Evaluation of systemic endothelial functions by flow mediated vasodilatation method in polycythemic individuals

Polisitemik bireylerde sistemik endotelyal fonksiyonların akış aracılı genişletme yöntemi ile değerlendirilmesi

Muzaffer Keklik[1], Uğur Kalan[2], Eyüp Özkan[3], Bahadır Şarlı[3]

1 Kayseri Training and Research Hospital, Department of Hematology

2 Kayseri Training and Research Hospital, Department of Internal Medicine

3 Kayseri Training and Research Hospital, Department of Cardiology

Corresponding Author: Muzaffer Keklik, M.D. Kayseri Training and Research Hospital, Department of Hematology, Kayseri, Turkey. E-mail: muzafferkeklik@yahoo.com

Received: 06/06/2016

Accepted: 20/07/2016

ABSTRACT

Aim: Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases. Flow-mediated vasodilation (FMD) is a non-invasive ultrasound method of evaluating endothelial function. The aim of this prospective study was to compare the systemic endothelial functions by FMD method in patients with polycythemia with control group.

Method: The present study included 50 patients with polycythemia (hemoglobin (Hb) ≥16.5 g/dl) and 61 healthy control group aged ≥ 18 years at Kayseri Education and Research Hospital were included. In all individuals, the following laboratory evaluations were performed: complete blood count (CBC), serum iron indices, and routine biochemical tests. Also, all participants underwent the FMD study at Cardiology department with Doppler ultrasound.

Results: The results of 111 participants were analyzed. No significant difference was found between the groups with regard to age, gender, body mass index, hypertension and diabetes mellitus rates ($p>0.05$). On the other hand, the rate of smoking was 76% in the polycythemia group, while it was 36.1% in the control group ($p<0.005$). Likewise, Hb, hematocrit and serum lipid parameters were significantly increased in polycythemia group except high density lipoprotein (HDL) cholesterol ($p<0.001$). Also, FMD was significantly reduced in polycythemia group compared with control group ($7.78 \pm 4.83\%$ vs. $10.98 \pm 7.86\%$, respectively) ($p= 0.010$).

Conclusion: The results of the study indicate that polycythemia is associated with endothelial dysfunction and hyperlipidemia. As the noninvasive ultrasound method for peripheral endothelial testing is generally accurate and reproducible, and can be performed serially, FMD can be used safely in patients with polycythemia to evaluation of endothelial functions.

Key words: endothelial function; flow-mediated vasodilatation; policythemia

ÖZET

Amaç: Polisitemi, kan volümünün bir parçasını teşkil eden eritrosit miktarındaki artışla karakaterize bir patolojik durumdur. Akış aracılı genişletme (FMD) yöntemi, sistemik vasküler fonksiyonların değerlendirilmesinde giderek önemi artan bir non-invaziv tetkik olup, çeşitli hastalıklarda bu yöntem ile vasküler endotel hakkında önemli bilgiler edinilmiştir. Bu prospektif çalışmada amacımız, polisitemik bireylerde FMD yöntemi ile vasküler fonksiyonları değerlendirmek ve sağlıklı kontrol grubu ile karşılaştırmaktı.

Metod: Bu çalışmaya; Kayseri Eğitim ve Araştırma Hastanesi'ne başvuran 18 yaş ve üzeri, 61 sağlıklı ve Hb değeri normal sınırlarda olan gönüllü ile, 50 polisitemi olgusu alındı. Polisitemi tanısı için Hb>16.5 g/dL baz alındı. Bireylerin tam kan sayımları, demir parametreleri ve rutin biyokimyasal tetkikleri istendi. Katılımcılara Kardiyoloji kliniğinde Doppler ultrasonografi eşliğinde FMD işlemi uygulandı.

Bulgular: Toplam 111 olgunun değerlendirildiği çalışmada, gruplar arasında yaş, cinsiyet, vücut kitle indeksi, hipertansiyon ve diabet oranları bakımından fark yoktu. Buna karşın, sigara içim oranı polisitemi grubunda kontrol grubuna göre anlamlı şekilde yüksekti (%76 ve %36.1, sırasıyla) ($p<0.005$). Hb, hematokrit ve serum lipid parametreleri de yüksek densiteli lipoprotein (HDL) kolesterol dışında polisitemi grubunda anlamlı şekilde yüksekti ($p<0.001$). Ayrıca, FMD oranı polisitemik grupta kontrol grubuna göre anlamlı şekilde düşük bulundu (% 7.78 ± 4.83 ve %10.98 ± 7.86, sırasıyla)($p= 0.010$).

Sonuçlar: Bulgularımızla, polisitemik bireylerde vasküler endotelyal fonksiyonların olumsuz etkilendiği ve hiperlipidemi birlikteliği ortaya konmuştur. Non invaziv ve kolay uygulanabilen FMD yöntemi ile, polisiteminin muhtemel endotelyal disfonksiyon gelişimindeki rolü öngörülebilir.

Anahtar kelimeler: endotelyal fonksiyon; akış aracılı genişletme; polisitemi

INTRODUCTION

Polycythemia is a condition that results in an increased level of circulating red blood cells in the bloodstream. Currently, it is considered when a hemoglobin (Hb) level of greater than 16.5g/dL in men and women (1, 2). Polycythemia can be divided into two categories; primary and secondary. While in the primary polycythemia the increase in red blood cells is due to inherent problems in the process of red blood cell production, the secondary polycythemia generally occurs as a response to other factors or underlying conditions that promote red blood cell production. Recently, flow mediated vasodilatation (FMD) is the most commonly utilized non-invasive assessment of vascular endothelial function. With an ultrasound-based method, arterial diameter is measured in response to an increase in shear stress, which causes endothelium-dependent dilatation (3, 4). There is limited information about FMD study in patients with polycythemia (5-7). In this study, our aim was to compare the systemic endothelial functions by FMD method in patients with polycythemia and healthy individuals.

MATERIAL AND METHODS

In this prospective study, overall 110 data obtained by polycythemia patients and healthy individuals (n = 50, n= 61, respectively) at Kayseri Education and Research Hospital between February 2016 and May 2016. Participants were 18 years old and older. For polycythemia diagnosis, the baseline Hb had to be ≥16.5 g/dL (World health organization (WHO) 2016 criteria). In all participants, laboratory evaluations were performed before beginning FMD application: complete blood count (CBC), serum iron level, total iron binding capacity (TIBC), transferrin saturation (TS), serum ferritin level, and routine biochemical tests. At the time of FMD examination, information regarding coronary risk factors was recorded for each participant and included age, gender, body mass index, smoking status, and comorbidity. Participants smoking at least one cigarette/d for one year within the last 5 years were considered smokers. All blood tests were measured using available routine kits at hospital laboratory. All participants underwent brachial artery ultrasound after withdrawal of all drugs for at least 24 h, and they were investigated by the same operator. Brachial artery FMD was measured with a high-frequency (7.0- 13.0 MHz) ultrasound scanning probe to obtain longitudinal images of the brachial artery at a point 5 to 10 cm proximal to the antecubital fossa (Philips HD11 XE Ultrasound system). Two-dimensional images were obtained at baseline with Doppler ultrasound scanning to assess arterial diameter. Angle correction software was used during Doppler imaging to approximate a 20-degree angle of incidence to blood flow. Increased shear stress was achieved by producing reactive hyperemia. A pressure cuff placed on the right forearm was inflated up to 50 mmHg higher than systolic blood pressure for 5 minutes in order to induce ischemia by occluding arterial flow. Arterial diameter measurements were repeated within 60 seconds of cuff deflation. Arterial diameter was measured from the right arm at the intima-media interface of the clearest echocardiography line.

Images were acquired with electrocardiogram gating, with measurements made in end diastole corresponding to the onset of the R wave. Measurements were reported as % change in diameter. The study approved by the Kayseri Education and Research Hospital Training and Planning Committee (52332816/50- 09.02.2016) and written informed consent was obtained from all individuals.

Statistical analysis

Before the analysis, the continuous variables were evaluated with Shapiro–Wilk test to determine whether the distribution was normal or not and data were expressed as the median (range) (for skewed data) or mean± standard deviation (SD) (normally distributed data).

The results were statistically evaluated according to the independent-samples Student's *t*-test (normally distributed data) and Mann-Whitney U-test (for skewed data). A p value ≤ 0.05 was considered statistically significant. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) computer program version 22.0.

RESULTS

The results of 111 participants were analyzed (50 polycythemia group, 61 control group).

In the polycythemia group, the mean age was 40.28 $\pm$ 16.78 years. In the control group, the mean age was 39.28 $\pm$ 3.72 years. While there were 40 males and 10 females in the polycythemia group, there were 45 males and 16 females in the control group. Baseline clinical and laboratory characteristics of participants are provided in Table 1. There were no differences with regard to age, gender, BMI, hypertension and diabetes rates. On the other hand, percentage of smokers was significantly higher in polycythemia patients than control group (76% vs. 36.1%, respectively) ($p<0.001$). Hb, hematocrit and serum lipid parameters were significantly increased in polycythemia group except high density lipoprotein (HDL) cholesterol ($p<0.001$). No significant differences were observed in serum iron parameters, mean corpuscular volume (MCV) and mean corpuscular Hb concentration (MCHC) values between the groups. Also, there were no statistically significant differences in FMD values between the hyperlipidemic and non- hyperlipidemic groups ($p= 0.460$). Likewise, no significant differences were observed in FMD rates between the smoking and non- smoking groups ($p=0.051$). Figure 1 demonstrates that FMD rates in the polycythemia and control groups. The rate of FMD was 7.78 ± 4.83% in the polycythemia group, while it was 10.98 ± 7.86% in the control group ($p= 0.010$).

Table 1. Characteristics of patients and controls.

Variables	Polycythemia (n= 50)	Control (n= 61)	*P* value
Age, years[a]	40.28± 16.78	39.28 ± 3.72	0.681
Male/Female	40/10	45/16	0.504
BMI, kg/m^{2}[a]	25.3± 3.6	24.8± 2.8	0.638
Hypertension (%)	19 (38)	21 (34.4)	0.843
Diabetes Mellitus (%)	5 (10)	7(11.5)	1
Smoking	38 (76)	22 (36.10)	**< 0.001***
Hemoglobin, g/dl[b]	18.20 (16.80- 21.20)	14.40 (13.50-16)	**< 0.001***
Hematocrit,%[b]	53.60 (47.90-64.70)	42.10 (40.80-44.80)	**< 0.001***
Serum iron, µmol/l	98 (32- 305)	97 (84- 168)	0.090
TIBC,µg/dl[b]	251 (52- 485)	216 (164- 318)	0.571
TS,%[b]	39 (25- 267)	47 (28- 102)	0.093
Serum ferritin,ng/ml[b]	52 (21- 177)	63 (41- 301)	0.670
Total cholesterol, mg/dl[b]	188.50 (83-254)	145 (110-197)	**< 0.001***
Triglyceride, mg/dl[b]	170 (51-545)	108 (81-152)	**< 0.001***
LDL cholesterol, mg/dl[b]	99.50 (13-182)	65 (51-120)	**< 0.001***
HDL cholesterol, mg/dl[b]	43 (29-76)	41 (34-51)	0.184

BMI: Body mass index; TIBC: total iron binding capacity; TS: transferrin saturation; LDL: Low density lipoprotein; HDL: High density lipoprotein

[a] Mean ± standard deviation [b] Median (range) *statistically significant

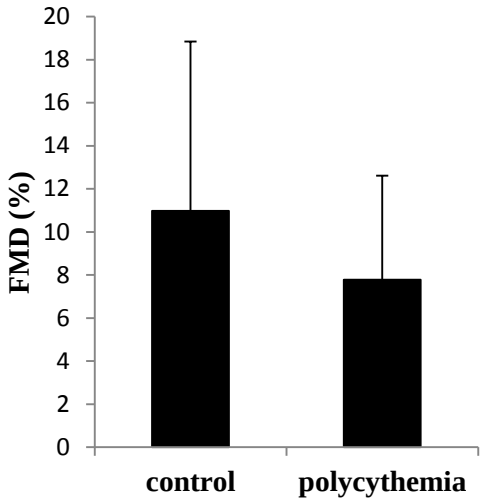

Figure 1. FMD as percentage change in the control and polycythemia groups (p=0.010).

DISCUSSION

Our study demonstrate that endothelium-dependent FMD is markedly impaired in patients with polycythemia than in gender- and age-matched control group. These findings suggest that polycythemia has to be considered as a pathogenic factor for cardiovascular events. Moreover, our results showed that polycythemia is associated with hyperlipidemia and smoking. Serum lipid parameters were significantly increased and percentage of smokers was significantly higher in polycythemic group. On the other hand, no significant differences were observed in FMD values between the smoking and non-smoking groups. In contrast to our study, Clarenbach et al. found that in their study, FMD was significantly impaired in smoking patients (3). Already, in the literature conventional cardiovascular risk factors such as hyperlipidemia, smoking, hypertension and diabetes have been shown to impair FMD in the brachial artery (8-11). Probably endothelial dysfunction has a key role in atherosclerosis and constitutes the first step of cardiovascular disease progression. However, data on potential biological factors underpinning the association between smoking, hyperlipidemia and endothelial function, such as hypoxia, systemic inflammation, oxidative stress and sympathetic activation, are mostly lacking. Likewise, in a few study the authors found that FMD was significantly impaired in patients with hyperlipidemia (8,10). On the other hand, in our study there were not significant differences in FMD values between the hyperlipidemic and non- hyperlipidemic groups. In another study, Manganaro et al. have found decreased FMD rate in coronary artery disease (12). However, there is limited information about FMD in patients with polycythemia. Neunteufl T. et al. showed that polycythemia vera is associated with endothelial dysfunction in their study (5). They found that FMD was impaired in polycythemia vera compared the control group (7.6± 2.9% vs 11.6± 5.7%, p= 0.009). In our study FMD was 7.78 ± 4.83% in the polycythemia group, while it was 10.98 ± 7.86% in the control group (p= 0.010). Our data were in accordance with their findings. Also, Yucel et al. investigated whether regular blood donation is associated with improved endothelial function in healthy adults (6). FMD was improved after blood donation (9.9± 3.8% vs. 10.75± 3.9%) and these findings were statistically significant (p= 0.006). They reported that regular blood donation improves endothelial function. In their study, participants were 18 years old and older healthy males. Patients with hypertension, diabetes mellitus or any other disease affecting any vascular territory were not included. Whereas in our study, we compared the endothelial functions in adult patients with polycythemia with adult control group both males and females. Also, patients with hypertension, diabetes mellitus or hyperlipidemia were included our study. For all that, there were no differences with regard to age, gender, hypertension and diabetes rates between the groups. On the other hand, in our study total cholesterol, LDL cholesterol and triglyceride values were significantly increased in polycythemia group. Already, correlation between increased levels of serum lipid parameters and cardiovascular events has been identified in previous studies (13,14). Also, in polycythemic patients,

hyperlipidemia and impaired endothelial function could potentially lead to increase in the progression rate of cardiovascular diseases.

As a conclusion, in the present study, we found a significant impairment in endothelial functions by FMD method in patients with polycythemia, which is a non-invasive tool for evaluation of vascular endothelial function. Although not yet recommended for routine clinical use, FMD testing has provided valuable insights into vascular changes associated with polycythemia.

REFERENCES

1. Posfai E, Marton I, Borbenyi Z, Nemes A. Myocardial infarction as a thrombotic complication of essential thrombocythemia and polycythemia vera. Anatol J Cardiol. 2016;16:397-402.

2. Lim Y, Lee JO, Kim SH, Kim JW, Kim YJ, Lee KW, et al. Prediction of thrombotic and hemorrhagic events during polycythemia vera or essential thrombocythemia based on leukocyte burden. Thromb Res. 2015;135:846-51.

3. Clarenbach CF, Senn O, Sievi NA, Camen G, van Gestel AJ, Rossi VA, et al. Determinants of endothelial function in patients with COPD. Eur Respir J. 2013;42:1194-204.

4. Pusalavidyasagar S, Sert Kuniyoshi FH, Shamsuzzaman AS, Singh P, Maharaj S, Leinveber P, et al. Comparison of Endothelial Function in Asian Indians Versus Caucasians. Metab Syndr Relat Disord. 2016;12:12.

5. Neunteufl T, Heher S, Stefenelli T, Pabinger I, Gisslinger H. Endothelial dysfunction in patients with polycythaemia vera. Br J Haematol. 2001;115:354-9.

6. Yucel H, Zorlu A, Kaya H, Yilmaz MB. Regular blood donation improves endothelial function in adult males. Anatol J Cardiol. 2015;30.

7. Zheng H, Cable R, Spencer B, Votto N, Katz SD. Iron stores and vascular function in voluntary blood donors. Arterioscler Thromb Vasc Biol. 2005;25:1577-83.

8. Clarkson P, Celermajer DS, Donald AE, Sampson M, Sorensen KE, Adams M, et al. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. J Am Coll Cardiol. 1996;28:573-9.

9. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation. 1993;88:2149-55.

10. Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. Circulation. 2002;105:546-9.

11. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39:257-65.

12. Manganaro A, Ciraci L, Andre L, Trio O, Manganaro R, Saporito F, et al. Endothelial dysfunction in patients with coronary artery disease: insights from a flow-mediated dilation study. Clin Appl Thromb Hemost. 2014;20:583-8.

13. Giannattasio C, Piperno A, Failla M, Vergani A, Mancia G. Effects of hematocrit changes on flow-mediated and metabolic vasodilation in humans . Hypertension. 2002;40:74-7.

14. Faxon DP, Fuster V, Libby P, Beckman JA, Hiatt WR, Thompson RW, et al. Atherosclerotic Vascular Disease Conference: Writing Group III: pathophysiology. Circulation. 2004;109:2617–25.