoxon paired \( t \) test.
The hematological characteristics of all the groups are shown in Table 1. Although thrombosis history was determined more in JAK2V617F mutation (+) patients than JAK2V617F (−) patients, there was no significant relationship between JAK2 mutation status and thrombosis history ($P > .05$).

### Flow-Mediated Dilatation Measurements
The median FMD result was 7.8 (0.0-30.5) for the 49 patients with MPD and 13.9 (6.15-26.8) for the control group. The patients with MPD had significantly lower FMD results than the control group (Table 3). When the FMD results were evaluated according to the disease subgroups, the median FMD result was 9.9 (0.0-21.6) for patients with PV, 7.3 (0.0-30.5) for patients with ET, 7.5 (0.0-18.0) for patients with PMF, and 13.9 (6.2-26.7) for the control group. The FMD results were similar for each of the disease subgroups, and all were lower than the control group but only to a statistically significant level in the patient with ET group ($P = .016$). The FMD results of all the groups are shown in Table 4. There was a negative correlation between JAK2V617F mutation status and the FMD results in all the disease subgroups but not of a statistically significant level ($P = .180$). In all patients with MPD, the FMD results were significantly lower in patients with a thrombosis history or thrombosis at the time of diagnosis compared to those with no thrombosis ($P = .026$, $P < .05$; Table 5). The thrombosis risk stratification at the time of diagnosis revealed that 11 of the patients with PV were low risk and 7 were high risk, and of the patients with ET, 15 were low risk and 9 were high risk at the time of diagnosis. There was no significant relationship between risk status and the FMD results of the patients with PV and ET ($P > .05$). The FMD result was 6.5 (0.0-29.7) in patients with at least 1 CVD risk factor and 12.8 (6.7-30.5) in patients with no risk factor. It was significantly lower in all patients with MPD with any CVD risk factor ($P = .001$, Table 6). For patients with PV, there was no significant difference in the FMD (%) results according to CVD risk status ($P = .251$). In the patients with PV, the FMD had no relationship with Hb, Hct, WBC count, Plt count, and thrombosis history, but there was a significantly negative correlation with age ($r = −0.500; P = .035$). In the patients with ET, the FMD had no relationship with any hematological parameter. In the patients with PMF, the FMD was only negatively related to thrombosis history ($r = −0.791; P = .034$). In the patients with PV and ET, there was a negative correlation between Hb levels and the following variables was performed using Pearson $r$ analysis for normally distributed data, and Spearman correlation analysis for nonnormally distributed data.

### Compliance With Ethical Standards
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval for this cross-sectional study was granted by Gazi University Faculty of Medicine Ethics Committee (number: 25901600-7580; date: November 11, 2013).

### Results
The study included 49 patients with newly diagnosed MPD, comprising 18 with PV, 24 with ET, 7 with PMF, and a control group of 30 healthy patients. The demographic characteristics of all the patients are shown in Table 1. JAK2V617F mutation was studied in all patients and was determined in 15 (83.3%) patients with PV, 16 (66.6%) cases with ET, and 4 (57.1%) cases with PMF. Of all the patients with MPD, 11 (22.4%) had a thrombosis history and 2 (4%) had thrombosis at the time of diagnosis. The diagnoses of patients with thrombosis were PV in 5 cases, ET in 6 cases, and PMF in 2 cases. Thrombosis was arterial in 10 cases and venous in 3 cases. Of the arterial thrombosis cases, 6 cases had involvement of the coronary arteries, 2 cases the cerebrovascular system, and 2 cases the peripheral arteries. Of the venous thrombosis cases, 2 cases were lower extremity deep venous thrombosis and 1 case was portal venous thrombosis. The thrombosis detected at the time of diagnosis was arterial in one case (coronary artery) and venous in the other case (portal venous thrombosis). In 2 cases of PV and 3 cases of ET, there was a history of bleeding. All the disease groups were similar according to the JAK2V617F mutation status, thrombosis, and bleeding history ($P > .05$). The hematological characteristics of all the groups are shown in Table 2. According to the normal distribution characteristics. The comparison of 2 independent variables of quantitative data was analyzed using the $t$ test and the Mann-Whitney $U$ test. For 3 or more independent variables, variance analysis or the Kruskal-Wallis $H$ test was used. The comparison of qualitative variables was performed using Pearson $\chi^2$ analysis for normally distributed data, and Spearman correlation analysis for nonnormally distributed data.

### Table 1. Demographic Characteristics of All the Groups.

|                         | PV, n = 18 | ET, n = 24 | PMF, n = 7 | Control, n = 30 |
|-------------------------|-----------|-----------|-----------|----------------|
| Age (years)             | 58.28 ± 14.15 | 48.42 ± 16.13 | 57.14 ± 17.72 | 50.70 ± 12.35 |
| Gender                  |           |           |           |                |
| Male (n, %)             | 6 (33.3) | 15 (62.5) | 2 (28.6) | 16 (53.3) |
| Female (n, %)           | 12 (66.7) | 9 (37.5) | 5 (71.4) | 14 (46.7) |
| CVD risk status         |           |           |           |                |
| Risk (+) (n, %)         | 15 (83.3) | 12 (50.0) | 4 (57.1) | – |
| Risk (−) (n, %)         | 3 (16.7) | 12 (50.0) | 3 (42.9) | – |

Abbreviations: PV, polycythemia vera; ET, essential thrombocytopenia; PMF, primary myelofibrosis; CVD, cardiovascular disease.
and Plt count ($P < .05$). In all patients with MPD, the FMD was negatively related to age, WBC count, Plt count, and thrombo-
sis history. In the patients with PV, there was a significant
positive correlation of thrombosis history with age and Plt
count. In the patients with ET, thrombosis history was signif-
icantly negatively related to Hb and Hct. In the patients with
PMF, thrombosis history was positively related to WBC count.
In all patients, there was a significant positive correlation
between thrombosis history and age ($r = 0.414, P = .03$).

**Discussion**

Cardiovascular complications are the most important cause of
morbidity and mortality in patients with MPDs. Of these
complications, thrombosis is the most serious and the most
frequently seen, so it is important clinically to explain the
relationship between thrombosis and disease. There have been
many studies about the pathogenesis of thrombosis in MPDs. It
is generally agreed that the mechanisms are ED, Plt activation,
Hct levels, the effect of erythrocytosis, leukocytosis, and

**Table 2. Hematological Characteristics of All the Groups.**

|                | PV, n = 18 | ET, n = 24 | PMF, n = 7 | Control, n = 30 | P    |
|----------------|------------|------------|------------|-----------------|------|
| Hemoglobin (g/dL) | 17.16 ± 1.86 | 13.93 ± 1.64 | 12.26 ± 1.91 | 14.07 ± 1.33 | .000^a|
| Hematocrit (%)    | 52.9 (40.7-67.0) | 42.0 (29.0-48.0) | 34.1 (31.5-46.8) | 40.3 (12.6-49.9) | .000^b|
| Leukocyte (×10⁹/L) | 10300.0 (5000-15020) | 9720.0 (5000-15020) | 7820.0 (2500-12180) | 6470.0 (4080-9600) | .000^c|
| Platelet (×10⁹/L)  | 499266.67 ± 316663.51 | 854375.00 ± 396164.41 | 339371.43 ± 343302.14 | 228233.33 ± 448888.89 | .000^d|

**Table 3. Comparison of the FMD Results Between the Disease and Control Groups.**

|                | MPD, n = 49 | Control, n = 30 | P    |
|----------------|------------|-----------------|------|
| N = 79         | 49         | 30              |      |
| FMD (%) (median) | 7.8 (0.0-30.5) | 13.9 (6.15-26.8) | .002 |

**Table 4. Comparison of FMD Results Between Disease Subgroups and the Control Group.**

|                | PV, n = 18 | ET, n = 24 | PMF, n = 7 | Control, n = 30 | P    |
|----------------|------------|------------|------------|-----------------|------|
| N = 79         | 18         | 24         | 7          | 30              |      |
| FMD (%) (Median) | 9.9 (0.0-21.6) | 7.3 (0.0-30.5) | 7.5 (0.0-18.0) | 13.9 (6.2-26.7) | >.05 |

**Table 5. Comparison of FMD Results According to Thrombosis Status.**

|                | Negative, n = 36 | Positive, n = 13 | P    |
|----------------|-----------------|-----------------|------|
| N = 49         | Arterial (n = 10) | Venous (n = 3)  |      |
| FMD (%) (median) | 10.6 (0.0-29.7) | 4.0 (0.0-12.5) | 9.4 (5.7-30.5) | .022^a|
| FMD (%) (median) | 10.6 (0.0-29.7) | 5.7 (0.0-30.5) | .026 |

**Table 6. Comparison of FMD Results According to CVD Risk Factor Presence.**

|                | CVD Risk Factor (+) | CVD Risk Factor (−) | P    |
|----------------|---------------------|---------------------|------|
| PV (n = 18)    | 9.82 (6.58)          | 14.90 (7.39)        | .251 |
| ET (n = 24)    | 5.1 (0.0-29.7)       | 12.5 (6.7-30.5)     | .003 |
| PMF (n = 7)    | 4.36 (3.29)          | 16.87 (1.16)        | .002 |
| All (n = 49)   | 6.5 (0.0-29.7)       | 12.8 (6.7-30.5)     | .001 |

Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis.
^aDifference between PV and the other 3 groups.
^bDifference between PV and the other 3 groups.
^cDifference between ET, PV, and control groups.
^dDifference between PV, ET, and control groups.

thrombocytosis on the endothelium and abnormalities of the coagulation cascades. In the current study, the FMD method was used to assess the ED of patients with MPD. To the best of our knowledge, this is the first study assessing ED with FMD in all 3 MPD subgroups (PV, ET, and PMF), respectively. In this method, the vasodilatation response of the brachial artery to shear stress caused by reactive hyperemia is assessed. In literature, this vasodilatation response is generally accepted to be 10% to 20%, while some have suggested 5% to 15% for healthy patients.

In the current study, the measurements revealed that the FMD (%) of all the patients with MPD was significantly lower than that of the control group. This result supports the opinion that ED is present in MPDs. However, in the consideration of disease subtypes, the FMD (%) was statistically significantly lower than that of the control group only in the patients with ET. This result may have been due to the limited number of patients in the other 2 disease groups (PV and PMF). In the first study in which the FMD method was used to assess ED in MPDs, Neunteufl et al reported a lower FMD (%) demonstrating ED in 20 patients with PV compared to a control group. In that study, none of the patients had arterial thrombosis that could have been evidence of ED presence before the emergence of arterial complications. CD40 ligand is a glycoprotein expressed by activated Plt s, which binds CD40 to the endothelial cell surface. CD40 ligand has been reported to play a role in the initial development and prognosis of atherosclerotic lesions. Lessiani et al studied the relationship between FMD (%) measurements and CD40 ligand in patients with PV. According to the results of that study, FMD was markedly impaired and CD40 ligand levels were significantly increased in patients with PV compared to the control group. The only factor independently affecting the CD40 ligand level was found to be FMD (%).

It has been previously explained that the levels of endothelial cell markers, E-selectin, and thrombomodulin have been seen to be higher in patients with thrombosis than in those with no thrombosis. Increased levels of E-selectin may play a role in endothelial activation and thrombosis, so it has been suggested as a marker for thrombotic risk. In another study comparing patients with MPD and a control group, similar to recent study results, it was determined that increased levels of thrombomodulin levels may demonstrate endothelial cell damage. All these results show that there may be endothelial cell activation in patients with MPD, which has importance in the pathogenesis of thrombosis, and measurable markers in the plasma can be used to detect this activation. In the current study, during assessment of ED with FMD, a significantly lower FMD (%) was found in patients with thrombosis history compared to those with no thrombosis. This finding may show that ED in patients with thrombosis is more significant, so it may contribute to thrombosis formation or become evident after thrombosis occurs. To the best of our knowledge, our study is the first to assess ED with FMD in patients with MPD who have thrombosis complications.

The relationship between the pathogenesis of thrombosis and hematological parameters has been studied for many years. In a study in patients with PV, the thrombosis risk was found to be 38-fold higher in patients with Hct levels >60% compared to those with 40% to 44%21. It is still unclear whether Hct levels increase the thrombosis risk via the formation of ED or by another mechanism. Similar to the current study, 2 previous studies reported no relationship of FMD (%) with Hct levels and Plt counts when the FMD method was used to detect ED in patients with PV. In an in vitro assessment of adhesion to subendothelium of Plt s in patients with PV and a healthy control group, there was no difference between the groups. Other studies have also reported no relationship between thrombosis and thrombocytosis. However, leukocytosis has been found to be a thrombosis risk factor for patients with PV and ET in recent studies. It has also been previously shown that there is a positive relationship of thrombosis with WBC count but no relationship with thrombocytosis and JAK2 mutation status. In the current study, the lower FMD (%) was associated with an older age in patients with PV. In the patients with ET, FMD had no relationship with any hematological parameter. The FMD (%) was observed to have a negative correlation with thrombosis history. A lower FMD (%) was seen in all patients with MPD, demonstrating ED, which is associated with older age, thrombocytosis, leukocytosis, and thrombosis history. Similar to these results, in a recent international study of 891 patients with ET, after a median follow-up of 6.2 years, 109 (12%) patients experienced arterial (n = 579) or venous (n = 537) thrombosis. In multivariable analysis, the predictors of arterial thrombosis included age >60 years, thrombosis history, cardiovascular risk factors, leukocytosis (>11.3 × 10^9/L), and the presence of JAK2V617F. In PV, only thrombosis history was found to be a major risk factor for recurrent vacular events. The relationships between thrombosis and leukocytosis have been examined by different groups of investigators with conflicting and inconclusive findings. In the current study, 35 of 49 patients were determined with JAK2V617F mutation, and in all 3 disease subtypes, there was no correlation of mutation status with FMD results and thrombosis history. Thus, no relationship was determined between JAK2 mutation and ED. Therefore, because of these controversial results, the relationship of ED and thrombosis with hematological parameters is still unclear.

Nitric oxide (NO)–related dilating function is very important for endothelial normal function. It was shown before that aging decreased NO-related vasorelaxant function. Several studies suggest that ED in older patients is a progressive condition caused by many different pathophysiological mechanisms. In the current study, lower FMD (%) in all patients with MPDs, demonstrating ED, is associated with older age similar to recent studies. The NO donor, L-arginine, was shown as an effective agent for prevention of anthracycline-induced myocardial injury in a study. The authors suggested that L-arginine is an effective medication for prevention of ED. Endothelial progenitor (EPCs) and
circulating endothelial progenitor cells (CPCs) are also important for normal endothelial function.\textsuperscript{37} The agents that will increase the levels of NO, EPCs, and CPCs might be a potential target in the future to prevent ED. Controlled studies in which these markers analyzed are needed.

It has been previously reported that cardiovascular risk factors markedly impair FMD.\textsuperscript{11,13} In the current study, the FMD (\%) of patients with at least 1 CVD risk factor was significantly lower than that of patients with none. Therefore, this result suggested that ED in MPDs may have a relationship with CVD risk factors. There are conflicting results in literature as to whether or not CVD risk factors have any effect on thrombosis in patients with MPD. The most important study about the relationship between thrombosis and CVD risk factors is the European Collaboration on Low-dose Aspirin in Polycythemia Vera study.\textsuperscript{38} In that study, age and thrombosis history were found to be independent risk factors for thrombosis in patients with PV and ET. It was also reported that smoking and diabetes mellitus affect CVD mortality and prognosis. Another study of patients with ET showed a relationship between vascular risk and hyperlipidemia and others have reported a relationship with smoking.\textsuperscript{39,40}

To conclude, in this study, the endothelial function and the effect of ED on thrombosis were assessed in patients with MPD using FMD, which is an effective and acceptable method. Markedly impaired FMD (\%) demonstrating ED presence was seen in MPDs with thrombosis history. The FMD (\%) was associated with CVD risk, older age, leukocytosis, thrombocytosis, and thrombosis history. The small number of patients in the disease subgroups can be considered a limitation of this study. In addition, FMD (\%) was significantly lower in patients with any CVD risk factors compared to those with none. Therefore, how these CVD risk factors affected the results is not clear. Larger patient population-based studies in the future may provide a more robust assessment of ED independent of CVD risk factors and give more accurate results. Else, measurements of FMD (\%) after therapy at the targeted levels of hematological parameters may allow a comparison of the results with the initial data and show the relationship with Hb, Hct, Plt, and WBC counts.

**Authors’ Note**

A. Yildiz, K. Acar, and M. Gürçüldür made substantial contribution to concept and design; A. Yildiz, M. Gürçüldür, M. Yazol, and S.O. Oktar contributed to analysis and/or interpretation of data; A. Yildiz, K. Acar, and M.S. Pepeler done critical writing or revising the intellectual content; and final approval of the version to be published was made by A. Yildiz and K. Acar.

**Declaration of Conflicting Interests**

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